Incrimination of Mayflies as a Vector of Potomac Horse Fever in an Outbreak in Minnesota

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Mayflies pose a significant risk to horses because they are vectors of Potomac horse fever (PHF). Investigation of a cluster of cases associated with a large horse show in early July in southeastern Minnesota provided positive identification of *Neorickettsia risticii* through polymerase chain reaction (PCR) in dead mayflies inside the show facility. Competitors reported vast numbers of dead mayflies that blew into the facility and into horse trailers at the show grounds, which contaminated the stalls, hay supplies, and water of many horses. Veterinarians should educate their clients on ways to minimize their horse's exposure to mayflies in regions near waterways. Authors' addresses: Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, 1365 Gortner Avenue, St. Paul, MN 55108 (Wilson); Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, 1 Shields Avenue, Davis, CA 95616 (Pusterla); Town & Country Veterinary Hospital, 29161 Wilson Frontage Road, Winona, MN 55987 (Bengfort); and Route 1, Box 168, 380th Street, Dorchester, IA 52140 (Arney); e-mail: wilso011@umn.edu (Wilson).

1. Introduction

Potomac horse fever (PHF), caused by *Neorickettsia risticii*, has been frequently diagnosed in case clusters of illness in horses near waterways in the summer. Studies of the life cycle of the organism in California and eastern United States have revealed that the reservoir is most commonly trematode species with two life stages in intermediate hosts. In its first stage, the trematodes parasitize water snails in endemic areas.[1–5] During periods of warm water temperatures, cercaria infected with the organism are released from the snails, infecting and developing into metacercaria in the second intermediate host (aquatic insects). Caddisflies, mayflies, damselflies, dragonflies, and stoneflies have all tested positive for the organism using polymerase chain reaction (PCR) techniques.[2,3] Clinical disease has been experimentally produced in horses by feeding infected caddisflies, and clinical signs developed 10–15 days later.[3,4] Additionally, DNA of *N. risticii* has been found in adult trematodes, which infect the intestines of bats, birds, and amphibians.[6,7] This observation suggests that insectivores, like bats and swallows, may be both the definitive host for the trematodes and a reservoir for *N. risticii*.[8]

In Minnesota, case clusters of PHF are very unusual, despite the wide distribution of rivers, lakes, and ponds. Six Quarter Horses that attended the same 5-day horse show near Winona, Minnesota developed signs compatible with PHF within 3 wk of the show, and they were tested for evidence of the

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disease using serology or PCR. All of the owners commented on the unusual presence of large numbers of mayflies at the show grounds on July 10th, prompting a site visit to investigate the likelihood of an epidemiologic linkage of the mayflies to the disease.

2. Materials and Methods

On August 5, 2005, 27 days after the mayfly swarm, the show facility was inspected for proximity to waterways, and potential vectors of *N. risticii*. Banks of a small pond on the farm, two small streams ~2 mi away, and the Mississippi River (5 mi away) were examined for snails and aquatic insects. Specimens collected were bagged, labeled, and cooled, and they were then sent by overnight courier to California for molecular testing for DNA of *N. risticii* using real-time PCR.[5]

The equine veterinarian servicing the facility (Bengfort) was consulted to determine if other cases of PHF were observed in the region. Blood samples obtained before oxytetracycline treatment were submitted from two cases with compatible clinical signs in this practice as well as a fecal sample from one. Two additional veterinarians to the south of the region were also consulted about case clusters and encouraged to submit samples from suspect cases. Blood samples[6] and feces[3] were submitted for PCR testing from six presumptive PHF cases in northeastern Iowa 2 wk after the onset of clinical signs. Two of the three premises affected in northeastern Iowa were located very close to the Mississippi River and had previously experienced PHF cases.

