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Research Article

Limitations of Diagnostic Tests Using Rabies as an Example

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Abstract

Rabies is an acute, progressive and fatal encephalomyelitis, transmitted via the bite contact of infected animals, primarily the domestic dog *Canis lupus familiaris*. The true burden and public health impact of this fatal but preventable disease is greatly underestimated. Globally, an estimated 59,000 human deaths occur annually. Rabies therefore remains a neglected disease in most low-income countries. The rabies virus (RABV) is the prototype species of the *Lyssavirus* genus (*Rhabdoviridae* family, order *Mononegavirales*) and is non-segmented, negative sense RNA virus. In South Africa and the southern African sub-region, RABV is maintained in both (domestic) dogs and wildlife carnivores. The yellow mongoose *Cynictis penicillata* maintains the mongoose rabies biotype and on the other hand, the domestic dog, the black-backed jackal (*C. mesomelas*) and the bat-eared fox (*Otocyon megalotis*) all maintain the canid rabies biotype.

The World Organisation for Animal Health (OIE) has recommended specific methods for agent identification and detection of immune responses to lyssaviruses. In this perspective, the principles of the test methods for rabies diagnosis will be outlined briefly but more importantly the factors that generally lead to the underreporting of rabies cases on the African continent will be discussed. The test methods for rabies diagnosis for both agent identification and detection of immune responses include the direct fluorescent antibody test (DFA), the direct rapid immunohistochemical test (dRIT), enzyme linked immunosorbent assay (ELISA), virus isolation (both cell culture and mouse inoculation tests), immunohistochemical tests and conventional reverse-transcription PCR. This perspective highlights that for rabies diagnosis laboratories should not limit their scope to a single antigen detection method, but should consider other recommended methods in order to eliminate false negative results and consequently underreporting of the disease.

Keywords: *Rabies; Lyssavirus; Africa; Diagnosis Underreporting*

Abbreviations

DFA: Direct Fluorescent Antibody Test; dRIT: The Direct Rapid Immunohistochemical Test

Introduction

Rabies is a neglected disease in many tropical regions of the world [1]. The disease is commonly encountered in dog populations in many Asian and African where the disease burden is highest. At least 59 000 human deaths occur annually and billions of dollars are lost

due to rabies spread by domestic dogs [2]. One of the huge problems with many infectious diseases including rabies is underreporting. The underreporting arises due to a number of factors: from absence of local laboratories for both human and animal (medical and veterinary infrastructure) able to diagnose rabies, difficulties encountered in the shipment of samples from low access areas to national reference laboratories, and the lack of qualified and experienced personnel and suitability of methods currently in use [3]. At present, there are 17 lyssavirus species [4], with rabies being the most commonly encountered globally.

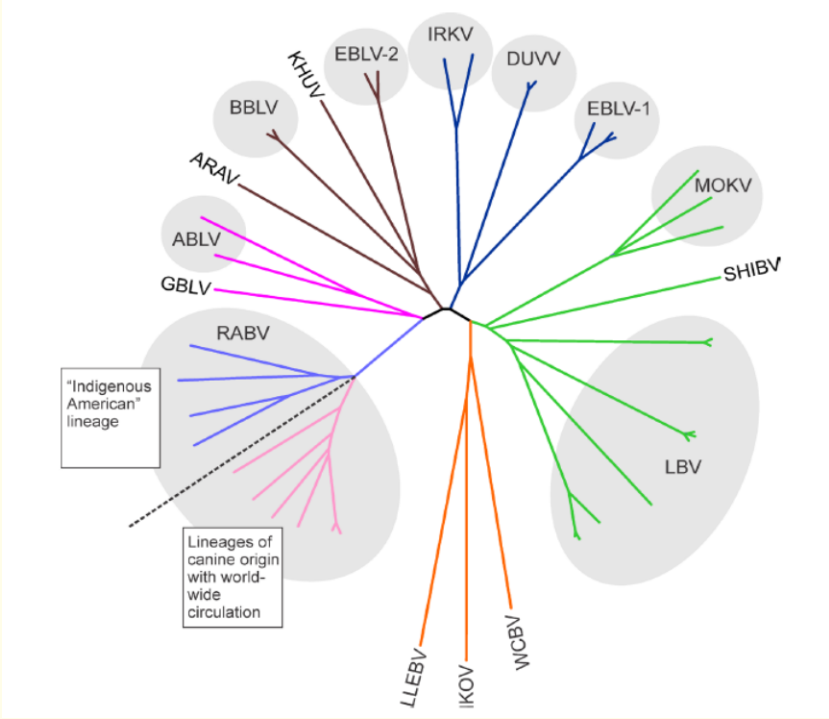

Figure 1: *Unrooted phylogenetic tree of currently recognised and putative lyssaviruses [in Laboratory Techniques in Rabies, 2018].*

Materials and Methods

Rabies specimens are submitted to diagnostic facilities from the field by personnel including veterinarians and animal health technicians. The specimens are submitted in the form of extracted brain tissues, as preserved in glycerol-saline or as fresh. In some cases particularly where post-mortem facilities are not available or inadequate, specimens are submitted as whole carcasses.

