PROCEEDINGS OF, THE

SOUTHERN AND EASTERN AFRICAN RABIES GROUP INTERNATIONAL SYMPOSIUM

PIETERMARITZBURG, SOUTH AFRICA

29-30 APRIL 1993

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FOREWORD

During the Rabies Conference held in Lusaka in June 1992, the Southern African Rabies Group was formed. It was decided at that meeting to hold the following conference in South Africa a year later. A committee comprising Mr G.C. Bishop, Dr G.R. Thomson and Dr G.K. Brückner set up a series of three separate but related meetings which were held in Pietermaritzburg and Pretoria. The Training Course (26-28 April, 1993) and Symposium (28-29 April) were held in Pietermaritzburg and these proceedings deal with those two events.

Most of the 40 papers presented at the Research Workshop (3-5 May) held at Onderstepoort (Pretoria) are soon to be published in a special edition of the Onderstepoort Journal of Veterinary Research.

Despite the logistical difficulties experienced in splitting these events between two cities some 600 km apart, the result was successful. The Training Session was attended by 25 delegates from 14 countries and the Symposium by 188 scientists. All of our training delegates and many other overseas visitors were housed at the Midmar Resort situated on the outskirts of Pietermaritzburg. The gatherings took place in a spirit of great conviviality and many friendships were made and cemented. The enthusiasm of our international rabies experts was truly infectious. In this regard, the efforts of our "Three (Training) Musketeers", Jacques Barrat, Arthur King and Alex Wandeler must be mentioned. They cheerfully and willingly undertook a very heavy training load and worked extremely long hours to produce an outstanding course.

The generous and indispensable financial and administrative contributions of our various sponsors (listed below) are gratefully acknowledged.

The speakers who delivered papers at the Symposium covered the field of rabies comprehensively. Certain countries who did not present reports at the Lusaka Conference were invited to do so on this occasion.

These Proceedings have not been edited to a strict format and the papers submitted for this publication have not been altered significantly. Some of the information which was distributed during the Training Session is also included.

We thank the various speakers for their presentations and for their permission to publish these contributions in a rather "loose" format

During a business meeting of SARG, several resolutions were agreed on :

1. It was decided to change the name SARG to SEARG (Southern and Eastern African Rabies Group) as this describes more accurately the membership of our Group.
2. Dr Paula Dias was elected to succeed Dr Peter Sinyangwe as Chairperson and Mr George Bishop was re-elected as Secretary.
3. The control of rabies and epidemiological surveillance were identified as thrust areas for technical training and discussion at the next meeting to be held in Mozambique in two years time ~this venue has subsequently been changed to Harare.
4. The sociological basis for the unwillingness of people to have their dogs vaccinated should be actively researched.
5. A six-monthly Newsletter will be produced by Mr George Bishop
6. Dr Arthur King will serve as "roving technical expert" (finances permitting).
7. Drs Arthur King, Jacques Barrat and Alex Wandeler will produce a laboratory manual which will detail the basic recommended procedures to be followed in rabies diagnostics.

ACKNOWLEDGEMENTS

The generous and indispensable contributions of the following made the Symposium and training session possible and we express our sincere thanks and appreciation to :

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PROGRAMME

Thursday 29 APRIL 1993

0730-0830 REGISTRATION

FIRST SESSION CHAIRMAN : DR P. BOSMAN (S.A.)

0830-0850 OFFICIAL OPENING : DR A.I. VAN RIEKERK, MINISTER AGRICULTURE, S.A.

0850-0915 THE HUMAN PERSPECTIVE : DR B. NGUBANE, MINISTER OF HEALTH, KWAZULU, S.A.

0915-0945 RABIES : THE INTERNATIONAL : DR A. WANDELER (CANADA)

0945-1015 RABIES IN SOUTHERN AFRICA : PROF. R. SWANEPOEL (S.A.)

1015-1045 TEA

SECOND SESSION CHAIRMAN : MR G. BISHOP (S.A.)

1045-1115 CANINE RABIES : DR A. WANDELER (CANADA)

1115-1145 HUMAN RABIES-CASE STUDIES IN CHILDREN : DR J. GODLONTON (S.A.)

1145-1215 DEMOGRAPHIC TRENDS IN THE NATAL REGION : MS D. KRIGE (S.A.)

1215-1345 LUNCH

THIRD SESSION CHAIRMAN : DR G. BRdCKNER (S.A.)

1345-1405 ECOLOGY OF DOGS IN EASTERN AND SOUTHERN AFRICA AND IMPLICATIONS FOR RABIES CONTROL : DR B. PERRY (KENYA)

1405-1425 VARYING CONTROL STRATEGIES FOR DIFFERENT COMMUNITIES : DR M. BACHMANN (S.A)

1425-1445 PROBLEMS ASSOCIATED WITH RABIES DIAGNOSIS IN A BUSY PRIVATE PRACTICE : DR P. KRETZMANN (S.A.)

1445-1505 CANINE RABIES IN KENYA : DR W.K CHONG' (KENYA)

1505-1525 RABIES CONTROL: GENERAL ASPECTS : DR C. SCHUMACHER (FRANCE)

1525-1555 TEA

FOURTH SESSION CHAIRMAN : DR B. PERRY (KENYA)

1555-1615 RABIES CONTROL IN SOUTH AFRICA : DR G. BRÜCKNER (S.A.)

1615-1635 RABIES CONTROL IN NATAL : DR P. KLOECK (S.A.)

1635-1655 RABIES CONTROL IN KWAZULU : DR B. McCULLOUGH (S.A.)

1655-1715 RABIES CONTROL IN TRANSKEI : DR L. AMORAL (TRANSKEI)

FRIDAY 30 APRIL 1993

FIRST SESSION CHAIRMAN : DR A. WANDELER (CANADA)

0830-0900 VIDEO ON RABIES RHONE HERIEUX (FRANCE)

0900-0945 GENOMIC VARIATION IN LYSSA-VIRUSES : DR N. TORDO (FRANCE)

0945-1030 ANTIGENIC VARIATION IN LYSSA-VIRUSES : DR A. KING (U.K.)

1030-1100 TEA

SECOND SESSION CHAIRMAN : DR A. KING (U.K.)

1100-1130 BAIT VACCINE DEVELOPMENT : RECOMBINANT VIRUSES : DR B. BROCHIER (BELGIUM)

1130-1215 ADVANCES IN DIAGNOSTIC METHODS : DR H. BOUHRY (FRANCE)

1215-1230 RABIES RESEARCH AT ONDERSTEPOORT : DR G. THORSON (S.A.)

1230-1400 LUNCH

THIRD SESSION CHAIRMAN : DR G. THOMSON (S.A.)

1400-1500 PANEL DISCUSSION ON RABIES CONTROL IN SOUTHERN AFRICA

1500-1530 THE FUTURE OF SARG

1530-1600 TEA

FOURTH SESSION CHAIRMAN : DR P. SINYANGWE (ZAMBIA)

1600-1700 ADOPTION OF RESOLUTIONS

(a) FUTURE OF RABIES CONTROL IN SOUTHERN AFRICA

(b) FUTURE OF SARG

OPENING ADDRESS

A.I. Van Niekerk [1]

Mr Chairman, honourable guests, ladies and gentlemen.

I am indeed proud and honoured to be in the privileged position to welcome so many of our friends from Africa and abroad to this memorable occasion in South Africa. I am also proud that South Africa can play host to so many of our former contacts and friends with whom we have had such good relations in the past. Your attendance here today affirms our common commitment to promote and improve animal and human health in Africa to the advantage of all the peoples of Africa.

I am especially pleased to share this podium with my honoured friend Dr Ben Ngubane, Minister of Health of KwaZulu, as it is often argued that the control of rabies should really be the responsibility of the Department of Health. The fact that we are both here today to demonstrate our concern over the increased occurrence of this extremely dangerous disease for both humans and animals, confirms our mutual commitment to control and if possible, eradicate this disease from within our borders and in fact in as many countries of Africa as possible.

We are honoured by the attendance of so many experts in the field of rabies control and research from countries such as Canada, the United Kingdom, the USA and Europe. Your willingness to share your expertise with us, not only during this symposium but also during the training session which started on Monday as well as the scientific workshop next week, is highly appreciated.

It will not serve any purpose at this stage for me to elaborate on the alarming increase of rabies in dogs in Natal and KwaZulu. The mere fact that a symposium such as this was deemed necessary to address the problem in detail, is sufficient proof of our concern. You will be able during the course of the next two days, to listen to various experts in this field and you will have the opportunity to express your ideas and concerns with the aim to formulate a strategy for further action in this regard. I beg of you to not only make full use of the opportunity to expand your knowledge but also to share your expertise and experience with all so that at the end of this symposium, you can all go home with the assurance that it was a worthwhile and rewarding experience.

It is true that our main focus on rabies control in South Africa is currently centred on Natal and KwaZulu even though there is enough reason for concern about the incidence of rabies in the rest of our country. The statistics, however, gives us a grim picture of the current situation in Natal and KwaZulu :

- almost 80% of all the districts in Natal and KwaZulu are currently regarded as infected with rabies
- the number of known or reported human deaths due to rabies has increased from 6 deaths in 1985 to 29 during 1992
- more than 800 people had to be treated during 1992 after exposure to rabid dogs
- the urban and semi-urban areas in Natal are regarded as amongst the most densely populated areas in Africa with an estimated human to dog ratio of 6:1
- it is estimated that less than 30% of dogs in the whole of Natal/KwaZulu are immunised against the disease

You will without doubt hear more about this during today and tomorrow. It is, however, necessary to draw your attention to these facts at the very onset of this symposium to stress the importance of the realities - not only of the South African situation but for that matter, the realities pertaining to the whole continent as regards animal disease control.

You are all familiar with the epidemiological concept that animal diseases have a total disregard for international borders or man made geographical entities. This has been proven all over the world and

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[1] Minister of Agriculture, Republic of South Africa

also very clearly closer to home, right here in Natal and KwaZulu. To the same degree that South Africa is committed to guarantee the movement of non-diseased animals across its borders, do we rely on our neighbours to honour the same commitment. This is in my opinion what this symposium is all about - to seek solutions and to support each other in fulfilling this commitment not only with regard to the control of rabies but the control of all animal diseases that pose a threat to the economy of our continent.

We must all guard jealously over our ability to provide the guarantees expected from our neighbours and trade partners and which is necessary to enhance and promote the international trade in animals and animal products and we must continuously strive to improve our expertise in this regard.

It is common knowledge that African countries in general have limited international trade in animals and animal products. In comparison with world figures, imports to African countries amount to 11,1% of milk, 1,9% of meat, 4,7% of fish, 0,6% of wool and 0,3% of honey. Exports represent 1,12 % of milk, 0,46% of meat, 0,33% of fish, 6,28% of wool and 0,08% of honey. Thus, Africa imports more than we export. It is therefore essential that we make full use of the technological advantages to be gained from meetings such as this - especially to enhance the trade in animals and animal products and to avoid unnecessary hindrances by way of non-tariff barriers based on a lack of knowledge of health factors or disease occurrences.

However, looking at the realities and future prospects for animal disease control in southern Africa, it is obvious that one is dealing with a unique and complex socio-political situation. Most of the land in many African countries is held by tribal stockmen under communal ownership and any attempt to effect changes and improvement in animal disease control, depends on their co-operation.

In a way these stockmen hold the key to Africa's development and its future.

There is a desperate need to develop its third world agricultural sector and to maintain its first world high technology sector (Hofmeyr, 1990). As Africa south of the Sahara continues to move towards more effective use of higher technology, the opportunities for sharing South African developed skills and technology in animal disease control and livestock production, are increasing dramatically. Our present export of feeds, vaccines, veterinary pharmaceuticals and livestock will in the next century have to be supplemented or replaced by the export of skills and technology, specifically developed in and for African conditions. The technology developed during this process will have to be adapted for cost effective utilisation in the developing economies to the north (Griessel, 1990). I know that South Africa has much to offer to the rest of Africa, but we must on the other hand be willing to learn from experiences elsewhere in Africa and the rest of the world.

One of the most important realities that we will have to cope with, not only in South Africa but also in the rest of the continent, is that animal health and animal husbandry are inseparably interrelated. An animal production service which is competent in both spheres, is the best guarantee for obtaining enhanced productivity by a combined effort. Veterinary services are, however, subjected to a wide range of constraints, which often have a cumulative effect. Developing countries are affected more than any other by the world economic recession. Faced with limited resources, they still have to cater for the fundamental priorities, all of which are important: education, health, infrastructure, importation of goods, equipment and food, payment of interest on foreign debt, and so on. The effects of these constraints are particularly evident in the sphere of livestock production, mainly affecting the means available to animal health services.

It is unfortunately true that the promotion of animal health, has to compete with the aforementioned fundamental priorities, necessitating a re-evaluation of state supported veterinary services by redefining its fundamental functions. It has become necessary to redefine the level of state intervention and to phase out tasks better done by other semi-government or private structures. In South Africa we were also compelled to take such action in respect of animal health, thereby ensuring the availability of funds for the control of economically important diseases such as foot-and-mouth-disease and diseases posing a threat to human health - the most dangerous of these being rabies.

The development and utilisation of better technologies to enhance our disease control strategies is absolutely essential - not only to stay ahead of dangerous diseases such as rabies but also to even the cost-benefit ratio in respect of disease control.

It may well be argued that the loss of human lives cannot be measured in monetary terms - an argument which I fully support. However, in enhancing our knowledge of animal diseases and disease control methods, we can decrease the risk to human health as far as zoonotic diseases are concerned

thereby fulfilling your mission as veterinarians to create a buffer between animal vectors of disease and human beings. I am sure that this symposium will do much towards the achievement of this goal.

To the welcomes already extended to you, allow me to add that of the Republic of South Africa. May your deliberations be fruitful and pleasant. I wish you all a very successful symposium and a most enjoyable stay in South Africa.

RABIES : THE HUMAN PERSPECTIVE
KEY NOTE ADDRESS

B. S. Ngubane [1]

Mr Chairman, the Honourable Minister of Agriculture, distinguished guests, ladies and gentlemen.

The disease of rabies is probably as old as recorded history itself. It was recognised as a disease entity more than 2000 years ago by the civilizations of the Nile, the Euphrates and the Indus. Babylon, in 2300 BC was aware of the problem and made provisions for its control in law. The association of bite-wounds and rabies was known to Hippocrates who called attention to mad dogs, which destroy themselves and all they encounter. Aristotle knew that mad dogs could infect all animals that they met, except man! However, Pliny a Roman historian recognised the transmission of rabies from dog to man. So the association between rabies, dogs and man was well established many centuries ago.

From ancient times climatic conditions, especially heat and drought, were thought to be responsible for outbreaks of madness in dogs. In the New World, a collection of rare documents gives evidence of "a most extensive and malignant epizootic of urban canine rabies" in Mexico City in 1709, apparently in the stray dogs of the city, which spread all over the ancient territories of New Spain, involving the cattle of ranches and haciendas; some human deaths were recorded. This sounds all too familiar to us, who have been and are going through a similar experience! The pathogenesis of the Mexican epidemic was attributed to the malignant influence of Sirius, the Dog Star, linked to the summer weather. In Britain the term "the Dog Days" is sometimes still used to describe the period in July and August associated with the reappearance of Sirius and believed to be the hottest and most unwholesome part of the year.

Coming closer to home and the present day - there are reports of rabies in South Africa in the late 18th and 19th centuries, including an association with "genet cats" reported by du Toit as a belief in the Vryburg area for many years. In 1892-93 there was an outbreak of rabies in dogs in Port Elizabeth and the surrounding area, resulting from an imported dog. It was eliminated within a year by isolation and confinement of suspected dogs, and the destruction of dogs found unmuzzled and at large. These measures were consolidated into the Rabies Act of 1893, the first specific anti-rabies legislation in this country. In 1902 an epidemic in Zimbabwe (then Southern Rhodesia), mainly in dogs, was controlled by shooting-out about 60 000 strays and unattended dogs.

In this country, until about 1950, rabies was identified mainly in wild animals, with very few dogs and none of them in the Eastern Transvaal or Natal. From 1950, however, dogs were found positive for rabies in Northern and Eastern Transvaal, Rhodesia, Swaziland, Mozambique, and finally, in 1961, in Natal. Rabies was epidemic in the Midlands and the North Coast the mid-60's, but had disappeared by 1968, according to authoritative sources. Of course, it had not, or we would not be gathered here today.

Consider the following figures for South Africa as a whole.

Between 1953 and 1961 there were 585 human contacts of proven rabid animals with 19 deaths, 14 following dog bites - remember this 8-year total.

Barnard analysed bite-wounds inflicted by rabid animals on various categories of victims, and found that 923 animals, excluding dog and cats, bit 86 people, or 9% of all their victims; 281 dogs and cats bit 150 people, or 71% of all their victims.

So it can safely be said that rabid dogs and cats, of all vectors, represent the greatest threat to man.

Dogs and cats live in close association with people: in Natal, from 1961 to the present day, with few exceptions, rabies has been confirmed only in dogs, cats and domestic animals. So the human rabies situation in Natal/KwaZulu depends on the level of rabies in dogs and cats.

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[1] Minister of Health, Kwazulu Government

May 1961 saw the first reported human death from rabies in Natal, in the Ingwavuma district. Within a year a further 10 had been confirmed; there may have been more, because the disease was new to the area and could have been mis-diagnosed. Compare this number with the figure of 14 in 8 years I have just quoted. Within the same year rabies had been confirmed in 13 districts of the Province, transforming it from a previously rabies-free region into the country's most notorious region for the disease, a status it has, regrettably, maintained to this day.

In 1980, Durban and the adjoining areas to the south experienced the first epidemic of urban rabies since 1892. A number of professional people attempting to bring the outbreak under control, and to cope with the flood of human contacts, pleaded with the Central Government for more effective and enforceable legislation - immunise and confine dogs, shoot out strays and unaccompanied animals - measures which had proved their worth in other parts of the world. With these powers we might just have been able to avoid the situation we are in now. Higher authority turned a deaf ear; an apathetic dog-owning public ignored the legislative constraints which did exist, and the responsible authorities did too little to enforce them. In 1980, 159 animals were confirmed rabid, 87 of them within the Durban metropolitan area; 13 human deaths were reported. Since then, circumstances have overtaken us - the vast influx of people to the uncontrolled and unserviced informal settlements around the major urban centres, where control of dog is impossible except for the most dedicated of owners; a veterinary service which had been divided through the implementation of the homelands policy, and weakened by inadequate salaries and shortage of funds, and latterly a political situation which has made it hazardous for officers of government departments to enter many areas for rabies immunisation.

The figures tell the Natal/KwaZulu story :

- In 1984, 168 animal cases, 69 in the Durban metropolitan area, 10 human deaths.
- In 1987, 239 animals, 16 humans.
- In 1988, 154 animals, 26 humans.
- In 1990, 251 animals, 13 humans.
- In 1991 295 animals, 20 human deaths.

Last year 338 cases of animal rabies were confirmed, in 39 of our 59 districts in Natal and KwaZulu; both are the highest numbers recorded.

So is the number of human deaths. Twenty eight does not seem a large number in comparison with deaths from some other causes, but each one represents a death which Dr Bill Posthumus, Regional Director of Veterinary Services for Natal in the 1980's and, sadly no longer with us, described as "one of the most painful and horrible ways to die". A recent editorial in the Natal Mercury, reporting the death of an innocent youngster following a scratch by a stray dog, talked of "an ugly disease" and "a merciless illness". Rabies is all of these. The distress and grief of the affected families can only be imagined. The tragedy is that these deaths could have been prevented; they occurred in people who did not receive post-bite prophylaxis. Hundreds have received it, and for many it has averted inevitable death, although at considerable cost to the services administering it.

In the outbreak in Mexico City which I referred to earlier, the miraculous patronage of St Quinteria against rabies was advocated. So, if all else fails, remember, his name! However, we will do better to pin our hopes on seeking more earthly solutions to our problems.

I would like to heartily commend the Southern African Rabies Group for their initiative and enterprise in organising this truly international Symposium and the Training Workshop, to wish the Symposium every success and to express my fervent hope that from its deliberations will come a renewed initiative and new inspiration for the control of rabies.

RABIES : THE INTERNATIONAL SITUATION

A. I. Wandeler [1]

1 LYSSAVIRUSES AND THEIR GEOGRAPHICAL DISTRIBUTION.

The genus Lyssavirus contains numerous distinct viruses isolated from mammals, fish and arthropods. Some Lyssaviruses have been grouped into 4 serotypes: rabies (serotype 1), Lagos bat (serotype 2), Mokola (serotype 3) and Duvenhage (serotype 4). Serotypes 2, 3, and 4 have so far been found in Africa only.

The serological classification is slowly becoming obsolete with the accumulation and phylogenetic analysis of virus genome sequence data. Based on such information it has been suggested to group the rabies-related viruses into 6 genotypes (Bouhry et al., 1993). Genotype 1 accommodates the old serotype 1, the classical rabies virus strains. It occurs on all continents, except Australia and Antarctica. Genotype 2 replaces serotype 2: Lagos bat virus. Mokola viruses are genotype 3 (serotype 3). Duvenhage viruses are genotype 4 (serotype 4). European bat lyssavirus type 1 (EBL1) is genotype 5. European bat lyssavirus type 2 (EBL2) is genotype 6. EBL viruses are restricted to Europe (Eurosiberia?).

With more and more sequence information on different isolates accumulating, it is to be expected that Lyssavirus taxonomy and classification will undergo more changes in the near future.

2 RABIES IN TERRESTRIAL WILDLIFE SPECIES.

The areas for which the association of rabies viruses with populations of wild Carnivora is well documented are limited to North America, Europe, and parts of Southern Africa. The principal rabies hosts of the order Carnivora are all small to medium size (0.4 - 20kg) omnivores, scavenging, and foraging on small vertebrates, invertebrates, fruit, and refuse produced by humans. They reach high population densities (often several individuals/km2) in and near human settlements. High intrinsic population growth rates allow rapid recovery of populations decimated by persecution or disease. They all are able to support initial epidemics of high case density and thereafter an oscillating prevalence over many years.

A particular species may serve as a principal host only in a limited part of its geographical distribution, while in other parts of its range other species are responsible for maintenance and spread of rabies. The disease occurs regularly in a number of other mammalian species in addition to the species recognized as principal host. The occurrence of rabies in these other species may have little or no influence on the course of an epizootic; however, their role is often not so easy to define.

Each principal host species has its specific life history pattern and specific means of social interactions. These host qualities determine what virus variants are capable of survival. It is essential that the virus be transmitted by an infected animal during a period of virus excretion to enough other susceptible individuals. For this to occur, lyssavirus strains must be adapted to the physiological traits and population biology of their hosts (Bacon, 1985; Wandeler, 1991a). They must have a host specific pathogenicity and pathogenesis (length of incubation period, duration and magnitude of virus excretion, duration and extent of clinical illness). We assume that each principal host has its own virus variants adapted for persistence in its populations. With the development of monoclonal antibody technology it became possible to demonstrate that indeed antigenically distinct variants circulate in different host populations (Rupprecht et al., 1991: more recently these epizootic variants can also be characterized on the basis of genome structure, see e.g. Sacramento et al., 1991; Bouhry et al., 1992; Nadin-

[1] Rabies Unit, Animal Diseases Research Institute, Agriculture Canada, Nepean, Ontario, Canada

Davis et al., 1993). That distinct rabies virus strains circulate in different principal hosts and in separate geographic areas may be considered as support for the hypothesis.

3 BAT RABIES.

Lyssaviruses have been isolated from bats (Chiroptera) from Africa, the Americas, Europe, and Asia. The African bat Lyssavirus isolates are of genotypes (serotypes) 2 and 4, while those from bats in Europe where identified as genotypes 5 and 6. American bat rabies viruses have been categorized as serotype 1, but a more detailed analysis of the large diversity of distinct isolates is still good for surprises.

Chiroptera have life history traits that are quite different from those of carnivoran rabies hosts: they are small, long lived, have low intrinsic population growth rates, and are ecological specialists. Properties of lyssaviruses adapted to bats must therefore be different from those of Carnivora rabies. This statement remains a hypothesis since the population biology and epidemiology of bat rabies is insufficiently explored.

An interesting feature of bat rabies is the large diversity of isolates. In Canada alone, 7 distinct variants of serotype 1 are recognized with monoclonal antibodies. Several variants occur in a single species, and geographical distribution of variants are overlapping. This is in sharp contrast to the pattern of rabies in Carnivora, where very little epitope variation is recognized over very large areas, so synonymous base replacements in the viral RNA lead to geographical patterns of genome variants (Nadin-Davis et al., 1993).

4 DOG RABIES.

In large parts of Asia, Africa, and Latin America, the bulk of diagnosed rabies cases is seen in dogs (Baer and Wandeler 1987). For a brief overview see Wandeler on "Canine Rabies" in these Proceedings.

The rabies viruses isolated from wild Carnivora of a particular location all resemble the isolates from wild Carnivora from other parts of the world, but the differences among strains from wild Carnivora are greater than those among the isolates from dogs from different continents. This has been recognized previously with monoclonal antibodies (e.g. Wandeler, 1991a), and has now been confirmed by genome analysis (Smith et al., 1992). Similarities may indicate a common origin (transport of infected dogs), however they may also be the consequence of selective constraints imposed by the host.

5 HUMAN RABIES.

Worldwide about 35 000 people die from rabies every year. The number of people receiving postexposure treatment - mostly after dog bites - is about 3.5 million per year (Bögel and Motschwiller, 1986; Bögel and Meslin, 1990). Almost all human rabies deaths and the vast majority of treated bite exposures occur in developing countries (Acha and Arambulo, 1985). This may in part be due to a high rate of exposure to biting rabid dogs, but even if this assumption is correct, it does not fully explain the high number of rabies casualties. In view of the high efficacy of modern postexposure treatment, nearly all human rabies cases must be considered as failures of the medical system; the correct treatment was not applied, or not applied in time. The approp-riate treatment may not be universally available (spatially, temporally, socially, economically), or the appropriate treatment is not in compliance with traditional (religious) beliefs. It is also possible that the necessity of the appropriate treatment is not recognized because other treatments are considered equivalent or superior, or because the disease entity is not recognised (Wandeler et al., 1993).

6 RABIES CONTROL.

The ultimate purpose of rabies control is the protection of man from both infection and economic loss. 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 immunization 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.

For a brief review of dog rabies control see Wandeler on "Canine Rabies" in these Proceedings.

Wildlife rabies control by decimating host populations has been attempted in nearly all recognized major terrestrial hosts. However, the resilience of these opportunistic Carnivora to persecution and their reproductive potential, together with high carrying capacities of rural and urban habitats, often render control efforts unavailing. A more promising approach is mass vaccination of the main hosts, although immunization 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.

A vaccine to be used for oral immunization of free-living wild animals should comply with a number of requirements (WANDELER 1991b). If a safe, efficacious, and sufficiently thermostable vaccine is available, then a suitable bait needs to be selected. Efficacy and safety of candidate vaccines has been properly tested for only a limited number of target situations. The most important qualities of baits for proper vaccine delivery are that they should be attractive for the target species, and that they should be avoided by other species. All baits tested so far have been picked up not only by various domestic and wild Carnivora, but have been taken up by ruminants and rodents as well. In the event that vaccine and bait are found to be suitable, then the next goal is a vaccine delivery system that assures mass immunization of target species. This requires temporal and spatial bait distribution strategies. When deciding on these strategies it is important to take into consideration technical resources, administrative structures, and manpower needs, as well as constraints imposed by safety requirements, terrain, climate, etc.

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RABIES IN SOUTHERN AFRICA

R. Swanepoel

ABSTRACT.

The first confirmed outbreak of rabies in Africa, believed to have followed the importation of an infected dog from England in 1892, occurred in the eastern Cape Province of South Africa, and was brought under control in 1894. An unconfirmed epidemic of rabies in dogs occurred in western Zambia in 1901. By the following year the disease had apparently spread along a major trade route, to cause an outbreak in Zimbabwe which engulfed most of the country before being eradicated in 1913.

The existence of endemic rabies of viverrids (mongooses and genets) was confirmed in South Africa in 1928, and since then the vivverid disease has continued to occur widely on the interior plateau of the country with spill-over of infection to cattle and a variety of other animals. From about 1947 onwards, an invasive form of dog rabies spread from southern Zambia and/or Angola into Namibia, across northern and eastern Botswana into Zimbabwe and the northern Transvaal by 1950, entered Mocambique in 1952, and spread from there to Swaziland in 1954. Dog rabies extended from southern Mocambique into Natal in 1961 to cause a major epidemic which was brought under control in 1968.

The disease re-entered northern Natal from Mocambique in 1976 and since then dog rabies has proved difficult to control in the peri-urban settlements of Natal KwaZulu. The disease spread from Natal to Lesotho in 1982, and into the Transkei region of the eastern Cape Province in 1987, to reach the Ciskei by 1990. The spread of the disease in dogs was followed by the emergence of rabies of jackals and cattle in central Namibia, northern Botswana, Zimbabwe and the northern Transvaal. A unique outbreak of rabies in kudu antelope occurred in central Namibia from 1977 to 1985, apparently involving oral spread of infection between individuals. A few cases of rabies in the bat-eared fox were recognized each year in Namibia from 1967 onwards, and from the 1970's occurrence of the disease in the fox has emerged as a distinct problem in the northern Cape Province and spread to the west coast.

The rabies-related viruses, Lagos bat, Mokola and Duvenhage, associated with bats, shrews and rodents in Africa, are known to have caused isolated cases of disease in South Africa, and on one occasion a small outbreak involving six cats and a dog in Bulawayo, Zimbabwe. However, the results of monoclonal antibody tests on numerous specimens, indicate that the rabies-related viruses are not a major cause of disease in southern Africa.

The full text is to appear elsewhere: Swanepoel, R., Barnard, B.J.H., Meredith, C.M., Bishop, G., Foggin, C.M. & Hübschle, 0.J., 1994. Rabies in southern Africa. Onderstepoort Journal of Veterinary Research, in press.

CANINE RABIES

A. I. Wandeler [1]

1 CANINE RABIES.

Rabies has a widespread occurrence in Africa, the Americas, Asia, and Europe. One can distinguish between areas in which dogs are the predominant recognized hosts and areas where rabies is maintained by wild animals. In those latter situations only 0.1-5% of the rabies cases reported annually are in dogs. In large parts of Asia, Africa and Latin America, rabies in dogs is much more common, making up 95% or more of all diagnosed rabies cases. Rabid dogs are also responsible for the vast majority of human cases. Most of them occur in developing countries, where in some places the recorded number may exceed 0.5 per 100,000 inhabitants per year. The number of people receiving post-exposure treatment after dog bites is between 10 and more than a 100 times higher than the number of recorded fatalities. Dog keeping practices, high rates of exposure and a number of cultural factors related to health systems lead to a high human rabies mortality rate. Even though dog rabies is often termed "urban rabies", it is clearly a rural problem in many developing countries.

In industrialized countries of North America and Europe, where the epizootic is maintained and spread by wild carnivores, three factors may account for the low incidence of rabies in dogs: most dogs are restricted in their movements; they are kept indoors or in enclosures and leashed when outside; dog vaccination is strong-ly recommended or even compulsory. Human rabies is rare due to low rates of exposure, high standards of health education, and relatively easy access to post-exposure treatment with potent vaccines.

Dogs are kept and tolerated at very high numbers in most human societies. Dog population densities may reach several thousand per km2; this is considerably more than any wild carnivore population ever achieves. Their abundance is not explained by their limited economic usefulness. The tolerance granted to them must find explanation in processes of socialization and psychology. Cultural conventions determine the level of supervision of their social interactions and access to resources (food, water, shelter, mates), which is partially a function of the density and structure of human settlements. 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.

For more details see Beran (1991), and Wandeler et al. (1993).

2 DOG POPULATION MANAGEMENT.

Rabies has a high incidence in dogs in areas where dog populations reach high densities and where the animals are poorly supervised. Attempts to reduce dog numbers and to educate owners toward responsible ownership should therefore be attempted. For this purpose the WHO/WSPA "Guidelines for Dog Population Management" should be consulted. Recommended control measures include movement restrictions, reproduction control, habitat control, and removal of straying dogs. The control of movements is intended to limit social contact and access to resources (both leading to disease transmission and uncontrolled reproduction). Reproduction control may be achieved through mating restrictions, surgical sterilization, and drugs (injectable, oral). Habitat control is meant to reduce the availability of resources (litter, waste, shelter). These approaches to population management are dependent on the promotion of responsible dog ownership; their implementation may often be too costly for achieving perceivable effects on a dog population. The removal of straying dogs usually has only

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insignificant impacts on population densities and is therefore not an productive method of population control, but it may serve law enforcement and is an aid to education in responsible ownership.

3 DOG VACCINATION IN AREAS WITH WILDLIFE RABIES.

In European and North American nations with predominant wildlife rabies dog vaccination is recommended or compulsory. Owners have to register (or license) dogs. Registration can be made dependent on the production of a certificate that the animal has been vaccinated against rabies when over 3 months old and has been revaccinated at periods of not more than 2 (or 1) years. Vaccinations should be done by parenteral inoculation of a product recognised by the National Authorities, usually an inactivated vaccine conferring two years of immunity after one injection.

4 RABIES CONTROL IN AREAS WITH CANINE RABIES.

Rabies control in areas with canine rabies is usually not a simple application of regulations on dog ownership. Their enforcement is impeded by a number of ecological and cultural constraints. But well planned and executed vaccination campaigns may reduce rabies incidence in dogs drastically and may even eliminate the disease in areas where it is not maintained by wildlife. Taking the cost and benefits of a campaign into consideration, we suggest that disease 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. Comprehensive national, rather than temporary, local plans are imperative. These plans have to identify a goal, and they have to consider national structures and resources. Effective intersectorial cooperation is necessary. Useful guidelines for programme management are :

- WHO, 1983: Guidelines for Dog Rabies Control
- WHO/FAO, 1990: Guiding Principles for Planning, Organization and Management of Veterinary Public Health Programmes

Both documents give detailed guidance on the planning and management of control programmes, on legislation, and on techniques in local programme execution.

For planning a comprehensive control programme it is necessary to consider a number of dog population parameters (size, turnover, accessibility). A vaccination coverage of about 75% of the total population should be attempted. This goal should be achieved in a particular area (of limited size) within a relatively short time period (a few weeks). A number of different approaches can be taken, e.g. temporary 'neighbourhood clinics', door-to-door vaccination. Pilot projects may help in assessing: 1) dog accessibility, 2) ways of cooperating with local residents and 3) avenues to provide information and education. Plans for large scale operations, vaccination strategies and logistic aspects can then be adjusted according to findings in the pilot phase. An effective maintenance programme must be part of the plan. Operational research for monitoring campaign efficiency is strongly recommended.

5 VACCINES.

5.1 Vaccines for oral use :

At present there are no vaccines available for oral immunization of dogs. Such vaccines are under development. They must meet higher safety standards than the presently used oral wildlife vaccines.

5.2 Vaccines for parenteral use :

Inactivated nerve tissue vaccines may be prepared from brains of lambs or suckling mice inoculated new-born i.c. with fixed viruses. They may be adjuvanted. These vaccines do not always have an efficacy comparable to the efficacy of inactivated tissue culture vaccines.

Whenever possible modern inactivated tissue culture vaccines should be used. They combine safety with high immunogenicity. Cell lines and primary cell cultures are used as substrates for a number of

virus strains. Several manufacturers include a variety of different antigens (distemper, adenovirus, leptospirosis, parainfluenza, parvovirus) in combined vaccines. No indication of competitive inhibition have been noted, but every new product should be investigated for its overall immunogenic potency.

The use of modified live (attenuated) vaccines is no longer recommended for dog immunization, except for special situations (e.g. national campaigns under economic constraints). Live recombinant vaccines and other products of genetic engineering will soon become available.

It is recommended that vaccines be completely innocuous, even for very young animals, and that they confer immunity for one (preferably two) years after one injection in all dogs above an age of three months.

There are no treatment schedules or vaccines licensed for post-exposure treatment of dogs.

For more details see Bunn (1988, 1991) and Precausta and Soulebot (1991).

6 QUESTIONS, RESEARCH NEEDS, OPERATIONAL RESEARCH.

Rabies in dogs is a significant threat to human health. An estimated 30,000 people are dying from dog transmitted rabies per year. The widespread occurrence of human rabies is not only due to the frequency of exposures, but also to the failure of applying proper treatment after bites from rabid animal. More inquiries into health systems and the ethnology and sociology of preventing and curing dog transmitted diseases are clearly indicated.

Dog rabies epizootiology is not well understood and it is rather unfortunate that more thorough studies have never been done. It is sometimes questionable if canine rabies is really independent from a wildlife reservoir. Structural constraints and a shortage of resources may often preclude a suitable epizootiological surveillance. On the other hand, the easy access to dog populations should allow collection of valuable data, e.g. detailed case histories (possible source of infection, other animals/humans exposed, etc.).

A number of dog populations in different parts of the world and in different ecological and cultural settings have been studied in recent years (Wandeler et al., 1993). We feel that dog population biology is reasonably well explored. Although, one has to remember that tolerance, supervision, availability (accessibility) of resources, and other aspects of the "habitat carrying capacity" are human cultural traits that vary dramatically from area to area. Attributes of culture not only determine dog population characteristics, but also their accessibility for control operations. Questionnaire surveys produce information on dog:human ratios, dog keeping practices, reproduction, morbidity and mortality, etc. However, such data relate only to the owned segment of a dog population. If there is a suspicion that there are ownerless dogs, one should resort to an experimental approach as used by wildlife biologists (for a description of techniques see e.g. Caughley, 1977, or Davis and Winstead, 1980). Modified mark-recapture techniques can be implemented without too much difficulties during mass vaccination campaigns. Such "operational research" conducted in conjunction with pilot projects may provide a large amount of useful data.

