

Detecting a Novel Circovirus in Foxes with Meningoencephalitis

How custom RNAscope® assay probes are enhancing viral understanding



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The burden of infectious diseases on human health worldwide has markedly increased over the past few decades, and most of these emerging events are caused by viruses originating from the animal world. Understanding the roots of viral behavior in animal hosts is therefore vital, and this is a primary focus of Sarah Getu's and Saskia Smits's work at the Department of Viroscience, Erasmus MC. They here explain how the creation of a custom RNAscope® in situ hybridization (ISH) probe allowed them to localize a newly discovered circovirus to neurologic lesions in foxes, providing valuable information to support causation.

Can you explain your research focus?

Following transmission between species, viruses usually go through a period of adaptation to the new human host, after which they may spread to cause epidemics or eventually a true pandemic. At the Department of Viroscience, one of the main pillars of research focuses on emerging and re-emerging virus infections. We work to identify and characterize viral pathogens in clinical and public health settings in both humans and animals, with our optimized approach resulting in the identification of a plethora of previously unknown human and animal viruses.

Establishing disease causation in the molecular era has been debated since the start of the use of molecular detection techniques in biomedical research laboratories. Associating newly identified viruses with disease relies on many features that aid in studying viral pathogenicity, including:

- Comparing viral prevalence in closely matched patients versus healthy control individuals
- Seroconversion (the detection of an antibody response)
- Detecting viral antigen/nucleic acids in affected tissue

It is this last point where RNAscope® technology is utilized in our laboratory, providing evidence to support a link between the novel virus and disease¹.

How did you discover the Fox Circovirus?

In this particular example, we were asked whether we could look for a potentially new viral etiology in adult foxes that were brought into the RSPCA Norfolk Wildlife Hospital (United Kingdom), suffering from a neurological disorder with signs including abnormal behavior, lack of fear and reduced alertness. All animals had similar histological findings consisting of chronic multifocal/diffuse lymphoplasmacytic meningoencephalitis orientated on the forebrain, yet many of the usual causative organisms were excluded. Using viral metagenomics assays on serum from a subset of diseased foxes, we revealed the presence of fox circovirus genomes.

How did you move from identification towards characterization of this novel circovirus?

Since we identified a novel virus, a custom RNAscope® ISH probe design was required in this case. Working with ACD, the custom RNAscope® assay probe was designed against the Rep coding RNA of this novel virus, and the assay subsequently showed multifocal fox circovirus RNA signal clearly associated with the

1 - [Detection of Circovirus in Foxes with Meningoencephalitis United Kingdom, 2009-2013.](#)

Bexton S. (2015). *Emerg. Infect. Dis.*; 21(7):1205-8.

2 - [Novel cyclovirus in human cerebrospinal fluid, Malawi, 2010–2011.](#)

Smits S.L. et al. (2013). *Emerg. Infect. Dis.*; 19:1511-3.

3 - [Identification of a new cyclovirus in cerebrospinal fluid of patients with acute central nervous system infections.](#)

Tan L.V. et al. (2013). *MBio.*; 4:e00231–13.

forementioned histologic lesions in the cerebrum. The ability to provide custom assay service is aiding tremendously in our research and the support of the ACD team was very good, with fast responses and expert advice.

At present it remains unclear whether the fox circovirus is associated with the observed neurological disease. Showing the fox circovirus present at the sight of histopathological lesions is suggestive of disease involvement but not formal proof, and work is still ongoing in this area.

How does RNAscope® ISH compare to alternative viral detection methods?

RNAscope® ISH has provided us with a unique opportunity to examine the expression of viral RNA in a highly sensitive and specific manner at the sight of histopathological lesions. This contrasts with in-solution methods such as RT-PCR techniques that don't provide the spatial information that is so crucial when studying which cells are targeted by viruses. Regarding the fox circovirus and neurological disease, the localization of the fox circovirus to cells in histopathological lesions of the brain provides indirect support for a causative role of the virus in disease. The assay also has the advantage of a one-day workflow and we have received excellent technical support from ACD.

Ideally one would use both ISH and immunohistochemistry to have independent confirmation of virus localization results. However, for many new viruses no antibodies are available and ISH provides a very good alternative.

How does RNAscope® ISH compare to traditional RNA *in situ* hybridization methods?

RNAscope® ISH provides us with consistent results, while the sensitivity and specificity has led to new discoveries and a publication. Moreover, classical RNA ISH is very labor intensive and

difficult to set up for a new target, while we've been setting up RNAscope® assays for over 20 targets, and it has proved to be quick and easy.

Do you use RNAscope® ISH for FFPE samples?

Yes, this was previously quite difficult due to degradation of nucleic acids, and now we use RNAscope® ISH frequently on sub-optimally preserved tissue successfully (e.g. wildlife animals). We have found the RNAscope® assay protocol to be generally effective on many of these archival samples, and therefore we can gain valuable insight into viral gene expression in these poor quality samples.

What do you see for the future of using RNAscope® ISH in your research?

It is of note that cycloviruses, belonging to a proposed new genus in the family Circoviridae, were recently found in serum and cerebrospinal fluid (CSF) of humans with paraplegia and acute central nervous system infections^{2, 3}, suggesting that viruses from the family Circoviridae may more commonly have neurological tropism than previously anticipated. However, a causal link between circovirus infection and disease in man and animals remains to be proven. To clarify the epidemiology and pathogenicity of circoviruses in foxes, additional circovirus surveillance is warranted, as the prevalence of circoviruses in foxes was relatively high and closely related circovirus species seem pathogenic for both dogs and foxes.

We will continue to use RNAscope® ISH probes in our research, and look forward to examining other targets of interest in archival FFPE samples. In fact, we would certainly recommend RNAscope® technology to researchers wanting to examine a target of interest for which there is not a reliable antibody available.

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