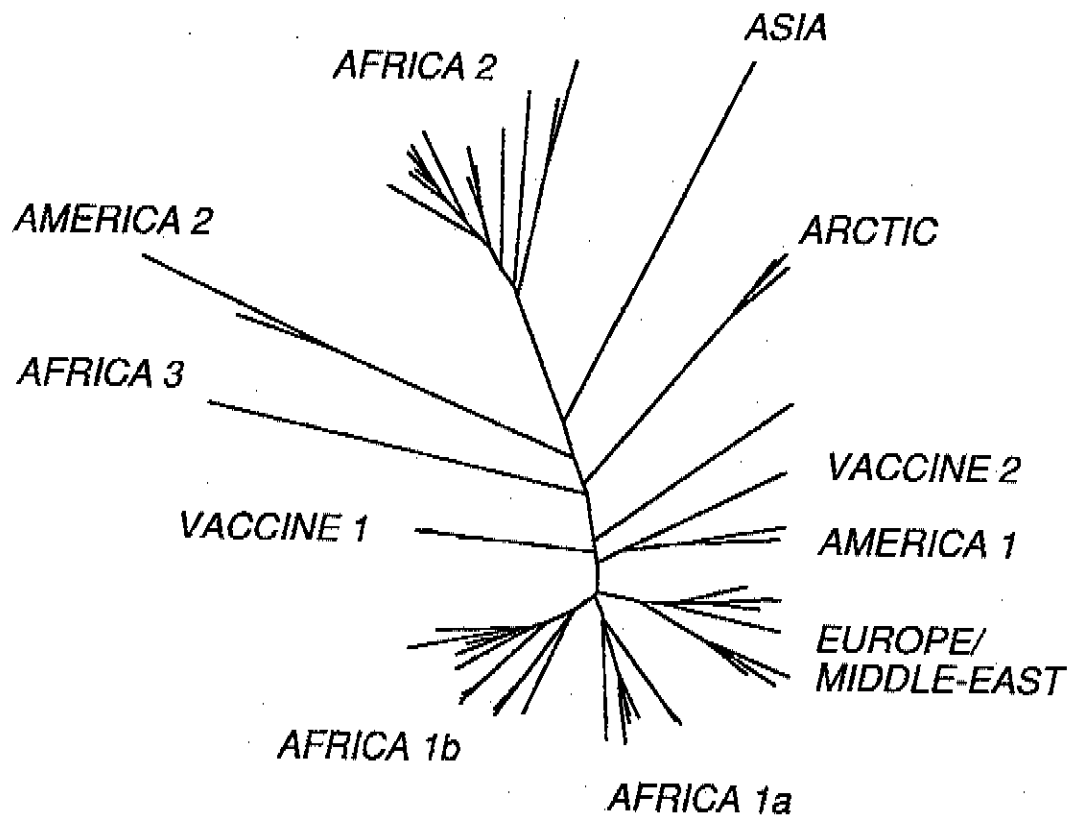


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WHO WORKSHOP ON GENETIC AND ANTIGENIC MOLECULAR EPIDEMIOLOGY OF LYSSAVIRUSES

NIAGARA FALLS, CANADA
17 NOVEMBER 1994



Radial phylogenetic tree showing the relationships between the different geographical lineages of Lyssaviruses and rabies vaccine strains.
(Furnished by H. Bourhy, WHO Collaborating Centre for Reference and Research on Rabies, Pasteur Institute, Paris, France.)