Pilot projects in general may help in assessing 1) dog accessibility, 2) ways of cooperating with local residents, and 3) avenues to provide information and education. Plans for large scale operations, vaccination strategies and logistic aspects can then be adjusted according to findings in the pilot phase. We also suggest that in future programmes, some operational research be conducted in order to monitor campaign efficiency.

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A PAEDIATRICIAN'S PERSPECTIVE OF RABIES

J. Godlonton [1]

Working as a full-time paediatrician at Edendale Hospital, a large local hospital serving the local black communities of Kwazulu, one views the rabies problem from a number of different perspectives.

1 THE MEDICAL PERSPECTIVE

Most patients presented with rabies do not constitute a diagnostic problem. The child usually has the classical features of the "furious" form of rabies encephalitis and in most cases there is a positive history of a dog bite in an endemic area. The futility of the disease as a whole has been the cause of fear and fascination for many thousands of years. Attempts to alter the inexorably lethal course of the disease have met with little or no success. There is always an element of therapeutic frustration and indecision at the outset in view of the patient's generally good condition and one is tempted to resort to heroic and/or unusual therapeutic and supportive measures. In all cases heavy sedation and intravenous feeding are the rule. After death, an attempt is always made to obtain virological and histological confirmation of the disease.

There are a number of deeply frustrating components to the clinical rabies scenario. Many of our rabid children have been treated at a hospital or clinic in a rabies endemic area at the time of the bite and had not received the recommended prophylactic rabies therapy. Many parents or custodians seem unaware of the nature or importance of the rabies problem. The correct "management" of the biting animal is frequently not done.

Yet the disease has a certain fascination. It is difficult to reconcile the advanced cerebral pathology with the relatively delayed onset of clinical warning symptoms. Why are there so comparatively few diagnosed human rabies cases in an area of high endemicity? Are we missing other forms of rabies encephalitis? Shouldn't we be looking harder for them? The answer to all these questions is yes. Why is there a burning pain at the bite site during the prodromal stage of the disease? This is thought to be due to viral replication and invasion of the CNS at the level of the spinal cord, yet it is difficult to reconcile this fact with the often long interval between the infective bite and the subsequent early symptoms of the disease.

2 THE BUREAUCRATIC/ADMINISTRATIVE PERSPECTIVE

There are many complexities and unknowns as far as rabies administration is concerned. Rabies is a notifiable disease and the follow-up action is a somewhat futile exercise because the illness is always fatal and the bite has usually taken place a considerable time before. The form GW17/7 should be completed in all instances of possible rabies exposure. This form is mostly an unknown entity to medical practitioners and it is not available at most casualty stations. The protocol pertaining to deaths suspected of being caused by rabies is difficult to implement. Policy and other circumstances dictate that such cadavers must be examined some 100 kilometres away in Durban and, in addition, there is a cultural resistance by the local communities to post-mortems being carried out.

3 THE PUBLIC PERSPECTIVE

There is a frustrating and wide-spread ignorance of the importance of immunisation of animals in an endemic area. There is also uncertainty as to what to do with the patient and the animal when a bite incident takes place. It is almost impossible to implement rigorous follow-up action in the third world environment where rabies is rife. The apparent apathy of the people at risk is difficult to reconcile with

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the picture of a child in the terminal stages of the disease. Yet it is difficult to impress on the local population the seriousness of the situation when they themselves are living in life-threatening times (for reasons other than rabies). Their day to day priorities are more often a question of making some sort of living in an increasingly more hostile world. One of the most vital pieces of information that is required when the child presents at the hospital with a dog bite is fate of the dog itself. It is often almost impossible to trace the dog because the disease destroys its strong territorial instincts and they tend to disappear into areas where they are not recognised. More than 70% of the diagnosed cases in dogs are labelled as "stray" or "unrecognised". It is certainly true that all these dogs could be termed unsupervised.

The disease has taught us many things, one of the most important of which is that close co-operation between the veterinary and medical professions can and does bear fruit. We have struck up an excellent understanding and working relationship with Allerton and the Veterinary Department. We intend to, and will, develop this collaboration into a success story.

DEMOGRAPHIC TRENDS IN NATAL/KWAZULU

Dulcie Krige [1]

1 INTRODUCTION.

Why think about demography (the science of population statistics) at a rabies conference? Dogs, the vector in the strain of rabies found in Natal/KwaZulu, are very closely associated with people - hence human population growth and movement tells us a great deal about numbers and concentrations of dogs. Before looking at this in detail let us consider just one statistic - since rabies first became a problem in Natal/KwaZulu in 1960, the human population in the area has grown from around 3m to 8m i.e. it has considerably more than doubled. If we accept research which indicates a dog:person ratio of 1:6 then the number of dogs in 1960 was 500 thousand and in 1992 1,3 million. Hence if it is necessary to vaccinate 70% of dogs in order to contain the spread of rabies then 350 thousand rabies vaccinations were necessary in 1960 and 910 thousand are necessary now - whereas 250 thousand dogs were vaccinated in 1961 and only 350 thousand were vaccinated in 1992.

2 HOW ACCURATE ARE THE FIGURES WE WORK WITH?

The answer to this is unfortunately "not good at all" - but let us look at this more closely :

- The person:dog ratio is an essential component necessary to calculate the number of dogs in Natal/KwaZulu, and different ratios for various types of areas and degrees of poverty of the population are essential to estimate sub regional totals. But the ratios available are not sufficiently accurate, at the sub regional level, to be used with the fullest confidence. Even if accurate human population figures are available for an area but the person:dog ratio is in fact 3:1 instead of 6:1, then the target population will be thought to have been achieved when in reality only half that target has been reached!
- The degree of accuracy of the 1991 SA population census is also not as high as we would like - the black population was undercounted by 17%, Asians by 12% and coloureds and whites by 11%. A post census validation exercise by the Bureau of Market Research at UNISA (in conjunction with Central Statistical Services CSS), and for which I was responsible for the Natal part of the exercise, shows that 1 black person in 4 was missed in the areas enumerated by CSS, that this figure was higher in the self governing homelands, 37%, and that in certain districts the undercount was as high as 50%! This adjustment (by race, age, gender and district) is built into the published census results. Not all areas however were enumerated in the traditional manner by CSS - in certain areas (including Soweto in the Transvaal) and especially the five KwaZulu census areas which form part of the Durban Functional Region the methodology was a combination of aerial photographs and random samples and the result of this was a very high level of accuracy in these areas.

It should be noted that this high degree of attention to validation was not given to previous censuses and although they were known to be far from accurate (as are all censuses even in the USA) it is impossible to go back now and estimate how much the undercount varied by area.

The purpose of this depressing overview of the level of accuracy of data on which decisions are based is that we need to be constantly aware of the problem and to :

- keep updating (e.g. by adding a question to other surveys) regarding the accuracy of person:dog ratios;

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- get advice from experts when using census figures - e.g. for Ndwedwe the figures are very good, for Vulindlela there is a 50% undercount.

3 RATES OF POPULATION GROWTH AND MOVEMENT.

The 1991 census estimated the Natal/KwaZulu population at just under 8 million - of this 7.5% of the population was white, 9.5% Asian, 1% Coloured, 12% Black resident in Natal and 69% black resident in KwaZulu. I make these comments on race not to return to the racial divisions away from which we are trying to move but simply because these divisions still are in RSA very much a proxy for socio-economic status and this gives a useful indication of the sections of the population which can afford to vaccinate their dogs themselves (or get them to the SPCA) and these who cannot afford to do this themselves. I'll return to this point later under a discussion on the political fragmentation of Natal/ KwaZulu.

There are a number of demographic aspects which need to be examined :

- Population growth in RSA is certainly high and (as touched on in the introduction) it is essential that health services including rabies vaccinations keep pace with this growth. It is necessary however to note that fertility rates are dropping more rapidly than demographers had foreseen - such decreases ("declining increases" more accurately!) are a basic part of the demographic transition and are determined by levels of urbanisation and of decreasing levels of poverty. Hence it would appear that the population growth rate has peaked and the South African growth rate is some 2.3% p.a. and Natal KwaZulu's 2.1% p.a. - at which rate the Natal KwaZulu population will take some 33 years to double.
- The assessment of the degree of urbanisation in Natal/ KwaZulu varies from that based on the Central Statistical Services definition of 25% of the black population of Natal/KwaZulu to the Urban Foundation's figure of 46%. It is more realistic to accept the Urban Foundation's figure and this tells us that the level of urbanisation is quite far advanced. This results in the fact that 32% of the total population of Natal/KwaZulu is presently resident in the Durban Functional Region and that this percentage will rise over the next twenty years and the actual population will increase from almost 3 million to 6 million people. This does not mean that there will be depopulation of rural areas but rather that population growth will result in rapid metropolitan growth. Unfortunately much of this movement will simply take the form of moving rural poverty to urban areas and will lead to large informal settlements. The advantages of this for rabies vaccinations is that it will be possible to locate new points fairly close to Durban!

4 THE IMPLICATIONS OF POLITICAL FRAGMENTATION.

The map of Natal/KwaZulu shows the high degree of administrative fragmentation - the jig saw puzzle nature of the area. This means that unless there is co-operation between the veterinary services of the two administrations - by which I mean joint policy decision making and on-going consultation which includes accurate statistical records of numbers of vaccinations per magisterial district - it is perfectly pointless to vaccinate in one area while only lOkm away there is no attempt at rabies control. Political solutions are likely ultimately to resolve the problem but in the short term the kind of co-operation which presently exists on the ground between SA and KwaZulu health services would make some considerable headway towards resolving the problem.

If all vaccine (as a free service) is to go to the poor it should be noted as discussed earlier that 15% will need to be distributed in Natal and 85% in KwaZulu.

5 AWARENESS OF THE NEED FOR RABIES VACCINATIONS.

It is clear that even where a free service is provided to vaccinate dogs, and even where much has been done to raise awareness of the need for action it remains true that often there is little response. This is not at all surprising. People are fighting an all-out, continuous battle against poverty. In Kwa-Zulu there are disproportionate numbers of the elderly, children and women; men are away (if they are lucky in this period of economic decline) working as migrant labourers. Children walk often lOkms to school each day - leaving before dawn and returning at dusk. Women walk kilometres to collect water

and firewood, they work in fields, they cook and care for their families and now somebody from government comes and says they must walk l0kms or more and waste a whole busy day to take their dog to be vaccinated against a sickness they have never heard of and that they've never known anybody to die from!

So perhaps we should think more creatively about how to get dogs to inoculation points. Certainly there should be broadcasts over the radio (95% of people have access to radios), and stories about rabies in the schools, but it would also make sense to play videos at the two places where people gather regularly and to make these inoculation points since there would not be any additional effort for people to attend. Women do take their babies to clinics because they are well aware of the need for immunization; and the elderly attend pension pay-out points every two months. Certainly it might require extra innovative measures - like paying a labourer to sweep up afterwards so that clinic sisters are not annoyed by the mess dogs leave behind and is there any way of designing collapsible cages to take along so that dogs are kept apart and don't infect one another with other diseases? After I had written the first draft of this paper I discussed these suggestions with a friend who has done much health-related research in Natal/KwaZulu and she tells me that it is never women who take dogs for rabies vaccinations but only men adults and youngsters). This would mean that although pension points might be viable as vaccination points, clinics would not be!

My point is that if we realise the constraints under which the poor live, explore the socio-economic realities, and work together to find solutions, possibilities soon spring to mind.

Rabies is quite clearly a disease which has a disastrous ability to spread widely but the onus for control rests with the state, with those who are aware of the frightening consequences, and not with those who have never seen anyone die of the disease whereas they have all seen babies die of diarrhoea and measles and people of all ages die of TB and they will soon start seeing them die of AIDS.

6 CONCLUSION.

I have attempted to show that if the number of dogs in a region is directly related to the number of people then it is necessary to understand population dynamics, to know how many people there are, what their growth rates are and how fast urbanisation is occurring. But it is also necessary to understand the constraints of poverty so that planning can take these into account.

My suggestion, and it is clearly a biased one considering my research interests, is that all the vaccination points (together with the number of dogs vaccinated at each) in Natal/KwaZulu be put into a Geographic Information System (GIS), as well as all rabies incidents. Also in the GIS would be the 1991 census data together with person:dog ratios collected from surveys. This would make it possible accurately to monitor the extent to which rabies vaccinations are reaching the necessary 70% of the dogs of an area - particularly in areas where rabies has been rife. It would permit a policy to be developed with regard to short and long term vaccination priority points by including pension points (and possibly shops) in the GIS.

Table 1 : 1991 census undercount, bureau of market research.

Whites	11%
Coloureds	11%
Asians	12%
Blacks	17% *

* in CSS areas 25%, in self governing homelands, 37%

Table 2 : Durban functional region population.

1991	3 MILLION*
2010	6 MILLION

* Durban municipality 720 000

Table 3 : Natal/Kwazulu

Year	Number of people	Number of dogs	Rabies vaccine needed	Rabies vaccine used
1960	3000000	500000	350000	250000
1991	8000000	1300000	910000	350000
2010	12000000	2000000	1400000	?

DOG ECOLOGY IN EASTERN AND SOUTHERN AFRICA : IMPLICATIONS FOR RABIES CONTROL [1]

B.D. Perry [2]

1 INTRODUCTION.

The domestic dog is by far the most important species in the maintenance and transmission of rabies in Africa, although the degree of it's importance varies from region to region. In general, it appears that rabid dogs constitute a greater proportion of confirmed rabies cases in eastern Africa than in southern Africa (reviewed by Perry, 1993). In the latter region, certain wildlife species, notably the yellow mongoose (Cynictis penicillata), the black-backed jackal (Canis mesomelas), the side-striped jackal (C. adjustus) and the bat-eared fox (Otocyon megalotis), play significant roles in certain areas (Barnard, 1979; Bingham, 1993; Thomson & Meredith, 1993).

Perry (1993) discussed the possible reasons for regional differences in species associations, suggesting that they were a result of both real and artificial phenomena. Real phenomena include the differing wildlife species distributions, population densities, ecologies and land use systems between the regions which may affect the species associations of rabies. In addition, the rabies virus isolated from the yellow mongoose can be differentiated from the dog-associated rabies virus prevalent in the region on the basis of differing reactions with a panel of antinucleocapsid monoclonal antibodies (King, 1991; 1993). This suggests that differences in species susceptibility to rabies viruses demonstrating different antigenic characteristics may exist, similar to the phenomena reported in several wildlife species in North America (Smith, 1988; Smith, Reid-Sanden, Roumillat, Trimachi, Clark, Baer & Winkler, 1986). Artificial phenomena include possible regional differences in dog rabies control, with apparently better control in southern Africa than in eastern Africa; in Europe and North America wildlife species became increasingly important in the epidemiology of rabies following the control of the disease in dogs (Steck & Wandeler, 1980; Perry, 1987). The recent dramatic increase in the proportion of rabid dogs in South Africa, reportedly as a result of a decrease in the dog vaccination coverage in Natal and Kwazulu (Bishop, 1993), is a clear illustration of this.

Not only is the domestic dog the most important species overall in eastern and southern Africa, but also the disease in dogs has been reported with apparent increasing incidence over the last twenty years or so, with many countries of the region currently reporting record numbers of cases (Perry, 1993). Given our understanding that there is a close relationship between the rates of increase in population sizes of dogs and humans, it is likely that the dog population has increased significantly over this period. There are very few estimates of dog population growth in the region, but in Zimbabwe, Foggin (1988) calculated a rate of growth of the dog population of 5.15% per annum over the period 1954-1986. It is likely that the detection rate of rabies (i.e. the number of confirmed positives as a proportion of the total rabies cases) has declined in many parts of the region over the same twenty year period, due to the decreasing financial resources at the disposal of government veterinary services in many countries and the resulting constraints on the effective delivery of diagnostic services (de Haan & Nissen, 1985; de Haan & Bekure, 1990; WHO, 1984). The combination of increases in reported disease incidence, apparent increases in dog population sizes and assumed decreases in rabies detection rates leads one to believe that dog rabies is a substantially greater problem in the region than is documented.

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[1] The complete version of this paper has been submitted to The Onderstepoort Journal of Veterinary Research. The inclusion of this document in these proceedings in these Proceedings has been done with the permission from that Journal's editor, to whom we express our thanks

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Is it possible to reverse this trend and if so, how? There are numerous inactivated rabies vaccines now available on the international market that have been shown to be highly efficacious in dogs, and it is generally believed that the constraints to effective dog rabies control are economic and logistical rather than technical, namely the poor accessibility of dogs to vaccination, the inadequate availability of rabies vaccines in many countries and the high cost of vaccines. For this reason, there is a strong argument for new approaches to be taken in the delivery of these effective vaccines to dog populations. The common theme of such approaches is effective targeting of rabies vaccine, a concept that has received considerable attention in the control of human diseases such as hepatitis B (Anderson, 1992) and this raises two important questions :

1. What are the high risk components (segments) of the dog population in terms of their capacity to transmit rabies?
2. Is it possible to develop methods to access these components with vaccination and other control measures, both effectively and at the appropriate frequency?

Studies of dog ecology can provide much of the information required to answer these questions, and this approach has been identified by several workers (Beran & Frith, 1988; Wandeler, Budde, Capt, Kappeler and Matter, 1988) and pursued by WHO (WHO, 1988; WHO/WSPA, 1990). This paper will discuss the potential contributions of dog ecology studies to improved delivery of rabies control in eastern and southern Africa, summarise the requirements of such studies and briefly review data currently available from the region.

2 TARGETING THE DELIVERY OF DOG RABIES CONTROL MEASURE.

Effective dog rabies control requires the immunization of a large proportion of the dog population in order to reduce the contact rate between rabid and susceptible dogs to a level too low to sustain rabies transmission within the population. In broad terms, this requires high levels of vaccination coverage and WHO (1984) has proposed a target level of 70% of the dog population. However, required immunization levels to control endemic dog rabies will vary considerably from place to place depending on numerous factors, including the potential contact rate between infected and susceptible dogs (a function of dog densities, dog movement restriction and dog dependency on owners for food). Thus in spite of the ideal requirement of vaccinating high proportions of all dogs, in an economic environment currently characterised by severely limited resources, it is likely that the cost effectiveness of dog rabies vaccination could be improved considerably by identifying those sub-populations at greater risk and concentrating resources on them to achieve required immunisation levels. But how can high risk dog populations be identified? Although in general terms these are likely to exist where contact rates exceed "a certain threshold", little work has been done on defining what such threshold levels might be and what factors contribute to differences in contact rates (although such work has been carried out for some viral and bacterial infections of humans; Anderson and May, 1991, and for rabies in some wildlife species; Bacon and Macdonald, 1980, Bacon, 1985, and more recently for feral dog populations; Macdonald and Carr, 1993). It is likely that in many cases high risk components of the dog population are also those that require special consideration as far as accessing vaccination. In some communities, such as the more affluent urban and rural societies who keep dogs in confined compounds as pets, rabies risk is probably lower and in addition high vaccination coverages can generally be achieved in these communities with the minimum of effort, due to their access to the media and financial resources to purchase quality veterinary services. High risk dog populations are likely to exist in many high density urban suburbs, where close proximity between households leads to high dog densities; lack of financial resources to adequately feed dogs in these communities, resulting in extensive scavenging, combined with little dog movement restriction results in high dog to dog contact rates. Clearly numerous intermediate situations exist. Figure 1 illustrates the hypothetical difference in spatial terms between dog population densities and overlapping home-ranges (and thus potential contact rates) between a high density, low income, urban suburb setting and a rural setting. It is intuitively apparent that the vaccination coverage levels required to interrupt rabies transmission would be different for these two examples.

The WHO has recommended the use of a matrix classification system for dogs, based on the level of dependence on human beings for food, shelter and companionship, and on the level of restriction or supervision imposed (WHO, 1988). It is designed to improve the targeting of dog rabies control measures and to provide a framework for their delivery (Fig.2). Under this classification, dogs fall into four broad categories: restricted (fully dependent and fully restricted); family (fully dependent and semi-

restricted); neighbourhood (semi-dependent and semi-or un-restricted); and feral (independent and unrestricted). Although very simplistic, this framework can be adapted to specific conditions for practical use in a country or area with the input of appropriate dog ecology data. For example, the restricted dogs and the family dogs are likely to be highly accessible for immunization by traditional injectable means, but family dogs may also be the target for dog removal if not immunised in a vaccination programme. Furthermore, owners of restricted dogs are more likely to have the resources to seek private veterinary services. Neighbourhood dogs might be the target for oral rabies vaccine distributed at key dog assembly points (Perry and Wandeler, 1993). True feral dogs should be removed if possible. The term stray dog, commonly used in the past, is considered a misleading term and WHO (1988) recommends it be used only to describe a dog not in compliance with local regulations. Under the WHO classification, a stray dog may thus be a feral dog, an abandoned or lost animal or merely a free-roaming family dog.

An important component of identifying high rabies risk dog populations is a well documented understanding of rabies epidemiology, including species distributions and interactions, age and sex incidence rates. Furthermore, in order to develop quantitative approaches to determine levels of vaccination cover required in different dog populations, data on the prevalence of rabies infection, incubation period, duration of virus excretion, etc. are required. Although there are broad understandings and perceptions of these parameters from most of the countries in the region, there are few examples of the effective utilisation of accurate diagnostic records to provide good, quantitative, epidemiological data on rabies occurrence in dogs. A notable exception is Zimbabwe, where among other things Foggin (1988) 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 in animals under three months of age. Relating the age-specific incidence to the results of the Zimbabwe dog census carried out in 1986, which 20% of dogs were found to be under three months of age (Brooks, 1990), Foggin concluded that the low incidence of rabies in dogs of this age group, a group that is not legally required to be vaccinated in Zimbabwe, is unlikely to pose a significant threat to the control of dog rabies. Of 687 rabid dogs for which data were gathered for the period 1982-1986, 193 were observed attacking and biting other dogs. Foggin (1988) suggested that this observation of one dog in five biting at least one other dog was 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.

3 DATA REQUIREMENTS ON DOG ECOLOGY.

The types of data required for effective targeting and delivery of rabies control measures have been summarised by WHO (1984; WHO/WSPA, 1990), and these documents provide selected methods for obtaining such data. Of particular importance are total dog population size and structure, proportion of dogs accessible for a given control strategy, and dog population turnover. However, the types of data required depend on the questions being asked, and in a rabies control programme these questions are likely to centre around three potential control measures: vaccination by traditional injectable means; oral vaccination by bait delivery; and dog control (principally by dog removal). Immunization with injectable vaccines has been and will continue to be the most important method for the control of dog rabies in eastern and southern Africa. Oral vaccines administered by bait delivery to dogs are still in the early stages of development, but it is likely that they will play an increasingly significant role in the future, given their potential to allow immunization of a proportion of the dog population not easily accessible by injectable vaccines (Perry and Wandeler, 1993). Dog control in the form of quarantine, movement control and dog removal has in the past been a valuable adjunct to vaccination programmes. However, in recent years it has rarely been applied effectively in the region and in some cases its use has been counterproductive, removing dogs which are easily accessible for immunization and provoking public antipathy to rabies control programmes (Perry, 1993). Nevertheless, if properly targeted and applied in conjunction with immunization and at the appropriate frequency it may be valuable in reducing the contact rate between high risk sub populations of dogs.

Table 1 lists three major questions for each of the three control options mentioned, and identifies the data requirements in each area. For each parameter, a series of techniques have been described for gathering or assembling data, and the reader is referred to two publications by the WHO where these procedures have been well reviewed and documented (WHO, 1984; WHO/WSPA, 1990). In broad

terms there are three types of approach: those using calculations based on human population size, those using observational techniques derived from studies of wildlife populations, and those using questionnaire surveys of dog owning communities. Techniques for gathering data on the major dog ecology parameters are summarised in Table 2 and results of the few studies carried out within the region are given below. Other results from studies carried out in Machakos, Kenya and Lusaka, Zambia are presented in this volume (Kitala and McDermott, 1993; de Balogh, Meslin and Wandeler, 1993). In all dog ecology studies, it is important to consider the units of measuring dog population sizes and structure, in order that the results can be compared from one study to another, and used for decision-making purposes. Dog population sizes, for example, are usefully expressed as dogs/human, dogs/household and dogs/square kilometre, and calculated by administrative units (e.g. province), by land-use category (e.g. urban, rural) and, if possible, by the socioeconomic status of owners and keepers.

3.1 Dog population size.

There are very few studies of dog population sizes reported for eastern and southern Africa. Using the estimated range of dog:human ratios of 1:8 - 1:11 presented by Bögel, Andral, Beran, Schneider and Wandeler (1982), Perry (1993) calculated the dog population sizes of Kenya, Tanzania and Malawi for 1990, using projected human population figures of the World Bank (1986). These were estimated to be 2.3 - 3.2 million (Kenya), 2.5 - 3.4 million (Tanzania) and 0.73 - 1.0 million (Malawi). When compared to rabies vaccine issue statistics published for the three countries (Luusah, 1988; Machuva, 1988; Msiska, 1988), estimated vaccination coverage rates of 2.4% (1979), 3.9% (1988) and 5.0% (1987) were calculated for Kenya, Tanzania and Malawi, respectively (Perry, 1993).

However, the most comprehensive study to date of dog population size at a national level in eastern and southern Africa was that of Brooks (1990), in which a full national dog census for Zimbabwe was carried out. The total dog population was found to be 1.3 million, providing an overall dog:human ratio of 1:6.3 (based on an estimated human population of Zimbabwe for 1986, the year of the dog census, of 8.5 million; World Bank, 1986).

At the sub-national level, dog population sizes and dog:human ratios have been estimated in studies in South Africa and Kenya. In South Africa, Arbuckle (personal communication, 1990) carried out a random sample survey of households by ethnic group in 9 regions of Natal and Kwazulu, and estimated the number of dogs per household and the dog:human ratio (Table 3). In Kenya, Perry, Kyendo, Mbugua, Price and Varma (submitted) used a visual capture/recapture method to estimate the dog population size in a high density, low-income suburb of Nairobi. During a rabies vaccination campaign, vaccinated dogs were fitted with a nylon collar. One week later, a team traversed the study area observing and counting collared and uncollared dogs. Using the Lincoln index method of calculation (Seber, 1973; WHO/WSPA, 1990) the dog population was estimated to be within the range 580-635 (with 95% confidence), and the vaccination coverage was calculated as 68-75%.

Further sub-national level studies have now been carried out in Kenya and Zambia, the results of which are reported in this volume (Kitala and McDermott, 1993; de Balogh et al., 1993) so it is possible that a clearer understanding of dog population sizes in several parts of the region, particularly in relation to human populations sizes, will emerge.

3.2 Dog population structure.

In addition to the studies in Kenya and Zambia reported elsewhere in this volume (Kitala and McDermott, 1993; de Balogh et al., 1993), studies of dog population structure have been carried out at a national level in Zimbabwe, and at local levels in South Africa and Kenya.

The dog census of Brooks (1990) in Zimbabwe provided valuable and detailed information for the difference provinces, and a summary of the findings is presented in Tables 4 and 5. However, in the current economically-constrained environment, it is not envisaged that such a detailed national census will be easily repeated in other countries, and sample surveys or highly geographically-restricted studies are likely to be more cost-effective. The dog population studies carried out in South Africa and Kenya were both very site specific, thus their relevance to other situations even in the same countries is unknown.

In South Africa, Rautenbach, Boomker and de Villers (1991) gathered some data on the dog population of Maboloka town in Bophuthatswana while studying their health status, and a summary of their results is presented in Table 6. In Kenya, the study of Perry et al. (submitted) in a high-density suburb of Nairobi found the mean age of dogs to be 3.1 years (± 2.5) and the male : female ratio to be 6.4 : 3.6. About 80% of dogs encountered had not previously been vaccinated against rabies.

4 CONCLUSIONS.

In much of eastern and southern Africa, the major constraints to dog rabies control are logistical and economic rather than technical. These logistical and economic constraints comprise principally the accessibility of dogs to vaccination, the availability of rabies vaccines and the cost of vaccines. It is considered that the accessibility of dogs to vaccination can be improved substantially through better targeting of rabies vaccine delivery to those sectors of the dog population of greatest importance in their capacity to transmit rabies. Studies of dog ecology will permit such sectors of the dog population to be more clearly identified, and will facilitate the development of more efficacious methods of accessing them with parenteral and, in the future, oral rabies vaccines. Well structured dog ecology studies will also generate data, which, in conjunction with data on the epidemiological characteristics of rabies in dog populations, will allow the development of quantitative models of dog rabies and its control, which could form the basis for decision support systems for use by governments, rabies control officers and veterinarians in the future.

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Table 1 :A summary of the questions raised in dog rabies control programmes that require dog ecology data and the categories of dog ecology data required

Questions	Data requirements
1a) How many doses of vaccine are required ? - injectable – oral 1b) How many dogs require to be removed?	- Total dog popln size ? population size by risk group (? high, medium, low), by dog type (family, neighbourhood etc). - % coverage required in population (? and risk groups, dog types) - Dog population structure - (Habitat)
2. How can coverages of each of the three methods be optimised?	% accessible of risk groups, dog types by each control method - ownership - responsibility - supervision - attitudes to dogs, rabies and rabies control
3. How often do these measures need to be applied?	Dog population turnover

Table 2 : A summary of the methods used to collect and derive data on different parameters of dog ecology. Modified from WHO/WSPA (1990)

Parameter	Method
Dog population size	1 Inference and calculation from human population statistics and estimated human: dog ratio
	2 Total or direct counts.
	3 Estimation from rate of capture.
	4 Estimation from rate of recapture.
	5 Estimation from photographic recapture.
Dog functions and accessibility	1 Questionnaire surveys.
	2 Observational studies.
Dog population structure	1 Questionnaire survey.
	2 Observational studies (sex).
	3 Tooth wear of handled/killed dogs (age).
Dog movement	1 Observational studies.
	2 Tracking with markers (dyes, radiotelemetry)
Population turnover	1 Observational studies.
	2 Questionnaire of dog owners

Table 3 : Summary of dog population sizes in Natal and Kwazulu (from Arbuckle, 1990)

Ethnic group	Human : Dog ratio	Dogs/household
Asian	7.6:1	0.6
Black urban	15.6:1 (11-23)	0.48
Black rural	8:1	1.10
Coloured	8:1	0.58
White	2.9:1	1.20

Table 4 : distribution of the characteristics of the dog population surveyed by province in Zimbabwe (from Brooks, 1990)

Province	Number of households interviewed	Number of dogs in interviewed households	Average number of dogs per household	dogs per household in dog owning households	Number of households with dogs (% of total)	Number of puppies in interviewed households (% of total)	Number of adult male dogs in interviewed households	Number of adult female dogs in interviewed households	Sex ratio (male:female)	Extrapolated number of dogs in 1988	Average dog to people ratio	Average number of dogs per sq km
Manicaland	614	744	0.91	2.3	325(53%)	141 (19%	353	250	0.59:0.41	233 102	1:5.8	0.7
Mashonaland central	495	583	0.79	2.3	161(33%)	62(27%)	157	119	0.54:046	93,297	1:6.6	0.4
Mashonaland east	660	400	0.60	2.1	194(29%)	78(19%)	157	155	0.51:0.45	85 374	1:8.6	0.9
Mashonaland west	595	445	0.83	2.5	168(28%)	125(28%)	155	135	0.58:042	158,139	1:5.6	2.6
Masvingo	735	765	1.04	2.8	353(48%)	114(15%)	383	271	0.50:0.41	240,263	1:4.6	5.4
Matabeleland north	540	548	1.02	2.4	150(47%)	91(28%)	132	125	0.51:0.49	159 679	1:5.2	1.4
Matabeleland south	379	375	0.99	2.1	115(45%)	60(24%)	100	116	0.59:0.41	102 509	1:5.1	1.6
Midlands	748	825	1.11	2.7	307(46%)	192(16%)	374	319	0.54:046	267 761	1:4.2	4.5
Zimbabwe	4714	4271	0.91	2.2	1933(41%)	857(20%)	1815	1563	0.58:0.44	1 308 577	1:5.5	2.4

Table 5 : distribution of the characteristics of the dog population surveyed by land use in Zimbabwe (from Brooks, 1990)

Land use sector	Number of households interviewed	Number of dogs in interviewed households	Average number of dogs per household	Average number of dogs per dog owning household	Number of households with dogs (%)	Number of adult male dogs in interviewed households	Number of adult female dogs in interviewed households	Sex ratio (male:female)	Number of puppies less than one month old in interviewed households (% of total)
Urban	868	855	0.98	1.7	536 (61%)	112	84	0.57:0.43	39(17%)
Communal lands	2738	3092	1.12	2.3	1345 (49%)	1471	1100	0.57:0.43	670(21%)
Large scale commercial farms	841	720	0.85	2.4	49,7%)	52	76	0.64:0.36	60(31%)
Small scale commercial farms	340	449	1.32	2.7	192 (57%	163	150	0.53:0.50	77(17%)
Resettlement areas	1127	918	1.22	1.6	111 (10%	95	65	0.59:0.41	76(13%)
Zimbabwe	4714	4271	0.91	2.2	1937(41%)	1815	1563	0.58:0.44	859 (20%)

Table 6 : Summary characteristics of the dog population of Maboloko town, Bophuthatswana (from Rautenbach et al., 1991)

Human : Dog ratio	11.1 : 1
Dogs/dwelling	0.68
Male : Female ratio	5.6 : 4.4
Mean age	30.8m (range 3-96)
Unrestrained	58%
Chained at times	15%
Permanently chained	26%
Permanently in cages	1%

Figure 1 : A hypothetical diagrammatic representation of the difference in potential contact rates between dogs in urban and rural settings. The points represent the location of dogs, and the circles represent their home-ranges. For simplicity each home range is the same, but in reality this is unlikely to be the case.

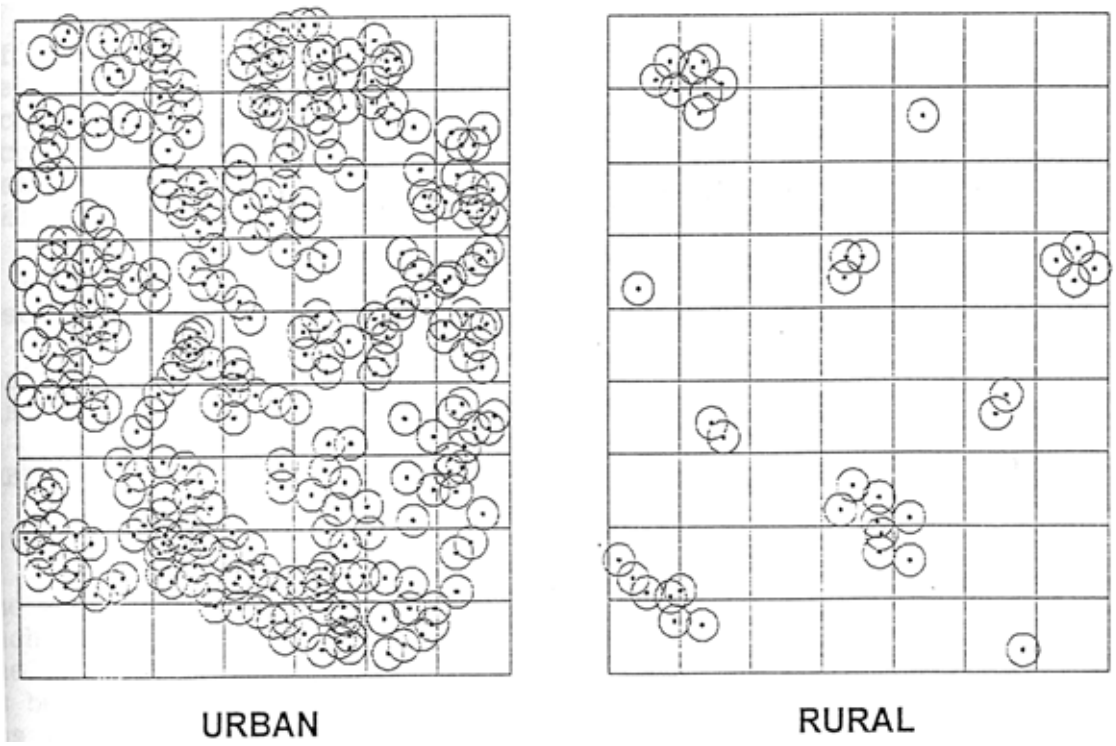

Figure 2 : A matrix classification of dogs based on their dependency and restriction. Developed by WHO (1988).

	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	
SEMI-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

RABIES CONTROL - VARYING STRATEGIES FOR DIFFERENT COMMUNITIES

M. Bachmann [1]

1 OVERVIEW.

The Pietermaritzburg State Veterinary area covers eight magisterial districts. There is one city, 3 towns, 7 villages and 1864 farms. The 1992 corrected population census indicated that 428 000 people live in the area. As parts of several districts are attended to by the KwaZulu Veterinary Service it is estimated that Pietermaritzburg area of responsibility has 350 000 persons. These persons own an estimated 40 000 dogs.

Official control of rabies is by means of dog vaccinations. Each year 26 to 28 000 vaccinations are administered officially. Six vaccinators known as Animal Health Technicians administer the vaccinations. Of these three are trainees. Funds available to meet the cost of vaccine, travelling and equipment are R40 000 (salaries not included).

In addition to the above private practitioners administer ± 6 000 doses of vaccine and the SPCA 2 000.

During the past year 33 cases of rabies were diagnosed. There was also one case in a human.

2 RABIES PREVALENCE PIETERMARITZBURG STATE VETERINARY AREA.

(350 000 People, 40 000 dogs, 34 000 vaccinations)

The bar charts in Fig 2 indicate the number of cases diagnosed each month over the past 24 months, the highest being 9 cases and the lowest being 0 cases. The average number of cases per month is between 2 and 4. During 1989, 1990, 1991 and 1992 prevalence has remained at this level.