Members of the Minnesota Quarter Horse Association with Minnesota addresses (n = 576) were mailed an informational letter about PHF and a 3-page questionnaire. The questions were designed to delineate vaccination practices, exposure to waterways, aquatic insects, night lights, bats, and barn swallows, and signs of disease compatible with PHF in their horses (fever, depression, diarrhea, laminitis, limb swelling, and/or abortion).

Additional information on cases was solicited from attending veterinarians or gleaned from the medical record of one case presented to the Large Animal Hospital of the University of Minnesota.

For purposes of data analysis, horses were considered cases either if the diagnosis was confirmed by serology (single very high titer or four-fold change in titer) or PCR testing or if the veterinarian attending the horse made the diagnosis based on observation of clinical signs and response to treatment with oxytetracycline.

3. Results

Dessicated adult mayflies were found in scattered cobwebs near doors and windowsills in the show facility, particularly along the southern and eastern sides. These were identified as members of the *Hexagenia* genus, family Ephemeridae, on the basis of morphology (Fig. 1). Using real-time PCR, *N. risticii* was detected in one of 10 pools of mayflies, each containing four adult insects. No other specimens collected at the site visit tested positive.

Fig. 1. *Hexagenia* spp. mayfly. (Courtesy of Alex Wild.)

Discussions with equine practitioners from three practices in the region of the show facility and to the south revealed multiple cases of illness highly suggestive of PHF during the same time period. Only one blood sample from the local clinic was PCR positive, but two Iowa horses had positive indirect fluorescent-antibody assay titers (>1:40) on samples analyzed at the National Veterinary Services Laboratories.[a]

Ninety-five questionnaires were returned promptly (17%) as well as five others with no forwarding address or from owners with no horses in Minnesota. None of the Minnesota respondents, who collectively owned 557 horses, had ever had PHF diagnosed in any of their horses or on the home farm, which reinforces the unusual nature of the PHF outbreak in Minnesota. In a few instances, the respondents could not answer one or two specific questions about the horse's environment, particularly if the horse was boarded at a commercial stable. Four of six cases from the Winona show were represented, and similar information was attained from the medical record of a fifth horse seen at the University of Minnesota. There were five additional cases identified by the questionnaires during the same time period that had not attended the show but lived just south of that region.

Location of Positive Cases

Based on feedback from attending veterinarians and the questionnaires, cases were confirmed as PHF from four different sites in late July. Six cases were associated with the Winona show grounds; two cases were asssociated with Caledonia, Minnesota. Three cases were associated with La Crescent, Minnesota, which is along the Mississippi River. Five cases were associated with New Albin, Iowa, just south of the Minnesota border and also along the Mississippi River, and one case was from Waukon, Iowa, close to a branch of the Iowa River (Fig. 2).

Fig. 2. Location of affected farms for horses developing PHF in late July of 2006. Red, Winona; yellow, Caledonia; green, La Crescent; purple, Waukon; white, New Albin.

Onset of Illness

The first signs of illness in the Winona horses were observed on July 24, 15 days after the swarm of mayflies were observed. The three horses in La Crescent, Minnesota first showed signs of illness on July 27 (one horse) and July 31 (two horses), whereas the horses in Caledonia, Minnesota manifested signs on July 26 and 31. In the six Iowa cases, signs were first observed in both New Albin and Waukon on July 28 and in subsequent cases during the next 2 days. Horse owners and veterinarians reported other suspect cases in the area, but further information was not available.

Vaccination Status

Only one of six cases from the horse show was currently vaccinated against PHF, having received its annual booster in June. One had been vaccinated in previous years, and two were never vaccinated. Vaccination status of the other two was unknown. Of the five Minnesota Quarter Horses that became ill because of other exposures, three had been vaccinated that spring and two had been vaccinated in previous years. Two of the Iowa cases were vaccinated in May, just 2 mo before developing signs of PHF.

