Novel Rickettsial Species Causing Equine Neorickettsiosis

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1. Introduction

Neorickettsia spp. are Gram-negative, obligatory intracellular bacteria. This bacterial species is widely distributed in nature and can be found in a variety of trematode species, including Fasciola hepatica, the liver fluke of sheep, cattle, and humans, and hosts of unknown trematodes worldwide.1 The life cycles of the digeneans are complex and in their developmental cycle stages they can parasitize different host species. The Neorickettsia bacteria infect the fluke along the developmental stages of their life cycle and are therefore vertically transmitted. Neorickettsia are unique among the Family Anaplasmataceae because both vertical transmission and horizontal transmission (at least from fluke to vertebrate) have been documented.2 The typical trematode life cycle involves aquatic snails (e.g. Elimia spp.) as the first intermediate host, an arthropod as the second intermediate host, and a vertebrate as the definitive host (i.e., bats). Neorickettsia risticii was detected by PCR in metacercariae larvae infecting immature and adult aquatic insects (caddisflies, mayflies, damselflies, dragonflies, and stoneflies) collected from a pasture stream in northern California, Pennsylvania, Kentucky, and Ohio.3 It was demonstrated by Koch’s postulate then that horses develop Potomac Horse Fever (PHF) by ingesting infected cercariae in water or insects infected by metacercariae harboring N. risticii.4,5 Because PHF has now been confirmed in many countries, and due to the wider global distribution of the disease, equine neorickettsiosis (EN) is considered a more appropriate designation.6 Equine neorickettsiosis has been documented in the United States, Canada, Brazil, Uruguay, France, and The Netherlands.7 Adult flukes may pass their neorickettsial endosymbionts to the definitive vertebrate hosts by an unknown mechanism, and once inside the vertebrate, are capable of invading and multiplying within a variety of cells. At present, there are four distinct diseases attributed to Neorickettsia spp., in which N. risticii, N. sennetsu, and N. helminthoeca are horizontally transmitted to definitive or accidental mammalian hosts of trematodes and subsequently cause disease.8 In a 16S rRNA sequence analysis of isolates of N. risticii from horses with clinical signs of PHF, a uniquely different organism was identified and it was proposed that this isolate may be a new and distinct species of Neorickettsia.9 This hypothesis could not be confirmed until recently when this novel species, designated N. findlayensis, was isolated from two horses with clinical signs of EN in Ontario, Canada.8,10
2. Discovery of Novel Rickettsial Species Causing Equine Neorickettsiosis

Equine neorickettsiosis is an acute, seasonal disease of horses characterized by fever, lethargy, anorexia, dehydration, diarrhea, laminitis, and/or abortion, that is commonly known as PHF.¹¹ Neorickettsia risticii (formerly Ehrlichia risticii) had been recognized as the etiological agent of PHF since being recognized in the mid-1980s.² In Canada, PHF has been confirmed in at least five provinces. In the province of Ontario, there is compelling published data of an endemic disease resembling EN that has been present since the late 19th century.⁷,¹³ Every summer, horses are presumptively diagnosed and treated for PHF in Ontario, but the etiological agent is not routinely confirmed by the recommended molecular testing in blood and feces. Blood samples (n = 41) from horses with typical clinical signs such fever, lethargy, inappetence, and diarrhea, that resided at various locations in Ontario, Canada, were tested by PCR and cultured from 2015 to 2020 at the Molecular, Cellular, and Environmental Rickettsiology Laboratory, Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University. Sixteen of 41 (39%) samples yielded a positive culture for Neorickettsia spp. organisms, of which 12 culture isolates were analyzed by PCR for four genes, 16S rRNA, Ssa3, and Ssa1, followed by sequencing.¹⁴ Phylogenetic analysis confirmed that 10 of the isolates were strains of N. risticii, whereas the remaining two isolates (Fin17 and Tom16) were a previously uncharacterized Neorickettsia spp.⁸ These two Canadian isolates were closely related to Neorickettsia spp. 081.³ The results were corroborated by whole-genome sequencing of Fin17 and genomic comparison with N. risticii, N. sennetsu, and N. helminthoeca. Further, the phylogenetic analysis of 12/16 of these Ontario isolates demonstrated clustering according to the geographic area of origin.

3. Experimental Infection with Novel Rickettsial Species in Horses

To demonstrate that the isolated organism was in fact the cause of the clinical signs, an experimental study to fulfill Koch’s postulate was undertaken. Two ponies were intravenously inoculated with the uncharacterized Neorickettsia spp. (Fin17-infected P388D1 cells).⁸ Pony 1 developed intermittent fever and lethargy, tachycardia, anorexia, and watery diarrhea, on Days 14 to 18 post-inoculation. Pony 2 also developed mild fever on Day 7, and lethargy on Day 11 postinoculation. Seroneconversion was detected by Day 6 for both ponies by using indirect fluorescent antibody assay. Neorickettsia spp. was detected by quantitative PCR (qPCR) in the peripheral blood specimens from both ponies by Day 9 post-inoculation and blood culture was positive on Days 9 and 16 in both ponies. Fecal samples were not tested for the molecular detection of the inoculated bacteria during this experiment. By using PCR and sequencing for 16S rRNA and ssa3V gene, the identity of the inoculated strain (Fin17) was confirmed for the culture isolates from both ponies as identical to each other and to the original Fin17 horse isolate. The experimental inoculation of Fin17 demonstrated that this isolate is capable of infecting horses and causes typical clinical signs of PHF or subclinical infection, and therefore fulfills Koch’s postulates as a novel causative agent of PHF.⁸

4. Summary

Neorickettsia spp. are a small group of Gram-negative, endosymbiotic of digenean flukes with a complex life cycle that involves aquatic and terrestrial environments of vertebrates and invertebrate hosts. The recent discovery of the novel species, N. findlayensis, underscores the importance of cultural isolation and in-depth molecular analysis of the isolated strains. There is currently no commercial molecular test for the diagnosis of N. findlayensis-associated EN. PCR amplification and amplicon size comparison of ssa3 gene can be used to distinguish new Neorickettsia spp. from N. risticii strains. In addition, based on the whole genome sequencing, a new PCR test to detect both species is currently being developed. Concomitant submission of ethylenediaminetetraacetic acid (EDTA) blood and feces is recommended to maximize the diagnosis of EN, nevertheless it has been observed that fecal samples are more likely to yield a positive result. Therefore, in the event that only one sample can be submitted, a fecal sample would be preferred.¹⁴ Further studies will be conducted to investigate the natural history of this new species, the pathogenesis of the disease, and to improve laboratory diagnosis and potentially vaccine development.

Acknowledgments

This research was supported in part by The Ohio State University and Equine Guelph.

Declaration of Ethics

The Authors have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors have no conflicts of interest.

References