This is not a totally effective means of measuring the dynamics of the rabies situation. Many infected dogs die without a diagnosis being made. However, in this area the transport and communication infrastructure is good. There are many private practitioners, several animal health technicians and a large number of well informed farmers. A very good diagnostic service is situated within easy reach. The information resulting from examination of specimens of animal brains is therefore more meaningful than is the case with more remote districts having poor infrastructure.

3 OFFICIAL VACCINATIONS.

The line graph in Fig 2 represents the number of vaccinations each month over the past 24 months. Note that these vary between a few hundred and over 6 000 per month. Also note that the greatest numbers of vaccinations are done between June and September each year because dry conditions at that time of the year lead to far better turn out at vaccination points.

It appears from fig 2 that there is a slight decline in prevalence following large numbers of vaccinations in winter and spring but cases nevertheless tend to be occur at any time.

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Table 1 : Human and dog population. Dog vaccinations 1992 and positive animals 1992.

	Population census	Dog population	Dog vaccinations
Camperdown	36315	3892	3801
Kranskop	7565	954	359
Lions River	43060	2889	1820
Impendle	2815	947	994
New Hanover	38207	2117	1576
Pietermaritzburg	228549	21678	11797+2200+6000 (SPCA) (PP)
Richmond	23476	3915	2871
Umvoti	41160	2891	2299
	421147	39283	25377+2200+6000
	421147	39283	33577

Table 2 : positive animals 1992

Camperdown	Cattle	4	
	Dogs	4	(2 strays)
Kranskop	Dogs	2	
Lions River	Dogs	1	(stray)
New Hanover	Dogs	6	(3 strays)
Pietermaritzburg	Dogs	6	(2 strays)
Richmond	Dogs	4	(3 strays)
	Horse	1	
	Human	1	
Umvoti	Dogs	3	(3 strays)
	Horse	1	

(we see a small proportion of the total "stray" cases and a high proportion on "owned dogs").

One is therefore led to make certain assumptions such as the following :

1. The levels of vaccinations appear to be inadequate.
2. As ± 35 000 vaccinations annually for ± 40 000 dogs does not appear to be effective (the vaccine provides 3 years of cover) one can assume that there must be far more than 40 000 dogs.
3. Far more vaccinations may have to be done. However, if more pressure is applied to the public to produce dogs for vaccination it is likely that increased vaccination figures will result from far more frequent vaccinations of individual immune dogs. The present budget will then become inadequate. The exercise may not be very effective in controlling the disease.
4. It is likely that even 100% vaccination levels annually would have only a minimum effect on the number of animals found to be infected i.e. the stray dogs, cattle, horses, etc. because of the regular influx of non-vaccinated and infected dogs from areas immediately adjacent.

I intend, in the rest of this talk, to indicate that several of the assumptions but not all, thus far are incorrect and that an entirely different scenario is more representative of the situation.

The information set out in Fig 2 is the type of information routinely used for rabies control assessment and evaluation. I submit it is not representative enough because if used alone it gives a distorted impression of the actual situation in different parts within the total area.

4 RABIES PREVALENCE PIETERMARITZBURG DISTRICT ONLY.

(150 000 people, 22 000 dogs, 20 000 vaccinations)

In Fig 3 the bar chart indicates prevalence by month. The line graph indicates monthly vaccinations in thousands of dogs only. The Pietermaritzburg district alone is reflected.

The prevalence peaks in June and July of 1991 were markedly reduced by two peaks of vaccination in June and September. The June peak of vaccination alone did not eliminate positive cases. Positive

cases only reached nil following the September peak in vaccination. The June peak was low because of stay away action in the area at the time.

It is very important to comprehend that all the positive diagnoses were derived from dogs having black owners who lived in the Edendale, Imbali and Willowfountain community. There have been no more positive dogs from that area since October 1991.

The January 1992 peak in prevalence was reduced to nil by the vaccination peak in February. This relates to a black community known as Sobantu. There were no further cases in that area after February 1992.

The September 1992 peak of vaccinations reduced the prevalence peak of the same month to nil in an Indian community known as Bombay Heights. There have been no further cases there since that time.

The last peak (only 2 cases) occurred in a white area (Prestbury) south of Pietermaritzburg in February 1993.

Figure 1 : Pietermaritzburg state vet. Area.

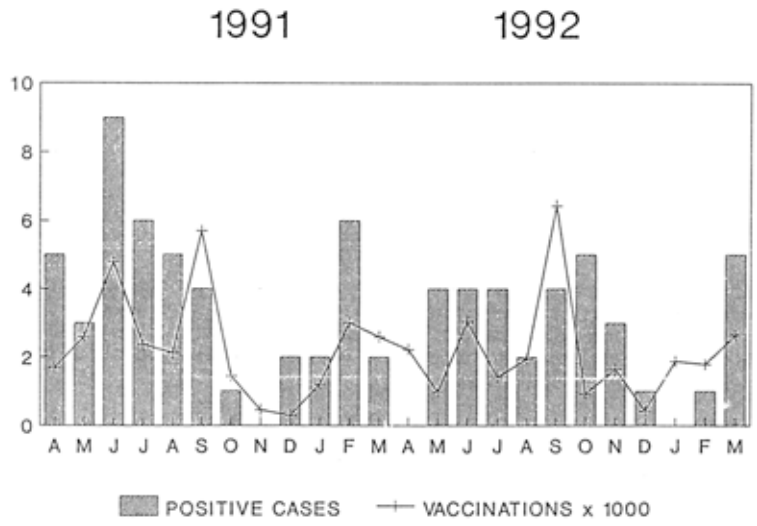
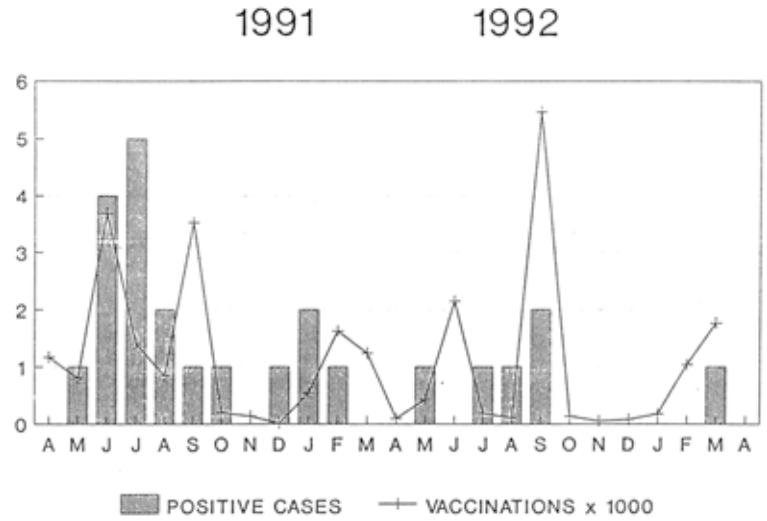


Figure 2 : Pietermaritzburg district.



INTERPRETATION OF FIG 2 : If too much data is collected and attempts are made to incorporate that data into one set of information, i.e. for a whole region or a whole province, interpretation becomes impossible as an average picture is represented which does not represent individual component areas. Therefore each outbreak MUST be considered on its own merits. If each outbreak is analysed separately it will be clear that many outbreaks can be brought under total control. In those areas where that is not possible the reasons must be understood.

However, new outbreaks tend to occur regularly for the following reasons.

1. Infection is regularly introduced by stray dogs from across the border where unrest makes vaccination a very dangerous process.
2. Regular prophylactic vaccination clinics alone cannot totally prevent the spread of rabies because clinics do not have total public support at all times planning and organisation is of inferior standard.
3. Campaigns get far more public support especially when infection is present. In campaigns vaccinators must achieve a target level of vaccinations. If this target is achieved the spread of infection becomes impossible and the reservoirs of infection die out. In routine prophylactic vaccination clinics, targets are seldom achieved because they are unknown. Potential future infection can then not totally be prevented.

5 SPATIAL DISTRIBUTION OF POSITIVE CASES (FIG 4).

When each positive rabies case is regularly plotted very accurately on a map a few very important observations become possible.

1. Rabies tends to occur in "clusters" or "clumps"
2. Rabies regularly occurs in a very limited part of the total area. This area can be defined
    - It is close to, or part of high density urban type black settlement
    - Vaccination levels in areas immediately adjacent may be inadequate because of danger to vaccination teams
    - Dog population recruitment into the area is high.

Such information tells us where our best efforts should be directed. We can plan and organise so that dog turnouts are high

Figure 3 : Spatial distribution of incidence in different communities.

6 VARYING VACCINATION STRATEGIES TO MEET THE NEEDS OF DIFFERENT COMMUNITIES.

We have seen thus far that if we collect all information with regard to rabies prevalence and incidence trends and also pay attention to the spatial and temporal distribution of incidence we can analyse each outbreak very realistically. We have also realised that certain communities are more prone to high levels of rabies infection than others.

We now need to look at different communities far more analytically. For the purposes of this talk a community can be defined as a group of people who together occupy a specific space or area with distinct borders. They behave and live in ways peculiar to their community and treat their dogs in a similar way. Different vaccination strategies must be devised to meet the needs of each community in order to achieve the best results.

7 THE WHITE FARMING COMMUNITY.

(150 000 people, 15 000 dogs, 6 000 vaccinations p.a.)

This community physically covers the greater part of the geographic area concerned. People with their dogs live comparatively far apart from each other. Dog numbers are controlled. Stray dogs are shot. Suspect dogs are produced for diagnosis. Animal Health Technicians visit these farms frequently. In consequence new dogs are soon vaccinated. Individual dogs need only to be vaccinated once in every three years. Therefore the annual vaccination level need not be more than 50 to 60% of the population.

A rabid dog would have to travel a long distance to come in contact with other dogs because of the sparse dog distribution. These would probably be vaccinated. The rabid dog would have a good chance of being shot. Rabies is therefore infrequent because the rabid dog cannot readily infect another dog.

8 THE WHITE FARMING COMMUNITY IN HIGH RISK AREAS.

(50 000 people, 8 000 dogs, 8 000 vaccinations p.a.)

These communities are in all respects similar to the foregoing but with one exception. They regularly have rabid dogs on their farms. The majority of positive animals are derived from these areas every year. In consequence their dogs are vaccinated annually in order to have the level of immunity as high as possible in the whole dog population. Puppies are vaccinated as soon after 3 months as possible. When a rabid dog is found we make a fuss. Because the number of affected people is small we can account for and vaccinate every single dog within a specific radius of the infected farm. In these areas more than 90% of dogs are vaccinated annually.

9 THE URBAN WHITE COMMUNITY.

(50 000 people, 12 000 dogs, 8 000 vaccinations p.a.)

These people keep many dogs. However, these are well cared for, population recruitment is low, dogs are largely confined behind fences, vaccination levels are high in general but many owners do not bother to have dogs vaccinated. These people are catered for in the city, towns and villages by regular vaccination clinics. They use private practitioners to a large extent. Rabies is infrequent but when it does occur a small campaign accompanied by the intensive spread of information is used. Rabies is seldom a serious problem.

10 THE INDIAN COMMUNITY.

(53 000 people, 5 000 dogs, 4 000 vaccinations p.a.)

All comments are as for the urban white community except that control by the community itself of its dogs is less satisfactory. Clinics are not well patronised. Consequently periodic campaigns are necessary, more especially when rabies occurs. Rabies can, however, be effectively dealt with and is seldom a serious problem.

11 THE BLACK URBAN AND PERI-URBAN COMMUNITIES.

(60 000 people, 8 000 dogs, 5 000 vaccinations p.a.)

It is in these communities that infection occurs frequently and spreads rapidly. The community itself applies none of the control measures mentioned. They are heavily politicised, poorly informed, highly mobile. They do not support clinics well.

Campaigns are the best means of dealing with these communities. Very well organised spread of information is essential. Vaccination venues have to be close together, i.e. not more than 2,5 km apart because people have to walk their dogs to the vaccination points on leads.

Accurate estimates of dog population is essential. When campaigns are conducted the target vaccination figures must be attained. If they are not attained the campaigns must be repeated. (See Fig 3. July and September 1991 vaccination levels and July and September 1992 vaccination levels. In both cases repeat vaccinations were required in order to reach target levels).

12 ATTAINING TARGET VACCINATION LEVELS.

For every outbreak a certain number of vaccinations must be attained in order to reduce that outbreak to nil. If and when the required level is attained in the face of an outbreak, the reduction of infection is dramatic. This has been demonstrated many times.

It is essential that as many persons in a given community must be well informed with regard to the time and date of all vaccination venues in order to get the best response. If people do not produce dogs for vaccination all else is futile. Every effort must be made to ensure that dogs are produced and that the community is accommodated in every way.

Table 3 : percentage response in communities to warning of rabies vaccinations by different means.

	Black Comm.	Indian Comm.	White Comm
Loud Hailer	65%	0%	Low
Word of Mouth	26%	33%	High
Advice at Schools	2.3%	8.4%	Not used
Radio Advice	1.3%	0%	Not used
Posters	3.6%	6.7%	Not used
Newspapers	1.8%	41.8%	Very High

Fig 5 is based on surveys among people who presented dogs for vaccination. It was necessary to know which type of information had resulted in their producing the dogs. Each person who brought dogs was asked only one question. "How did you know you must bring your dog here today for vaccination?" The table represents approximately 1000 replies.

By making use of the information presented it has proved possible to vaccinate at required levels in the face of outbreaks (control vaccination campaigns). Prophylactic vaccination, using regular clinics, especially when rabies is not present has been less successful.

We intend putting more effort into advising school children in future and also into placing information in Zulu in the media.

13 CONCLUSIONS.

- By vaccinating ± 85% of the estimated dog population annually, rabies can be controlled but not eliminated in this State Veterinary area.
- Both regular clinics and control vaccination campaigns are essential but they are used for different reasons in different communities.
- The personnel available here can cope with the situation as it is at present and could probably cope with a population of 500,000 people and 60,000 dogs if infection was not permitted to get out of hand, but not much more.
- Each community is distinct and unique. It must be recognised and constantly re-evaluated in order to arrive at an understanding of that community. Control measures must then be tailored to that community's specific requirements.
- The information that we use is not totally accurate and efforts require to be made to acquire more accurate information. The situation is dynamic and changes constantly.
- We need more accurate methods of determining dog population more especially in the high risk areas.
- Political instability has proved to be a most important factor in the increase in prevalence of many livestock diseases in Africa. It is the most important component of the deteriorating rabies situation in Natal. The direction in which matters are moving at present indicates a further deterioration in the immediate future.

PROBLEMS ASSOCIATED WITH RABIES DIAGNOSIS IN A BUSY PRIVATE PRACTICE

P.M. Kretzmann[1]

1 INTRODUCTION.

Some years ago I was presented with a dog that, according to the owners, had been hit by a car. It was a Saturday morning and I remember well the little limp figure that lay cradled in the arms of the owner in our waiting room. An initial clinical examination revealed no major trauma to the body and the dog was treated for shock and hospitalized. Later that afternoon the dog started chewing its water bowl - by the next morning the water-bowl and the cage door were severely mutilated. The dog was put down and rabies confirmed.

By far the majority of vets in practice in our area share anecdotes about cases with rabies presented to them. These stories commonly share one or both of 2 themes.

1. The nature of the clinical symptoms shown by the animals.
2. Their initial inability to make a conclusive clinical diagnosis.

Rabies is arguably the most publicised disease relating to dogs. The most sensational symptoms pertaining to the furious form are most extensively used in advertising campaigns and consequently public perception of the disease is based on the recognition of these symptoms.

It is widely recognised, however, that a large proportion of animals don't display these typical forms. The 2nd edition of "Rabies, The Facts" (2) indicates that only about 25 per cent of dogs with rabies develop the fully furious form of the disease - in the rest the disease is either paralytic from the outset, or rapidly becomes so. Fekadu (3) in the Natural History of Rabies (2nd edition), confirms that the diagnosis of rabies by clinical signs alone is inadequate since many dogs develop dumb rabies. He also quotes, in this book from a recent experiment that 24% of dogs, which were inoculated intramuscularly with various strains of rabies virus, had died without showing any signs of illness.

In the field, the longer the time lapse before establishing a clinical diagnosis of rabies, the greater the exposure to the owner of the animal, the diagnostician, his staff and all others in contact with the animal.

The aim of this paper, then, is to establish the difficulties with rabies diagnosis in private veterinary practices in the Natal region.

2 MATERIALS AND METHODS.

1. Statistics were assimilated from records kept by the Regional Veterinary Laboratory at Allerton, the Rabies Unit of which is the only diagnostic unit in Natal.
2. Eleven selected veterinarians in selected private practice in those areas in Natal where rabies is most frequently seen were asked to fill in a questionnaire and their input analysed (Fig.1).

These veterinarians were all experienced in rabies diagnostics. Four practices specialised in companion animals, the rest were involved in rural practice as well as with companion animals.

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3 RESULTS.

The private practitioners submit a considerable proportion of the specimens received by the regional laboratory (Figure 2). This graph highlights 2 important facts.

1. Private practitioners submitted 1974 specimens for rabies diagnosis to the Rabies Unit at the Regional Veterinary Laboratory at Allerton between the end of 1982 to the end of 1992 compared with 1178 by non practitioners (40.3% less) indicating the importance of the private practitioner as a screen in the Rabies Control programme.
2. A larger proportion of the number of specimens submitted by the private practitioner (58%) were negative compared with the 51% from non private practitioners.

This figure is unexpected if one considers that the vet is (or should be) an expert in the field of disease diagnosis.

Why then, this excessive proportion of overkill? A number of factors are immediately apparent.

1) Non private practitioners are usually presented with cases showing typical symptoms. These are often feral dogs, dogs from indigent homes or farm animals. These animals will often be destroyed on the farm and (often after consulting with the private vet) the specimen submitted to the laboratory. Private practitioners deal less with stray animals and more with anima1s with responsible owners, who are more likely to respond to early or unusual symptoms (Figure 3).
2) Veterinarians in small animal practice are more likely to err on the side of caution than rural or mixed practices, (verbal communication with eleven practitioners). The reasons for this are manifold but include :
   a) Specialization creates a greater incentive to achieve a conclusive diagnosis, hence submission of more borderline specimens.
   b) Easier access to the veterinary laboratory for the urban vets.
   c) Vets in the rural areas where rabies is rampant are exposed to greater numbers of cases and therefore more proficient diagnosticians.
3) The State pays the private practitioner R50.00 per specimen submitted irrespective of whether it is positive or negative - this relieves any financial incentive for ensuring that only highly likely specimens are submitted for confirmation.
4) The number of differential diagnoses that could be confused with rabies in Natal. These include:-
   a) Distemper - this disease has effectively been controlled in suburbia but remains a problem in the townships and rural areas, areas that are also hotbeds of rabies.
   b) Climatic and geographical conditions in Natal favour flea and tick development. Misuse of organophosphate and other insect and acaricide poisons frequently gives rise to CNS symptoms.
   c) Strychnine and other malicious poisons.
   d) Babesiosis in dogs (and cattle) sometimes results in encephalitic symptoms.
   e) Trauma.
   f) Behavioural changes, such as territoriality and jealousy.
   g) A further complication is that, with the present untenable security situation in South Africa big vicious dogs are replacing lap dogs in the homes. This results in more attacks on humans.

These factors would all contribute to making a clinical diagnosis of rabies by the private practitioner difficult. Those animals displaying typical symptoms will often be dealt with by others - less typical cases will be presented to the private practitioner.

In order to quantitate the problem a questionnaire was sent to eleven prominent veterinarians in private practices in the rabies areas in Natal and a number of answers were forthcoming.

The private veterinarian in general sees few cases of rabies in species other than dogs. (Table 1). This is confirmed by statistics from the Rabies Unit at Allerton. The practitioner generally does not experience any diagnostic problems in these other species. It was acknowledged, however, that rabies in cattle could be confused with Cerebral Redwater and in horses, colic. This is in contrast with the diagnosis of rabies in canines in private practice. (Table 2).

From this table a number of points become apparent :

1. All practitioners questioned (bar one) are completely happy with the liaison between the laboratory and private practice, indicating no realistic breakdown between clinical diagnosis and confirmation. The one practice who suggested some concern acknowledged a great improvement since the Rabies Unit was established at Allerton.

2. Most of the practitioners questioned had adequate hospitalization facilities. The importance of this as a diagnostic tool was emphasized time and again by these practitioners indicating that an initial conclusive clinical diagnosis was often impossible. Those practitioners who were concerned about the hospitalization of animals in their practices were apprehensive about the responsibility of having the animal escape

3. Respondents in general indicated that the limited time that was sometimes available for a detailed diagnostic build up was not a realistic deterrent if the hospital facilities were adequate

4. The table does indicate that most practitioners are concerned with recognising symptoms. The respondents further indicate that they see a far higher percentage of "dumb" rabies than the "furious" form. (Table 3).

Finally, the symptoms most frequently seen by the respondents are indicated in Table 4 and are:

1. A sudden change in disposition.
2. A watchful apprehensive expression of the eyes.
3. Drooling of saliva.
4. Paralysis of lower jaw and tongue.
5. If restrained, attacks objects within reach.

4 CONCLUSIONS.

The practitioners selected for the questionnaire are all experienced in rabies diagnosis and professionally respected. Their inability to recognise the symptoms of rabies in some cases, and their reliance on time in a proper hospital facility to achieve a diagnosis, is disturbing. Often the primary symptom they describe is a "sixth sense, a disturbing stare from the dog". This subjective symptom is something that the diagnostition can only learn through experience.

If one considers the volume of cases to which veterinary private practitioners are exposed (and this is likely to increase with the present political upheaval, resulting in inadequate vaccination in certain rural areas and increased number of feral dogs). The inexperienced private practitioner, particularly if he does not have access to adequate hospitalization facilities, will always be at risk.

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RABIES IN KENYA

W.K.Chong' [1]

1 INTRODUCTION

Rabies incidence in Kenya has remained high and widespread over the last ten years. It is most prevalent in dogs, followed by livestock, cats, wildlife and man in decreasing order. Wildlife rabies has been observed in five of the eight provinces with a total of thirteen districts affected. Of the wildlife species with confirmed rabies, the jackal, mongoose, wildcat, squirrel, honey badger and the fox contribute the bulk of the case incidence. Seasonal occurrence varies with years with 1991 showing two distinct peak periods. Control is largely directed at the dog population.

A rabies-like disease was first reported in South Nyanza District, Nyanza Province in Kenya in 1902 just a year after the Department of Veterinary Services was started. However, it was only in 1912 when rabies was first confirmed in Nairobi in a dog bitten by a jackal. This was two years after Kenya's first Veterinary Pathologist and his team of research workers had set up their equipment at Central Veterinary Research Laboratories, Kabete (7). Rabies in Kenya is believed to be caused by the true rabies virus (serotype 1) and probably one or more of the other three Lyssa virus serotypes, namely; Lagos bat virus (serotype 2), Mokola rhabdo virus (serotype 3) and Duvenhage rhabdovirus and European bat Lyssaviruses (grouped as serotype 4). However no work has been done to determine not only the occurrence or otherwise of these other serotypes, but also on determining the characteristics of the circulating strains of rabies virus.

The diagnosis of the disease is based on clinical signs, history and confirmation in the Laboratory (6,8). The latter is carried out at the Central Veterinary Research Laboratories, Kabete and Mariakani Regional Veterinary Investigation Laboratory. However, for disease security and documentation purposes, isolation of virus and other laboratory based rabies work is done at Kabete. Consideration is being given to decentralise diagnosis to involve the other Regional Laboratories at Eldoret, Kericha, Karatina and Nakuru. This is in order to cut down the long distances samples have to travel as well as easing other transport associated problems.

Diagnostic tests in use are Fluorescent Antibody Test (FAT), mouse inoculation test (MIT) and histopathology (6,8). M1T is done on all cases that test negative on both FAT and histopathology (6). Tissue culture is at the moment being adopted using neuroblastoma cells. Attempts have also been made at using BHK21 cells (6). Diagnostic capability has been greatly hampered by inadequate operational funds to run the services as well as low public awareness of the seriousness of rabies. Despite the above problems, the Central Veterinary Research Laboratories and the Regional Laboratory has been able to provide rabies diagnostic services, whenever called upon. The following presentation, is mainly a case report of rabies cases handled between 1983 and 1992 at Kabete (1).

2 RABIES CASES

Official recorded figures of laboratory confirmed cases (1) provide an indication and not a true extent of rabies occurrence in Kenya. This is attributed mainly to: non-presentation/or reporting of suspected cases, non-availability of local district diagnostic facilities, arrival of specimens at the diagnostic laboratories in a state of decomposition that no diagnosis can be made, as well as, general lack of awareness amongst the public.

From reviews on rabies early history, Kenya experienced varying degrees of rabies epidemics between 190O to 1969. No cases were recorded in years 1917-27, 1930, 1956, 1959, 1961, 1964 and 1966 - 67. There were one to five cases between 1912-16, 1928, 1929, 1931 and 1937-39. Waves of

[1] Dept of Veterinary Services, P.0. Kabete, NAIROBI, KENYA.

outbreaks went up to sixteen to twenty in 1933-35, 1947-48, and 1951. In 1932, 1945, 1950 and during the emergency period 1952-54, twenty one to twenty five cases were recorded. The figures went up, twenty six to thirty in 1946 and thirty one to thirty five in 1941 and 1949 (2,3,4). Between 1970 to 1975, there was relative quiet marked with low and stable incidence. This picture changed between 1975 and 1978 when there was an epidemic and then a brief decline period from 1978 to 1979. However, from 198O's onwards, the country experienced a dramatic rise, peaking at 290 cases in 1987. From 1983 to 1992, the case incidence has remained high, averaging 216.3 cases per year confirmed at Kabete, representing a ten year (1983-1992) average of 58.19 percent positive cases per year. (Figure 1, Table 1).

3 DISTRIBUTION OF RABIES

Although there are currently forty seven districts (Nairobi City, the capital included), the map of Kenya (Figure 2) and (Table 2) shows only forty one. The recently created districts (Nyamira from Kisii, Migori from South Nyanza, Bomet from Kericho, Makueni from Machakos, Tharaka-Nithi from Meru and Vihiga from Kakamega) which are omitted are represented as part and parcel of the mother districts for purpose of this reporting. Rabies has been widespread in the country over the last ten years. To date, all the eight provinces and thirty seven of the forty one (old districts boundaries) have at least had rabies. Generally, the number of districts with positive cases has ranged from seventeen to twenty nine (I8 - 1983, 1990; 25 - 1984; 29 - 1985; 26 - 1986, 1987; 21 - 1988, 1989; 17 - 1991, and 19 - 1992).

Eastern, Rift Valley, Central and Nairobi Provinces contributed slightly over ninety five percent of total rabies cases over 1983 - 1992 period. The highest Incidence was in Machakos in Eastern, followed by Nairobi, then Nakuru in Rift Valley, Nyeri and Kiambu in Central, and, Kericho in Rift Valley in that order, contributing nearly seventy two percent of the outbreaks. There has been no laboratory confirmed rabies recorded in Garissa and Mandera in North Eastern, and, Lamu and Tana River in Coast Provinces (Table 3).

The fact that rabies appears to be widespread in the country seems to be confirmed by the wide distribution in many species in which it has been confirmed (Table 4). The disease incidence is very high in the dog (62.83%) followed by livestock (cattle, sheep and goats) (28.39%), cat (3.19%), wildlife (2.82%), and other domestic stock (pig, horse and donkey) (2.04%). The human cases (0.74% confirmed may not portray a complete picture, given that many cases are diagnosed in human hospitals and still a number go unreported/ or unconfirmed at our laboratories.

4 WILDLIFE RABIES

In the country, wildlife rabies has been confirmed in Kericho, Laikipia, Nakuru, Narok (Rift Valley), Nairobi (Nairobi) Kiambu, Muranga, Nyeri (Central), Isiolo, Meru, Machakos (Eastern), and Kilifi, Taita-Taveta (Coast) (Table 5). Out of the districts with wildlife rabies, Kericho, Laikipia, Nakuru, Narok, Kiambu and Nyeri recorded more livestock than canine rabies. This is unlike Isiolo, Meru, Taita-Taveta, Machakos and Nairobi with more canine than livestock cases, the latter two districts reporting the highest case incidence. Kilifi recorded only canine rabies. Individual wildlife species experienced low rabies incidence, which could be attributed to the fact that there has been no efficient and sustainable method of wildlife sample collection and submission. In 1992, there was an increase in number of rabies cases, owing partly to contribution of a project undertaken within the National Museums of Kenya.

Although the true incidence of wildlife rabies has not been completely determined, there is a reflection (Table 6) that the jackal/wild dog, mongoose, wildcat, squirrel, honey badger and the fox which contribute approximately seventy four percent of wildlife rabies cases, have a role to play in rabies epidemiology. Other wildlife species may also have a part to play.

5 SEASONAL OCCURENCE

An attempt to depict the pattern of rabies occurrence annually from 1990 to 1992 is shown in Figure 3. The graph indicates varying number of peak periods for the years plotted, with 1991 clearly showing the high and low peaks in April and November respectively. This corresponds to the normal long and

short rainy seasons. The reasons for the varying rabies peak periods with apparent association with rain seasons, may be unravelled by the proposed and ongoing ecological and epidemiological studies (4,5) relating to the disease. This should lead to a better understanding of rabies occurrence and transmission.

6 CONTROL

Currently four methods are in use. These are: vaccination targeted mainly at dogs, destruction of stray dogs, restriction of dog movement and post-exposure immunisation of humans. The main target of control measures is the dog population. Unfortunately, dog population dynamics and exact figures are little known and/or understood, hence the percentage vaccination coverage is difficult to gauge. This is indeed a big setback in the successful control of the disease.

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Figure 1 : animal rabies in Kenya : 1970-1992

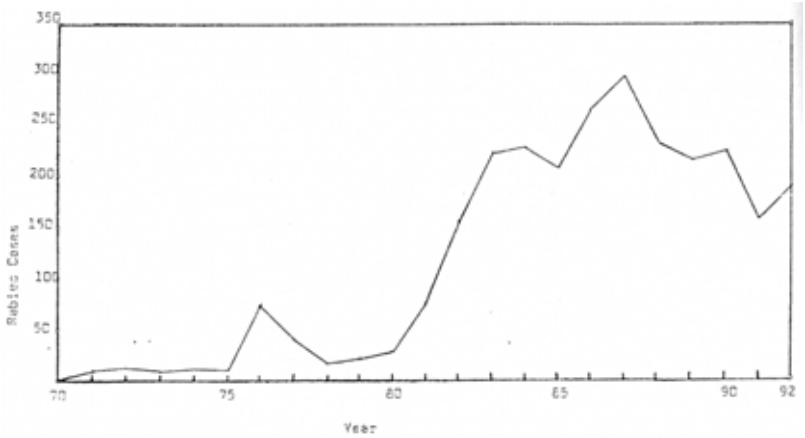


Figure 2 : map of Kenya

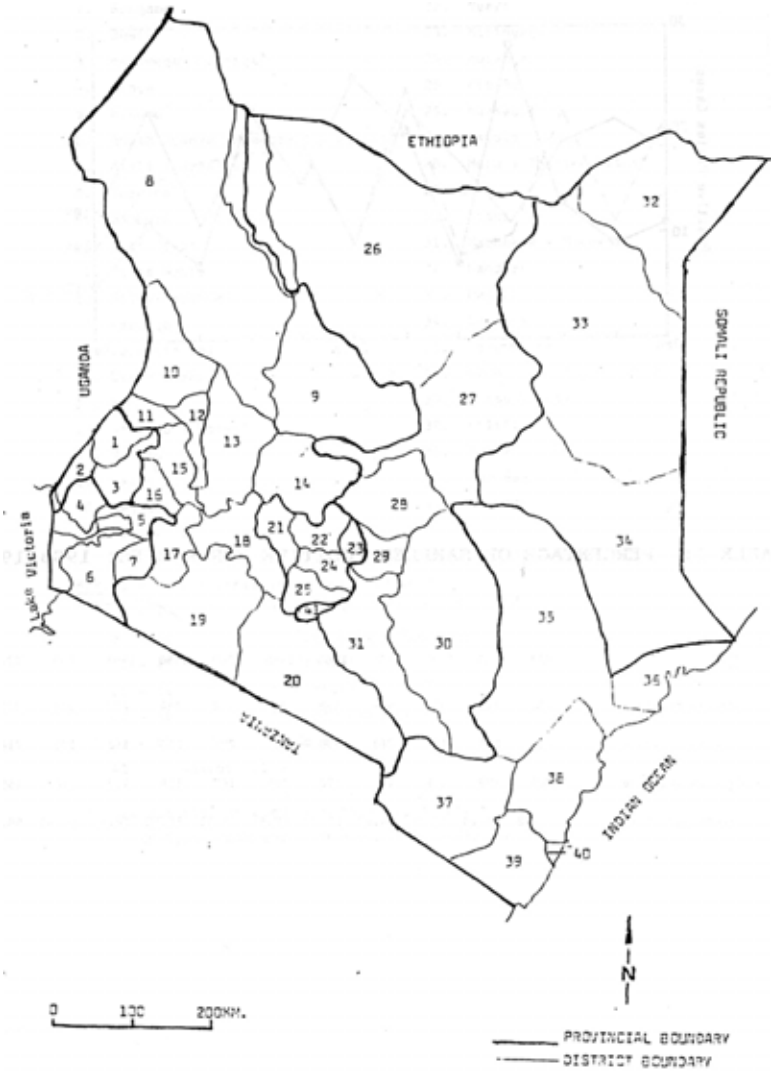


Figure 3 : seasonal occurrence of rabies : 1990-1992

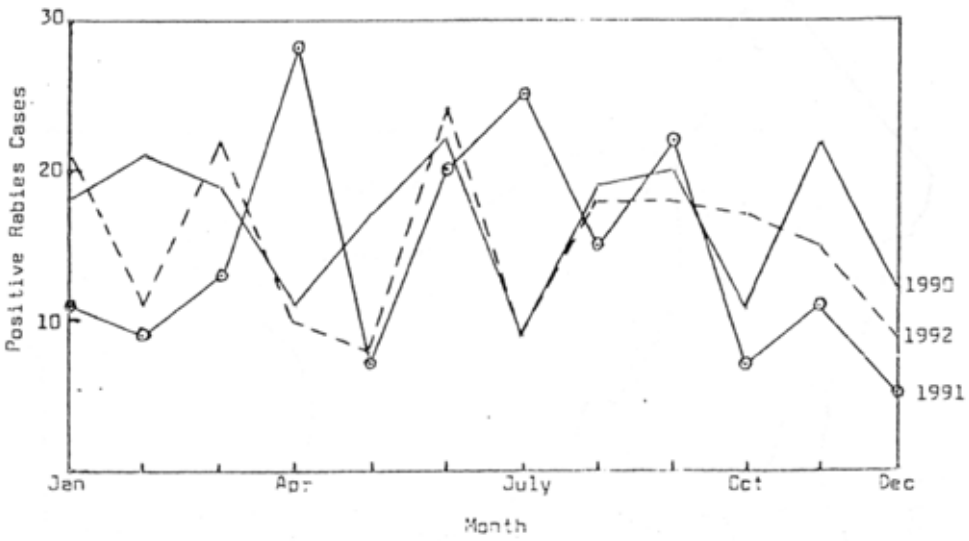


Table 1 : percentage of samples positive for rabies 1983-1992

YEAR	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	TOTAL
Samples submitted	450	425	321	404	468	352	372	354	270	301	3717
Samples Positive	224	226	195	261	290	213	205	200	158	191	2163
Samples Negative	226	199	126	143	178	139	167	154	112	110	1554
Percentage Positive	49.78	53.18	60.75	64.60	61.97	60.51	55.11	56.50	58.52	63.46	58.19

Table 2 : districts of Kenya

1. Bungoma	22. Nyeri
2. Busia	23. Kirinyaga
3. Kakamega + Vihiga*	24. Muranga
4. Siaya	25. Kiambu
5. Kisumu	26. Marsabit
6. South Nyanza (Homabay** + Migori**)	27. Isiolo
7. Kisii + Nyamira*	28. Meru + Tharaka-Nithi*
8. Turkana	29. Embu
9. Samburu	30. Kitui
10. West Pokot	31. Machakos + Makueni*
11. Trans Nzoia	32. Mandera
12. Keiyo-Marakwet	33. Wajir
13. Baringo	34. Garissa
14. Laikipia	35. Tana River
15. Uasin Gishu	36. Lamu
16. Nandi	37. Taita-Taveta
17. Kericho + Bomet*	38. Kilifi
18. Nakuru	39. Kwale
19. Narok	40. Mombasa
20. Kajiado	41. Nairobi
21. Nyandarua	

KEY: 1 - 3 Districts in Western Province
4 - 7 " " Nyanza "
8 - 20 " " Rift Valley Province
21 - 25 " " Central "
26 - 31 " " Eastern "
32 - 34 " " North Eastern "
35 - 40 " " Coast "
41 Nairobi City

* New district created from existing district
** South Nyanza was split into two new districts

Table 3 : distribution of rabies by region 1983-1992

REGION/DISTRICT	Canine	Cattle	Sheep & Goats	Porcine	Camel	Horse & Donkey	Cat	Wildlife	Human
EASTERN PROVINCE									
MACHAKOS	518	22	31			5	22	25	
KITUI	19	7	11			6	5		
EMBU	31	12	4				2		
MERU	16	10	2					1	
ISIOLO	2		1					3	
MARSABIT	1								
SUBTOTAL	589	51	49			11	29	29	
RIFT VALLEY PROVINCE									
NAKURU	89	75	16			9	2	3	
KERICHO	27	52	6			3	3	2	9
UASIN GISHU	26	26	6		1	3	1		1
BARINGO	26	25	14						
TRANS NZOIA	12	15	1				1		
LAIKIPIA	11	10	4			2		2	
KAJIADO	13	7	1						
NAROK	8	6	3					4	1
ELGEYO MARAKWET	3	3							
TURKANA	4								
NANDI	2	1							
WEST POKOT	2								
SAMBURU	1	1							
SUBTOTAL	224	222	51		1	17	7	11	11
NAIROBI PROVINCE	391	39	8	1		9	27	8	7
CENTRAL PROVINCE									
NYERI	62	55	11			1	2	2	
KIAMBU	50	49	4		1	1	3	6	
MURANGA	53	29	1			1		3	1
KIRINYAGA	11	8	3			1			
NYANDARUA	4	11	2						
SUBTOTAL	180	152	21		1	4	5	11	1
COAST PROVINCE									
MOMBASA	19	1	2				1		
KWALE	10								
KILIFI	9							1	
TAITA TAVETA	4	1	2					1	
SUBTOTAL	42	2	4				1	2	

REGION/DISTRICT	Canine	Cattle	Sheep & Goats	Porcine	Camel	Horse & Donkey	Cat	Wildlife	Human
NYANZA PROVINCE									
SIAYA	10	1	1						
SOUTH NYANZA	2	2							1
KISII	2	3							
KISUMU	5								
SUBTOTAL	19	6	1						1
WESTERN PROVINCE									
BUNGOMA	3	5				2			
KAKAMEGA	7	2							
BUSIA	4								
SUBTOTAL	14	7				2			
NORTH EASTERN PROVINCE									
WAJIR			1						
SUBTOTAL			1						
TOTALS POSITIVE	1359	479	135	1	2	43	69	61	16

Table 4 : animal species diagnosed positive for rabies 1983-1992

SPECIES	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	Total	% of Total Positive
Human	3	1	1	2	2	1		3	2	1	16	0.74
Dog	189	156	113	168	186	105	127	124	95	116	1359	62.83
Cattle	26	47	49	51	61	72	55	50	32	46	479	22.15
Goat	9	13	11	20	18	14	8	4	1	2	100	4.62
Cat	3	3	10	6	10	6	2	7	12	4	69	3.19
Horse/Donkey	4	4	6	5	7	6	2	6	3		43	1.99
Sheep	3	2	2	6	2	8	5	1	3	3	35	1.62
Pig										1	1	0.05
Total Domestic	214	225	191	256	274	211	199	192	152	172	2086	96.44
Total Wildlife	7		3	3	14	1	6	5	4	18	61	2.82
Total Animal Positve	221	225	194	259	288	212	205	197	156	190	2147	99.26
Total Positive	224	226	195	261	290	213	205	200	158	191	2163	100.00

Table 5 :districts with positive rabies cases in wildlife 1983-1992

REGION/DISTRICT	Dogs	Livestock*	Monkey	Jackal/ Wilddog	Honey Badger	Bat	Hamster	Wild Cat	Squirrel	Leopard	Fox	Hyena	Mongoose	Total
RIFTVALLEY PROVINCE														
KERICHO	27	58	1	1										87
LAIKIPIA	11	14		1							1			27
NAKURU	89	92				1					1	1		184
NAROK	8	9		1						2		1		21
SUBTOTAL	135	173	1	3		1				2	2	2		319
CENTRAL PROVINCE														
KIAMBU	50	54		4				1	1					110
MURANGA	53	30		2										85
NYERI	62	66						2						130
SUBTOTAL	165	150		6				3	1					325
EASTERN PROVINCE														
ISIOLO	2	1						3	1					7
MERU	18	12						1						31
MACHAKOS	518	53		9	3				4		2		7	596
SUBTOTAL	538	66		9	3			4	5		2		7	634
COAST PROVINCE														
KILIFI	9							1						10
TAITA TAVETA	4	3			1									8
SUBTOTAL	13	3			1			1						18
NAIROBI PROVINCE	291	48		2	1	1	1		2				1	347

* Livestock include cattle, sheep, goats, camel and porcine

Table 6 : rabies distribution in wildlife 1983-1992

SPECIES	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	TOTAL	% OF TOTAL WILDLIFE
Jackal/Wilddog*	4				9		1	1	1	4	20	32.79
Mongoose			1	1	1		1	1		3	8	13.11
Wildcat					2		2		1	3	8	13.11
Squirrel							2		1	5	8	13.11
Honeybadger			1	1				1		2	5	8.20
Fox	1				2			1			4	6.56
Hyena	1		1								2	3.28
Leopard	1									1	2	3.28
Bat						1		1			2	3.28
Monkey				1							1	1.64
Hamster									1		1	1.64
TOTAL WILDLIFE	7	0	3	3	14	1	6	5	4	18	61	100.00

* Majority of the samples identified were from jackal

THE CONTROL OF RABIES IN SOUTH AFRICA

G. K.. Brückner

1 INTRODUCTION

According to available information, rabies has been present in South Africa for many years, although the first definite confirmation by subinoculation was made in 1893 by Hutcheon (Mansvelt, 1962). Somewhat rare reports follow up to 1928 when infection was discovered in a yellow mongoose (meercat) (Cynictus penicillata) that led to more intensive investigations in wild animals, disclosing widespread infection in viverridae in the central regions of the country.

