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Review

Laboratory diagnostics in dog-mediated rabies: an overview of performance and a proposed strategy for various settings

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SUMMARY

The diagnosis of dog-mediated rabies in humans and animals has greatly benefited from technical advances in the laboratory setting. Approaches to diagnosis now include the detection of rabies virus (RABV), RABV RNA, or RABV antigens. These assays are important tools in the current efforts aimed at the global elimination of dog-mediated rabies. The assays available for use in laboratories are reviewed herein, as well as their strengths and weaknesses, which vary with the types of sample analyzed. Depending on the setting, however, the public health objectives and use of RABV diagnosis in the field will also vary. In non-endemic settings, the detection of all introduced or emergent animal or human cases justifies exhaustive testing. In dog RABV-endemic settings, such as rural areas of developing countries where most cases occur, the availability of or access to testing may be severely constrained. Thus, these issues are also discussed along with a proposed strategy to prioritize testing while access to rabies testing in the resource-poor, highly endemic setting is improved. As the epidemiological situation of rabies in a country evolves, the strategy should shift from that of an endemic setting to one more suitable for a decreased rabies incidence following the implementation of efficient control measures and when nearing the target of dog-mediated rabies elimination.

1. Introduction

The development of rabies diagnostic tests began after the routine inoculation of rabbits with rabies virus (RABV)-infected brain and saliva samples in 1880,[1] and the identification of Negri bodies after 1903.[2,3] Several different assays and diagnostic approaches are now available, and these represent important assets in the renewed global efforts to eliminate dog-mediated human rabies.[4] But how should these tests be used in the operational setting, especially in countries with a high caseload of dog-mediated rabies? In what sequence? For which expected level of performance?[5] This review comprises an overview of currently available assays and their strengths and weaknesses. Furthermore, a rabies testing strategy based on the authors' field experience is proposed. The assays are examined by type and in terms of the type of sample used and their usefulness in the endemic and non-endemic settings. The aim of this review is to guide virologists on the constraints and priorities of surveillance and detection, as well as animal and human rabies programme managers on the diagnostic methods and their performance.

2. Principles of RABV diagnostic tests

The various available reference diagnostic approaches resulting from international and academic initiatives have been described

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extensively in previous publications.[2,6–8] Their principles, as well as those of non-reference techniques – some recently developed[9–11] – are summarized in Table 1.

2.1. Detecting virus: inoculation tests

Historically and in the research setting, RABV infection is identified by infecting cells and detecting virus. This can be done either through the mouse inoculation test (MIT) or by inoculation of samples onto cultures of murine neuroblastoma or other cells (rapid tissue culture infection test, RTCT).[8,12] Following intracerebral inoculation of mice aged 3–4 weeks, MIT test results are available after an incubation period of up to 28 days. Some strains are associated with a longer incubation period. In laboratories with cell culture facilities and an appropriate level of bio-containment, the RTCT provides results within 24–48 h, which is far quicker than intracerebral inoculation.[13,14] Although it is more sensitive to toxic or bacterial contaminants, its sensitivity is comparable to that of

Table 1
Summary of RABV laboratory techniques and their advantages and limitations

Test	Principle	Advantages	Limitations	Ref.
Detecting viral replication				
Mouse inoculation test (MIT)	Intracerebral inoculation into young mice for virus amplification	Sensitive; amplifies virus for identification; easily performed; possibility of isolating infectious virus	Delayed results (up to 28 days); more expensive than RTCT; not recommended by WHO; requires animal facilities and adequate containment; potential animal ethics issues, as alternative methods exist	12
Rapid tissue culture infection test (RTCT)	Inoculation of sample onto cell cultures (e.g., neuroblastoma cells)	Faster and cheaper than mouse inoculation test; sensitivity comparable to MIT; no mice sacrificed	Requires training and manpower, as well as cell culture systems and fluorescence microscopy facilities; sensitive to toxic and bacterial contamination; amplification of live virus may require adequate biosafety (safety cabinets and BSL-3 laboratory)	12,15
Detecting viral RNA				
Reverse-transcriptase PCR (RT-PCR)	Transcribes viral RNA to cDNA and amplifies it using specific primers with further detection of PCR products in agarose gel	Applicable to any sample; highly sensitive and specific; PCR products can be used for further nucleotide sequencing	Time- and resource-intensive; cross-contamination and false-positives are a risk; no commercial diagnostic kits currently available	2,12,29
Real-time reverse-transcriptase PCR (RT-qPCR)	Transcribes viral RNA to cDNA and amplifies it using specific primers and probes with detection of PCR products in real time	Less cross-contamination; applicable to any sample; sometimes more sensitive than conventional RT-PCR; highly specific probes	Single sequence mismatch between the primer or probe sequence and the target viral sequence may alter the sensitivity of the test and even cause false-negative results; PCR products are too short and unsuitable for nucleotide sequencing; no commercial diagnostic kits currently available	12,29,30
Detecting viral antigens/proteins				
Direct fluorescent antibody test (DFAT)	Use of polyclonal or monoclonal FITC-conjugated antibodies for detection of rabies virus antigens by means of fluorescence microscopy	Gold standard for fresh or fixed brain samples; high sensitivity and specificity, even on fixed specimen; results obtained quickly; commercial diagnostic kits are available	Interpretation requires well-trained personnel (results highly observer-dependent) and a costly fluorescence microscope; less suitable on degraded samples	2,8,12
Antigen capture ELISA: rapid rabies enzyme immunodiagnosis (RREID)	Immunohistochemical technique based on the capture of various rabies antigens by specific antibodies labelled with enzyme	Highly specific but less sensitive than DFAT (96% agreement between DFAT and RREID test results); usable even on partly degraded brain samples; qualitatively readable with the naked eye; a large number of samples can be tested at the same time (screening)	Can be used on brain tissues only; requires great care to preserve specificity; no commercial diagnostic kits available	12,29,35–41
Rapid immunodiagnostic test (RIDT)	Immunochromatographic assay based on monoclonal antibodies to capture rabies antigens	Highly sensitive and specific but usually less so than DFAT; usable on brain and saliva samples from animals; results obtained rapidly	Dedicated for research purposes only; need for further validation before either OIE or WHO can recommend its use	11,43,44
Direct rapid immunohistochemical test (dRIT)	Biotinylated monoclonal antibodies for the detection of rabies virus antigens by means of normal light microscopy	High sensitivity and specificity; no need for fluorescence; results obtained quickly	Reagents difficult to obtain: need to identify an uninterrupted supply chain of quality-controlled monoclonal antibodies for sustainability	12,29,45

RABV, rabies virus; WHO, World Health Organization; BSL, biosafety level; FITC, fluorescein isothiocyanate; OIE, World Organization for Animal Health.