jove

Methods Collection In Lyssavirus Detection, Diagnosis and Research

Charles E. Rupprecht[1]

[1] College of Forestry, Wildlife and Environment, Auburn University

Corresponding Author

Charles E. Rupprecht
charleserupprechtii@gmail.com

Citation

Rupprecht, C.E. Methods Collection In Lyssavirus Detection, Diagnosis and Research. *J. Vis. Exp.* (185), e64453, doi:10.3791/64453 (2022).

Date Published

July 12, 2022

DOI

10.3791/64453

URL

jove.com/video/64453

Editorial

aRabies is an acute, progressive, viral encephalitis. The bullet-shaped etiological agent belongs to the genus *Lyssavirus*, at least 17 of which cause rabies around the world (except in Antarctica). Rabies virus is the most important member of these diverse RNA viruses. All mammals are susceptible, with bats and mesocarnivores being significant reservoirs. Transmission occurs primarily after a bite. As quintessential neurotropic agents, virions progress centripetally after deposition *via* axons from the periphery to the central nervous system (CNS) for replication, thereafter to the salivary glands, and are excreted into saliva. Globally, dogs are responsible for the greatest number of human deaths from rabies, causing tens of thousands of deaths annually. Considering zoonotic dimensions, laboratory methods are essential to applied research and disease notification, prevention, and control in humans, domestic animals, and wildlife. The objective behind this collection of nine interrelated articles is to introduce methods providing introspection to rabies occurrence, detection, pathobiology, epidemiology, management, and improvement in biologics.

How is rabies assessed? Rabies should be a notifiable disease, with appropriate collection, analysis, and epidemiological interpretation of data. Today, most cases are reported from passive surveillance, based upon animals that expose people. While it might appear reasonably straightforward to envision the safe capture, restraint, sedation, and euthanasia of a clinical suspect (i.e., a biting dog for rabies diagnosis), this is not as easy for some wildlife. For example, Taiwan was an area considered to be 'free' of rabies, originally based upon extensive surveillance in domestic animals. However, recognizing the importance of other animal reservoirs, investigators on the island turned their attention to wildlife. As Hsu et al. describe, the targeted collection of moribund and dead bats provides an opportunity for the detection of unique lyssavirus species in Taiwan[1]. Such efforts expanded the taxonomic breadth of this viral genus and provided a reasonable scheme for other assessments in Asia.

What tissues are needed for lyssavirus diagnosis? In addition to the derivation of relevant surveillance methods and correct species identification, safe and appropriate tissue selection is paramount to the sensitive and specific diagnosis of rabies. Since the 19th century, the central nervous system

(CNS) was appreciated as a primary tissue. Animals were euthanized, their carcasses were sent to the laboratory, skulls were opened, brains were removed, and portions examined microscopically for viral inclusions. However, the shipment of whole animals is not practical, particularly with large-bodied mammals such as livestock. Moreover, not all areas of the brain are equally useful for diagnosis. Jarvis et al. describe safe and practical necropsy methods for both small and large mammals that minimize exposure risk and reduce processing times[2]. The application of such biosafety recommendations and the concentration on the brainstem and cerebellum will improve practices for modern laboratory-based surveillance of animal rabies.

What options are used for viral antigen detection? For routine lyssavirus diagnosis, a direct fluorescent antibody (DFA) test for the observation of viral inclusions in CNS tissue has been used since the mid-20th century. While highly sensitive and specific, the DFA test requires a fluorescent microscope and uses acetone as a tissue fixative. Patrick et al. report the use of a direct, rapid immunohistochemical test (drit) that relies upon brainstem collection, formalin fixation of tissue impressions, the use of monoclonal or polyclonal antibodies conjugated to biotin, and the detection of viral inclusions by light microscopy, in less than 1 hour[3]. The drit has similar sensitivity and specificity as the DFA test, and is suitable for decentralized, enhanced laboratory-based surveillance under field conditions. For resource limited settings, a linear flow assay (LFA) is another method for rapid diagnosis that requires minimal technical expertise, as reported by Mauti et al.. Positive test strips from the field can be sent to a central laboratory for confirmation[4].

Besides antigen detection, are other methods suitable for lyssavirus diagnosis? The DFA, drit, and LFA tests are useful for the detection of viral antigens in postmortem CNS tissues. However, brain tissues may be decomposed. Moreover, in suspected human cases, antemortem tests are more desirable and can include alternate clinical samples such as CSF. As such, molecular assays are very useful to detect small quantities of viral nucleic acids. Marston et al. and Wang et al. describe a real-time and nested, respectively, polymerase chain reaction technique, respectively, that is rapid, sensitive, and specific[5,6]. Such careful attention to detail to minimize sample contamination and proper primer design allow primary diagnosis across the spectrum of divergent lyssaviruses.