During the middle of 1950 the disease suddenly made its appearance in the Northern Transvaal, an area far removed from the already known source of infection. The principal vectors proved to be dogs and jackal, which spread the disease rapidly through the more densely dog-populated areas of the Northern and Eastern Transvaal and from there to Natal (Mansvelt, 1963). Four enzootic forms of the disease exist, namely:

a) "Street" - virus in the Natal and Northern Transvaal areas where the dog and jackal are the most important disseminators of rabies.

b) Mongoose fixed virus in the central plateau where viverridae like mongoose and other wild felidae are the primary vectors.

c) Duvenhage bat virus in localised areas in the central Northern Transvaal.

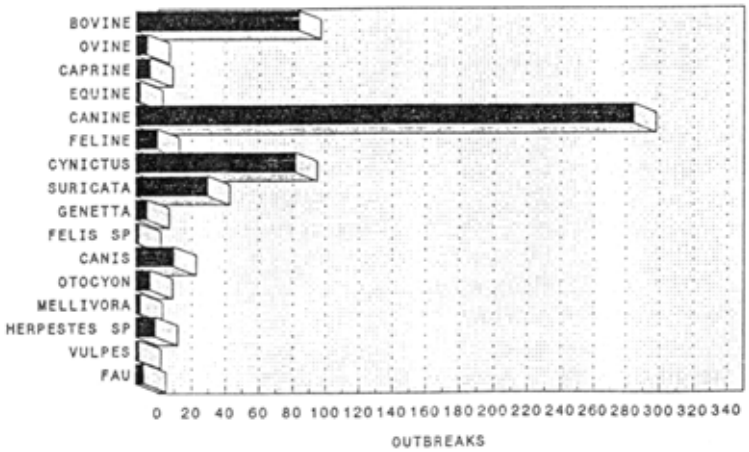
d) Bat and rabies related virus in the Natal area and most Northern districts of Northern Transvaal.

Such epidemiological differences are difficult to explain as they cannot be wholly correlated with regional population densities of animal species. Immunological differences in the virus are unknown but it could be suggested that questions of adaptation and invasiveness to species could be considered.

From a disease control and eradication point of view, the distinction is important as it involves the application of the most suitable measures. The extent of area placed under restrictions, as well as the most effective use of canine vaccination, come into play.

The primary vectors involved in the dissemination of the disease, are illustrated in Figure 1. The data is a reflection of the positive cases diagnosed in animals during 1992. The relation between the animal species involved remained almost, or less constant, for the past twenty to thirty years with a slight increase in domestic canine rabies.

Figure 1 : rabies in South Africa, species positive



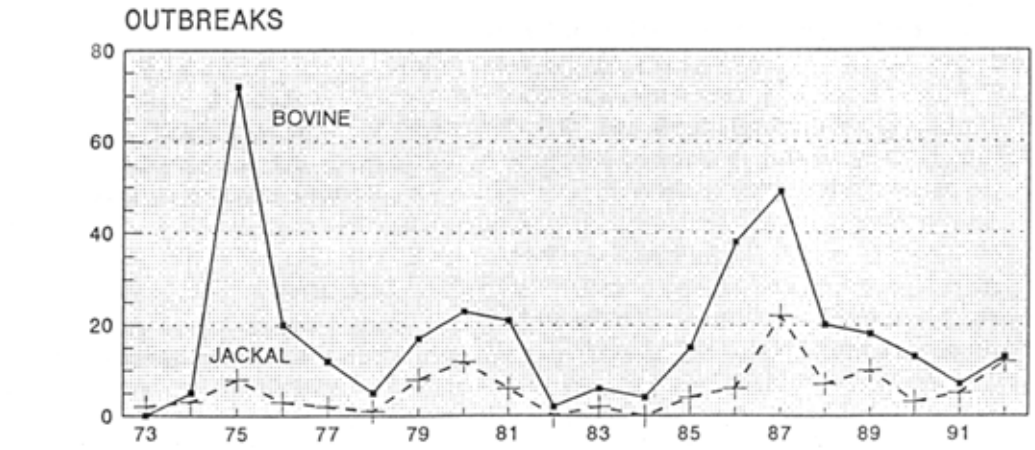
2 SEASONAL AND CYCLIC INCIDENCE OF RABIES IN SOUTH AFRICA

Seasonal fluctuations in the occurrence of the disease have been observed by Maré (1962), Barnard (1979), Zumpt (1969) and Brückner (1978). According to an analysis made by Maré, the highest incidence is during winter and early spring, while the least number of cases was recorded in summer and autumn. The explanations offered that infected viverridae are more readily seen during periods of sparse vegetation and, secondly, that bovines, through their inquisitive nature, are more likely to be bitten by these animals during such times, are borne out by field observations of increasing infection during periods of prolonged drought. Zumpt (1969) and Brückner (1978), however, correlate the increased incidence of the disease with the breeding habits of the yellow mongoose and the black backed jackal. Bueler (1969) states that the mating of Canis mesomelas in South Africa takes place from May through July, while whelping occurs from July through September. The prevalence of rabies during this period seems to coincide with the increased migratory behaviour in jackal and mongoose, especially as far as the male member of the species is concerned.

In the Natal region, where dogs are the primary vector of the disease, available data also suggests a higher incidence of rabies during the months June to September. No explanation can be offered for this observation as breeding habits and contact with wild animal species play no role in the dissemination of the disease in this area.

In areas where wild animals are the primary vectors of the disease, a cyclic trend in the incidence of the disease is evident. In Figure 2, data from the Northern Transvaal area where the black backed jackal is the primary vector of rabies, is presented to show a tendency towards a 5 to 6 year cycle in the occurrence of rabies.

Figure 2 : cyclical incidence of rabies from 1973 to 1992, bovine vs jackal



	73	75	77	79	81	83	85	87	89	91
BOVINE	0	72	12	5	23	21	6	4	38	20	18	13	7	13
JACKAL	2	8	2	1	12	6	2	0	6	7	10	3	5	12

3 THE PRINCIPLES OF CONTROL OF RABIES IN SOUTH AFRICA

The control measures recommended by the Expert Committee on Rabies of the World Health Organization form the basis of the regulations applied in South Africa. They are:

a) Registration, licensing and taxation of dogs.

b) Elimination of stray animals.

c) Restraint of dogs while the control campaign is under way.

d) Mass vaccination of dogs free of charge.

e) Provision of adequate facilities for diagnosis.

f) Reduction in number of wildlife species where these are a reservoir of disease.

g) A continual and energetic publicity campaign (Mansvelt, 1962).

3.1 The control of rabies is based on three principles:

Legislation : The creation of rabies control areas, import and movement control and compulsory vaccination in certain areas. Rabies is a controlled animal disease in accordance with the Animal Diseases Act, 1984 (Act 35 of 1984). The Act makes provision for compulsory notification of any outbreak or suspected outbreak of the disease, the procedure for submission of samples, the declaration of controlled rabies areas, quarantine or destruction of infected animals, compulsory vaccination, movement control and import control.

Availability of diagnostic services : The service must be reliable, easy to access by the public and a speedy notification of results.

Disease surveillance : The surveillance system must be reliable, the planning and execution of surveillance exercises must be based on sound epidemiological principles and the surveillance system must be supported by a reliable diagnostic service.

3.2 The primary aims of control are:

- To create an immune buffer between animal and man.
- To immunise enough of the susceptible canine population to lower the possibility of disease occurrence below an acceptable threshold level. In some areas, e.g. urban areas, it could be as high as 70% to 80%, while in certain rural areas a vaccination level of 40% of the canine population can still succeed in suppressing epidemics of the disease).
- The prevention of introduction of the disease from other infected areas or from infected countries by means of movement and import control.
- The prevention of the spread of the disease from infected areas to other non-infected areas.

The degree of control varies between endemic areas (e.g. in the central plateau where rabies are primarily transmitted by viverridae) and the traditional canine rabies areas or epidemic rabies areas (e.g.. in Natal and the Northern Transvaal). In the latter stricter control measures are applied in respect of frequency of vaccination and movement control.Those areas where the dog and jackal play the major role in the transmission of rabies, namely Natal and certain districts in the Northern Transvaal, have been declared rabies controlled areas as depicted in Figure 3.

Figure 3 : control areas : animal diseases act

Dogs in such an area must be vaccinated with an inactivated rabies vaccine between 3 and 7 months of age and after that at least once every three years. In high risk areas such as Natal, vaccination of all

dogs annually is practised as a routine control measure. A movement permit is required for dogs, cats, wild carnivores and ground squirrels within, into and out of the control areas.

Where outbreaks occur outside the control area, dogs and cats are vaccinated within a reasonable radius around the outbreak. Population reduction of meercats with phostoxin in known infected burrows, were undertaken by Animal Health Officers until the late 1980's. In areas where the black backed jackal is the primary vector of the disease, population reduction by poisonous bait is used only when the incidence in cattle becomes epidemic. Cattle are vaccinated free of charge in these areas by Animal Health Officers.

Vaccination of dogs and especially cats, outside the rabies control areas, breaks the chain between sylvatic rabies and human infection and therefore safeguards public health, although it does not control the spread of the disease in wildlife.

The necessary provision has been made in the Animal Diseases Act, 1984 (Act 35 of 1984), to safeguard the country against the importation of rabies. The Act requires that imported animals be accompanied by permits issued by the Chief of Veterinary Services of the exporting country, and that they may not be introduced otherwise than according to conditions stipulated in the permits.

3.3 Factors that may influence the achievement of satisfactory control

The control of rabies, more than most other zoonotic diseases, is influenced by a variety of factors from the external environment - especially the socio-economic environment. The nature and epidemiology of the disease cause sporadic moments of increased public awareness that quickly subsides in the presence of other more important factors affecting the socio-economic well-being of people. Thus the creation of a continuous awareness of the danger of the disease for human beings demands an intensive and costly input by public authorities. Yet the results are often disappointing for those involved in the control of the disease, if the outcome of their extension efforts is measured against, for example, the number of dogs vaccinated.

In South Africa the control measures, in those areas where sylvatic rabies is most prominent, are aimed at maintaining the prevalence of the disease within epidemiological acceptable levels. In these areas it is accepted that the disease manifests itself in seasonal and cyclic patterns and that control measures to prevent the further spread of sporadic outbreaks in urban areas are at most only a public relations exercise, with very little impact on the epidemiology of the disease. However, the increase in the occurrence of the disease in canine rabies areas like Natal, remains a major reason for concern. Very often the traditional means of control (e.g. mass vaccination campaigns or mobile vaccination clinics) are not sufficient to achieve satisfactory control. More radical methods of creating public awareness and to satisfy the needs of a wide spectrum of different communities, must then be implemented. Cognisance must be taken of basic needs of the community, e.g., the means of transport available to dog owners, the distance between dwellings and vaccination points, the cultural beliefs and disbeliefs of the community and the ability of decision makers in such communities to influence the people to present dogs for vaccination.

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RABIES CONTROL IN NATAL

P. Kloeck [1]

1 INTRODUCTION

Rabies was first introduced into Northern Natal in 1961. The density of the rural population along the coastal strip favoured the spread of the disease in dogs and the epidemic rapidly spread from Mozambique along a narrow strip approximately 8O km wide and 300 km long. In the rest of Natal, the disease incidence was low and sporadic (Figs 1 and 2).

No cases were diagnosed between 1969 and 1975 (Figure 3). In 1976 rabies reappeared in northern Natal and this reappearance co-incided with political instability in Mozambique where upon thousands of refugees fled to Natal. Since 198O, socio-political turmoil and instability locally, poverty, drought, starvation and poor living conditions have resulted in massive urban migration.

This uncontrolled migration coupled with an ever increasing human population concentrated on the fringes of towns and cities has led to peak numbers of recordings on a cyclical basis (Figure 4).

Presently rabies is Natal's most serious zoonosis with confirmed cases now averaging almost 30 per month. (Fig 5) The distribution by district has increased from 48% in 1989 to 76% in 1992. (Figs 6, 7 and 8)

Human deaths although still under reported are steadily increasing. Last year 26 human deaths were confirmed in Natal (Fig 9).

The breakdown according to species in the last 17 years reveals that canine rabies is responsible for 89 % of all cases diagnosed in Natal (Figs 10 and 11). One very definite peak is noticed in August/September and a leaner one in February (Figs 12 and 13).

Our directorate was structured to serve formal agriculture and until recently was not well organised to deal with endemic rabies in an escalating process of disorganised urbanisation. Control of rabies is hampered by political violence, faction fighting, cultural objections, death of dogs through Parvo, Distemper and fear of gatherings. The public is showing general apathy and our efforts to vaccinate in many problem areas are resisted, occasionally with the threat of force.

We admit that we have made mistakes, and may continue to do so but we have also attempted to address what we perceive to be the two most important aspects, namely:

A. increasing awareness through education.

B. adapting strategy to accommodate uncontrolled urban migration.

2 INCREASING AWARENESS OF RABIES - BY EDUCATION

We have developed and produced a high impact, cost effective communication and education programme with the prime objective of educating the public at large and children in particular. We are concentrating on less affluent urban and peri-urban communities. We believe that we can educate and motivate our nation through our children.

Our message is: RABIES KILLS - THERE IS NO CURE

PREVENT RABIES - VACCINATE YOUR DOG

[1] Directorate of Animal Health, Natal Region, P/Bag X 02, CASCADES, Pietermaritzburg.

Our objectives are to:

I. create awareness of rabies in general,

II. educate the nation - particularly children,

III. ensure participation in rabies vaccination campaigns.

Our campaign appeals to all ethnic groups and will be sustainable at Provincial level for a number of years. We believe we are capable of delivering a strong message, with high recall through short duration, high frequency visibility.

Our first objective is :

2.1 Create awareness of rabies in general.

a) We have developed a logo which we believe is friendly, fun, versatile and highly credible. All ethnic groups can readily associate with our character. (Fig 14)

b) We have printed T-shirts bearing our logo for distribution in areas worst hit by rabies. This provides a travelling message which is often worn for extended periods of time.

c) The province has been flooded by bumper stickers in bright luminous colours. They are affixed to cars, bicycles, suitcases etc. and are extremely popular, particularly amongst the youth.

d) Stamps with the same cartoon and logo in luminous colours are used on correspondence and large pink posters for shop windows and offices. (Fig 15)

e) Balloons bearing our logo and our sponsoring company are distributed at schools and shopping centres.

f) Taxi operators carry more passengers than the combined national bus and rail system. 2500 taxis are given audio tapes with recordings of all the current favourite pop music hits. Rabies awareness adverts and essential Rabies facts with background sounds coming from a howling dog and crying human baby, get the message across to 3 million commuters monthly.

g) Factual information and case studies are regularly circulated through the printed media and

h) Talk and phone-in programmes are held with electronic media from time to time.

Our second objective is to educate people :

2.2 Education:

i) We have developed a concept to communicate rabies prevention, action and control through the use of cartoon characters. The message is delivered graphically in the form of a cartoon strip, telling the visual story of the danger and prevention of rabies (Fig 16). These leaflets are distributed prior and during vaccination activities, through schools, public functions and supermarkets.

j) Education by means of visual aids could prove to be the most effective method of fighting this killer disease and assisting in its control. Every school in Natal should have suitable material with which to educate the local population and encourage them to vaccinate their animals. We have videos filmed and used extensively by our staff, but Africa's most urgent need in rabies education and control today is a new modern rabies video with reference to circumstances on this continent. Millions of people in Africa could benefit from it.

k) About 60 % of our population is made up of children and this is why we have directed a massive campaign through schools, distributing information brochures, talks and videos. We believe that the public need to be well and accurately informed. Good information will lead to good cooperation and good results.

We have linked this with a simple dog:cat population survey questionnaire. Human population dynamics are being used to reflect dog numbers in Natal more accurately. The co-operation and assistance from all headmasters and schools visited so far has been quite outstanding and given us some hope for the future.

Our survey results reflect the following:

GROUP	AVERAGE HUMAN : DOG RATIO
Asian	6.58 : 1
Black	5.49 : 1
Coloured	5.75 : 1
White	2.44 : 1

2.3 Ensure participation in rabies vaccination campaigns

To assist us in this objective we sought additional help not linked to any government or quasi-government structure.

The 0.K. Bazaars is by far the most popular chain store serving the needs of our less affluent communities.

Once the social implications of rabies in the community and the horror of rabies were demonstrated, the 0.K. Bazaars had no hesitation in putting their full weight and support behind the project.

They designed a number of eye-catching aids to highlight the importance of vaccination and control. This awareness package has been designed to help educate children and increase community involvement. By way of a promotional competition using colouring-in sheets and posters, prizes such as grocery vouchers, portable radios and a colour television set are being sponsored. In addition, each child who brings a dog for vaccination will receive a peak hat, bumper sticker and a rabies colouring-in chart.

The 0.K.'s team of artists have produced graphic illustrations on "The Story of Rabies". This could be circulated throughout Africa in magazines and newspapers. Thousands of lives could be saved.

They have kindly made their facilities available for vaccination clinics and are contributing to community participation, which forms the basis of a successful rabies control program.

Rabies with all its implications and consequences is too great a threat to combat alone. By joining forces with the private sector we have added oomph to our campaign.

3 ADAPTING STRATEGY TO ACCOMMODATE UNCONTROLLED URBAN MIGRATION

1. Five new permanent posts have been created in Durban.

2. A full time inoculation team has been introduced in the Durban metropolitan area.

3. Members from all ethnic groups have been enlisted to assist in sensitive areas.

4. Temporary vaccinators, drawn and selected by the local community to serve that community in "hot spot" areas, has been used with great success.

5. Vaccine pressure has been intensified. We have increased the number of clinics in the worst affected areas and reduced the distances between vaccination points.

6. Follow up operations involving teams vaccinating dogs on a door to door basis has been introduced.

7. Scramblers have been requisitioned for use in the inaccessible semi-urban and rural parts of Natal.

8. Basic epidemiological studies have been done and rabies questionnaires circulated. One of the most important findings was that >80 % of the respondents had walked one kilometre or less to have their dog vaccinated. This observation was probably the single most important piece of information that enabled us to motivate for more staff. Previously our clinic vaccination points were 7 - 10 kilometres apart.

9. Preliminary bait-vaccine trials are being conducted by Onderstepoort.

10. Most private practitioners are including rabies fractions in their routine inoculations.

11. This has allowed our directorate to spend less time on affluent dog owners and to concentrate our efforts on the less affluent dog owners and in areas where rabies is at its worst.

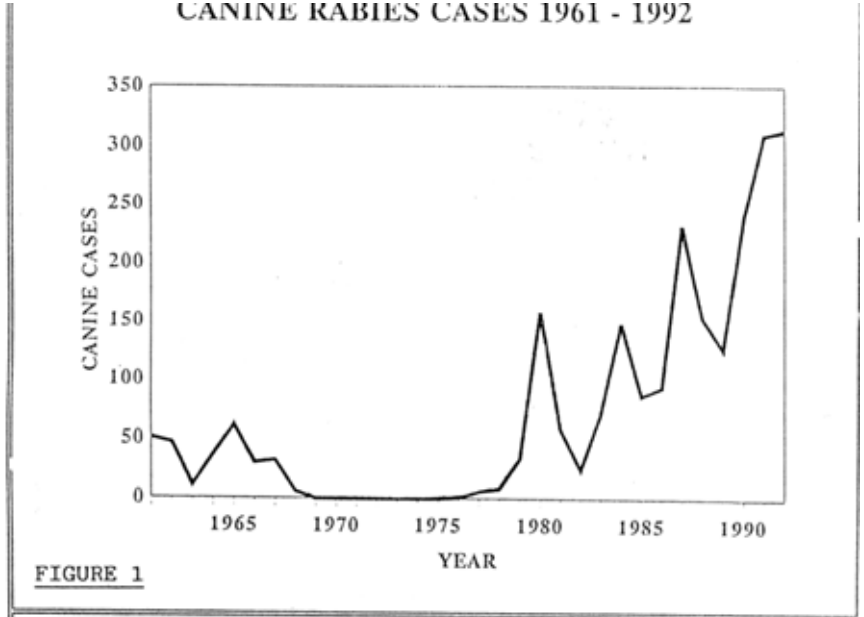
12. The S.P.C.A. and certain private practitioners in endemic rabies areas receive free vaccine.

13. Intensive campaigns were launched in various areas using sugar farms, farmers associations and health and education infrastructures. Vaccine was given to community leaders to initiate their own campaigns. Several large companies issued company policy statements insisting on vaccinated dogs among the work force.

14. In "hot spot" areas vaccination of all dogs irrespective of age after weaning is encouraged.

15. We have shifted emphasis from elaborate certification to a more simplified proof of vaccination.

Mr Chairman, I have given you a brief overview of the activities of Animal Health Technicians in Natal. It appears as though our efforts are producing some favourable results. We have vaccinated more dogs in the past year than ever before, but we are still a very long way off achieving the desired levels of vaccination. It is my firm belief that we will ultimately resolve our problem but this will obviously hinge on political solutions in the near future.



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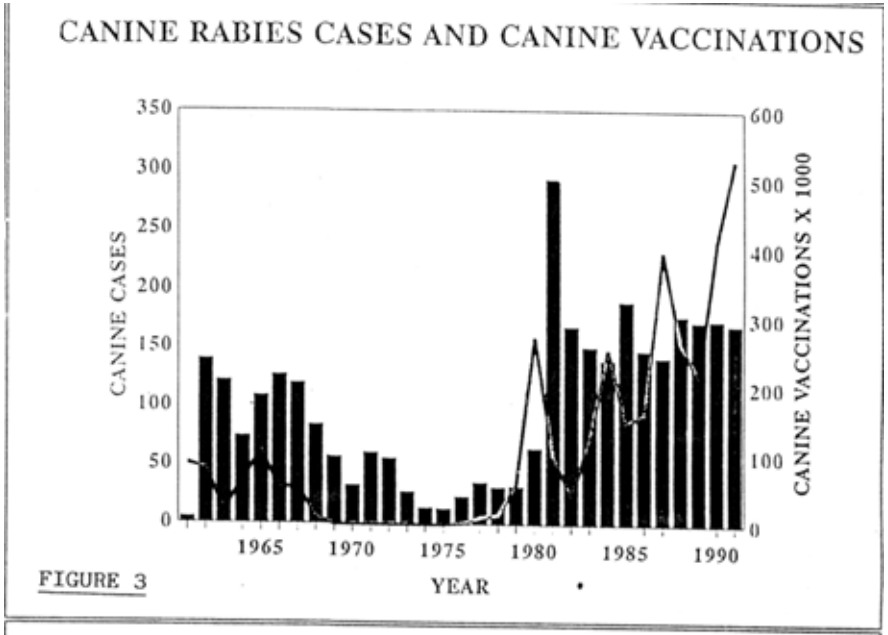

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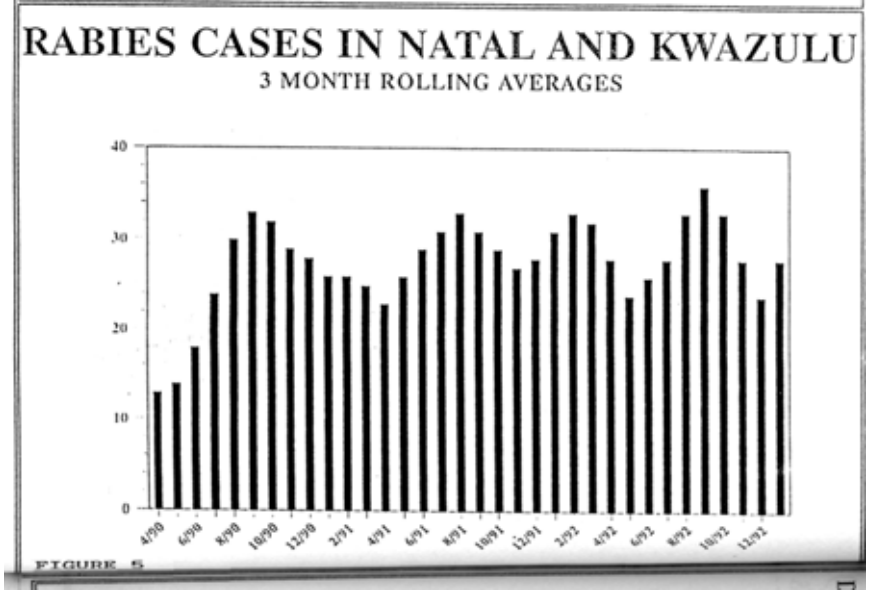

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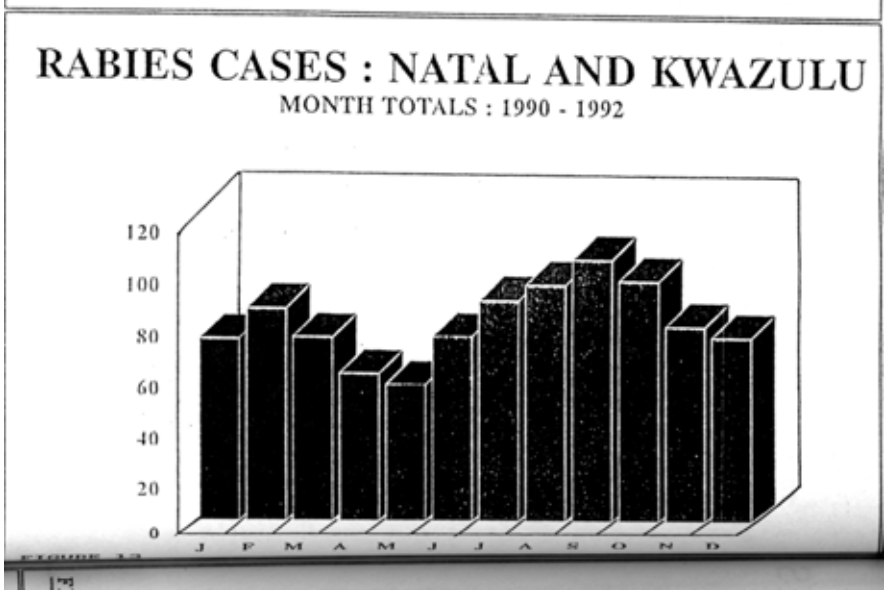

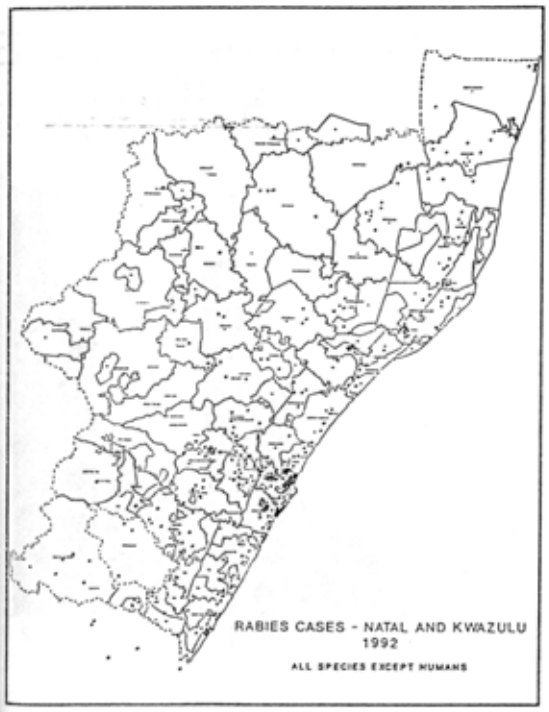

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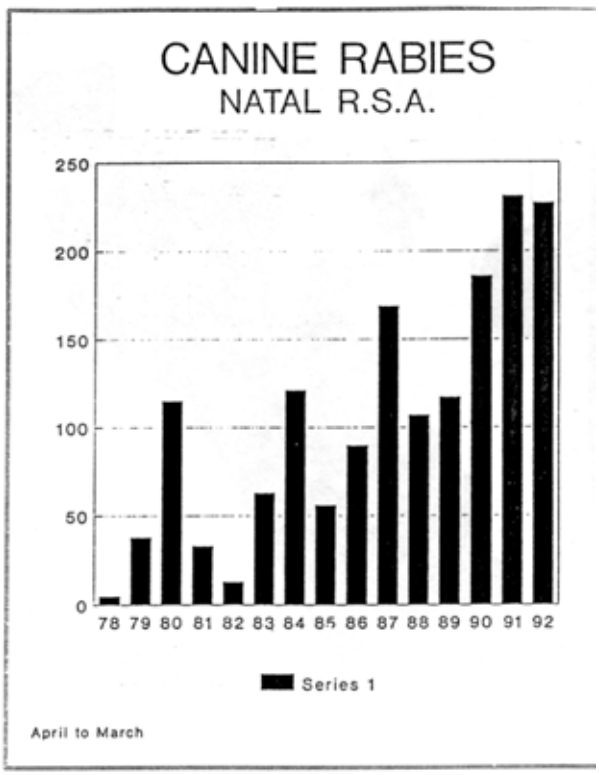

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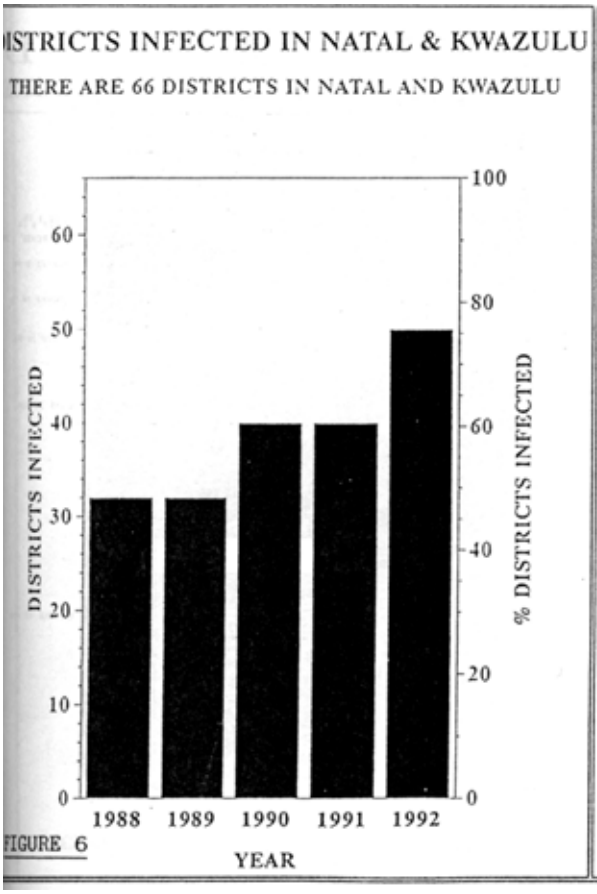

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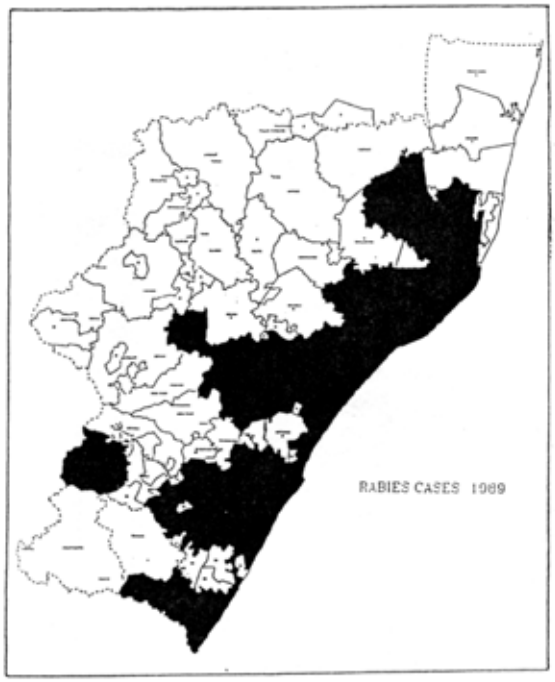

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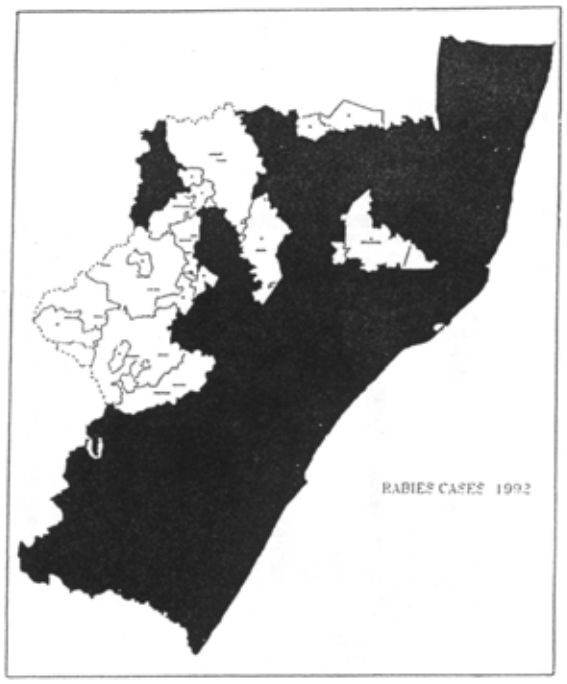