Vaccination rates were low in non-ill horses attending the Winona show. Only 7 of 20 (35%) horses were vaccinated preceding the show, and two of those horses had been vaccinated twice before the show. Another seven horses were vaccinated in late July or August after the outbreak was attributed to PHF. Among other respondents, 28 of 62 (45%) indicated that horses were vaccinated before the outbreak; eight horses were vaccinated after the outbreak. Vaccination status was unknown for three horses.

Water Exposure

As expected, the majority of the horses represented by owners who completed the questionnaire had waterways within 2 mi of their farm, and many horses were also near water at shows or parks. Only 7 of 95 (7%) questionnaire respondents claimed no water exposure, and none of the horses owned by these respondents were ill. Six believed that their horse's only water exposure occurred at shows, one of which was a case from the Winona show. All of the Iowa cases had water on the farm or within a 2-mi radius.

Vectors

Two-thirds of the owners attending the Winona show commented on the large number of mayflies they observed. Several inches of mayfly carcasses accumulated overnight beneath a night light overhanging the southeast entrance of the show facility. When the door was opened in the morning, thousands of dead mayflies blew into the barn and stalls, getting into the hay of a number of horses stalled near the entrance. Owners also reported large numbers of dead mayflies in the grass outside the building and in the back of pickup trucks on the grounds. Similarly, aquatic insects were seen around night lights by four of five owners of the regional cases that did not attend the show. Very few other horse owners reported seeing aquatic insects near their horses that summer, including the owners of the six affected horses in Iowa.

Bats were sighted on the home farm of 10 of 13 cases and 24 of 59 non-cases; odds ratio for bats as a risk factor was 4.86 (CI = 1.064–25.172, χ^2 p = 0.039). Barn swallows were more ubiquitous. They were present on 13 of 14 case farms and 43 of 65 non-case farms. This association was not statistically significant (odds ratio = 6.65, CI = 0.807–147.065, p = 0.094).

Night lighting, which attracts mayflies, was significantly associated with the risk of PHF, because it was reported on 13 of 15 case farms and 29 of 68 non-case farms (odds ratio = 8.74, CI = 1.664–61.036, p = 0.006). The types of lighting ranged from stall lights on timers to bright security lights left on throughout the night, usually on the outside of the barn or nearby the facility.

4. Discussion

This is the first outbreak of PHF with strong evidence to incriminate mayflies as the vector for *N. risticii*. Based on PCR positives by multiple investigators, mayflies have been postulated to be a potential source of organisms for horses; however, these insects have not yet been successfully used to reproduce the disease.[3,5] Like caddisflies, mayflies can hatch in waterways in huge numbers when environmental conditions suit the species, a fact well recognized by fly fishermen. The newly emerged adults swarm, typically maintaining a stationary position near an environmental marker that is usu-

ally an element of the local landscape and near water. The insects mate in the air above their emergence site, living only hours to a few days at most after the female lays her eggs on the water surface.[9] The southeastern portion of Minnesota is known to have massive hatches seasonally, and swarms are commonly observed on roadways. This has led to the labeling of mayflies as road hazards, and it has prompted construction of river bridges with open grate surfaces. Asphalt provides a reflective surface that is like water, and this attracts female mayflies to oviposit on the surface. The heat retention of this surface also increases the longevity of both the males and females. Mayflies also oviposit on the shiny body work and windscreen of cars as well as shiny black plastic.[9] For similar reasons of light polarity, genera that hatch at dusk may be attracted to night lights such as those used at the Winona show grounds.

The conjunction of weather conditions (heat and wind) with life cycles of trematodes and mayflies may have led to a massive mayfly hatch during the well-attended Winona show. The presence of thousands of mayflies at the facility was unexpected because the farm is >5 mi from the Mississippi River and high in the bluffs. Strong wind could have facilitated movement of mayflies from an aquatic environment into a remote area. This state-of-the-art show facility and arena are enclosed, and the barn aisle doors were closed overnight; however, bright security lights were left on outside the barn overnight, which attracted the insects that then died at the barn in enormous numbers. When the overhead doors were opened, the mayfly carcasses blew into the barn aisles as well as into many horse stalls. Competitors at the show perceived these as a gross nuisance rather than a disease threat to their horses, not knowing that three of six horses that subsequently developed PHF would die or be euthanized because of laminitis. Most swept the carcasses out of the aisles and stalls and tried to shake them out of the hay.