Results and Discussion

The DFA method was initially published in the Bulletin of the OIE in 1959 and is now widely used for rabies diagnosis [5]. The method is quick and has a sensitivity and specificity of 99.5% and 100%, respectively. The method is not different from how it was performed in the late 1950s [6], although some of the key steps such as acetone fixation of the smears are now much shorter. There is also variation in the quantity of antigen observed between the different regions of the brain [7] and therefore it is highly recommended to make a composite smear to avoid missing antigen particularly in those samples with low antigen. For instance, the thalamus has a 97.8% prevalence and therefore reliable region for the DFA, particularly for rabies lyssavirus [7].

In this perspective, we will describe three examples in which the DFA missed low lyssavirus positive samples. The first case involves a panel of 28 brain tissues obtained from dogs and received from the University of Nigeria (Nsukka). DFA performed in both Nigeria and the OIE Rabies Reference Laboratory (Onderstepoort, South Africa), confirmed 23 of the 28 samples as lyssavirus positive. The 5 supposedly negative samples were subsequently shown to be rabies positive by hemi-nested PCR. In the second situation, 5 samples from a panel of 8 brain tissue specimens received from the Central Veterinary Laboratory (Maputo, Mozambique) were positive for rabies. In both these instances, the samples purportedly found to be negative were subsequently shown to contain lyssavirus RNA by the sensitive quantitative reverse transcription polymerase chain reaction (PCR) (the 8 false negative samples had an average of 600 gene copies/µl). In the third situation, and in October 2010, a dog brain tissue was diagnosed as rabies negative. However, since the dog was involved in a human contact, a further biological test was performed in laboratory Balb/C mice. In brief, a 10% brain suspension of the dog brain tissue was inoculated into a family of suckling mice and some mice succumbed 6-days post-inoculation (Figure 2). The brain tissues of the mice that succumbed were subsequently confirmed to be lyssavirus positive by DFA.

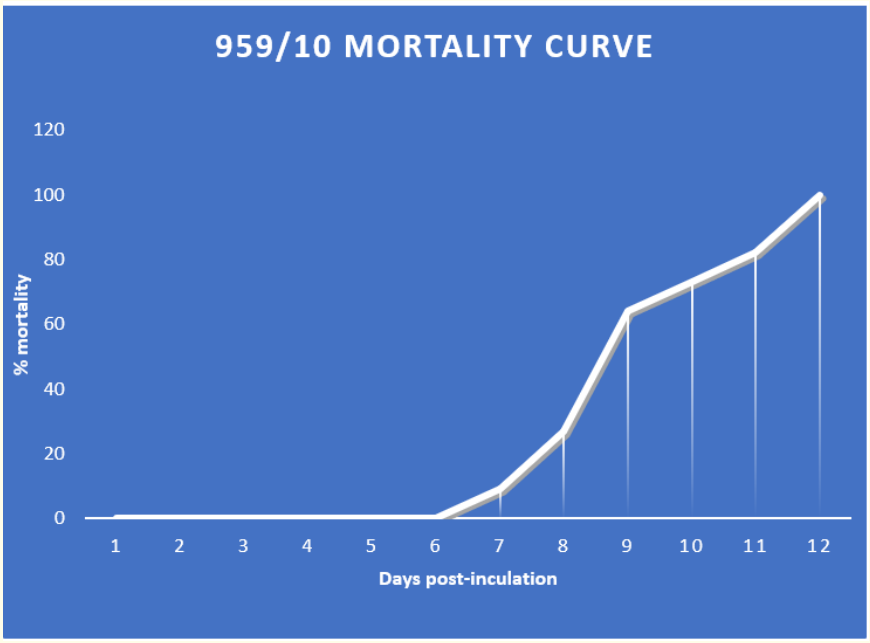

Figure 2: *Diagram showing mortality rate of suckling mice (Y-axis) versus the days post-inoculation. Mice started succumbing 6-days post-infection.*

The condition and quality of the tissues for rabies diagnosis is important given that partially decomposed central nervous tissues are not suitable for the DFA test. The sensitivity of the DFA is dependent on the degree of autolysis of the specimen, how comprehensively the brain specimen was sampled, the lyssavirus type and the proficiency of the personnel. Molecular tests though detect presence of genetic material and can be useful as in the case of the false-negative samples described above.

Conclusion

Underreporting of infectious diseases including rabies is common place in regions where there are inadequate facilities for laboratory diagnosis. It is evident from the perspective that confirmatory methods such as molecular assays be introduced in to complement existing diagnostic methods and minimize reporting false negative results. It is crucial to note that the experience of the readers underpin good and efficient rabies diagnostics. The sensitivity may be lower in samples from vaccinated animals due to the localization of antigen which is confined to the brain stem. In addition, the specificity and sensitivity of the anti-rabies fluorescent conjugates for locally predominant virus variants should also be checked before use.

Conflict of Interest

No conflict of interest exists.

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