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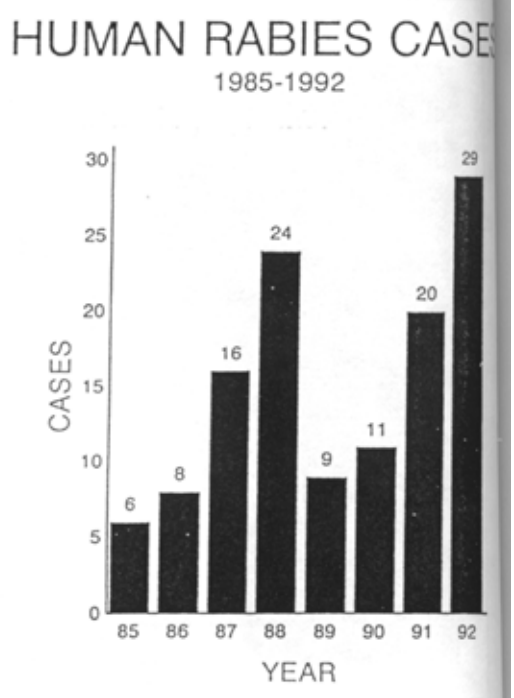

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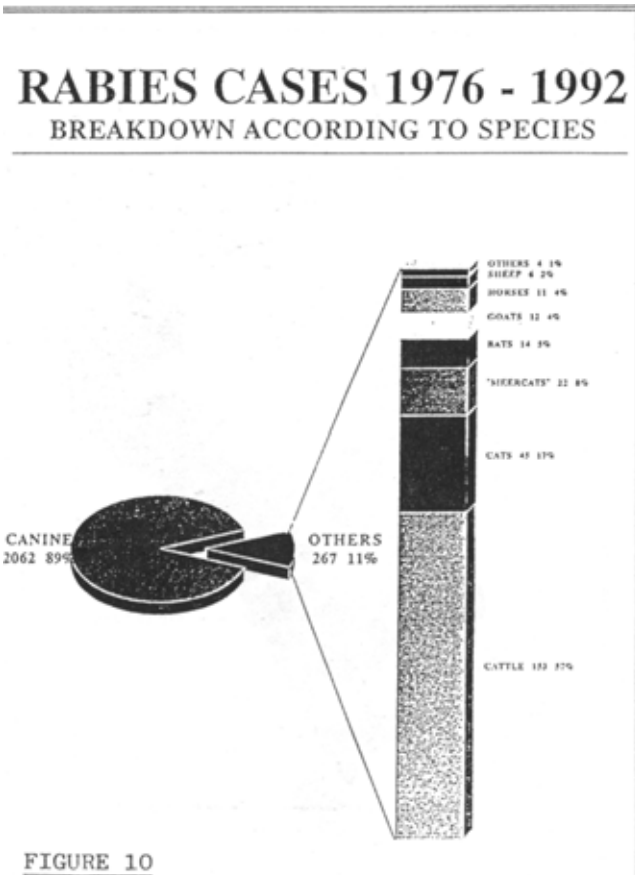

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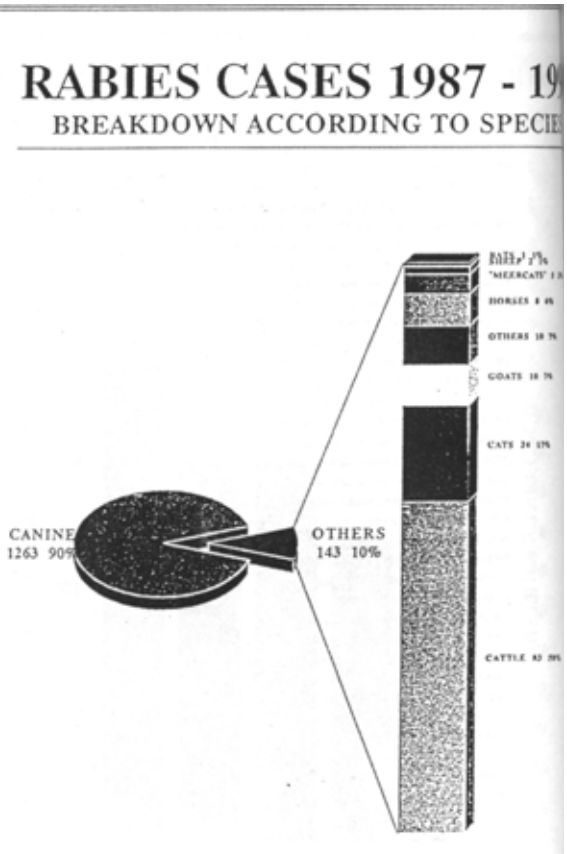

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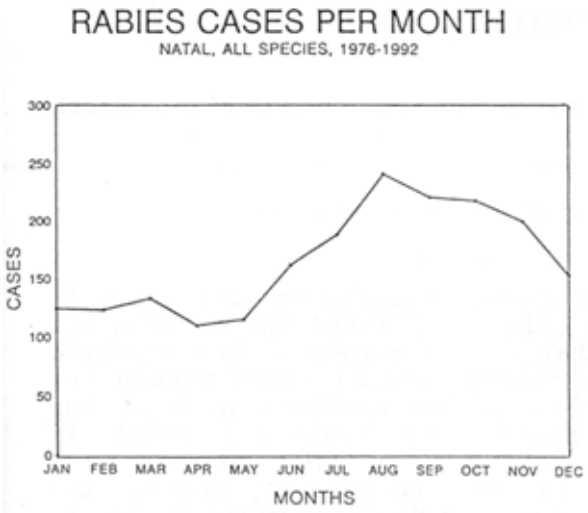

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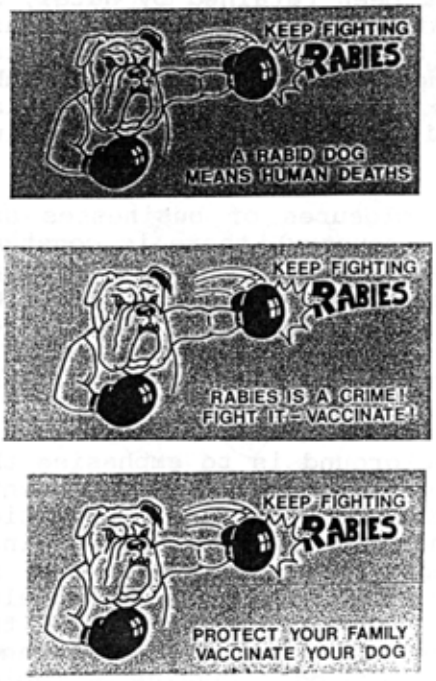


FIGURE 15

FIGURE 16

RABIES CONTROL IN KWAZULU

B. McCulloch [1]

1 INTRODUCTION.

Natal, KwaZulu and Transkei are closely related or integrated in terms of geographical location (Fig. 1). Natal and KwaZulu are both parts of South Africa. Natal is a province of the Republic of South Africa. KwaZulu is a self-governing state within the Republic of South Africa. Buried in the southern part of the Natal/KwaZulu complex is a section of Transkei, which is physically separated from the main body of that country by a piece of Natal. Transkei is an independent state. The Natal/KwaZulu complex stretches from the Mozambique border in the north to the Transkei border in the south, some 550 km as the crow flies. The complex stretches some 150 to 250 km inland from the coast. The overall area measures some 9 million hectares and of this KwaZulu covers some 3.6 million hectares, that is about 40% of the whole.

In terms of black population concentrations, the myriad of boundaries between Natal and KwaZulu are beginning to lose meaning. Black townships are long established in the Natal/Kwazulu complex. Many of the older or earlier townships such as Clermont and KwaDabeka have been retained by Natal, as representinq the Republic of South Africa, others, such as Umlazi and KwaMashu have been handed over to KwaZulu. All of the Natal black townships are close to the industrial and commercial hubs of the Province and satellite to the white and indian residential areas. In general, the black townships, administered by KwaZulu, lie in the more peripheral parts.

With the present economic crisis, closures of businesses and drought conditions over much of the country, there is constant movement of black squatters into all the black townships of Natal and KwaZulu. It could be said, at this time, that black squatting in and adjacent to the black townships and white and indian residential areas that lie close to the industrial and economic hubs of Natal, is the fastest growing real estate industry in the country.

The purpose of this demographic background is to emphasise the weight of black population growth and black population movements on the changing face of Natal and KwaZulu. The black population of Natal grows on a daily base and much of what Natal gains, KwaZulu loses. For all practical purposes there are no significant weights of whites, indians or coloureds in KwaZulu; these population groups are resident in Natal. To generalise, the white population has some understanding of rabies, understands the need for rabies control and understands the value of rabies vaccinations. Likewise, the more affluent sectors of the indian and coloured populations have some understanding of rabies, understand the need for rabies control and understand the value of rabies vaccinations. To generalise further, most of the less educated sectors of the black populations of Natal and KwaZulu and, for that matter many of the better educated sectors, are aware of rabies, have some understanding of the need for control, but, certainly in KwaZulu, have distinct reservations about the use of anti-rabies vaccine. Objections to vaccination vary from area to area. Formal and semi-formal objections to vaccination are most evident in the rural and semi-urban areas and from township people who have game hunting interests.

[1] KwaZulu Veterinary Division, Private Bag X05, ULUNDI 3838, South Africa.

Figure 1 : an outline map of Natal and Kwazulu to indicate the distribution of their respective districts

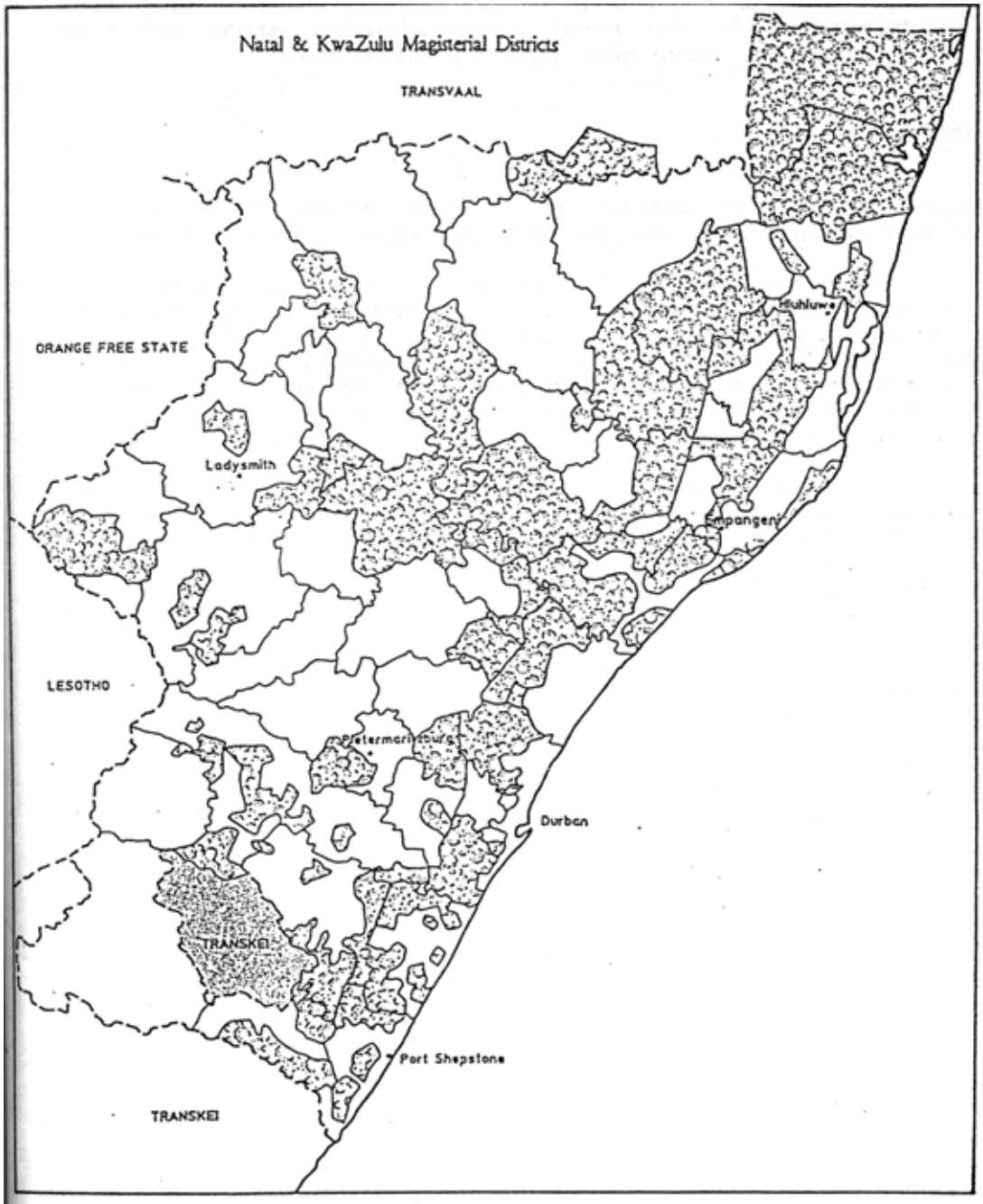


Fig. 1. An outline map of Natal and KwaZulu to indicate the distribution of their respective Districts:

Natal ☐ KwaZulu ▨ Encompassed portion of Transkei ▨

2 CONTROL.

A 70 to 100 km wide coastal strip encompasses those parts of Natal and KwaZulu most seriously affected with rabies (Fig. 2).

To the south the main high risk rabies areas of KwaZulu are Isingolweni, Umlazi, Mpumalanga and Umbumbulu. Moving north the next high risk rabies areas are the peripheral parts of the Durban/Pietermaritzburg corridor and KwaMashu and Ntuzuma, which lie just to the north west of Durban. Further north are the high risk rabies areas of Enseleni, Ongoye and Enkanyezi, satellite to the Empangeni, Richards Bay and Eshowe triangle.

It is almost certain that the incidence of animal rabies recorded in KwaZulu is below the true incidence of the disease. In particular, lack of all kinds of communication infra-structure, a general lack of motor transport and distances per se play roles here. To determine the actual shortfall is virtually impossible and any estimate thereof would be sheer guess work. There is not a great deal, if any, deliberate hiding of occurrences but, notwithstanding on-going education, the extent of real or pretended indifference to the disease and its consequences is always a surprise. Actual known occurrences of animal rabies over the last 16 rears are shown in Table 1.

The basic control approach in KwaZulu is to mount free needle vaccination campaigns. In the high risk areas the aim is to vaccinate at 4 to 6 month intervals and in the low risk areas at 8 to 12 month intervals. Coupled to this vigorous attempts are being made to establish puppy vaccinations on an on-going base. This latter exercise however is not popular and as a result has not reached its full potential. All dogs and cats presented for vaccination are vaccinated, irrespective of where they come from and irrespective of the date of last vaccination.

Vaccinations are carried out by the Animal Health Technician cadre, who are assisted by the Dip Assistant cadre. Loud-hailers are used to facilitate call-up.

Vaccination centres are planned in advance and the Inkhosi (Amakhosi), Indunas, Local Authorities, local people, schools, health clinics and churches are advised in advance. Posters are placed at stores, bus stops and in mini-bus taxis. Vaccination centres range from cross roads, outside of trading stores, school gates, particular trees - in fact, any convenient and known point.

Primary schools, or rather primary school gates, are a particular target point as pupils are advised in advance by the Animal Health Technician, through the teachers, of the vaccination programme for the area. In addition, on the day of vaccination pupils are released from most schools, but not all schools, to bring their dogs and cats for vaccination. Vaccinations in the vicinities of high schools are noticeably less successful, due to the intransigent natures of some of the older pupils.

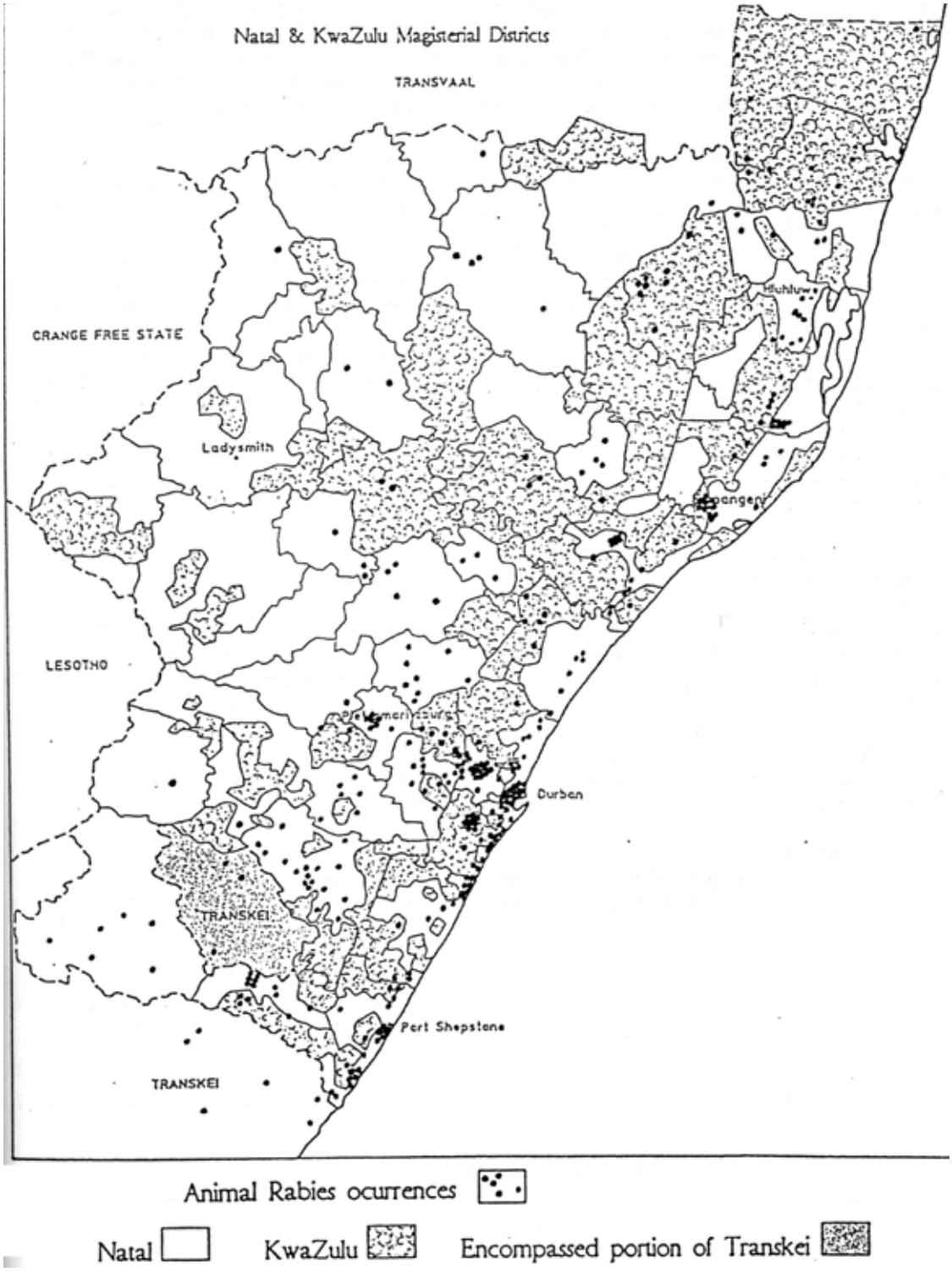
Coupled to the physical process of vaccinating on the ground, details of vaccination programmes are, and were, broadcast in the early morning on Radio Zulu, on the Zulu version of the agricultural advisory programme, "Calling all Farmers", which is one of the most popular programmes on Radio Zulu, occupying the peak time of 05:30 in the morning. Pamphlets are, and were, distributed in the target areas a week or so in advance of the actual campaign. These 2 approaches are now handled on a "stop, go" base, as it was found that these advertising approaches often lead to political friction and victimisation of people at the actual venues.

On a broader front, rabies and rabies control featured as a dominant issue in the annual policy speech of the Minister of Agriculture and Forestry, Inkhosi Dlamini. This speech is primarily directed at the Honourable Members of the House, who represent the top strata of the KwaZulu hierarchy and have great influence in their constituencies.

Further, KwaZulu participates equally with Animal Health (Natal) in the Star Taxi Music tape strategy as described earlier in the symposium. Great hopes are placed in the expectation that the tape will breakdown existing Zulu prejudices to anti-rabies vaccination.

Dog and cat vaccinations over the last 16 years are shown in Table 1. More recently, as of 1988/89, 1989/90, 1990/91 the trend was downwards but as of 1991/92 there has been an upturn which hopefully will continue into 1992/93, as the 1992/93 figures are extrapolating at some 142 500 dogs, 11 125 cats, a rise of some 6% and 17% respectively, over last year, the 1991/92 year.

Figure 2 : an outline map to indicate the distribution of animal rabies in Natal and Kwazulu per se, over the 1992 calendar year.



Zulus form the bulk of the population in both Natal and KwaZulu. Taking various population estimates into account, namely: Health Planning : Sub Regions in Natal and KwaZulu (1980); Urban-Econ (1989); Census Statistics (1991) and the Urban Foundation (1992), and taking on-going population movements into account, it is not unreasonable to currently estimate 1.5 -2.0 million Zulus in Natal and 4.0 - 4.5 million Zulus in KwaZulu; that is about one third of the total Zulu population in Natal and about two thirds in KwaZulu. It is considered that the weight of the Natal/KwaZulu dog population lies in Zulu hands. As just indicated, there is a massive Zulu population in Natal. This Natal Zulu population owns an impressive weight of dogs and this creates a problem in its own right, when we look at

the vaccination figures and consider the various sources of so-called "stray dogs". (As an aside here it is suggested that "owner-unknown dogs" would be a more fitting and realistic description than "stray dogs", as it is hard to believe that there is a significant stray dog population in Natal or KwaZulu). To continue with the main theme, on the base of a KwaZulu Zulu population of 4.0 - 4.5 million and a Natal Zulu population of 1.5 - 2.0 million, and taking cognisance of the possible ratios of dogs per person within the different population groups in Natal and KwaZulu (Arbuckle, 1990), KwaZulu probably vaccinates some 28% of its dog population, which compares to an equivalent figure in Natal, the Natal figure rising to about 35% with Natal private practitioner figures taken into account. Overall cover is probably about 30%.

Table 1 : The recorded occurrences of animal rabies in Kwazulu and dog and cat rabies vaccination figures for Kwazulu over a sixteen year period : 1976/77 to 1991/92.

Year	Recorded occurrences of rabies in animals	Rabies vaccination figures	
		Dogs	Cats
1991/92	116	134037	9501
1990/91	90	122897	7124
1989/90	39	129247	8215
1988/89	36	135186	9607
1987/88	64	136916	11814
1986/87	26	105873	7998
1985/86	25	118467	10769
1984/85	35	146914	11939
1983/84	23	94933	8336
1982/83	14	115148	8559
1981/82	20	141061	10756
1980/81	55	315234	36890
1979/80	29	49604	2811
1978/79	4	10352	271
1977/78	2	27820	1379
1976/77	16	22623	1588

3 PROBLEMS RELATED TO RABIES CONTROL IN KWAZULU

As stated earlier, most of the less educated portions of the Zulu populations are aware of rabies, have some understanding of the need for rabies control, but have distinct reservations about the actual use of anti-rabies vaccine. Many, many Zulus believe that the vaccine interferes with a dog's hunting ability, that the vaccine makes a dog run slower, that the vaccine prevents a dog from biting properly, that the vaccine makes a dog noisy, that the vaccine makes a dog attack sheep, that the vaccine makes a pregnant bitch abort, that the vaccine causes death and that the Government's purpose is to kill dogs, and, so on and so on. As one argument is countered, the community immediately brings forward another argument in its place.

Some of the other pertinent factors which contribute to low vaccination cover are related to time, distance, low incomes, lack of education, weather and communication infrastructure.

To enlarge on some of these aspects and, not necessarily in any specific order, the following are important.

Time is one of the most important commodities, if it can be called a commodity, in Kwazulu. The day begins at dawn and from then on it takes time to collect water, collect firewood and herd or tether livestock. These 3 areas are very much part of life on a daily basis, certainly in rural Kwazulu. For example most of the Kwazulu people have to walk somewhere for water and somewhere else to get fuel for domestic purposes. In particular livestock require to be herded or tethered in the day and kraaled at night, to prevent theft and, probably more important, to prevent livestock straying into growing and unharvested crops. In this respect, damage to another person's crops necessitates compensation payments, a consideration in a cash strapped economy. Thus to reiterate, collecting of water, firewood and herding of livestock are important time consuming activities not normally appreciated by the city dweller.

By and large, the main means of locomotion in Kwazulu is by foot. Travel by foot takes time. As far as rabies vaccination is concerned, it is not just a case of putting the dogs and cats in the car and "heigh-ho, off we go" on a 5, 10 or 15 km drive to the vaccination centre. That exercise is no sweat for the affluent motorist, it is an inconvenience, but not a sweat. For most people in KwaZulu getting the dog to the rabies centre means coaxing, leading, dragging or carrying the dog to the centre, uphill and downhill, often over rough terrain in areas where the hills are incredibly steep. Walking is a time hungry activity and in the KwaZulu context, walking is not altogether a leisure activity. This is especially so for the elderly person, who may be the only one free to take the household dog or dogs to the vaccination centre, as the other members of the family are committed to predetermined tasks, such as, paid employment, school attendance and, community, agricultural and rural activities. Quite often the elderly person can only control one dog at a time.

Now to the weather. Rain is a totally disrupting factor. People won't work with livestock in the rain, let alone drag dogs about in the rain. Even a few days of heavy mist, as is common in the high lying areas, especially in the southern half of Natal and KwaZulu, is a totally disrupting factor. As indicated earlier, KwaZulu operates from the road sides as much as possible, but the roads such as they are, are mainly earth roads and are often, excluding drought conditions, impassable in wet weather. Impassable conditions often pertain for a few days after the rain has cleared up, so while overhead weather conditions are indeed suitable for vaccinations, the vehicles however, can't get through.

Bearing in mind, the time, distance and walking factors, it can be understood that KwaZulu vaccination campaigns are protracted. Because KwaZulu campaigns are protracted, they are subject to constant, intermittent delay. Wet weather, is a main disrupting factor in this connection. Cancellations and readjustments of the programmes follow to cover the missed areas at a later date.

From the above it can be appreciated that the natural environment in its various aspects does, in itself, present KwaZulu with a number of problems.

Away from the natural environment per se KwaZulu has other problem areas which need to be appreciated. KwaZulu society is constructed very much on a hierarchical pattern. The Inkhosis (Amakhosi), the Indunas, the Local Authorities and Tribal Police all play authoritative roles, as do the different KwaZulu Government Departments, and in various areas, likewise, the South African Police and the South African Defence Force. In addition, the community in itself demands a conforming pattern of behaviour. In this latter connection, for example, everybody starts ploughing and planting at the same time, that is after the community is in agreement. To jump the gun and plough earlier than is accepted by the community, could well mean that the crops are destroyed by the conforming members of the community. This concept more or less leads to what often happens at Farmer Association, diptank and other community meetings. Sometimes these meetings go well. However those with a rabies content are quite often less successful. The usual condemnations of anti-rabies vaccinations, as mentioned earlier, are forwarded. Once this starts, others of the same persuasion join in, and the KwaZulu Animal Health Technicians, who are either addressing or translating at the meeting are up against a negative majority vote situation, which they can't win, as they can only keep denying the allegations. Their denials are dull and uninspiring, compared to the flamboyant descriptions of situations in relation to vaccine stricken dogs. Once the bit is in the dominant raconteur's teeth, he will embellish and repeat the same story again and again to everyone's delight; there is no time controlling factor at these meetings.

This negative majority opinion factor is a situation which is not widely experienced in the white sector, but with say town counsellors, politicians, club committee members and, those of the Directorate of Animal Health who address disgruntled Farmer Association meetings from time to time. However the KwaZulu Animal Health Technicians, have to cope with negative majority community opinion many times in the course of the working year. KwaZulu Animal Health Technicians are responsible for about 20 dip tanks, which represent at least 2400 stock owners at 120 stock owners per tank. Both stock owners and non-stock owning members of a particular community must be dealt with on a group base of one kind or another. The negative majority opinion factor, although a common experience in these group circumstances, is very demanding on morale.

To revert to the hierarchical situation, if the Inkhosi is against rabies vaccination as happens in some parts, it is difficult to get a good response to the vaccination campaign and, basically because the Inkhosi is frightened of losing face, he is not open to persuasion and the circumstances become virtually impossible to alleviate. Again in the hierarchical context, Tribal Police or Tribal Authorities often turn up at vaccination centres to collect dog tax. Vaccination activity is then stopped until the dog tax collectors are withdrawn, as an association of anti-rabies vaccinations with dog tax collection aggravates

the problems of obtaining adequate cover. Often the end result is, that this particular section of a campaign has to be rescheduled.

Overhanging these broad community related problems of distrust of vaccine, time, distance, weather, hierarchical structure, negative majority vote, and so on, and so on, there are the clouds of political unrest and the even more vicious palls of faction fighting.

Political unrest as we all know is at an unprecedented level in Natal and KwaZulu, with spill over into the Reef and other places where there are concentrations of Zulu migrants. Political unrest is currently on an on-going base and daily progress can be ascertained from any newspaper in the country, let alone a Natal or Zulu newspaper. Gang violence per se, which appears to have some links with political unrest, is totally out of hand in many areas. There is no doubt that political unrest has a major influence on KwaZulu vaccination campaigns. Vaccination teams have to look for opportunities to slip into unrest township areas such as Umlazi, Kwamashu, Gamalakhe, Kwa makhutha, Ezakheni, Ezikhawini, Ngwelezana and Wembezi, to carry out the campaigns and, even then, many people are frightened to bring their dogs for vaccination. Further, in the townships one of a dog's major functions is to guard the house. To take the dog to the vaccination centre leaves the house unguarded, and unguarded houses are vulnerable to immediate gang or political vandalisation, with ill-affordable losses to the affected family. Hopefully with political settlement in the future, current unrest will simmer down and remove these particular problems from the arena of rabies control.

Now to faction fighting, which is a completely different kettle of fish from political unrest, although as with political unrest its expression, involves the use of lethal weapons, such as the AK-47 assault rifle , the pistol, the home made rifle, the panga etc.. Faction fighting is mushrooming over extensive areas and is rife in the rural environment. Just one example, out of very many. In a rural area to the east of Ladysmith, there are at least 300 men on each side of a 2 sided faction fight. Only women are free to move. Any man who takes a job in Ladysmith, Durban, Johannesburg or wheresoever, is targeted and murdered by the opposite faction. Thus in this part, as in other parts, men moving out of their immediate environment can only move in groups. Clearly faction fighting has major influences on KwaZulu vaccination campaigns.

Indeed, overall in these faction fighting parts, actual movement is difficult and groups of people waiting to have their dogs vaccinated make good rifle targets. Unlike political unrest there is no hope, or no real hope, that faction fighting will abate. Faction fighting is on-going and the reasons for faction fights starting are multiple. Many start at weddings, many are stock theft related and, more recently many have started over taxi routes. In the Tugela basin for example faction fighting has been on-going for over 70 years.

With respect to the 1991/92 book year, of the 26 Districts which make up KwaZulu, 20 or 77% were affected with political unrest, or faction fighting, or both.

Working in the political unrest and faction fighting environments wrecks havoc with staff morale. Again looking at the 1991/92 book year, veterinary/animal health personnel did not escape unscathed from the turmoil. Two Dip Assistants were killed, the wife of one Animal Health Technician was murdered, and one Dip Assistant and one Animal Health Technician had to be transferred to save their lives. Urban unrest areas like Umlazi, KwaMashu, Gamalakhe, Kwa Makhutha, Ezakheni, Ezikhawini, Ngwelezana and Wembezi for example, varied and still vary from safe to explosive; often at a moment's notice. Animal Health Technicians originating from faction fighting areas can seldom be stationed in their home areas as they are pressurised to join one faction or another; either way, whether they join a particular faction or not their lives are at high risk.

There is no doubt that rabies, unrest and faction fighting exert influences, but differently weighted influences, on the outlook to life in the Zulu environment. There were 27 human deaths from rabies in Natal and KwaZulu in the 1992 calendar year (Fig.3). On the other hand 1 041 people died as a result of unrest in Natal and Kwazulu over much the same period, namely January 1992 to February 1993; 902 in Natal, 121 in KwaZulu (Fig. 3). Over the Republic of South Africa as a whole, a total of 6 025 people were killed or injured in unrest related incidents in the 1992 calendar year; 2 465 killed, 3 560 injured (Fig. 3). No data is available vis-a-vis human deaths related to faction fighting.

Figure 3 : to indicate human deaths in Natal and Kwazulu as a result of rabies and as a result of unrest.

4 SUMMARY.

On the dark side all the prejudices against the anti-rabies vaccine will be with the Zulus for some time. There are specific problem areas of determined majority opinion against rabies vaccination. In all areas fundamental problems are walking time, the individual's time per se, wet weather and at community meetings the vocal anti-vaccinationist who provides the entertainment highlights of the day. Political unrest and gang violence have reached unprecedented heights and working conditions in many areas are, for animal health personnel, life threatening and, to say the least, unpleasant. Hopefully and on the positive side a political settlement will expedite economic recovery in Natal and KwaZulu, and will lead to a sharp drop in political unrest, which in turn should help, albeit indirectly, to reduce gang violence. Again on the positive side, there are great hopes that the Star Taxi Music tape exercise will lead to a cultural shift in the attitudes of the Zulu populations of Natal and Kwazulu to rabies vaccination of dogs.

Finally in very sombre vein, there appear to be no solution to the problem of faction fighting and it effects on all aspects of community life.

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RABIES CONTROL IN TRANSKEI SOUTH AFRICA

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1 INTRODUCTION

Transkei is located in the north east of the Cape province of South Africa bordering Natal and Lesotho in the north with the Indian ocean to the east.

Rabies was recognized for the first time in this region in mid 1986. The initial outbreak of the disease was identified in Maluti and Mt. Fletcher districts, which adjoin Lesotho and Natal (Transkei Veterinary Services Annual Report, 1986/87). More outbreaks of the disease were confirmed the following year in Umzimkulu, which is completely surrounded by Natal. The disease now affects the entire Transkei area. The number of rabies cases diagnosed increase every year.

Control of rabies through vaccination of 70% to 80% of the dog and cat population is the commonest measure implemented world-wide (Tierkel, 1975). Vaccination of dogs and cats was implemented in Transkei as soon as the first outbreaks of rabies were confirmed and continued to be done on an annual basis. In Europe and North America the effective vaccination of dogs led to a reduction of the disease incidence in these species (Tierkel, 1975) but led to either a real or relative increase in wild animal rabies (Winkler, 1986). The same control measure has been applied here but has not stopped the spread or prevented the rise in the number of canine and other domestic animal rabies cases in Transkei so far. Reasons for this are uncertain but may be linked to the land use pattern practised in the area.

Transkei covers an area of about 41000 square kilometres with a population of about 3 million. The communal sector accounts for 93% of the land use patterns in the country. The other 7% consists of irrigation projects, commercial farms in areas recently acquired from South Africa, forestry plantations, nature reserves and municipalities. The communal sector has 1000 administrative areas divided into 4000 locations or villages. The majority of the human population and therefore of animals is to be found in this sector. Problems associated with control of animal diseases in communal communities are numerous but designing appropriate control programmes is probably the most difficult. The problem of disease control in these areas is complicated further by lack of accurate data on aspects such as dog population figures, dog population turn over rates and interaction of the animals with each other and with the community. Information on dog populations appears to be very limited in most parts of Africa (Brooks, 1990). In spite of these drawbacks, attempts to control rabies in Transkei continue to be made and the present paper outlines activities employed to date. A multidisciplinary approach involving disease diagnosis, extension work, field practices and subsequent evaluation is reported.

2 DIAGNOSIS OF RABIES.

Diagnosis of rabies was done using the standard method of demonstrating the rabies virus antigen in the brain of suspected infected animals by means of the Fluorescent antibody test (FAT) (Dean and Abelseth 1973). The Fluorescein Isothiocyanate (FITC) conjugate was obtained from the Onderstepoort Veterinary Institute.

3 RABIES VACCINES.

The lyophilized vaccine made by the Onderstepoort Veterinary Institute was used by the State for mass vaccination of dogs and cats until its production ceased in 1991. Inactivated vaccines were used thereafter and these were mainly Rabdomun (Pitman-Moore GmbH) and Rabvac TM 3 (Solvay) but

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also small quantities of Rabisin (Maybaker Animal Health). In addition, Rabguard TC (Smith-Kline Beecham Animal Health) was also used by the private veterinarians.

4 VACCINE STORAGE AND CONVEYANCE.

Vaccine was stored as much as possible according to the manufacturer's recommendations and to the extent to which the available facilities could permit. However, appropriate conditions of storage and conveyance of vaccines at the points of usage could not always be guaranteed.

5 VACCINATION OF ANIMALS.

Primarily dogs and cats were vaccinated during campaign periods between July and September each year. Inoculation of animals was done in teams which comprised of a State Veterinarian/Regional or District Stock Inspector, Area Stock Inspector (s) and one or several general assistants. In municipalities, vaccination of animals against rabies was conducted at points established for the purpose. In the field, the teams moved from one location to another vaccinating animals at designated spots.

Following confirmation of a case of rabies in an area, all dogs and cats within the area and surrounding areas were vaccinated against rabies. Previously vaccinated animals were given a booster injection. If the case was confirmed in an animal other than a dog or a cat, all animals of the same species were vaccinated as well.

6 RABIES CONTROL STRATEGY.

A strategy to combat rabies in Transkei was developed. This involved compulsory annual campaigns to vaccinate dogs and cats against rabies in order to inoculate 70 - 80% of the population, which is necessary to achieve control, and to mobilise and motivate the veterinary staff and the people at large for better response.