Once diagnosed, is conventional microscopy adequate for modern insights on lyssavirus pathobiology? Using two-dimensional microscopic methods alone may result in missing critical *in vivo* elements in context and complexity. However, using long free-working distance objectives, virus-infected tissues can be imaged by conventional confocal laser scanning microscopy at high resolutions, as detailed by Zaeck et al., to visualize pathogen distribution and better appreciate fine details on viral pathogenesis, spread, tropism, and neuroinvasion[7].

Are there alternatives for measuring rabies vaccine potency? The current methods to measure rabies vaccine potency require large numbers of laboratory rodents. However, costs, confidence intervals, and welfare issues necessitate an alternative. The rabies virus glycoprotein elicits virus neutralizing antibodies after vaccination. As communicated by Jallet and Tordo, an *in vitro* ELISA test may be used instead of animals to measure the relative content of rabies virus vaccines, using highly specific monoclonal antibodies[8].

Can inference on oral vaccine-bait consumption by wildlife be accomplished using biomarkers? Other than

parenteral vaccination of humans and domestic animals, oral vaccination of wild mesocarnivores has been operational since the 1970s. Besides measuring vaccine immunogenicity or the resulting efficacy among populations of foxes, raccoons, skunks, etc. by case detection, biomarkers can be used to infer contact and consumption of vaccine-laden baits. Historically, the detection of incorporated tetracycline from baits was done in the bones or teeth of animals. An alternative to the collection of such hard tissues is the serological measurement of a different biomarker, such as iophenoxic acid. Berentsen et al. report on the analyses of such derivatives in the sera of mongooses, an important natural reservoir in Asia, that was introduced into the Caribbean region[9]. After consumption of bait containing this biomarker by captive mongooses, they extracted iophenoxic acids into acetonitrile and analyzed animal sera by liquid chromatography-mass spectrometry. Adequate marking was detected for several weeks after bait consumption and such derivatives could be suitable for field evaluation of vaccine-laden bait uptake in free-ranging animals.

Due in part to the ongoing COVID-19 pandemic, improved virological methods in enhanced lyssavirus surveillance, pathogen discovery, confirmatory diagnosis, and variant characterization are anticipated. Reading about different techniques in the peer-reviewed literature is typically the first step for an interested individual. However, directly observing a sequence of steps, and hearing the context from an experienced operator, removes nuance and misinterpretation. Current and future laboratory and field protocols related to rabies, as recommended by the OIE and WHO, could benefit from additional visual methods collections to assist new investigators, especially within developing countries.

Disclosures

The author has nothing to disclose.

Acknowledgments

We thank all the authors for their contributions to this collection and to our colleagues for continuing progress in this field.

References

1. Hsu, W. C. et al. Standard operating procedure for lyssavirus surveillance of the bat population in Taiwan. *Journal of Visualized Experiments.* (150), e59421 (2019).
2. Jarvis, J. A., Brown, K. T., Appler, K. A., Fitzgerald, D. P., Davis, A. D. Rabies necropsy techniques in large and small animals. *Journal of Visualized Experiments.* (149), e59574 (2019).
3. Patrick, E. M. et al. Enhanced rabies surveillance using a direct rapid immunohistochemical test. *Journal of Visualized Experiments.* (146), e59416 (2019).
4. Mauti, S. et al. Field postmortem rabies rapid immunochromatographic diagnostic test for resource-limited settings with further molecular applications. *Journal of Visualized Experiments.* (160), e60008 (2020).
5. Marston, D.A. et al. Pan-lyssavirus real time RT-PCR for rabies diagnosis. *Journal of Visualized Experiments.* (149), e59709 (2019).
6. Wang, Y. et al. Evaluation of a universal nested reverse transcription polymerase chain reaction for the detection of lyssaviruses. *Journal of Visualized Experiments.* (147), e59428 (2019).
7. Zaeck, L., Potratz, M., Freuling, C. M., Müller, T., Finke, S. High-resolution 3D imaging of rabies virus infection

in solvent-cleared brain tissue. *Journal of Visualized Experiments.* (146), e59402 (2019).

8. Jallet, C., Tordo, N. In vitro ELISA test to evaluate rabies vaccine potency. *Journal of Visualized Experiments.* (159), e59641 (2020).
9. Berentsen, A. R. et al. Analysis of iophenoxic acid analogues in small Indian mongoose (*Herpestes auropunctatus*) sera for use as an oral rabies vaccination biological marker. *Journal of Visualized Experiments.* (147), e59373 (2019).