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WORLD HEALTH ORGANIZATION
VETERINARY PUBLIC HEALTH UNIT

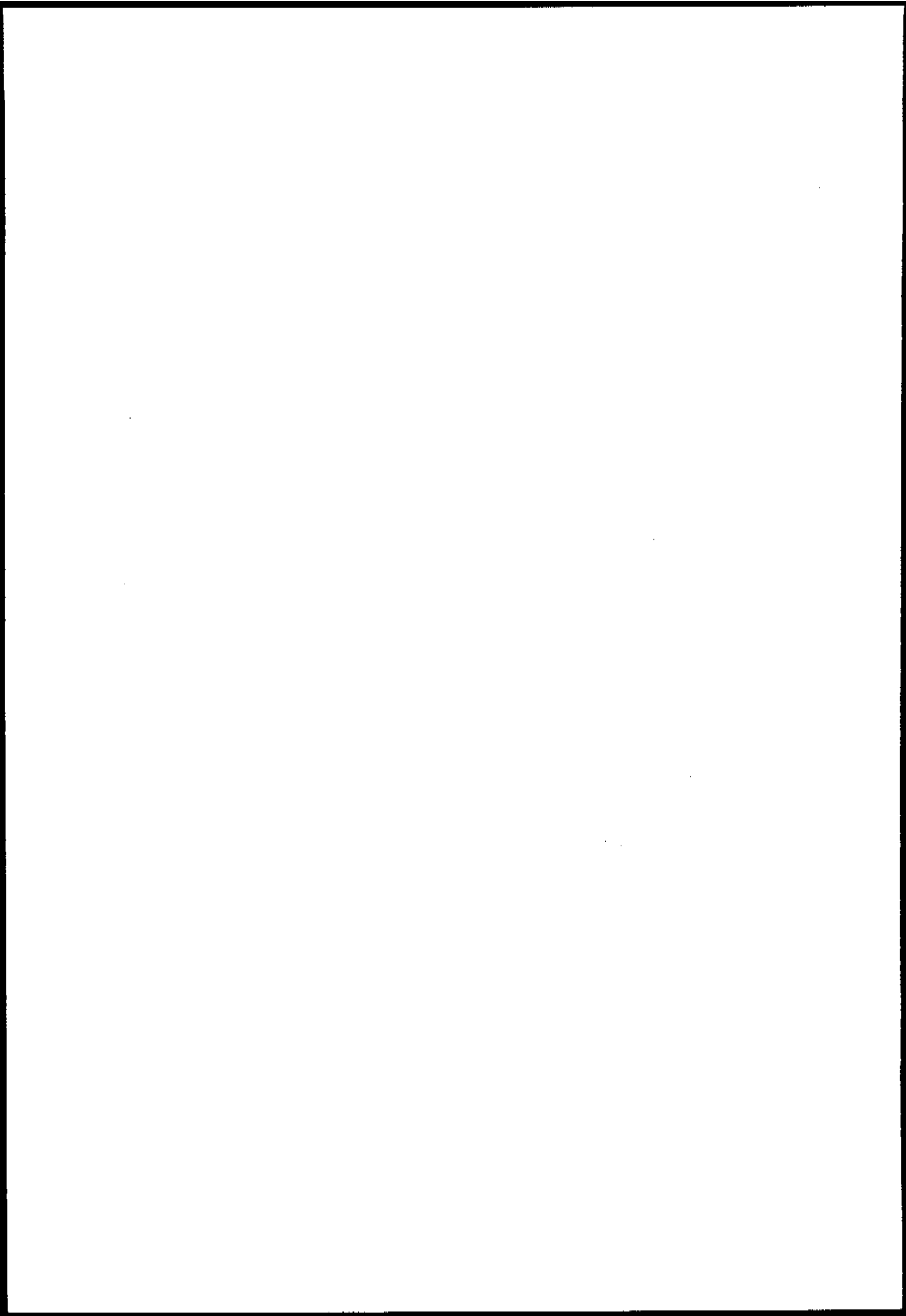
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1. INTRODUCTION

The meeting was opened by Dr F.-X. Meslin, Chief, Veterinary Public Health unit, on behalf of the Director-General of WHO. He thanked the organizers of the 4th International Symposium on Research towards Rabies Prevention in the Americas, 16-19 November, who hosted this workshop as a satellite activity. He recalled the activities coordinated by WHO in the field of characterization of Lyssaviruses using monoclonal antibodies from 1982 to 1990, and the progress made since in the application of genetic methods for virus identification to Lyssaviruses. He stressed the need to review the recent results and discuss the advantages/disadvantages of serological versus genetic typing techniques for both epidemiological and phylogenetic purposes as well as routine diagnosis. Dr A.A. King was elected Chairman, and Dr J.S. Smith Rapporteur.

2. CURRENT CLASSIFICATION SCHEME FOR MEMBERS OF THE LYSSAVIRUS GENUS

2.1 Current classification

Serological tests (monoclonal and polyclonal antibody studies) of rabies isolates led the WHO Expert Committee on Rabies in 1991 (Eighth report, Technical Report Series, 824, WHO, Geneva, 1992) to propose the following classification of the rabies group of rhabdoviridae, genus Lyssavirus:

Serotype 1 prototype strain Challenge Virus Standard (CVS); includes the majority of field viruses isolated from terrestrial mammals as well as isolates from insectivorous bats in North America and haematophagous bats in Latin America; also includes fixed virus laboratory strains.

Serotype 2 prototype strain Lagos bat, first isolated from pooled brains of bats in Nigeria.

Serotype 3 prototype strain Mokola, first isolated from shrews in Nigeria.

Serotype 4 prototype strain Duvenhage, first isolated from a human in South Africa.

(Further details on serotypes 2, 3 and 4 may be found in references 6 and 18, Annex 2.)

In addition, at the time of its meeting this Expert Committee stressed that a number of viruses still remained to be typed, including European bat lyssaviruses (EBL) isolated from *Eptesicus serotinus* bats (EBL1) and *Myotis* bats (EBL2), as well as those isolated from humans exposed to bats in Finland and Ukraine.