Simultaneous observation of cases of PHF in the surrounding area and in three communities to the south of Winona show that many mayfly hatches occurred along the Mississippi and Iowa rivers. Two of the Iowa farms with PHF cases in 2005 were reported to have had positive cases in preceding years; however, the attending veterinarian had not noted mayflies around these farms. The recurrence of cases at these farms strongly suggests an endemic focus that may bear further epidemiologic scrutiny of the mayfly vector. Because the informational letter they received included a picture of a mayfly, the Minnesota regional horse owners may have been biased toward reporting seeing aquatic insects by the well-publicized mayfly swarm at the Winona show.

The genus of mayfly, *Hexagenia*, has not been reported previously in the two counties (Winona and Houston) of southeastern Minnesota identified in this outbreak. There are confirmed records of both *Hexagenia bilineata* and *Hexagenia limbata* in the Wisconsin counties directly across the Mississippi River from Winona, La Crescent, and New Albin, Iowa. *Hexagenia limbata* has been confirmed in the county south of New Albin and Waukon, Iowa.[10]

Night lights may play an important role in attracting mayflies to equine farms during massive hatches, and they were significantly associated with the risk of being a PHF case in this outbreak.[8] Many farms have outdoor security lighting that is left on all night, particularly at commercial stables. Stall lighting was kept on until late evening using timers on many premises to promote slick hair coats in the show horses. All six of the Iowa cases were on facilities with night lights on the barn or outside near the horse's pasture. If a mayfly hatch alert system were available, these lights could be switched off during periods of risk. Alternatively, horse owners can inspect the ground near nighttime lighting sources. Lastly, there may be alternative types of lights in which the polarity of light is less attractive to the mayfly species.

The roles of bats and barn swallows in these case clusters cannot be definitively determined. The increased proportions of bats and barn swallows observed on case farms versus non-case farms warrants further investigation because they may indicate that there are plentiful insects to support their populations. This observational study does not suggest that these animals should be eliminated from horse farms.

The efficacy of PHF vaccines in preventing clinical disease remains controversial.[8,11] Vaccination histories from horses in this outbreak showed that the vaccination gave poor protection from clinical illness; 37% of cases were currently vaccinated, which is similar to the vaccination frequency of non-case horses attending the Winona show. Two of six Iowa cases were vaccinated 2 mo before the onset of disease, and four of the Minnesota regional cases were vaccinated that spring. Interestingly, all three of the Winona showhorses that died were not currently vaccinated nor was the regional case that died. This perceived lack of efficacy may have led to the decision to not vaccinate horses on the two endemic farms in Iowa that were identified in this study. Because the five non-vaccinated, affected horses had resided on those farms for ≥2 yr, one can presume that they did not develop sufficient natural immunity from previous exposure; however, all of these horses recovered. These vaccination failures may represent variability in an individual's responsiveness to the vaccine antigens or lack of immunogenicity of the vaccines used. Until new vaccines become available, more strategic vaccination to target peak antibody production with highest likelihood of exposure to *N. risticii* may be the most useful tool for reducing disease mortality in endemic areas.

This unusual outbreak of PHF in Minnesota and Iowa horses is the first to incriminate mayflies as

significant vectors for horses. Horses may ingest large numbers of these organisms when swarms are attracted to a horse's environment, particularly by night lighting. Bats were also significantly associated with the risk of clinical disease, but their role in transmitting the organism to horses has yet to be determined. Veterinarians should advise horse owners of the risk posed by mayflies and the steps that can be taken to reduce exposure to *N. risticii*.

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