In mobilising and motivating staff, Stock Inspectors were urged to obtain reasonable dog population figures in their respective areas, the vaccination exercises were carried out in teams following drawn up itineraries. Staff were also expected to carry out vaccinations during weekends in Industrial and Urban areas and to continue vaccinating animals throughout the year outside the campaign period.

To mobilise and motivate the population at large, vaccination was done free of charge, vaccination certificates were issued in rural areas as well and as many sites as possible were established for inoculation of the animals.

The strategy further involved coordination of the vaccination campaigns with the neighbouring countries. Animal vaccination was preceded by a well orchestrated awareness campaign.

7 RABIES AWARENESS CAMPAIGNS (INFORMATION PACKAGE)

The initial task was to clearly identify the critical success factors (CSF's), vis a vis:

a. the Transkeian rural community and how it perceived rabies as a human disease, as a zoonosis, as well as the role of the dog as a pet and its interaction with them. The general belief that rabies is a disease of dogs, the absence of a well cut out extension programme from the Health Department, and the traditional way of keeping dogs have contributed to the spread of the disease in Transkei.

b. the Animal Health Officers awareness and thorough understanding of the disease and its control is pivotal in such a strategy. The Animal Health Officer should understand that he is an extension officer among other things (DBSA Publication 1992).

c. the style of information transfer should be 'tailor made' for a given area to facilitate easy assimilation. This involves clearly defining the problem, in this case rabies, designing the information materials, and then packaging in such a way that it is attractive to our target consumer, the community.

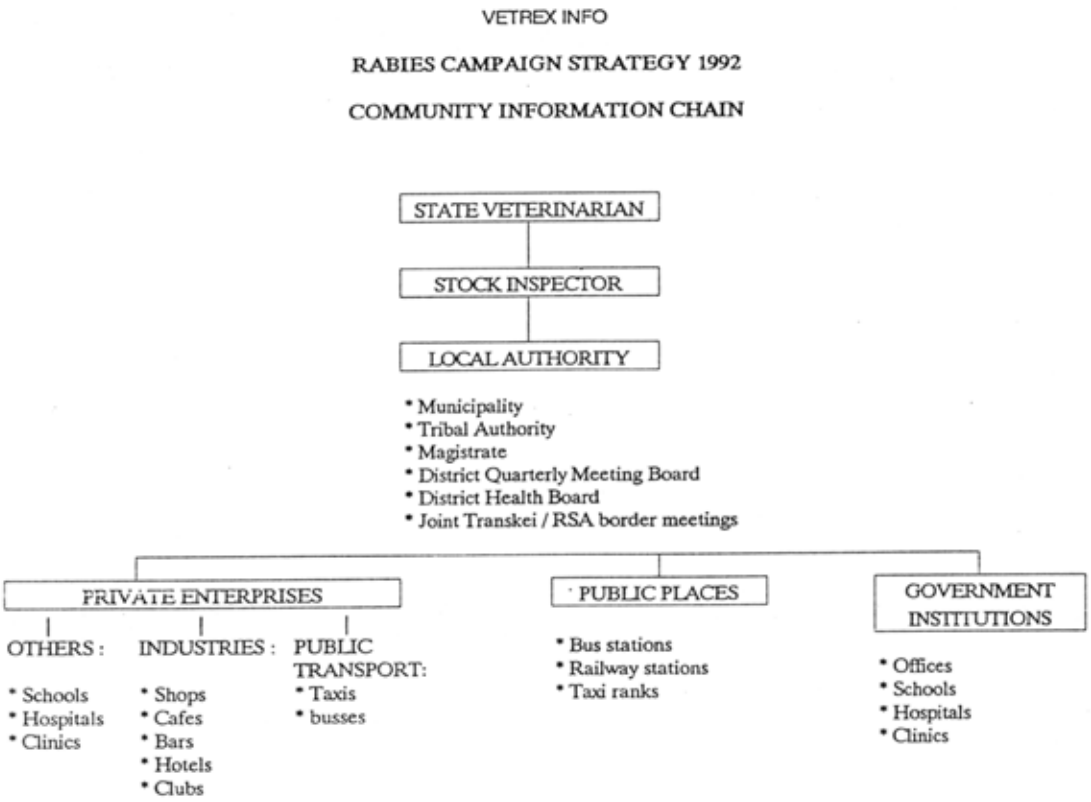
The Veterinary Services through its Training and Extension Unit (VETREX) was prompted to develop an "INFO - package" designed in such a way that it could address the above successfully and contribute to the general awareness of the disease in Transkei.

The strategy was to introduce a community information chain in which the key role players, possible physical resources and information centres, are clearly identified (Chart I).

The group approach of extension whereby occasions like Farmers days, Pension days, School visits and Health days were used effectively to change the communities perception of rabies as a disease of dogs and the image of a dog as a pet (Bembridge, 1991).

The officers directly involved, i.e. State Veterinarians , Animal Health Officers and Dipping Foremen were regularly refreshed, both in the subject matter and extension methods through short courses and seminars.

Chart 1 : rabies campaign strategy 1992



A centralized, uniform Info - package, easily adaptable for use in the field was vital. Hence book style methods of extension were adapted to meet the needs of the rural Transkeian community. Use has been made of posters , Info - leaflets, radio programmes, videos (local production) and the Press (Chart II).

Chart 2 : Extension Media

a) Farmers days
b) Special information days
c) Medical clinic days
d) Information leaflets
e) Films and Videos
   i) own , "in house " production
   ii) others, commercial
f) Posters
g) Press
   i) Radio talks
   ii) Radio Short announcements
   iii) Press releases in the National Newspapers

Another successful and unconventional method of extension was to use a converted minibus as a Mobile Info Centre. This vehicle, well equipped with a Public Address System and music for every taste has been very instrumental in the process of propagating information about rabies.

8 INCIDENCE OF RABIES IN TRANSKEI, MAY 1986 TO DECEMBER 1992:

Out of a total of 553 specimen of suspected cases of rabies submitted to the laboratory in Umtata for analysis between 1986 and 1992, 322 or 58.2% were positive for rabies (Table I).

Table I : Results of the laboratory diagnosis of rabies

Animal Species	Pos	Neg	Total	%Pos
Canine (Dog)	168	89	257	65.3
Bovine	108	36	144	75.0
Caprine	17	16	33	51.5
Ovine	8	31	39	20.5
Porcine	6	2	8	75.0
Feline (Cat)	5	21	26	19.2
Equine	5	2	7	71.4
Donkey	1	0	1	100.0
Zebra	2	10	12	16.7
Springbok	1	2	3	33.3
Eland	1	1	2	50.0
Red hartebeest	0	5	5	-
Impala	0	5	5	-
Blue wildebeest	0	4	4	-
Jackal	0	2	2	-
Mountain reed-buck	0	2	2	-
Genetta	0	1	1	-
Cape polecat	0	1	1	-
Blesbok	0	1	1	-
	322	231	553	58.2

The number of rabies cases has been rising every year since then (Fig. I). In 1986, 8 cases of rabies were diagnosed in the northern part of the country, then 33 more cases were confirmed in 1987 in which year 3 cases of rabies were also diagnosed in the central part of the country. The rabies incidence in the northern zone reached a peak of 26 cases in 1988 while 7 more cases occurred in the central part of the country. Rabies cases in Northern Transkei declined to 4 and 6 during 1989 and 1990 respectively but rose to 40 and 41 in the central sector during the same period. Rabies was identified in Southern Transkei in 1991. The number of cases diagnosed in the southern, central and northern sectors of the country in the same year were 5, 58 and 9 respectively.

By 1992, there were 24, 49 and 11 cases of rabies in the northern, central and southern zones of the country respectively (Fig. 2, Appendix I).

Table 2 : Species of animals in which rabies was diagnosed

Animal species	Number	% of total
Canine	168	52.2
Bovine	108	33.5
Caprine	17	5.3
Ovine	8	2.5
Porcine	6	1.9
Equine	5	1.6
Feline	5	1.6
Zebra	2	0.6
Donkey	1	0.3
Springbok	1	0.3
Eland	1	0.3

The dog accounted for 52.2 % and cattle 33.5% of all rabies positive cases (Table 11). The other domestic animals which included goat, sheep, pig, horse, cat and donkey constituted 12.9% of the cases. The remaining 1.5 % of the cases occurred in zebra, springbok and eland (Table I). Rabies was

detected in the dog population before it was diagnosed in other animals. The number of rabies cases in the dog reached the highest level one year before the incidence in other animals peaked. Furthermore, the disease trend in these animals followed and mirrored that in the dog population (Fig. 3). Rabies appeared to be more prevalent during winter months (Fig. 4).

8.1 Vaccination of animals against rabies: June 1986 - December 1992

During 1986 and 1987, the respective number of dogs in the northern part of the country vaccinated each year against rabies were 140456 and 155432 (Table III).

Table III : Annual rabies vaccination figures of dogs and cats

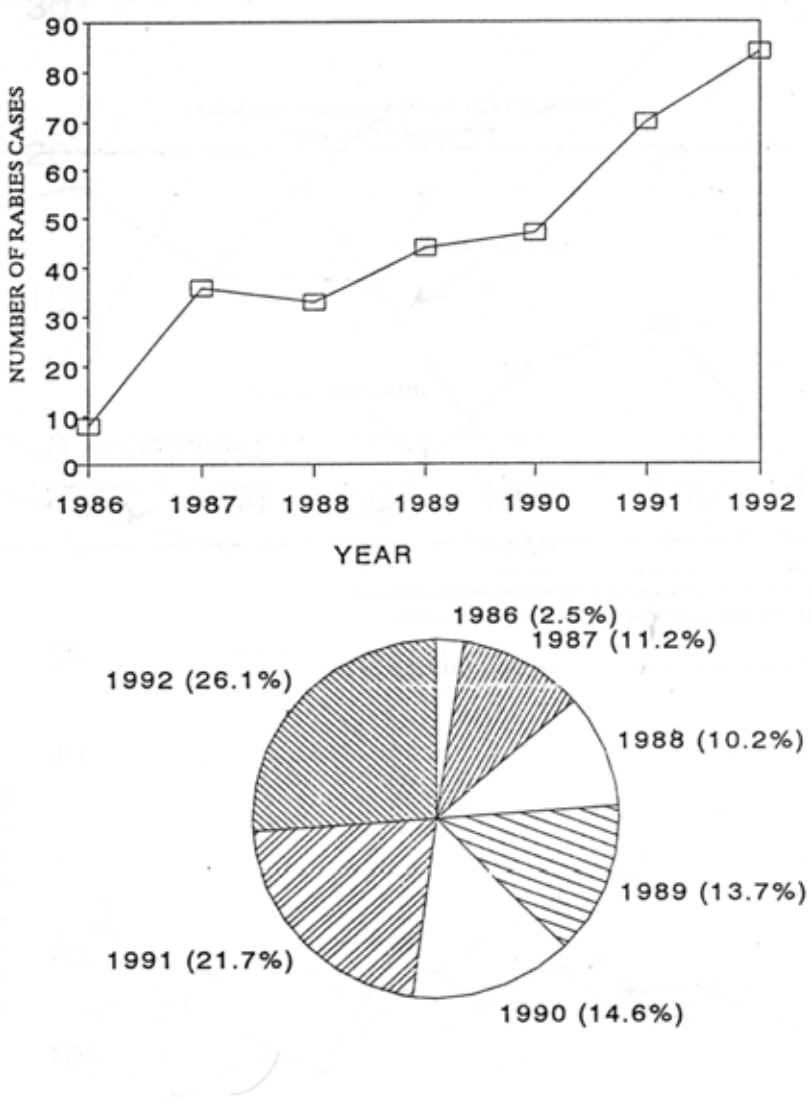
	canine			feline
Year	**Vaccinated**	**Census**	**% vacc.**	**Vaccinated**
1986 (N)	140456	220548 (130226)	63.7 (107.8)	20508
1987 (N)	155432	259988 (161039)	58.8 (96.5)	29295
1988 (C)	131170	257475	50.9	21174
1989 (C)	178442	256358	69.6	43604
1990 (C)	202990			26617
1991 (C)	207807			23958
1992 (C)	374315			37779

The corresponding dog population in this area in the same years was 130226 and 161039. Dogs in the rest of the country were also vaccinated against rabies in 1988 and 1989. The respective number of dogs inoculated each year were 131170 and 178442 compared to the dog population census in the country of 257475 and 256358 respectively. Annual vaccination against rabies was continued in 1990, 1991 and 1992 and 202990, 207807 and 374315 dogs were inoculated during each respective year. There was no dog population census during the same periods (Table III). The number of dogs vaccinated against rabies in the northern sector as a proportion of the dog population in the area was 107.8 % in 1986 and 96.5 % in 1987 . Compared to the dog population in the country, the number of dogs vaccinated against rabies between 1986 and 1989 ranged from 50.9 % to 69.6% (Table III).

Vaccination figures of individual districts available for 1989 and 1991 indicated that the number of dogs vaccinated against rabies as a proportion of the population in each district varied between 26% and 184% (Table IV).

The population of cats vaccinated against rabies varied from 20508 in 1986 to 43604 in 1989 and 3779 in 1992 (Table III).

Figure 1 : number of rabies cases in Transkei, totals for 1986-1992



THE NUMBER OF RABIES CASES CONTINUES TO INCREASE EACH SUCCEEDING YEAR .

Figure 2 : number of rabies cases in Transkei, period 1986-1992, Northern, central and Southern zones

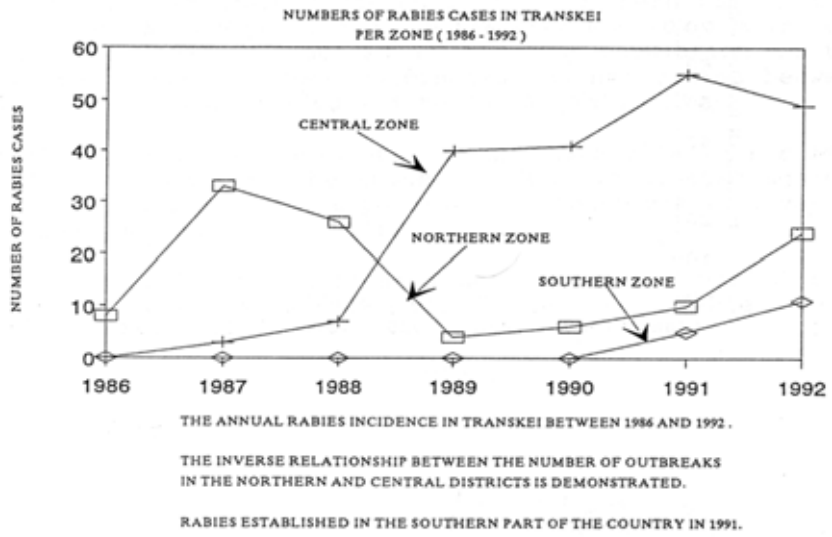


Figure 3 : total canine rabies cases vs total non-canine cases, central and Northern zones, 1986-1992

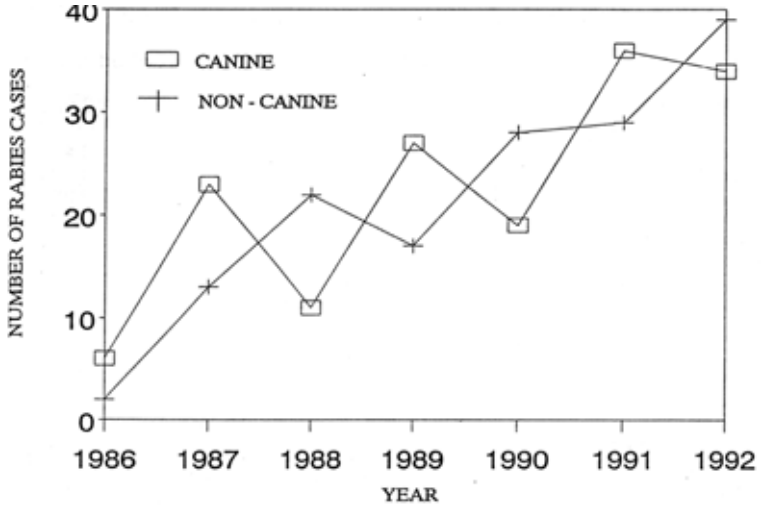


Figure 4 : seasonal incidence of rabies in Transkei

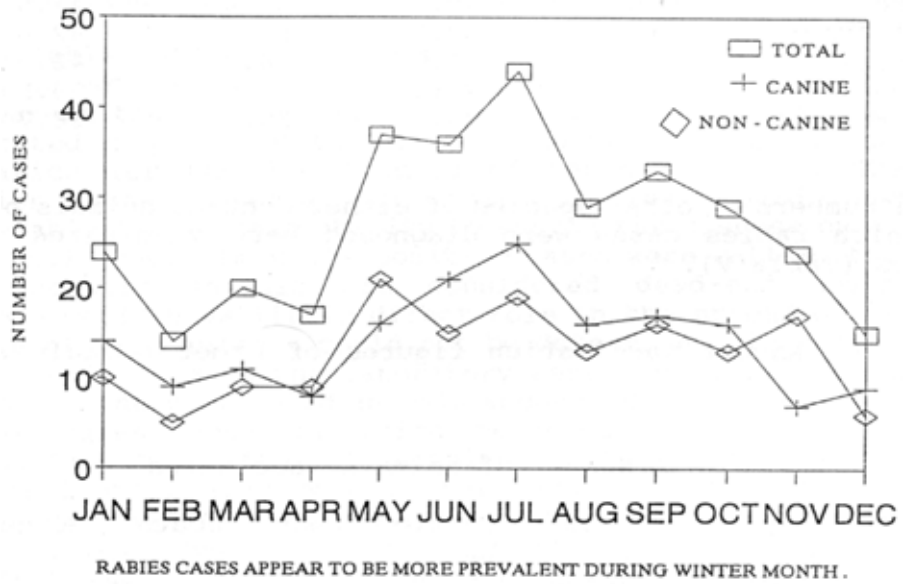


Table IV : The proportion of dogs inoculated against rabies in individual districts

DISTRICT	Percentage of dogs vaccinated for rabies		
	1989	1991	Average
Butterworth	184.0	1l0.7	147.3
Elliotdale	145.8	119.9	132.8
Mt. Ayliff	126.5	128.2	127.3
Kentane	161.0	65.7	113.3
Nqamakwe	152.4	71.8	112.1
Idutywa	124.1	96.6	1l0.4
Tsomo	138.3	57.6	97.9
Mqanduli	97.4	80.9	89.1
Cofimvaba	93.4	81.3	87.3
Nqgeleni	114.7	56.2	85.4
Willowvale	130.1	33.8	81.9
Herschel	78.2	83.7	80.9
Tabankulu	58.3	l02.7	80.5
Port St.Johns	94.7	62.2	78.4
Qumbu	25.2	1l0.6	67.9
Maluti	33.6	l00.0	66.8
Tsolo	39.6	88.9	64.2
Umzimkulu	79.2	40.1	59.6
Umtata	44.3	71.8	58.1
Flagstaff	49.0	58.6	53.8
Cala	58.2	46.7	52.4
Engcobo	43.9	53.9	48.9
Bizana	40.8	54.6	47.7
Mt.Frere	40.6	54.4	47.5
Mt.Fletcher	32.1	53.1	42.6
Lusikisiki	46.3	38.2	42.2
Libode	26.4	50.6	38.5
Lady Frere	33.6	39.8	36.7

Small numbers of other species of either contact animals or those in which rabies cases were diagnosed were vaccinated against rabies (Table V).

Table V : Annual vaccination figures of other animals against rabies

Year	Dog	Cat	Cattle	Sheep	Goats	Horses
1986	140456	20508				
1987	155432	29295	484			
1988	131170	21174	222			
1989	178442	43604	2228			
1990	202990	26617	610	240	49	
1991	207807	23958				40
1992	374315	37779	1967			

Between 88% and 95% of animals were inoculated duing the campaign periods (Table VI).

Table VI : The number of dogs vaccinated against rabies duing the campaign period as compared to the whole year

Year	Campaign Period	Rest of Year	Total	%
1990	291079	11911	202990	94.1
1991	198785	9022	207807	95.7
1992	329463	44852	374315	88.0

9 DISCUSSION

Since rabies established itself in Transkei in 1986, an ever increasing number of cases is diagnosed each year. Initially, rabies outbreaks were confined to the northern sector of the country where it was first identified. However, by 1987, the disease had spread southwards and cases were diagnosed in the central region. During 1991, rabies cases were confirmed in the southern part of Transkei as well. The spread of rabies all over Transkei has taken place amidst concerted efforts to control it by mass vaccination of dogs and cats throughout the country.

The available data appears to indicate that rabies in Transkei was dog driven, as over 50% of positive cases were from dogs. It was apparent that low numbers of cats were infected because they constituted only 1.6% of the positive cases. There was no obvious explanation for the low figures of rabies in cats. The low infection rate detected in this species would however, considerably limit their ability to spread the disease on a large scale. While over 33% of the positive cases were cattle, they and other domestic animals are considered dead-end hosts and therefore would have little direct role in the spread of rabies. The role of wild animals could not be determined since only 6.9% of all submissions to the laboratory were from this category of animals. Furthermore, wild animals accounted for only 1.5% of all positive cases, none of which belonged to the carnivorous species. Less than 1% of all submissions to the laboratory were wild carnivores. This was at variance with findings in the Republic of South Africa, where the latest figures indicate that rabies is more predominant in the mongoose and quite different from the situation in Europe and North America in which rabies is wild animal driven (Reid - Sanden et al., 1990).

Mass vaccination of dogs against rabies in the northern sector of Transkei was successful in curbing the disease prevalence for a brief period. The antirabies vaccination campaigns of 1986, 1987 and 1988 were followed by a marked reduction of the disease incidence in Northern Transkei during 1989 and 1990. However, the incidence of rabies in the country during the same period was rising. This was due to a dramatic rise in the number of cases in the central part of the country.

The fact that the rabies incidence in Transkei has continued to increase each year in spite of the efforts to control it may reflect deficiencies in the approaches employed to date. It would appear that an opportunity to block further spread of the disease from its initial focus in the north was lost through failure to implement an immediate antirabies vaccination campaign all over the country due to lack of funds. Country wide vaccination of dogs and cats against rabies was instituted from 1988 by which time the disease had probably spread to most parts of the country.

It was also quite clear from the available figures that there was considerable inconsistency between the number of dogs inoculated against rabies and population estimates for a given period and area. In some instances the vaccination figures exceeded the population estimate markedly. For example, almost twice as many dogs as the population estimate were vaccinated in one of the districts in 1989. On the other hand the proportion of the population vaccinated was as low as 26% in some districts.

This obviously indicated that accurate animal population census figures were lacking. Assessment of the effectiveness or the extent to which to implement control measures using data of this nature would not yield reliable results. It would be impossible to use such data to fix a target figure of animals to be inoculated in order to attain the minimum proportion of 70% required to achieve reasonable control of rabies (Tierkel, 1975). Besides being unreliable, the available data also showed that less than 70% of dogs in Transkei were vaccinated against rabies between 1986 and 1989. If these figures were reliable, this would be one major reason for failure to control the disease in Transkei. Furthermore, since the dog population turn over pattern in Transkei was unknown, designing an appropriate programme that could ensure successful inoculation of all dogs and cats against rabies throughout the course of the year could not be done reasonably.

On the other hand the use of the so called "INFO - package" made an impact to some extent in some areas, and as such contributed to the marked improvement in the response by the community to the call to have their dogs and cats vaccinated against rabies. It was also interesting to note that the use of a vaccine which was administered subcutaneously, issue of vaccination certificates, free vaccinations and a No-dog-tax approach did influence the whole exercise positively. The intensification of the awareness campaign in the month preceding the start of inoculations meant a lot in terms of dog turn out for vaccination as shown by the 1992 figures.

In the control programme carried out in Transkei, antirabies vaccination campaigns were held once a year between July and September and up to 95% of all dogs vaccinated against rabies each year were inoculated during this time. Although the percentage turn over of the dog population in Transkei was unknown, it was quite likely that a good proportion of dogs and cats liable for vaccination against rabies outside the campaign period were not presented for inoculation. In fact, towards the end of every vaccination campaign against rabies, most of the dogs diagnosed positive for rabies were young ones often under 6 months of age (Akol, personal observation).

There was little proof of vaccine failure but it could not be ruled out completely because of certain factors which prevailed under the operational conditions. First, it should be realized that quite a number of dogs in the rural areas were in poor nutritional state, were rarely dewormed and therefore must have had high parasite burdens. Such animals were likely to be immunocompromised and therefore would make poor candidates for vaccination. In addition, due to inadequate conditions of storage and conveyance in the field, deterioration of the vaccine could occur. This was particularly important at the time of use of the lyophilized rabies vaccine.

In conclusion, the present attempts to control rabies in Transkei appear to have fallen short of expectations. This could be attributed to the lack of definite, achievable and measurable targets in the current programmes. Biological factors involving vaccine storage, conveyance and administration and the poor condition of recipient animals, may also have negated the effectiveness of vaccination against rabies in this country. Lack of awareness and sheer negligence on the part of the community was an important factor. However, awareness campaigns were effective in improving turn-out for vaccination.

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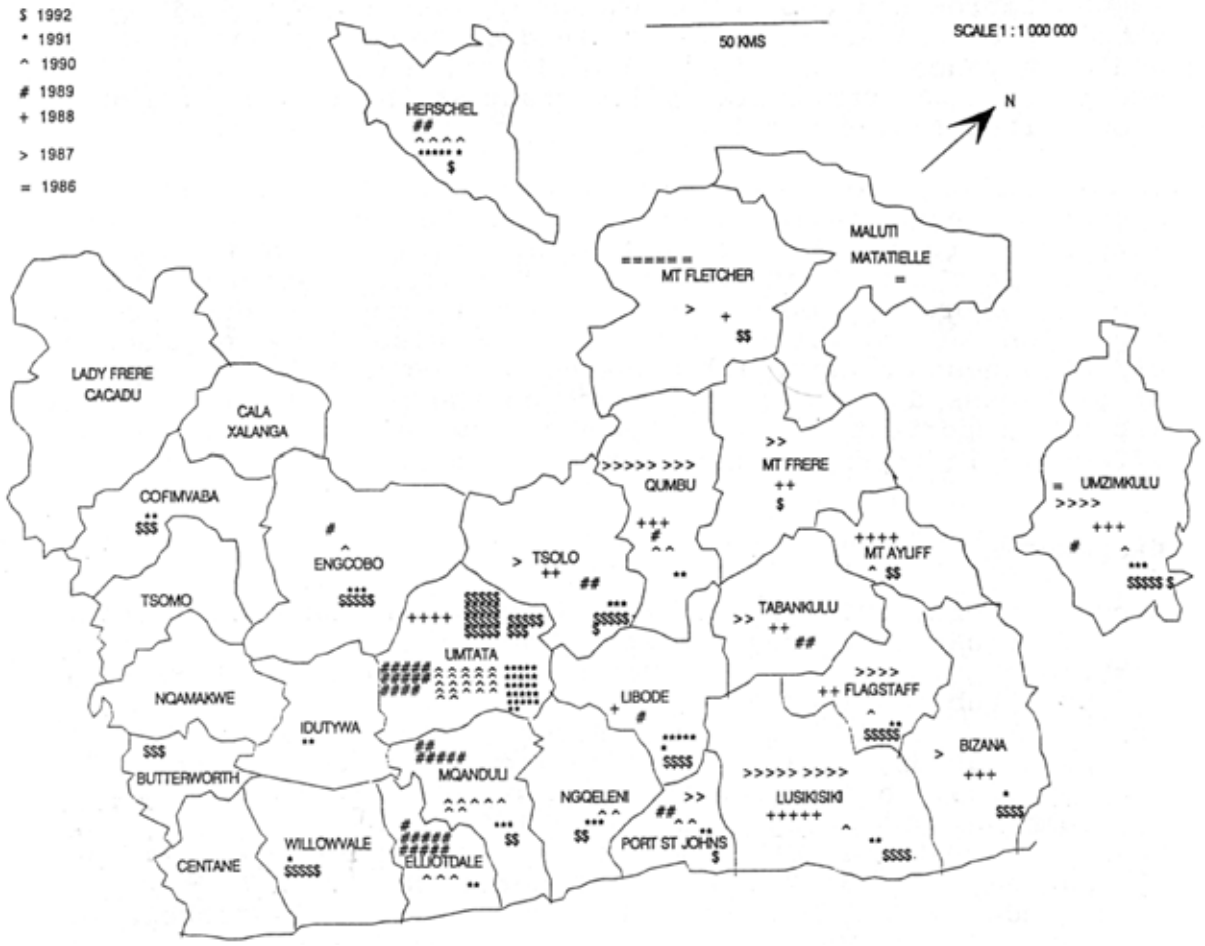
APPENDIX 1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BIZANA
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NGQELENI
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WILLOWVALE
ELLIOTDALE
PORT ST JOHNS
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ANTIGENIC VARIATION IN LYSSAVIRUSES

A. King [1]

Taxonomically, Lyssaviruses belong to the Order Mononegavirales, Family Rhabdoviridae (Pringle, 1991). The Rhabdoviridae are characterized by a negative-sense genome of single stranded RNA and the family is divided into two genera, Vesiculovirus and Lyssavirus, the latter of which includes rabies and the rabies-related viruses. The lyssaviruses are serologically distinct from other rhabdoviruses. A biological characteristic of many of them is that on primary isolation in animals they display a wide range of incubation periods, but following several passages the incubation period of these "street" viruses becomes shorter and of "fixed" duration.

Rabies virions are bullet-shaped with an average length of 180nm and diameter of 75nm. The cylindrical symmetry of the virion is formed by the approximately 165 x 50nm helical nucleocapsid of 30 to 35 coils with a periodicity of around 4.5nm. The surface of the virion is covered, except at the planar end, by 9nm "spikes" or peplomers at 5nm internals (Tordo and Poch, 1988). The frequently observed irregular shape of the planar end of the infectious virion may be due to the formation of a "tail" during budding from the host cell plasma membrane at the time of release from the infected cell (Wunner, 1991).

The genomic RNA of the infectious particle contains five genes, each of which codes for a structural protein of the virion. The nucleocapsid core is formed from the RNA and three of the proteins - N (nucleoprotein), NS (non-structural, but this is a misnomer - transcriptase associated phosphoprotein) and L (large, virion associated transcriptase). The lipid-containing envelope is a bilayer in which the M (matrix protein) lining is surrounded by G (glycoprotein). The G protein spikes are anchored within this bilayer.

The G and N are the two most extensively studied proteins. The G protein possesses the biological and immunological functions of cell surface receptors and antibody binding sites. These reside in the 439 amino acid region of the ectodomain G protein and this 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, lyssavirus group specificity is determined by its cross-reactivity and it has an important role in enabling diagnosis and virus identification by monoclonal antibody anti-nucleocapsid (Mab-N) techniques.

The amino acid sequences of the N proteins are 450 residues long and they display a high degree of similarity. For example, the N proteins of ERA and PV viruses differ by 4 amino acids (99 percent similarity) and those of ERA and CVS-11 viruses differ by 9 amino acids (98 percent similarity) (Wunner, 1991). It is this high degree of homology between the N proteins that allows differences in Mab-N reaction patterns to be used as a tool in epidemiological studies.

Currently, within the genus Lyssavirus four serotypes are recognized: serotype 1, rabies virus; serotype 2, Lagos bat; serotype 3, Mokola and serotype 4, composed of Duvenhage (Africa) and the European bat lyssaviruses EBL 1 and EBL 2. The prototype strain of serotype 1 rabies virus is Challenge Virus Standard-24 (CVS-24), derived from Pasteur's virus. The serotype includes other fixed viruses and street viruses, whether isolated from dogs or cats among domestic animals, or from wild animals such as foxes, skunks and raccoons. Viruses isolated from bats in the Americas are included in this serotype.

The initial typing of viruses of serotypes 1 to 4 Duvenhage (Africa) was established by animal cross-immunization experiments, but the more recently shown distinction between African Duvenhage and the European bat lyssaviruses EBL 1 and EBL 2 is based upon group-specific antigenic differences determined by Mab-N reaction patterns of the virus internal RNP antigens. Viruses of serotypes 2 to 4,

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colloquially known as the rabies-related viruses, have been recently reviewed (King and Crick, 1988; Rupprecht, Dietzschold, Wunner and Koprowski, 1991).

Although reports of bat rabies in Europe had predated the isolation in Africa of Duvenhage virus by some 16 years, similarities between this virus and later isolates from European bats led to the assumption that European bat rabies was of African origin. Some differentiation between these viruses has now been obtained by the use of anti-glycoprotein Mabs (Mab-Gs) (Rupprecht at al., 1991) and Mab-N analysis has shown clear distinctions between European bat viruses and Duvenhage virus of Africa (King, 1991).

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 Mab-Gs is usually carried out by a varying virus: constant antibody method using a dilution of Mab-G sufficient to neutralize 3 to 4 log10 of homologous virus. Analysis with nucleocapsid reactive 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, 1993).

By using Mabs it has been shown that rabies isolates from a given geographical area or species have unique reactivity patterns both in the N and G protein components of the virion (Wiktor, Flamand and Koprowski, 1980). 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, although the reason for such single-species involvement is not clearly understood. Although geographically separate outbreak areas allow the comparison of variants from terrestrial animals on the basis of their collection site, no geographical outbreak areas have been defined for bat rabies in the Americas. Indeed, infected bats of migratory species have been found throughout their natural range that may extend for thousands of miles (Smith and Baer, 1988).

All four rabies serotypes have been shown to be present in Africa. It has now been shown that there are at least five variants of serotype 2 Lagos bat virus and three variants of serotype 3 Mokola virus; no variation was found amongst the only three isolates of African serotype 4 Duvenhage virus analysed (King, 1991). Using the Wistar Institute, Philadelphia, Mabs panel to examine serotype 1 viruses, it was shown that viruses isolated from three slender mongooses of Zimbabwe differed in their G and N proteins from other serotype 1 viruses of Zimbabwe (Foggin, 1988).

In a more recent study (King, Meredith and Thomson, 1993), over 100 rabies viruses isolated from various species from Namibia and South Africa since 1980 were examined with a panel of 80 Mab-Ns consisting of 34 from the Wistar Institute, Philadelphia, 29 prepared at the CVL Weybridge from the rabies-related viruses and 17 from the Centre for Disease Control, Atlanta.

In the tests, 40 Mab-Ns were positive and 24 were negative with all isolates and 24 reaction patterns were obtained. Results supported the findings of Foggin (1988) in Zimbabwe that isolates of "mongoose" origin differed from other serotype 1 viruses. In general, the virus types predominantly from viverrids were found in mongooses, domestic and wild cats whilst those from canids were found in dogs, black-backed jackals and bat-eared foxes. A computer-based analysis identified three broad subgroups within the "viverrid viruses" while the "canid viruses" appeared to be homogeneous. Spill-over of "canid" virus into viverrid species and vice versa was shown. For examples, canid virus type was isolated from a civet and viverrid virus type was isolated from one of 13 dogs, three of 12 black-backed jackals and two of 16 bat-eared foxes. Interestingly, viverrid virus type was isolated from all of the 11 tested domestic and wild cats. Of the livestock animals examined, viverrid virus type was isolated from two bovines and canid virus type was isolated from seven bovines. The finding of canid virus type in the black-backed jackal population area and in the expanding bat-eared fox population area confirmed historical reports and surveillance data that the virus in these species was probably of canine origin; similarly, the preponderance of viverrid virus type in the mongoose population area confirmed the historical view that mongoose rabies on the central plateau of South Africa differs from that of canine origin.

In Europe, extensive oral vaccination campaigns have shown that in areas freed from fox rabies, rabies is not observed in any other species (except bats). Thus historical records and surveillance indicate that European rabies is a single epidemiological entity. In southern Africa, the biological distinction between canid and viverrid rabies, suspected from historical records and surveillance and now

confirmed by Mab-N analyses, indicates that rabies is not a single epidemiological entity. This biological distinction does not accord with the traditional epidemiological division between canine (urban) and sylvatic rabies: the canine virus appears also to be well established in wild canids (jackals and bat-eared foxes) which thus form a reservoir of that virus type. A study of antigenic variation in other areas of Africa and in other parts of the world where it is yet to be attempted would no doubt be rewarding.

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RABIES ERADICATION IN BELGIUM BY FOX VACCINATION USING VACCINIA-RABIES RECOMBINANT VIRUS

B. Brochier [1]and P.P. Pastoret [1]

1 INTRODUCTION.

In Belgium, as well 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 immunization of foxes, by the distribution of baits containing a suspension of vaccinia-rabies recombinant virus, has been experimentally assessed and subsequently engaged in the whole infected area of Belgium.

2 METHOD.

2.1 Vaccine.

A recombinant vaccinia virus expressing the immunogenic glycoprotein of rabies virus (V-RG) has been developed by Transgene in France. This vaccine is currently being used at a large scale level in France, the Grand Duchy of Luxembourg and Belgium. Field trials are also in progress in the USA for the oral vaccination of raccoons. The development of V-RG has been performed by five groups of collaborators: Rhone Merieux, CNEVA and Transgène in France, the Wistar Institute in the USA and the University of Liège in Belgium. Numerous experiments carried out in laboratory as well as in the field have demonstrated the efficacy, safety and stability of this vaccine.

