Identification of a previously undescribed divergent virus from the Flaviviridae family in an outbreak of equine serum hepatitis

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Theiler’s disease is an acute hepatitis of horses that is associated with administration of equine blood products. Although this association was first described nearly a century ago, its etiologic agent has remained unknown. A recent outbreak of Theiler's disease provided a unique opportunity to investigate the mysterious cause of the disease. Following treatment with an equine blood product raised against botulinum toxin, 8 of 17 horses developed clinical or subclinical signs of hepatitis. Serum from the horses, as well as the antitoxin used to treat the horses, was collected during the outbreak. As we later discovered, the batch of antitoxin given to the horses that developed hepatitis harbored a previously undescribed virus (Fig. P1).

To explore the possibility that a known or as-yet unidentified virus was the underlying cause of the equine hepatitis outbreak, we performed high-throughput sequencing on RNA extracted from the serum of two of the most clinically ill horses and from an aliquot of the antitoxin administered to these horses. We searched among the more than 60 million resulting reads from these samples for foreign, nonhorse sequences with similarity to viruses. Our analysis identified a 10.5-kb sequence from a previously undescribed and highly divergent virus of the Flaviviridae family that we designate Theiler’s disease-associated virus (TDAV). Notably, TDAV is significantly different from the recently discovered nonprimate hepativiruses in horses, which have yet to be linked to disease. TDAV was the only virus found in both index cases as well as the antitoxin, and the TDAV sequences identified in all three samples were virtually identical, suggesting that infection likely coincided with the administration of the antitoxin. Although phylogenetic analysis revealed that TDAV is most similar to the newly proposed Pegivirus genus, it shares only 35.3% amino acid identity with its closest relative, GB virus D. We also identified several distinguishing features within the TDAV genome, including previously unobserved large genetic insertions within the coding region of an essential protein (NS5A), and the absence of binding sites for a small regulatory RNA, microRNA-122, whose binding is critical for infection by other hepatitis-causing members of the Flaviviridae family.

A molecular epidemiologic survey of additional horses from three separate locations supports an association between TDAV infection and acute serum hepatitis. Eight of the 17 horses treated with the TDAV-containing antitoxin preparation...
developed hepatitis, whereas all untreated horses and horses treated with a different antitoxin were negative for TDAV. Although 9 horses treated with the TDAV-containing antitoxin were asymptomatic, every horse receiving this antitoxin showed some level of virus positivity, with 15 of the 17 having relatively high viral loads. Notably, it is not uncommon for human patients infected with hepatitis C virus (HCV), a distantly related member of the Flaviviridae family, to remain asymptomatic as well.

Follow-up studies of serum from horses residing at the outbreak site 1 y later provided evidence that TDAV, like HCV, establishes both acute and chronic infections, is not transmitted efficiently other than by i.v. administration, and is associated with a spectrum of outcomes ranging from no symptoms to severe hepatitis. Interestingly, horses with the most severe clinical cases of acute hepatitis were negative for TDAV when tested 1 y later, and evidence of chronic infection was detected in some subclinical and asymptomatic cases. To strengthen the link between TDAV and hepatitis, we inoculated healthy horses with the original virus-containing blood product implicated in the outbreak. By tracking viral load and liver enzyme levels during the study, we collected evidence that several weeks of viremia preceded liver injury and that liver disease may not be directly related to the level of viremia. Although we have not absolutely proved that TDAV alone causes acute hepatitis in horses, we have gathered substantial evidence to suggest that TDAV plays a central role in Theiler’s disease alongside as-yet unknown host determinants.

This study provides a compelling example of how the combination of unbiased metatranscriptomic sequencing approaches and classical epidemiology can elucidate the role of previously unknown viral agents in diseases of unknown etiology. Our study also presents opportunities to investigate a potentially important pathogen of horses and provides critical information for efforts directed toward the control and eradication of equine serum hepatitis.