Recent molecular phylogenies constructed from either nucleotide or aminoacid sequence data have described the Lyssavirus genus as six distinct genetic lineages:^{3,4,5,15,16,21}

Rabies	Genotype 1
Lagos Bat	Genotype 2
Mokola	Genotype 3
Duvenhage	Genotype 4
EBL1	Genotype 5
EBL2	Genotype 6

Although not all the existing non-rabies Lyssavirus isolates have been examined, the genetic lineages of those tested to date agree, for serotypes 1-4, with the virus groups to which these isolates belong following serological testing. Recent results, however, call for the classification of European Bat Lyssaviruses (EBL1 and EBL2) in two new and independent genotypes 5 and 6 respectively.²

Current molecular phylogenies for the Lyssavirus genus are constructed on nucleotide or aminoacid sequence obtained for the entire coding region of the nucleoprotein gene;³ however, identical phylogenies have been obtained from partial nucleotide sequence of the nucleoprotein gene or of genes encoding other viral proteins such as the glycoprotein.^{5,11,16,23}

2.2 Recommendations

Because the resolving power of genetic methods for virus classification is superior to serological methods, all non-rabies Lyssavirus isolates should be identified by nucleotide sequence data.

The amount of nucleotide sequence required to construct a phylogeny for a given sample may vary, but sequence data must be sufficient to produce statistically reliable results. The genome area to be sequenced should be one for which sequence data for reference strains are available. Investigators should be encouraged to make data for the reference strains available through gene banks or similar facilities.

3. SEROLOGICAL METHODS FOR TYPING LYSSAVIRUS ISOLATES

3.1 Panels of monoclonal antibodies

Different panels of monoclonal antibodies (MAbs) are currently available from a number of WHO Collaborating Centres and institutions (see Annex 3). Each of these panels has a different discriminating power. A selection of a limited number of these MAbs will be needed according to the area from which isolates originate. These WHO Collaborating Centres and other institutions are willing to assist in identifying within their own panel those MAbs which would best fit a particular situation. In addition, a panel of 10 anti-nucleoprotein MAbs was selected through WHO collaborative research (Report of the Sixth WHO Consultation on Monoclonal Antibodies in Rabies Diagnosis and Research, Philadelphia, USA, 2-5 April 1990, WHO/Rab.Res/90.34). These MAbs allow identification of various lyssavirus types, subtypes and the differentiation of major virus strains used for vaccine production from field isolates. The core of this panel comprises three MAbs 502-2 positive with all Lyssavirus types, C15-2 positive with all type 1 (classical rabies viruses) and 422-5 positive with types 2, 3 and

4. An FITC-conjugate from MAbs 502-2, C15-2 and 422-5 is available from the Centers for Diseases Control, Atlanta, USA.

3.2 Recommendation

Serological tests, especially with (MAbs),^{6,7,8,17} should be encouraged and expanded. These tests and reagents are now more widely available to the less-developed areas of the world from which new isolates are likely to be made. With a small number of monoclonal antibodies, laboratories can rapidly screen large numbers of samples. Interesting samples can be tested further by genetic analysis.

4. EPIDEMIOLOGICAL INVESTIGATIONS FOR RABIES WITH MAbs AND/OR GENETIC TYPING

4.1 General considerations

MAbs resolution power is sufficient for identifying the broad geographical boundaries of a particular variant or identifying the species affected by different variants within the same area. For example, cases of rabies resulting from contact with vampire bats can be distinguished from cases of dog rabies in Latin America by antigenic methods.^{15,16} Similarly, in southern Africa, cases of rabies associated with the reservoir in viverrid species can be distinguished from those associated with the reservoir in canid species.⁷ The resolving power of genetic typing is superior to that of MAbs. For example, genetic typing is needed to distinguish fox rabies from dog rabies along the US-Mexico border or to identify different populations of fox rabies variants in Ontario and in Europe.^{9,14,15}

4.2 Recommendation

The most appropriate method for virus identification should be selected according to the nature of the investigation and its purpose.

5. PCR FOR ROUTINE RABIES DIAGNOSIS

5.1 Current problems

PCR technology for lyssavirus characterization has been extensively described.^{19,24} The sensitivity of PCR increases the risk of false positive samples resulting from contamination during necropsy or sample preparation. False negative results may occur if primers for RT-PCR do not possess sufficient homology with all rabies variants present within the testing area. A region of the rabies genome broadly con-

served among all rabies variants has not been definitively identified; therefore, standardization of primers for RT-PCR is not possible at this time.

5.2 Recommendation

The group wished to reiterate the recommendation made by the Expert Committee on Rabies (WHO Technical Report Series 824, WHO, Geneva, 1992): «The use of molecular probes and PCR is not currently recommended for the routine diagnosis of rabies».

ANNEX 1

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ANNEX 2

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ANNEX 3

WHO COLLABORATING CENTRES AND INSTITUTIONS

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