ABSENCE OF PATHOGENICITY

ABSENCE OF PATHOGENICITY

54 ANIMAL SPECIES

		EUROPE		AMERICA
6 WILD TARGET SPECIES	*MAMMALIA*	RACCOON DOG	RED FOX ARCTIC FOX	RACCOON STRIPED SKUNK VAMPIRE BAT
36 WILD NON TARGET SPECIES	*MAMMALIA*	2 CARNIVORA 6 RODENTIA 1 ARTIODACTYLA		6 CARNIVORA 10 RODENTIA 1 ARTIODACTYLA 1 MARSUPIALA 1 INSECTIVORA
	AVES	2 PASSERIFORMES 2 FALCONIFORMES		1 CHARADRIIFORMES 1 FALCONIFORMES 1 STRIGIFORMES
6 DOMESTIC SPECIES	CATTLE	SHEEP	PIG HORSE	DOG CAT
4 LABORATORY SPECIES	MOUSE	FERRET	RABBIT	HAMSTER
2 PRIMATES	CHIMPANZEE		SQUIRREL MONKEY	

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2.2 Baiting system.

A suspension of V-RG at 108 TCID50 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 long term bio-marker of bait uptake and can be detected in bones by using fluorescence microscopy. The efficacy of this vaccine-bait system has been tested in captive foxes. Each fox of three experimental groups was fed one, two or three baits containing 108 TCID50 of V-RG. As shown by the incorporation of tetracycline, all the foxes voluntarily ingested at least one bait. One month after baiting, Fifteen of eighteen foxes developed rabies antibodies, 14 of 18 developed vaccinia antibodies and 16 of 18 foxes resisted a lethal rabies challenge.

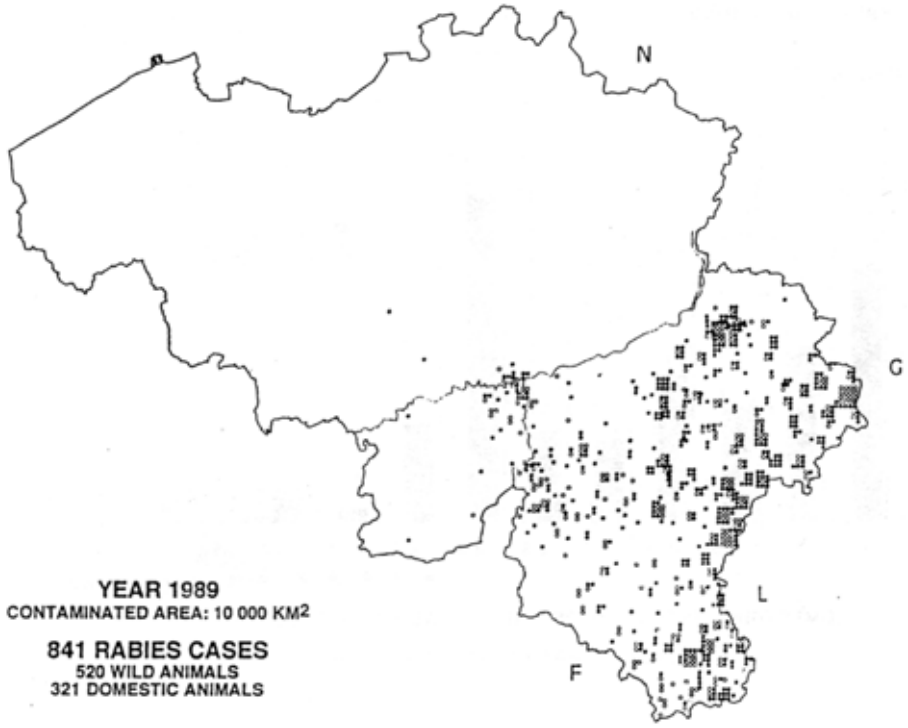
The VR-G vaccine – bait system :

- Is efficient (Immunogenicity + attractive power)
- Is safe for target and non-target species
- - Is stable (storage without freezing, durable activity in the field)
- can be dropped by air (mechanical resistance)
- is easily available

2.3 Campaigns of fox vaccination.

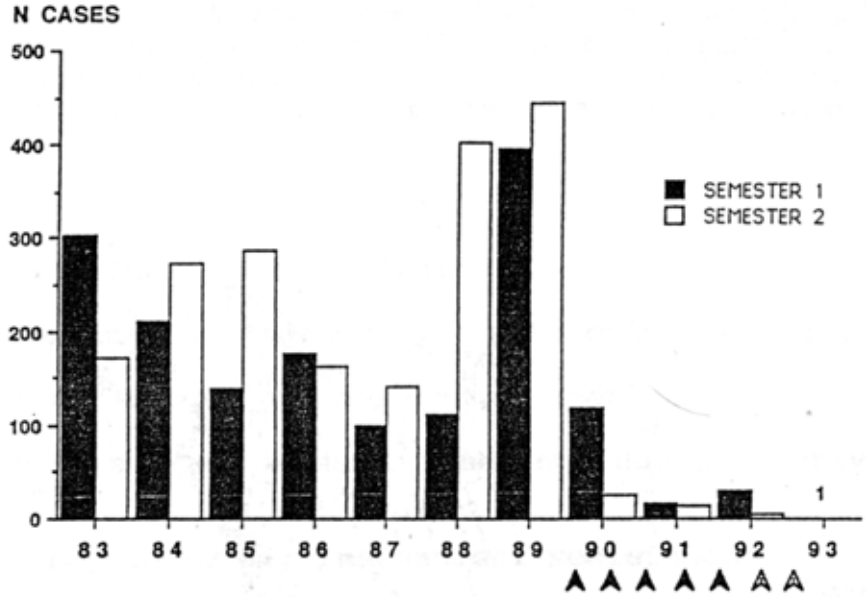
The rabies infected area covered 10 000km^2 in the southern part of the country and was bordered by a river and four neighbouring countries: the Netherlands, Germany, Grand Duchy of Luxembourg and France (see map 1). This region was heavily infected before the campaigns of fox vaccination - 840 animal rabies cases were recorded in 1989. That represents a mean rate of 1 case per 10km2.

In the autumn of 1989, the first campaign of fox vaccination was carried out in the whole of the infected area. From 1989 until 1991, four similar full campaigns were performed. Each time, 150000 V-RG baits were dropped by plane at a mean density of 15/km2. In 1992, a new vaccination area was defined to create an immune belt along political borders. Two defense campaigns were carried out in spring and autumn-winter 1992.



RESULTS

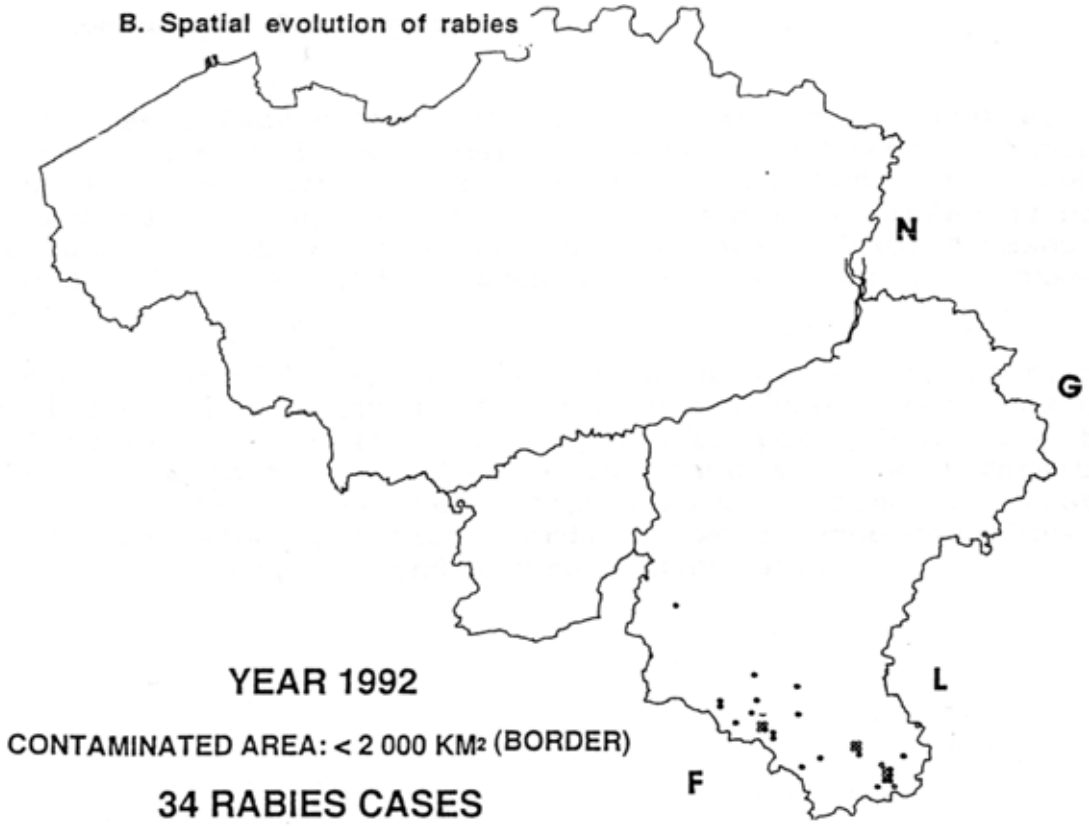
A. Rabies incidence

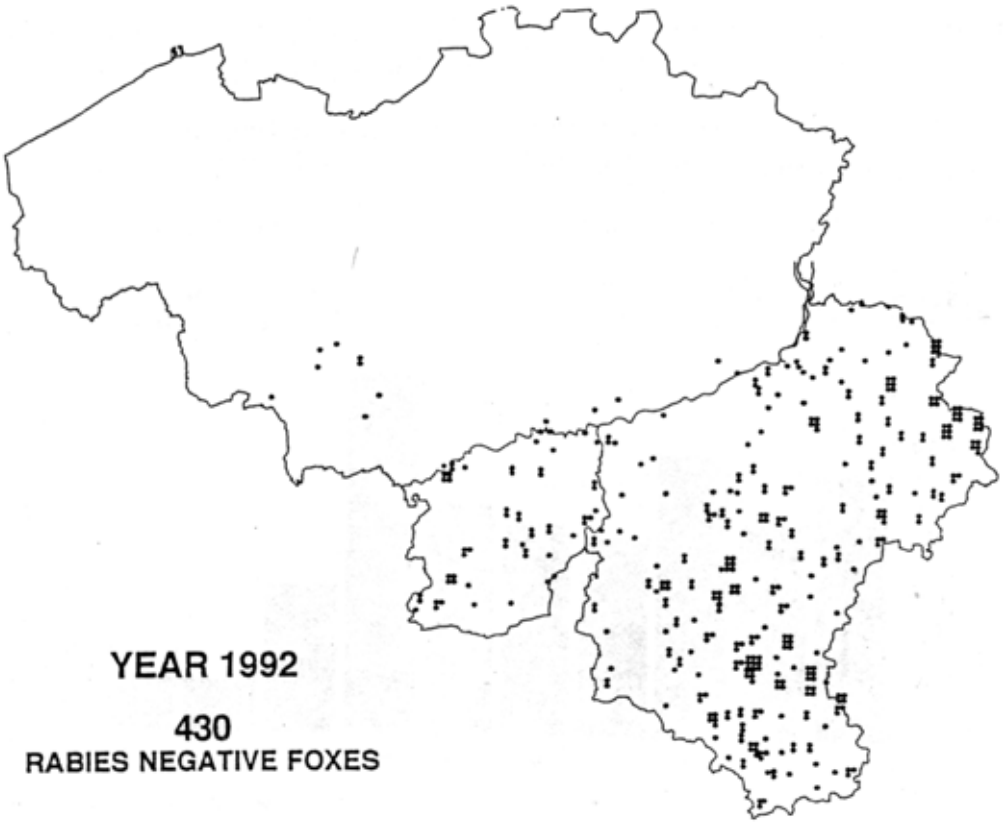


SEMESTRIAL EVOLUTION OF ANIMAL RABIES IN BELGIUM

Black arrows: full campaigns; white arrows: partial campaigns (along borders).

B. Spatial evolution of rabies





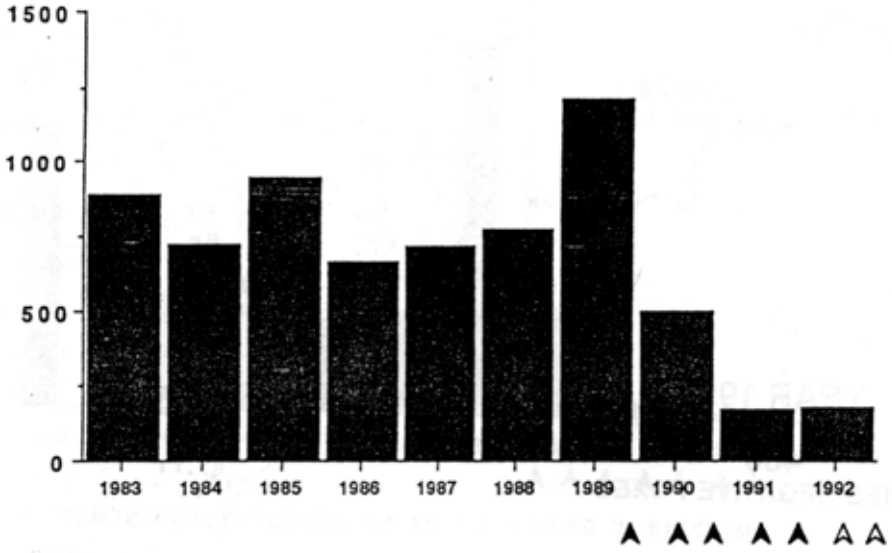
YEAR 1992

430
RABIES NEGATIVE FOXES

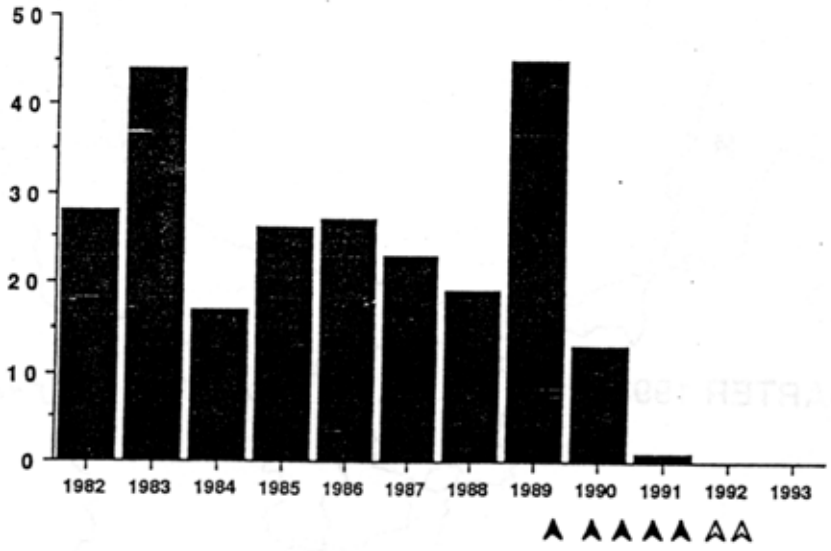
1st QUARTER 1993

1 RABIES CASE
1 badger
65/79 foxes TC+ (82%)

C. Consequences



EVOLUTION OF POST-EXPOSURE HUMAN TREATMENTS



EVOLUTION OF RABIES IN DOMESTIC CARNIVORES

Black arrows: full campaigns; white arrows: partial campaigns (along borders).

3 CONCLUSIONS.

Thanks to the vaccination of foxes, rabies disappeared from 80% of the initially infected area of Belgium.

The efficiency and stability of the vaccine-bait association made these results possible within two years.

The creation of an immune belt along borders is still required.

Vaccination will be completely interrupted when the defined surveillance area will be rabies-free for at least 1 year.

Cartes et annexes ~~page~~s 116-117-118-119

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ADVANCES IN DIAGNOSTIC METHODS AND TYPING OF RABIES VIRUS *[*]

H. Bourhy[1], B.Kissi [1] and N. Tordo[1]

1 INTRODUCTION.

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). Nevertheless, new laboratory methods for rabies diagnosis are still developed in order to increase the specificity and the sensitivity, reduce the time required for diagnosis, extend the detection to all the lyssaviruses, extend the sensitivity of the "intra-vitam" diagnosis and lower the cost of the diagnosis.

Today, there is also a need 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 has been developed recently (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; Smith et al., 1992). 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.

2 ROUTINE PROCEDURES FOR RABIES DIAGNOSIS (FIG.1)

2.1 Rapid methods of sample collection.

The opening of the skull for the collection of brain specimens for rabies diagnosis is a relatively long and delicate procedure that should be performed by well trained technicians. It also requires special precautions to avoid accidental exposure to the virus through wounds or by aerosol. Internal brain sampling without autopsy by the introduction of a disposable plastic pipette via the occipital foramen (Barrat and Halek, 1986) or via the retro-orbital route (Montano Hirose et al., 1991) appeared to be particularly rapid and safe in field conditions.

2.2 Virus isolation in cell cultures.

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) employing murine neuroblastoma cells (Portnoï et al., 1982) which is equally as accurate but which can be used to detect the products of virus replication in one or two days rather than in weeks (Bourhy et al., 1989).

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* This paper was also given at the Symposium on Rabies Control in Asia, Jakarta, Indonesia, April 27-30 1993.

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2.3 Detection of rabies nucleocapsid by immuno-enzymatic tests

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). The results are not very much impaired if the specimens have not been maintained imperfectly good conditions for transportation. It is strongly recommended as a back up procedure to corroborate the FAT results, as a simple technique for epizootiological surveys, and as a sensitive technique for laboratories which are not equipped for performing FAT.

The RREID can also be modified 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) 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. Compared to the RREID, the threshold of detection of the modified technique (RREID-lyssa) was 100 times lower (0.2 ng of viral nucleocapsid whatever the serotype). The sensitivity and the specificity were identical to the RREID. Consequently, RREID-lyssa can be a useful tool for diagnostic laboratories that receive specimens infected by rabies-related viruses.

3 CONTRIBUTION OF MOLECULAR BIOLOGY TO THE RABIES DIAGNOSIS AND TYPING

3.1 Diversity of the lyssaviruses.

Four serotypes of rabies and rabies-related viruses were determined on the basis of seroneutralization and monoclonal antibodies studies: classical rabies virus strains (serotype 1) and Lagos bat virus (serotype 2), Mokola virus (serotype 3), Duvenhage virus (serotype 4) (W.H.0., 1990). European bat lyssaviruses (EBL) were not classified. Rabies virus (serotype 1) is present worldwide except in several protected islands. Rabies-related viruses (serotype 2, 3, 4 and EBL) have a large geographic distribution in Africa and in Europe. All the rabies and rabies-related viruses are pathogenic for mammals including man and lead to a rabies-like encephalitis. Monoclonal antibody studies and cross protection experiments have demonstrated that Mokola virus was the rabies-related virus most distantly related to the vaccinal strains of serotype 1 (King and Crick, 1988).

3.2 Genetic study of the Mokola virus

A molecular study of Mokola virus was undertaken in order to appreciate the maximal diversity of the Lyssavirus genus. Viral RNA was extracted from purified virus propagated in BHK-21 cells. Two primers extrapolated from the PV genome sequence generated 5 molecular clones encompassing the complete Mokola virus genome (Bourhy et al., 1989). The cDNA sequence of the 5568 nucleotide of the 3' moiety and the predicted polypeptide sequence of Mokola virus antigenome was determined (Bourhy et al., 1993). The sequence encodes 5 major non overlapping open reading frames (ORF) corresponding from the 3' to the 5' end to the N, M1, M2, G and the beginning of the L polymerase, respectively. The order of protein conservation in lyssaviruses (nucleoprotein > matrix protein > phosphoprotein) is consistent with the vesiculovirus findings and seems more general in the order Mononegavirales. Nevertheless, whatever the protein, N, MI or G, a weak conservation of the AA sequence of the antigenic sites defined in the rabies virus was noticed in Mokola virus. These mutations observed in these immunogenic regions explain the lack of cross protection between vaccinal strains and Mokola virus (Tordo et al., 1993).

3.3 Detection of rabies virus nucleic acids

Comparison between the sequences of the Mokola virus (serotype 3) (Bourhy et al., 1993) and the PV strain (serotype 1)(Tordo et al., 1988), two lyssaviruses representative of the most divergent serotypes 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 previously described (Sacramento et al., 1991). Primers allowing the amplification of the whole nucleoprotein (N),glycoprotein or pseudogene were defined (Bourhy et al., 1992; Sacramento et al., 1992; Tordo et al., 1992). Each of these three genomic target 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. For the purpose of diagnosis (Fig. 1), the PCR products were diluted, heat denatured for 10 mn, 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 were air dried before covalent binding of the nucleic acids by UV illumination at 312 nm for three mn (Sacramento etal., 1991). For virus typing, 2-10 µl of the PCR products were digested by a panel of selected restriction enzymes and separated by electrophoresis on a convenient agarose gel in buffer containing ethidium bromide. The pattern was analysed on a print of the gel viewed under UV illumination at 312 nm (Bourhy et al.,1992; Sacramento et al., 1991).

3.4 Typing of rabies viruses after genomic amplification

3.4.1 Introduction

The N gene was chosen for molecular epidemiology studies for several reasons. First of all, in a comparative way, since most of the rabies-related viruses were identified according to their reactivity with antinucleocapsid monoclonal antibodies. Secondly, because of the important role of the nucleoprotein in inducing immunity, in particular against infection with heterologous lyssaviruses. Thirdly, the N gene seems to be a good target for comparison among isolates across a relatively long term evolution. Finally, the study of the N gene respects the progressivity of the PCR technique from a simple diagnosis which has already allowed the amplification of the N gene to a very precise typing by determining its nucleotide sequence. For this purpose, a rapid method to sequence selected genomic areas of wild isolates was developed (Sacramento et al.,1991)(Fig. 1). Where possible, we worked with the original infected brain. Otherwise, suckling mouse brains infected with the original virus at the lowest number of passage available were used. The viral RNA extraction, the cDNA synthesis, the PCR amplification, the purification of the amplified fragment on 0.7% NuSieve GTG agarose and its direct sequencing were performed as described by (Sacramento et al., 1991). Alignment of the deduced amino acid sequence of the N proteins was performed by the ClustalV package of multiple alignment programs (Higgins and Sharp, 1989). The phylogenetic trees were also generated by the ClustalV package of multiple alignment programs according to the neighbour joining method (Saitou and Nei, 1987).

3.4.2 Determination of genotypes

The N gene of seventy rabies and rabies-related viruses representative of the diversity of the Lyssavirus genus were analysed and a phylogenetic tree was constructed. Six tight genetic clusters named genotypes were distinguished (Fig. 2 and 3): (1) rabies virus, (2) Lagos bat virus, (3) Mokola virus, (4) Duvenhage virus, (5) European bat lyssavirus biotype 1 (EBL1) and (6) EBL2. Genotypes 1 and 4 corroborated the previous classification in serotype. However, the genetic grouping appeared more powerful than seroneutralization and NC-MAbs studies in establishing that EBL1 and EBL2 must be considered as independent genotypes. It is also more sensitive to appreciate inter-genotype relationships: genotypes 4 and 5 are related and genotypes 2 and 3 are the most phylogenetically distant from the vaccinal and classical rabies virus of genotype 1. The threshold of similarity below which a new genotype should be defined is in the interval of 96-93% AA similarity.

3.4.3 Determination of geographic lineages

An extensive analysis of rabies virus of genotype 1 collected all over the world was also conducted in order to further evaluate the diversity within the genotype 1 of lyssavirus and more precisely to detect wild reservoirs of canine rabies and to appreciate the divergence of the wild isolates with the vaccinal rabies strains. The nucleotide sequences (1531 b) of the whole nucleoprotein gene and the intergenic nucleoprotein -phosphoprotein region of seventy strains originating from forty different countries were determined. The lineages distinguished by the evolutionary analysis correlate with the geographic origin (Fig. 4). This analysis allowed us to distinguish nine groups of phylogenetically distinct viruses of genotype 1: Africa 1, Africa 2, Asia, Arctic rabies, Europa and Middle East, Latin America 1, Latin America 2 and two groups of vaccinal strains. The group Africa 1 can be divided into Africa la circulating in the North and the East of Africa and into Africa 1b, circulating in the South of the African continent. Viruses of the group Africa 2 are distributed in the central part of Africa. Further analysis of other strains would certainly have allowed us to define other groups. Nucleotide sequences evidence also a common ancestor for fox, raccoon dog, dog and wolf isolates in Europe.

3.4.4 Comparison with vaccinal strains

Genetic variability may complicate the efficacy of a vaccine intended to prevent rabies. If this proves to be the case, genetic heterogeneity, rapid evolution and consequently antigenic diversity will offer rabies virus ample opportunity to evade the host immunity induced by rabies vaccines. The % of similarity between vaccinal strains and wild isolates remains relatively high at the AA level (Fig.5). This is in favour of a good protection given by the vaccinal strains. Nevertheless, particular attention was given to some strains isolated from reported cases of vaccination or treatment failures in animals or in humans. In those cases the sequence at the antigenic sites were also very similar to those of the vaccinal strains and not different from field strains collected in the same area. The cases of vaccination and treatment failures do not seem to be due to a particular genetic variation.

4 CONCLUSION

New laboratory methods are now available for the diagnosis of rabies. The fluorescent antibody test should remain the reference technique. Virus isolation in cell culture and RREID were shown to be sensitive and specific confirmatory methods. They can be recommended as a back up procedure to corroborate the FAT results. RREID would also be very useful for laboratories which are not equipped for performing FAT. In the last few years, antigenic analysis has played a major role in viral classification by discriminating between variants. Nevertheless the advent of molecular biology brought more rapid and performant tools. Already, PCR applied to rabies provides a progressive technique starting from diagnosis and ending to a precise genetic characterisation by sequencing of viral limited genetic areas, advantageously replacing seroneutralisation and NC-MAbs studies. For this purpose the study of the nucleoprotein gene appears to be an efficient method discriminating between genotypes and defining phylogenetic relatedness between wild isolates. It allows us, for example, to characterise two different phylogenetic groups in Africa. It also provides a way of rapid analysis for mutations in the antigenic sites of strains isolated from reported cases of vaccination or treatment failures in animals or in humans.

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Figure 1 : Principle and time required for the different diagnosis and typing techniques

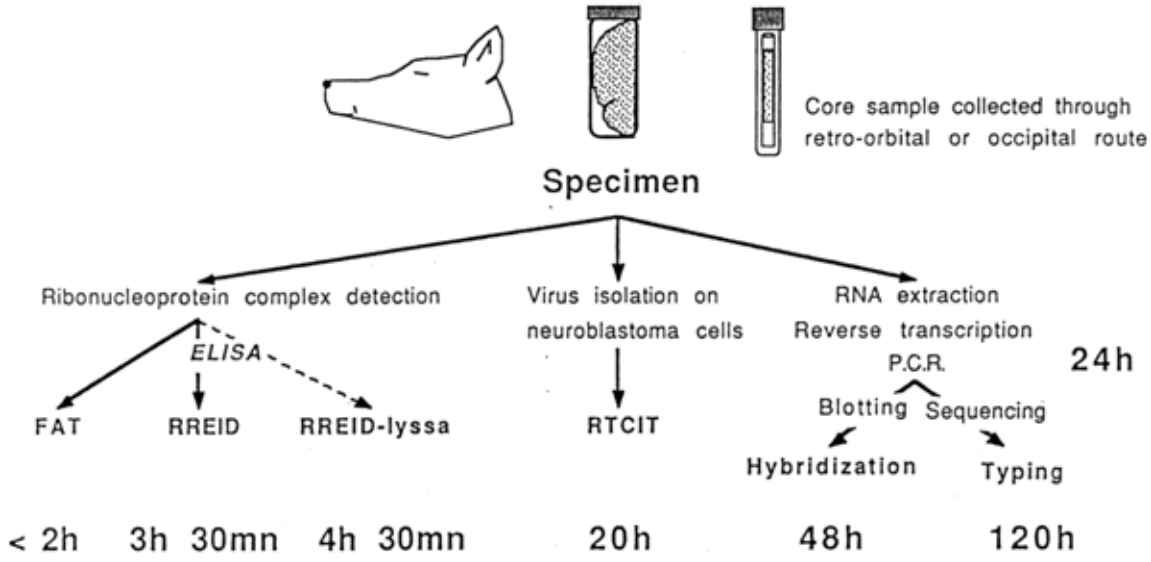


Figure 2 : Radial phylogeneric tree showing the relationships between the different genotypes of lyssavirus.

Alignments were performed by the ClustalV package of multiple alignment programs(Higgins and Sharp, 1989). The tree was generated according to the neighbour joining method (Saitou and Nei, 1987). The dotted line surrounds the branches corresponding to the strains of genotype 1. The lengths of the branches indicates the phylogenetic distance between the different viruses.

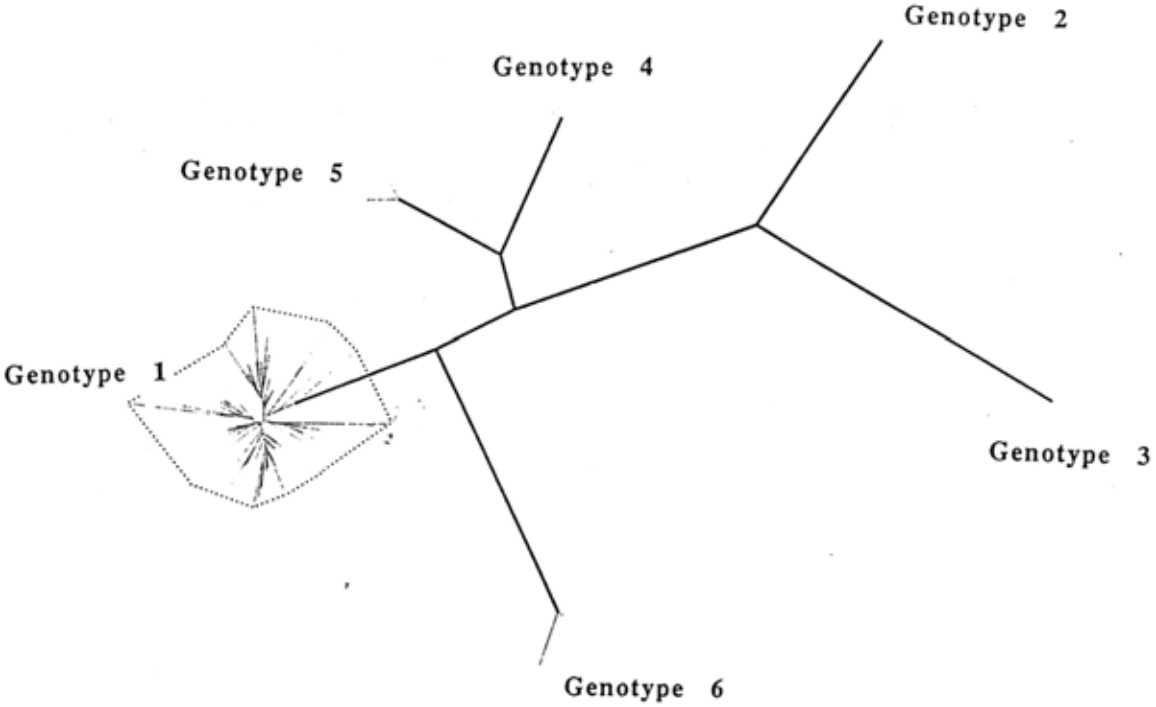


Figure 3 : Geographic distribution and host species of lyssaviruses (from King and Crick, 1988; Metbatsion et al., 1992; Bourhy et al., 1993)

RHABDOVIRIDAE FAMILY - *LYSSAVIRUS* GENUS

GENOTYPE	GEOGRAPHIC DISTRIBUTION	ANIMAL SPECIES
1. Rabies	Worldwide, except: Australia, British Islands, Ireland, New Zealand, Japan, Antarctica, Scandinavia, Hawaii	Man, wild and domestic carnivores and herbivores, bats
2. Lagos-bat	Nigeria, Central African Republic, South Africa, Zimbabwe, Guinea, Senegal, Ethiopia	Frugivorous bats, cats, dog
3. Mokola	Nigeria, Central African Republic, Zimbabwe, Cameroon, Ethiopia	Man, shrews, cats, dogs, rodents
4. Duvenhage	South Africa, Zimbabwe	Man, insectivorous bat
5. EBL 1	European countries	Man, insectivorous bats: genus Eptesicus, Pipistrellus
6. EBL 2	European countries	Man, insectivorous bats: genus Myotis

Figure 4: Radial phylogenetic tree showing the relationships between the different geographic lineages of lyssavirus. Alignments were performed by the Clusta1V package of multiple alignment programs (Higgins and Sharp, 1989). The tree was generated according to the neighbour joining method (Saitou and Nei, 1987). The length of the branches are not indicative of the phylogenetic distance between the different viruses.

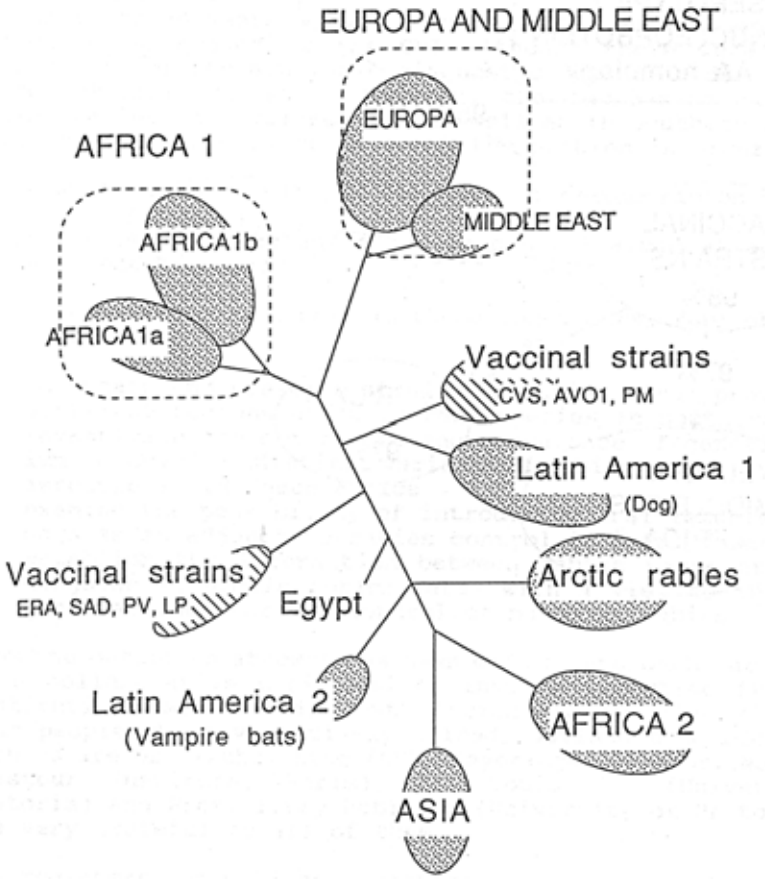
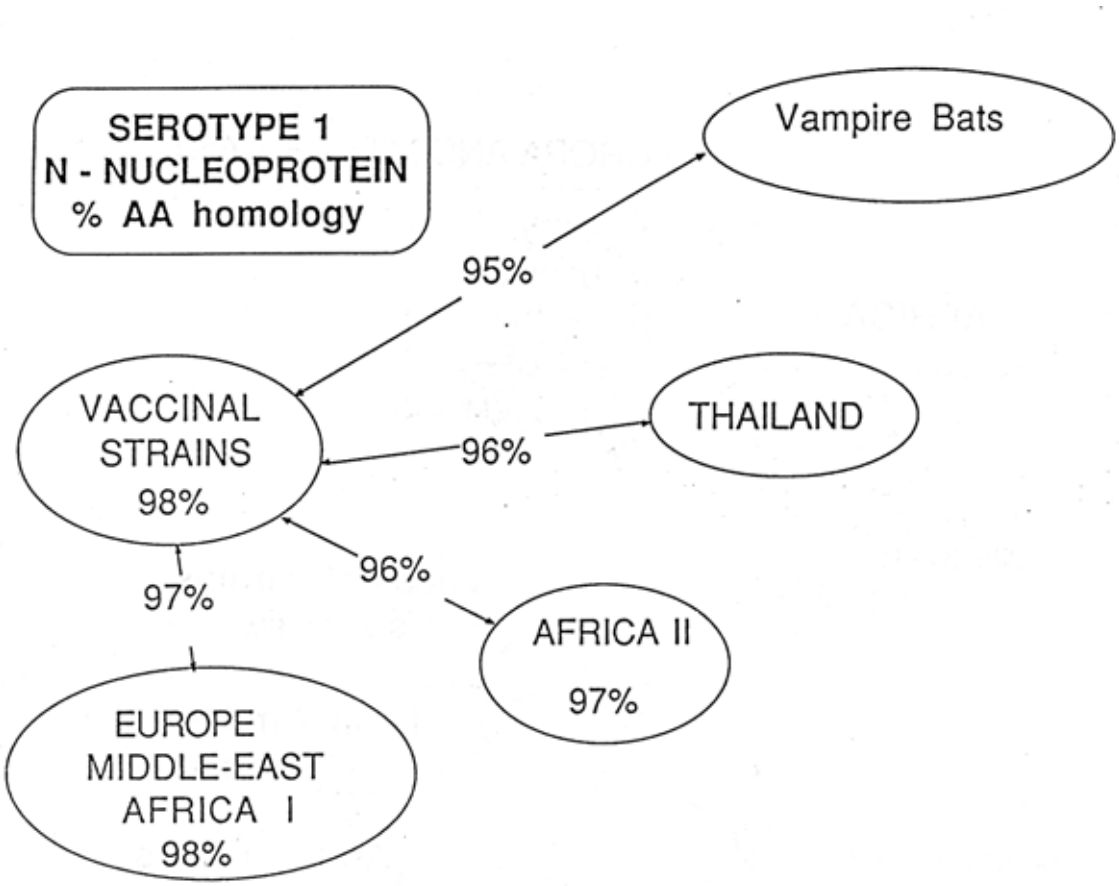


Figure 5: Schematic representation of the % of AA similarity between the different groups of viruses identified in Figure 3.



THE RABIES RESEARCH PROGRAMME AT THE ONDERSTEPOORT VETERINARY INSTITUTE (O.V.I.)

G.R. Thomson

The rabies research programme is one of the newer-programmes of the OVI, and has been in existence for approximately two years.

In the 15 to 20 years prior to 1991, little active research was conducted on rabies at the OVI although there were periodic assessments of the available diagnostic data in order to derive epidemiological trends. However, the increasing problem of canine rabies in Natal/Kwazulu as well as in southern Africa as a whole has forced us to research the problem in order to:

a) understand the causes of the current canine rabies epizootic

b) devise new approaches to rabies control aimed at augmenting those already in use.

In order to achieve success in these endeavours, four objectives were set, viz:

* to obtain and preserve strains of rabies virus prevalent in different regions and different species in southern Africa
* investigate the occurrence and prevalence of genetically or immunologically distinct variants of rabies virus (Lyssavirus serotype 1) in South Africa
* examine the possibility of introducing oral immunisation of dogs as an adjunct to rabies control in Natal/Kwazulu
* establish the interaction between rabies virus and yellow mongoose (Cynictis penicillata) with a view to the use of oral vaccines for the control of mongoose rabies

From the outset an attempt has been made to approach our research on a collaborative basis and to involve expertise from other institutions both within South Africa and in other countries. Four people who have actively helped, advised and collaborated with us are Dr. Arthur King (CVL, Weybridge, UK), Dr. Noël Tordo (Pasteur Institute, Paris), Dr. Louis Nel (University of Pretoria) and Prof. Terry Robinson (University of Pretoria). We are very grateful to all of them.

The registered as well as unregistered pilot projects currently in progress are summarized in Table 1.

Good progress has been made in collecting, storing (in freeze-dried form) and cataloguing rabies isolates from different species and different localities in southern Africa; more than 130 such stocks are now available.

The monoclonal (Mab) and genome sequencing activities have been particularly fruitful over the last year and it is now clear that rabies in South Africa exists in two different forms which are distinguishable both epidemiologically and virologically. On this basis we now classify rabies viruses as either "canid" or "viverrid". A publication on the Mab Studies is in press. Initial studies indicate that these two forms of the virus are both "covered" by current commercial vaccines.

A competition (blocking) ELISA test has been developed for the detection of antibodies to rabies virus nucleoprotein and has been shown to be both specific and sensitive. It has the advantage that it is able to detect antibodies in the sera of animals irrespective of species.

Appropriate to our international involvement has been the designation of the OVI by the OIE as a rabies reference laboratory. While this appointment is an honour, it brings with it no additional finance while carrying additional responsibilities. Until very recently the latter presented a problem in that the facilities for working with rabies virus at the OVI were far from ideal. Fortunately, finance has been made available for a total revamping of the rabies unit and we now are in the fortunate position in having a rabies unit which is adequate in all respects.

Table 1 : Rabies projects at the Onderstepoort Veterinary Institute currently in progress.

Project Leader	Project
Ms Cathy Kay	Development of an oral rabies vaccine for dogs
Dr Felipe Chaparro	Role of the yellow mongoose, Cynictis penicillata in the epidemiology of rabies
Dr Courtney Meredith	Differentiation of southern African rabies viruses using monoclonal antibody panels
Dr Louis Nel	Development of PCR-based genome sequencing for rabies epidemiological investigation
Prof Terry Robinson	Characterization of mitochondrial DNA variation and geographic population structure in the yellow mongoose, Cynictis penicillata
Mr Jan Esterhuyzen	Development of a blocking ELISA for the detection of antibodies to rabies virus N protein
Dr Gavin Thomson	The relative susceptibility of sheep and cattle to "canid" and "viverrid" rabies isolates.

SAFETY AND QUALITY CONTROL OF RABIES DIAGNOSIS

J. Barrat [1]

Any laboratory that performs rabies diagnosis must assess:

* the different measures taken to protect personnel(laboratory workers and others) from virus analysed in the laboratory,
* the reliability of the diagnosis itself, and
* the control of the information received and given by the laboratory. These two points are important considering the consequences of rabies diagnosis in terms of human and veterinary health.

1 BIOLOGICAL SAFETY

Rabies virus is a dangerous pathogen and laboratory work must not involve any risk to humans (both inside and outside the laboratory) animals or the environment. The pathogens that may be encountered in veterinary laboratories have been classified in 4 groups for human risk. The first one includes organisms unlikely to cause human disease and the fourth one, highly pathogenic organisms for which there is usually neither treatment nor prophylaxis. Rabies virus belongs to the third group.

The risk due to rabies virus depends on the kind of work that will be done in the laboratory. Rabies diagnosis on wildlife may cause more risk than producing fixed strains of rabies on well defined substrates, because these "street strains" may have characters not yet determined and because these specimens may carry other unknown pathogens.

Every laboratory has its own working routine when dealing with pathogens, but there are some general principles that can be summarized here. These classical "risk limiting" measures include:

Rabies virus is easy to inactivate physically (heating) or chemically. Iodine, quaternary ammonia and hypochlorite are some of the most commonly employed disinfectants but many others are efficient.

1.1 Isolation of the "at risk" area

The access to the laboratory should be restricted. This area may comprise only the necropsy room or it may include different rooms where rabies diagnosis is performed.

All articles entering this area are considered contaminated despite daily disinfection.

Any articles entering this area which is used for rabies diagnosis is then either incinerated or decontaminated. This room is disinfected after every "diagnosis session" and washed daily.

If the necropsy room is separate from the laboratory, the slides and/or inocula are transported to the laboratory either in isolation boxes or via a hatch.

1.2 No mixing of "safe" areas and "at risk" areas

This measure implies that there should be as little equipment in the necropsy room as possible. Generally, the different instruments in the necropsy room are dedicated to that place.

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[1] CNEVA, Malzeville, France

It must also be impossible for someone working in the autopsy room to go out with his or her protection clothes and without disinfection. A good way to prevent this risk is to have a "colour code" for the clothes worn: blue or green in the necropsy room and white in the other areas of the laboratory. The "inside" clothes must be sterilised before being washed.

1.3 Forward movement of the diagnosis chain

Generally the contamination risk decreases from the animal specimen submitted to the slide observed microscopically even if the fixed slide may still contaminate. So it is important to avoid any contact between a step and any previous one which may increase biological risk.

1.4 Passive protection of the staff

Passive protection is protection against virulent (infective) material and aerosols.

Wearing of special clothes, plastic eyeshade, mask, gloves, goggles, boots, ... will protect against splashing and spillages. All these protection systems are removed when leaving the rabies laboratory and stored in the hatch area. Safety laminar flow hoods may be used if there is a risk of aerosol formation (e.g. during grinding, homogenisation or centrifugation).

Storage of food or drink and their consumption, smoking or using cosmetics are not allowed in laboratories.

1.5 Immunisation of exposed staff

WHO recommends the preventive immunisation of the exposed staff. The immunisation protocol includes three injections at day 0, 7 and 28. The control of immunisation is made by serology 1 to 3 weeks after the last injection.

The subsequent testing of antibody levels is made every year at least and workers whose antibody level is under 0.5 IU/ml are boostered.

1.6 Elimination of biological waste

Diagnosis operations produce additional wastes from specimen submitted and laboratory work, e.g. mice, if mice inoculation test is used, and different disposable systems.

Treatment of these wastes is an important aspect of laboratory safety. Incineration is the most effective means of destroying animal and biological waste but it requires a lot of energy. An alternative technique is to bury this waste with quicklime in an isolated place, deep enough to be out of reach of carnivores that might pass through the fences of the laboratory premises.

2 QUALITY CONTROL OF THE DIAGNOSIS

Quality control of the diagnosis is of great importance because of the consequences of rabies diagnosis for man (in cases of human contact) and for animals (follow up measures in respect of contact animals).

These points have been or will be detailed in the literature but it is useful to summarize some points related to the safety notion as defined at the beginning of the paper.

Rabies diagnosis, especially the FAT, should preferably be carried out on a daily basis. Rabies diagnostics must be centralised at the country level by a national laboratory or at regional ones if the number of specimens received allows a regular daily practice for reading the FAT.

2.1 Permanent use of controls for the reactions

Brains of mice inoculated with CVS strain are used both to prepare the positive control impression smear slides for the fluorescent antibody test every week and a virulent suspension used as positive control for cell culture test. These brains may be collected from mice inoculated with CVS either when titrating a challenge virus (during vaccine potency test or seroneutralisation in mice) or using a suspension made specially for this purpose. It is possible to freeze-dry these brains.

If the laboratory has several positive specimens every day, one can use a positive sample from the previous day as a positive control.

2.2 Duplicating the tests

For the fluorescent antibody test two slides, each made from two areas of the brain (Ammon's horn and brain stem), must be prepared with every sample to be examined. Only one slide will be stained on the same day.

* If it is positive, the second one is not stained or it could be taken as second positive control for the next staining session (the following day).
* If it is negative, the second slide will be stained later for confirming the first negative result (most often on the following day).

This procedure provides more security to the fluorescent antibody test: any problem during one staining process will affect only one of the two slides. It allows also some saving of fluorescent conjugate because generally only one slide has to be stained for the rabid samples.

2.3 Keeping some sample of every specimen submitted for diagnosis

The practice of keeping for several weeks a frozen sample of every specimen submitted for rabies diagnosis is an useful safety measure because it allows for further examinations for rabies and for other differential diagnoses or isolations.

2.4 Duplication of every step of the diagnosis chain

The different steps are always performed by two persons separately who will confront their final results. In case of a disagreement, the step is immediately checked again and a third person is called in.

This control is essential for the interpretation of tests, screening of samples and transcription of results from the "laboratory notebook" into the results sheet and the database. This double check is important too for the control of data as it is detailed in the next part.

3 CONTROL OF DOCUMENTATION

Control of documentation may be considered as a part of the "duplicating habit". Every step that follows is performed by two persons independently, with regular control.

From the beginning to the end of the diagnosis chain, this involves:

* the exact agreement between the specimen form information and the details given on the specimen package itself,
* the recording of the specimens in the laboratory recording system,
* the identification of animals (species determination for wildlife, tattoo or tag numbers for domestic animals...),
* the identification of tubes, slides, plates etc. used for every single specimen,

* the exact agreement of specimen number with the plates, tubes (number when sampling the specimen in the necropsy room)

* the transcription of results from the "laboratory notebook" into the results sheet and the database,

* the final control of the information and the results on the form sent to the submitter who asked for the rabies diagnosis. This is the final step before being "officialised" by the signature of the responsible officer.

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BRAIN REMOVAL, SPECIMEN COLLECTION AND TRANSPORT

J. Barrat[1]

1 INTRODUCTION

Clinical signs of rabies are generally not highly characteristic and may vary greatly from one animal to another, depending on the areas of brain where virus is replicated and on individual variations.

As clinical observation may only lead to a suspicion of rabies, the only way to make a reliable diagnosis is to look for the presence of rabies virus or its components in samples collected from the suspected animal.

Rabies virus goes from the inoculation point to one nerve, then it reaches the central nervous system passively. Once the central nervous system is infected, rabies virus may diffuse towards peripheral organs (i.e. salivary glands, eyes, peripheral nerves).

Samples for experimental diagnosis are collected from the brain and sometimes from other organs. These are then submitted to laboratory techniques that detect either histologic or cytologic signs of viral replication, or viral antigen or virulence and pathogenicity of non-inactivated virus or viral nucleic acid.

This paper deals with the sampling and shipment techniques that can be used for rabies diagnosis. It will describe possible adaptation of techniques to hot or tropical conditions or to field conditions.

2 BRAIN REMOVAL

Once rabies virus has reached the central nervous system, it is replicated to reach high titres. Virus replication is not homogenous in the brain. The Ammon's horn and rachidian bulb are two areas where virus is easily found in high concentrations.

The classical way to collect brain samples is to open the skull. This procedure makes sampling easy but involves a laboratory structure with two persons for the opening of the skull.

2.1 Choice of opening system

Two points must be checked when choosing an opening system:

1. one must be able to change easily and rapidly the "blade" of the system. The "one blade per sample" principle avoids any cross-contamination from one sample to the following ones
2. if a mechanical system is chosen it must be resistant to the decontamination products used in the laboratory

Different systems have been tested in Malzéville, the one that has been selected is a shoeing-smith type blade used with a hammer. This system has been selected because such a blade is cheap (so the "one blade per sample" principle is easy to follow), easy to sharpen and is easy to decontaminate. This system is very convenient for any animal size from mongoose up to sheep and cows.

[1] CNEVA, Malzeville,France

2.2 Recording of samples received for diagnosis

The following procedure is used in Malzéville laboratory.

In a dedicated room outside the necropsy room, heads or animals received for rabies diagnosis are recorded and the duplication of the information indicated on the sample and on the specimen form used by the sending laboratory is checked by two persons.

The instruments and materials to be used for sampling are then collected and taken into the necropsy room. For every head or entire carcass, these consist of:

- one plate for the central nervous system,
- one scalpel and one toothless forceps (for brain removal after opening the skull),
- two microscope slides for the fluorescent antibody test,
- three plastic tubes, one for the cell culture test, another one to keep a sample of the brain and a last one for mice inoculation test when needed,
- a fixation vial when histological examination is performed,
- one blade to open the skull.

Some materials are used for all the specimens during one "opening session", they consist of:

- some scalpels used for cutting the skin and reclination of temporal muscles,
- one toothed forceps for holding the skin and muscle while cutting them,
- one hammer,
- a pair of holding forceps to hold the head on the necropsy table.

2.3 Opening of the skull

This operation is performed by two persons. One person holds the head on a plastic butcher's block using a forceps introduced into the eye sockets. The other person opens the head. After having checked that the number on the bag containing the specimen is the same as that on the plate, the opening of the skull is performed. The different steps are:

1. cutting of the skin
2. reclination of temporal muscle
3. opening the skull with the blade dedicated to this sample
4. brain removal
5. using the plate, forceps and scalpel dedicated to this sample, meningeal membranes are opened and the brain is put on the plate which is then passed to the person who performs the different tests used for rabies diagnosis.

2.4 Disposal of animals

Two methods may be used to dispose of the carcasses and the disposable materials after the diagnostic procedures:

- incineration
- burying in quicklime deep enough in a protected place not to be unearthed by carnivores.

The different reusable materials are decontaminated chemically the same day.

The necropsy room is washed and decontaminated every day.

3 COLLECTION OF SAMPLES

Experimental diagnosis of rabies may be performed on central nervous system samples or on samples collected from other organs (e.g. salivary gland).

3.1 Sampling of isolated central nervous system

The most important areas in the central nervous system are the Ammon's horn and the rachidian bulb.

Ammon's horn is a white semicylindrical body on the floor of the fourth ventricle. It may be exposed using different techniques:

- a longitudinal incision 1 to 2 cm lateral to the interhemispheric line
- a cross section made at the posterior third of the cerebral hemisphere. When this section has reached the Ammon's horn, a longitudinal one gives access to the entire organ
- a longitudinal section with scissors beginning at the posterior third of the brain and at the first third closed to the interhemispheric line.

Different parts are sampled depending on the technique used for experimental diagnosis.

The following table summarize this information:

	Ammon's horn	Rachidian bulb	Cortex	Cerebellum
Fluorescent antibody test	Y	Y		
Cell culture test	Y	Y	Y	Y
Mice inoculation test	Y	Y	Y	Y
Histology	Y			
RREID	Y	Y	Y	Y

Sometimes, when the central nervous system is autolysed, these parts may be difficult to identify. The experimental diagnosis is then performed from a general sample called "brain" without any further description.

3.2 Sampling the brain without opening the skull

In field conditions, for epidemiological studies for example, or when a laboratory structure is not available for sampling, two techniques may be used to collect brain samples without opening skull.

3.2.1 Occipital foramen route brain sampling

Sampling is done through the occipital foramen (BARRAT and BLANCOU, 1988) by using a drinking straw (5 mm in diameter) or a 2 ml disposable plastic pipette (BOURHY and SUREAU,1990).

The steps of this procedure are:

1. cutting the skin and neck muscles over the joint between the occipital bone (condylus occipitalis) and the atlas (atlas),
2. bend the head to give access to the occipital foramen,
3. screw the straw into the foramen in the direction of an eye. This route crosses the rachidian bulb, the basis of cerebellum, Ammon's horn and cortex,
4. the straw is pinched between the fingers and then drawn back gently,

A test was performed on foxes to estimate the sensitivity of this sampling technique compared with "classical" sampling. The results were as follows :

	rabid foxes	**non rabid foxes**
Classical sampling	36	7
Occitipal foramen sampling	36	7

3.2.2 Retro-orbital route brain sampling

This technique was developed by MONTANO HIROSE and coll. (1991).

The steps are successively:

1. push the eyeball aside
2. use a trocar to make an entry through the posterior wall of the eye socket
3. introduce through this hole a straw or a 2 ml disposable plastic pipette toward the occipital foramen.

The cerebral tissues sampled here are the same as the ones sampled by occipital foramen, but they are taken in the opposite order.

A test was performed to estimate the sensitivity of this sampling technique compared with "classical" sampling. The results were the following:

	rabid animals	**non rabid animals**
Classical sampling	51	12
Occipital foramen sampling	51	12

3.3 Salivary gland sampling

Sometimes it may be useful to check for rabies excretion i.e. presence of rabies virus in the salivary glands of the animal.

Sub-mandibular salivary glands are easy to sample and may be tested by different techniques such as fluorescent antibody test, cell culture test or mice inoculation test.

The removal of the sub-mandibular salivary glands is possible using the following technique:

1. the head is placed to one side
2. an incision is made in the skin covering the posterior angle of the lower jaw
3. the gland is superficial behind the posterior part of the jaw (it must not be confused with the submaxillary lymph nodes.)

4 SHIPMENT OF SAMPLES

Whatever transport is chosen, the sending of samples for rabies diagnosis must not induce risks :

- for man, i.e. there must not be any risk of human contamination during transport
- for the sample which must not arrive in the laboratory more autolysed than it was when it was collected

Classically heads or entire animals are sent rapidly with refrigerants to the laboratory. When these conditions cannot be followed, different preservation techniques may be used.

4.1 Transport without preservation

Transport without preservation is the classical way to send a specimen to the diagnostic laboratory.

The French specifications that regulate the transport of samples for rabies experimental diagnostics are as follows:

The veterinary authorities alone are authorized to rabies samples by post.

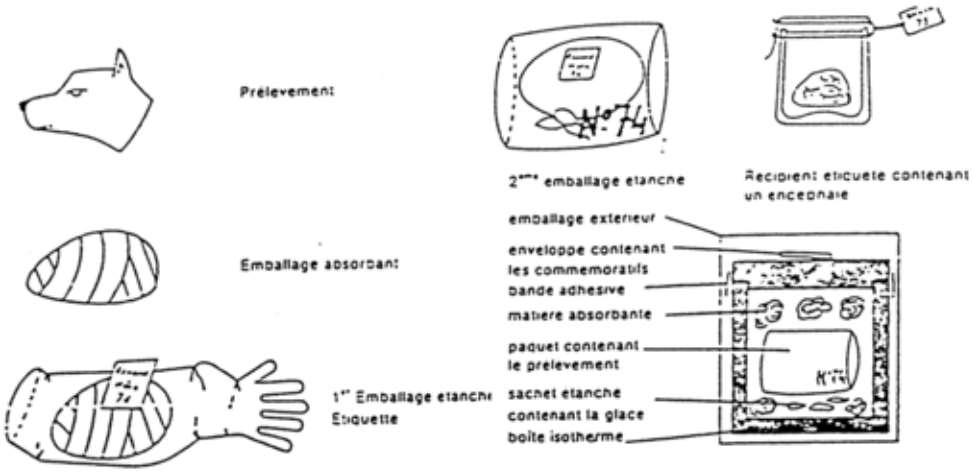
Four laboratories perform experimental diagnosis for rabies:

Diagnoses of an epidemiological nature are carried out in Malzéville

When human contacts are involved, diagnoses are made at one of the Health Ministry laboratories (i.e. The Pasteur Institutes at Paris and Lyon, and the Hygiene Institute in Strasbourg)

Brains are placed in a hermetically sealed, rigid container which is then identified. Heads or small animals are wrapped in absorbing paper and then placed in a resistant plastic bag which is also identified. This first package is then introduced into a second one, which is tightly closed and placed in an insulated box made of expanded polystyrene. This box contains absorbing and cooling material and it is sealed with an adhesive tape. An envelope containing all the information available on the samples is attached to the outside of the box. The box is then placed in a closed carton box where a label indicates clearly " Beware! Biological specimen for rabies diagnosis. Infectious hazard!". (see Figure 1).

Figure 1 : example of packaging used in France for rabies detection.



4.2 Transport using preservative solutions

The absorbing paper used around the head in the "classical" package may be impregnated with a 10% formalin solution. This is extra protection to the employees of the shipping services, but it does not protect the sample against autolysis.

The use of paper impregnated with a formalin solution is sometimes indicated as a mean to preserve specimens. The following test was performed in Malzéville. On day 0, some fox heads were placed in a 37°C incubator. Half of them were packed in an absorbing paper without formalin the other half were packed in an absorbing paper impregnated by a 10% formalin solution. On day 3, all the heads without formalin were completely autolysed. Those packed in an absorbing paper impregnated by a 10% formalin solution appeared much less autolysed. But the "preservation" related to the skin and superficial muscles only. The brains of both groups were totally liquefied. The preservation of the entire specimen with formalin is not efficient enough.

If rapid transport with refrigerants is not possible, preservative solutions may be used.

The choice of preservative depends on the laboratory techniques that will be used for diagnosis.

Formalin solution inactivates rabies virus. So transport is safe but the different inoculation tests cannot then be used. Diagnosis can then still be done by FAT and histology.

Glycerine solution does not inactivate the rabies virus rapidly. It is a preservative which inhibits temporarily the growth of contaminants. As rabies virus is not inactivated, all laboratory techniques (including inoculation tests) may be used on samples submitted in glycerine.

Results obtained when using these techniques are given in Annexure 1.

During the summer of 1982 it was quite hot in France and the local veterinary laboratories were asked to send half of the brain in 50% glycerine and the other half in 10% formalin. The inoculation was performed in mice from the glycerine treated part and fluorescent antibody test and histological examination were performed according to the procedure listed in Annexure 1.

The results were as follows:

Transportation delay	number of samples	fluorescent antibody test	histology	mice inoculation test
2 days	2	+	+	+
	2	-	-	-
3 days	3	+	+	+
	2	+	-	+
	3	-	-	-
	1	impossible	-	-
4 days	1	+	+	+
	1	-	-	+
	1	+	impossible	+
	2	-	-	-
5 days	1	+	+	+
	5	-	-	-
6 days	2	-	-	-
30 days	1	+	-	-

ANNEXURE 1: EXPERIMENTAL DIAGNOSIS ON PRESERVED SAMPLES

As stated above, if it not possible to send specimens rapidly and under cold conditions to the laboratory for rabies diagnosis, e.g. under field conditions, samples may be preserved by using either formalin or glycerine.

1 TREATMENT OF FORMALIN PRESERVED SAMPLES

Two techniques may be used with such samples: histology (Mann's staining for example) and FAT.

Histology is performed directly on a portion of a previously fixed sample.

We will give more details here on a technique that may be used to do the FAT on formalin preserved samples and on the sensitivity of this technique.

1.1 Fluorescent antibody test

The FAT can only be performed after treatment with proteolytic enzymes (UMOH and BLENDEN, 1981; BARNARD and VOSGES, 1982; BARRAT, 1986).

The samples consist of small pieces of brain, 3 mm long. They are stored in a 10% formalin solution.

The following procedure is used:

1. grind one piece in 5 ml of PBS w/o Ca or Mg pH 7.5
2. centrifuge the tube and discard the supernatant
3. resuspend the pellet in 5 ml of a 0.25% trypsin solution
4. keep the tube at +4 C overnight
5. centrifuge the tube and discard the supernatant
6. wash the pellet twice in PBS (without Ca or Mg)
7. smear the pellet, allow the smear to dry, fix and perform FAT as usual.

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

The test procedure was as follows:

On day 0 naturally rabid foxes were selected by FAT. The result of the FAT on the fresh sample was assessed for the intensity of fluorescence according to a 4 step scale. Different samples of brain were fixed in formalin.

On days 2, 5, 8 and 20, pieces of fixed brain were processed according to the previously described procedure and the results were scored according to the same scale as that used on the initial specimens.

The overall results were the following:

Recovery of rabid samples after 2, 5, 8 and 20 days in 10% formalin

	Days in formalin			
	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=74	85% N=71	62% N=71

This table clearly shows that grinding affords a better sensitivity to the technique. It also resulted in brighter fluorescence.

1.2 Histological examination

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

The test was performed on 50 samples. Histology recovered 50 positive samples after 2 an 5 days in formalin and 49 after 8 and 20 days.

1.3 Sensitivity of diagnosis using both FAT and histology

The overall sensitivity of techniques available on formalin preserved samples was estimated on 50 naturally infected rabid foxes. The results are given below.

	Days in formalin			
	2	**5**	**8**	**20**
Fluorescent antibody test	50	50	50	48
Histology	50	50	49	49
Number of positive samples to at least 1 technique	50	50	50	50

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

2 OCCIPITAL FORAMEN SAMPLING AND FORMALIN PRESERVATION

FAT applied after straw sampling through the occipital foramen and formalin preservation during 8 days at room temperature identified 102 out of 107 samples positive before fixation (95.3%) when trypsin treatment was done on a part of the sample corresponding to a 50 mcl pellet (i.e. a cube 3 mm long).

If the whole sample collected in the straw was treated by trypsin, only 14 out of 24 samples positive before fixation were found positive after trypsin treatment.

In the same preservation conditions, but without straw sampling, FAT identified 98% of the positive samples.

3 TREATMENT OF GLYCERINE PRESERVED SAMPLES

In order to eliminate as much glycerine solution as possible, the samples are washed in PBS before any other treatment.

After washing, the glycerine preserved sample is processed according to the usual techniques.

Glycerin has an antiseptic action, but it softens the brain tissues and make their handling difficult. If animals are sampled with straw or pipette, the sample must be kept inside the straw when kept in the glycerine so that only a small part of the sample on the open ends of the straw is softened.

3.1 Influence of temperature on diagnosis in glycerine preserved samples

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

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

The experiment was performed with 130 positive samples.

FAT and cell culture test have been used both on fresh and preserved samples. Fluorescence intensity was scored on a 4 step scale (1-25% of positive fields, 25-50% of positive fields, 50-75 and 75-100%).

3.1.1 Conservation out of the straw

18% of positive samples became negative after a 8 days conservation free in glycerine solution.

Fluorescence was assessed on 10 samples. It decreased by about 1/8 when held at 20 C.

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

3.1.2 Preservation inside the straw

130 samples were kept in the straw during the same 8 days conservation.

At 20°C, both FAT and cell culture were positive for all the samples. The average decrease of fluorescence was measured on 35 samples and was less than 1/8.

At 37°C too, both tests were positive. The average decrease of fluorescence was measured on the same 35 samples, it was less than 25%.

The same comparison was done between fluorescence in cells when sample was conserved at 20 and at 37°C. The average decrease of fluorescence was 12.5%.

3.1.3 Conclusion

When samples are to be preserved in glycerine solution, it is much better to keep them protected in a piece of straw. Otherwise diagnosis may become negative.

After 8 days in a glycerine solution with the brain sample inside the straw, the recovery rate was 100% for positive samples compared to fresh specimens. This result was obtained at temperatures of 20 and 37°C.

4 CHOICE OF A PRESERVATION TECHNIQUE

If samples cannot be sent rapidly and refrigerated to the laboratory, e.g. during epidemiological field studies, they must be preserved.

Two possibilities exist (formalin or glycerine), each of them allowing the use of different laboratory techniques. If samples are preserved in glycerine solution, they must be left inside the sampling straw. Samples preserved in glycerine solution must be pushed out of the sampling straw or pipette using a cotton swab or by means of positive air pressure by using of a rubber bulb.

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

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ELISA SYSTEMS FOR RABIES ANTIGEN DETECTION

J. Barrat [1]

1 INTRODUCTION

Several techniques are available for the diagnosis of rabies. These can detect either histologic or cytologic signs of viral replication, or viral antigen or virulence and the pathogenicity of non-inactivated virus.

The basic technique used in experimental diagnosis of rabies is fluorescent antibody test. It is used both directly on smears and to detect viral replication in mice or cells after inoculation tests. Sometimes the interpretation of fluorescent antibody test is not easy, particularly with autolysed samples. The most important point with fluorescent antibody test is the regular training of the persons who work with this technique.

Immunoenzymatic techniques were developed to detect viral nucleocapsid by another way: the antibody is not revealed by fluorescence of FITC but by the transformation of a substrate by an enzyme linked to the specific antibody.

* Immunoperoxidase on smears may be used instead of immunofluorescence. Both have the same sensitivity (Genovese and Andral, 1978), but as the test takes longer and is more expensive, it is not used in routine laboratory diagnosis.
* ELISA test: this paper deals with detection of rabies virus nucleocapsid in brain samples using an ELISA technique commercially available.

2 GENERAL INFORMATION OF THE TECHNIQUE

This technique is used to detect viral antigen whether the virus is inactivated or not.

The technique was developed in 1986 by P. Perrin. The Rapid Rabies Enzyme Immuno-Diagnosis is commercially available. It detects the presence of rabies nucleocapsid in the brain sample.

Antibodies specific for rabies nucleocapsid are adsorbed on the microtiter plate. The conjugate used has the same specificity.

2.1 Equipment needed

The kit itself is distributed by Diagnostic Pasteur (ref number 72201).

Plates may be read directly but the utilisation of a spectrophotometer makes it easier. The estimated investment for the laboratory is indicated in table 1.

2.2 Training of technicians

The interpretation of the test does not need any special training. The positive and negative controls provide clear-cut answers, the threshold is easy to determine and the reaction may be interpreted.

If the technicians do not know any other ELISA technique, a few days training are sufficient.

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2.3 Sensitivity of the technique

The correlation between fluorescent antibody test and RREID is between 96 and 99 %.

2.4 Delay of answer

Diagnosis is achieved in less than 4 hours by RREID. 40 samples may be analysed in duplicate using one single plate.

2.5 Cost of the technique

Table 1: Equipment needed for RREID (price were estimated in 1992)

Equipment	Price
Spectrophotometer	7300 US $
Titerplates washer	3700 US $
37 °C incubator	1100 US $
Refrigerator	550 US $
Multichannel pipette	750 US $
Miscellaneous	750 US $

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

3 PRACTICAL USE OF RREID

3.1 Composition of the kit

The different solutions and controls needed are included in this kit.

The microtiter plate may be divided into 6 trays of 16 wells. The volume of positive and negative controls given in the kit is sufficient for two reactions. If the plate has to be divided in more than 2 parts it will be necessary to prepare some 'home made' controls:

* the positive control is the supernatant of an 10% homogenate of rabid mice brains inactivated by BPL and then aliquoted and freeze dried,
* the negative control is the supernatant of an 10% homogenate of non rabid mice brains inactivated by BPL and then aliquoted and freeze dried.

The other solutions consist of

* washing solution (PBS + 0.05% Tween 20, pH 7.4),
* IgG anti-nucleocapsid conjugated with peroxidase,
* Substrate buffer (citrate - H2O2),
* OPD tablets,
* stopping solution (H2SO4, 4N).

The interpretation is made either by eye or using a spectrophotometer (492nm).

3.2 Results obtained with RREID

An evaluation has been made by 6 national or WHO reference laboratories on 1253 specimens from 27 animal species. Using fluorescent antibody test as reference, 97.4% of specimens (651 positive and 569 negative) gave identical results to both tests. 22 specimens (3%) were FAT+ / RREID-.

A second evaluation was made involving 12 field laboratories from Africa and Latin America on 818 samples. The following results were obtained: 96.7% of samples gave the same result (428 positive and 363 negative), 22 specimens (2.7%) were FAT+ / RREID- 5 specimens (0.6%) were FAT- / RREID+.

The test used in the French national reference laboratory for rabies on 2290 routine examinations correlated well with fluorescent antibody test: 99.2% of identical answers (287 positive and 1985 negative), 0.6% (15 specimens) were FAT+ / RREID- and 3 (0.1%) were FAT- / RREID+.

In Malzéville, 2572 specimens were tested by fluorescent antibody test, cell culture test and RREID. FAT and RREID gave the same answer for 98.9% samples (1853 negative and 692 positive ones), 12 specimens were positive only to FAT, 15 only to RREID (cell culture test was negative for these samples).

The estimated correlation between fluorescent antibody test and RREID thus ranges between 96 and 99%.

This kit can detect a concentration of 0.8 to 1ng of nucleocapsid of rabies virus per ml for serotype 1 strains.

This technique has two principal advantages:

1. it does not need any regular training for the interpretation of the test,
2. it is rapid: within 4 hours, 40 diagnosis may be performed in duplicate.

These two points make RREID a good technique for large scale epidemiological studies, but the test is more expensive than fluorescent antibody test: 4.5 US$ (in duplicate) for RREID and 1US$ for fluorescent antibody test.

4 RREID LYSSA

RREID detects a concentration of 1 ng of nucleoprotein per ml of serotype 1 virus. The sensitivity of the test is low for rabies-related viruses, despite the common antigenic properties of the nucleoprotein isolated from different serotypes.

In order to increase the sensitivity of the test for rabies related viruses (Perrin et al., 1992), 3 polyclonal antibodies were prepared against nucleoprotein of serotype 1 (PV strain), serotype 3 (Mokola strain) and EBL stain (not classified in serotype). These polyclonal antibodies were coated on microtiter plates and conjugated to biotin. The reaction involves the use a classical peroxidase-avidin conjugate.

These changes increase the sensitivity of the detection of nucleoprotein (concentration up to 0.2 ng/ml) and the range (nucleoprotein of the different rabies viruses are detected whatever their serotype).

5 CONCLUSION

The ELISA method is simple and rapid to perform in large scale epidemiological studies . The immunocapture phenomenon allows to perform easy and reliable diagnosis even on autolysed samples.

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ELISA SYSTEMS FOR RABIES ANTIBODY DETECTION

J. Esterhuysen[1] andJ. Barrat[2]

The reference method to determine the antibody level of a serum sample is seroneutralisation. The principle of this technique is to incubate a constant known amount of rabies virus with a constant volume of the serum to be tested.

The virus that has not been neutralised by the antibodies is quantified using an inoculation test either in vivo or in vitro.

This method is sensitive but time-consuming. Results are obtained within 1 to 5 days (with cells) and up to 14 days with mice.

Contaminated or putrefied serum samples are most often impossible to titrate because the bacteria (or their toxins) kill the mice or cells.

The immunocapture phenomenon used in ELISA tests is not susceptible to the contamination risk or to the toxin hazard as the techniques using a "living" final step. A second important point is that ELISA tests give their answer more rapidly than the classical seroneutralisation.

The antibody level may be determined in two different ways with ELISA techniques:

antibodies are directly quantified using microplates coated with the corresponding antigen

antibodies first react with a known amount of antigen. The remaining antigen is then quantified using a plate coated with the corresponding antibody.

One example of each procedure will be detailed here for rabies antibody titration using the ELISA technique.

1 ANTIBODY TITRATION USING A DIRECT ELISA

The example detailed here corresponds to the use of a commercially available kit: "Platelia" distributed by Diagnostic Pasteur (ref no. 72200).

In this test, the plate is coated with the rabies virus glycoprotein. It allows to determine the amount of serum anti-glycoprotein antibodies, which have neutralising activity.

Positive and negative control serum samples of human origin, are included.

The conjugate used is a proteinA - peroxidase one and the colour agent is OPD.

1.1 Preparation of the reference curve

Dilutions of both positive and negative controls are dispensed in duplicate into the wells.

The mean OD corresponding to the antibodies for every dilution is reported on the Y axis of a graph. The X one has a double scale: one is the inverse of the dilution and the other is the corresponding titre in IU/ml.

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1.2 Titration of sera

Every serum is diluted once at a predetermined working dilution. The corresponding OD is then reported on the reference curve and the titre determined.

If the measured OD is out of range of the reference curve, serum is diluted more or less so that the OD measured is within the confidence limits of the reference curve. The extra dilution is then taken into account for the calculation of the precise titre.

1.3 Advantages / disadvantages

This technique titrates the anti-glycoprotein antibodies which are the neutralising ones.

For species other than human, some problems may arise from the detection system that uses a protein-A peroxidase conjugate:

The affinity of proteinA for antibodies is species dependant and it is then necessary to prepare positive and negative controls for every species to be tested. It is also necessary to determine the correct working dilution.

ProteinA does not react at all with cattle immunoglobulins and ProteinA has then to be replaced by a proteinG conjugate.

2 ANTIBODY DETERMINATION USING A LIQUID PHASE BLOCKING SYSTEM.

The example cited here is a summary of J. Esterhuysen's work adapting a routine technique used for FMD serology to rabies.

In large scale epidemiological studies, the major problem is the diversity of species to be tested. In such a study, the former method supposes the use of controls from the different analysed species.

2.1 Principle of the method

A previously determined constant amount of virus is incubated with the diluted serum. After this step, the result of the reaction is transferred in a microtiter plate coated with rabies antibodies. A predetermined quantity of guinea pig anti-rabies serum is then added to every well. The fixation of this guinea pig serum is revealed with an anti-guinea pig immunoglobulin conjugate.

2.2 Advantages / disadvantages

The test described here is more "seroneutralisation like" than the previous one, but it is longer to standardise.

The main advantage of it is that it may be used on any species.

The correlation between the two techniques described here is close to 79%.

3 CONCLUSION

Although seroneutralisation is the recommended method to determine the antibody level for rabies, ELISA techniques are an interesting alternative, principally for large scale epidemiological studies.

Either of the two techniques described here may be used. Both correlate well, so the choice of which technique to use depends mainly upon the availability of species controls and upon the methods already used routinely in the laboratory.

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