Definitive Hosts of *Versteria* Tapeworms (Cestoda: Taeniidae) Causing Fatal Infection in North America

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We previously reported fatal infection of a captive Bornean orangutan with metacestodes of a novel taeniid tapeworm, *Versteria* sp. New data implicate mustelids as definitive hosts of these tapeworms in North America. At least 2 parasite genetic lineages circulate in North America, representing separate introductions from Eurasia.

Taeniid tapeworms (Cestoda: Taeniidae) comprise 4 proposed genera: *Taenia*, *Echinococcus*, *Hydatigera*, and *Versteria* (1). Until recently, genetic data were absent for *Versteria* sp. in North America. However, in 2014, we reported an unusual case of fatal metacestode (larval stage of tapeworm) infection in a captive Bornean orangutan (*Pongo pygmaeus*); the causative agent was identified as a novel *Versteria* genotype (2).

As previously described (2), the orangutan was born at a zoo in Colorado, USA, and was rejected by his birth mother. Approximately 10 months later, he was transported to the Milwaukee County Zoo in Milwaukee, Wisconsin, USA, for adoption by a surrogate mother. At ≈5 years of age, he died unexpectedly from acute respiratory distress due to disseminated infection with an unknown agent. A combination of metagenomics and gene-specific DNA sequencing revealed the etiologic agent to be a previously unknown *Versteria* lineage in its larval form.

We obtained wild mustelids (carnivores of the family Mustelidae) from Colorado, near where the animal was born, and Wisconsin, near where the animal died. We targeted mustelids because they are definitive hosts of *V. mustelae* tapeworms in Europe (3) and *V. brachyacantha* tapeworms in Africa (4). Colorado samples were submissions to the Denver Museum of Nature and Science, and Wisconsin samples were obtained from a local fur trapper. We also examined mustelids from Oregon, USA, as part of an ongoing investigation of *Versteria* spp. tapeworms in the Nearctic region and Eurasia.

From 4 mustelids from Colorado (1 otter [*Lontra canadensis*], 2 ermine [*Mustela erminea*], and 1 mink [*Neovison vison*]), we recovered 1 adult tapeworm from a female ermine collected 178 km from where the orangutan was born. From 17 mustelids from Wisconsin (1 mink, 5 long-tailed weasels [*M. f. frenata*], and 11 ermine), we recovered 1 adult tapeworm from a male ermine collected 56 km from where the orangutan died. From 17 mustelids from Oregon (1 mink, 1 ermine, and 15 long-tailed weasels), we recovered 1 adult tapeworm from an adult mink of unknown sex. All tapeworm specimens were fragmented, lacking intact scoleces, or both, which prevented complete morphologic description; however, microscope examination of mature segments from the Wisconsin and Oregon specimens revealed structures consistent with those of parasites in the genus *Versteria* (Figure 1).

We sequenced 396 bp of the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene from the 3 new adult tapeworm specimens according to previously published methods (2). The sequences of the tapeworms from the Colorado ermine and the Oregon mink were 99.5% and 99.2% similar, respectively, to the sequence from the orangutan, placing these new specimens confidently within the same *Versteria* lineage (Figure 2). By contrast, the sequence from the tapeworm from the Wisconsin ermine was only 90.7% similar to the sequence from the orangutan, making it a heretofore unrecognized lineage that clusters more closely with parasites from Eurasia (Figure 2).

To investigate the hosts from which adult tapeworms were recovered, we amplified and sequenced 751 bp of the mustelid cytochrome b (cytb) gene, using DNA extracts from adult tapeworm material. To do this, we used previously published methods (7,8) with modified PCR primers MVZ45_must_F (5′-CAGTNATAGCAACACAGCATTCATAGG-3′) and MVZ14_must_R (5′-GCTTCTCATTTTTGGTTTACAGAC-3′). This effort was successful, demonstrating that adult tapeworm material
Our findings shed light on the origins of the infection that proved fatal to the orangutan. We found an adult *Versteria* sp. tapeworm with a nearly identical DNA sequence in Colorado, where the orangutan was born; however, a *Versteria* sp. tapeworm from Wisconsin, where the orangutan died, was genetically divergent. Moreover, we found no evidence of *Versteria* tapeworms on the grounds of the Milwaukee County Zoo or nearby. Taeniid metacestodes encyst and can remain dormant for years before asexual multiplication (12). We therefore suspect that the orangutan became infected where it was born (Colorado) and
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Carried the latent infection to where it died ≈4 years later (Wisconsin). This animal’s sudden progression to disease remains a mystery, perhaps indicating immune deficiency or another precipitating factor, consistent with reports of disseminated taeniid infection in other hosts (13).

In general, our findings underscore that exotic animals in zoo settings are susceptible to infections harbored by local wildlife and that transport of such animals can complicate inferences about the origins of these locally acquired infections. We reiterate that taeniid tapeworms of the genus *Versteria* should be considered a threat to captive apes (2), and we recommend that wild mustelids, such as ermine and mink, be excluded or removed from the grounds of zoos where apes have access to outdoor environments. Given the close relationship between apes and humans, we also suggest increased vigilance for zoonotic infections.

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**Table 1.** Parasites in wild carnivore feces samples collected on the grounds of and near the Milwaukee County Zoo, Milwaukee, Wisconsin, USA, 2014*

| Parasite                  | Coyote, n = 3 | Long-tailed weasel, n = 6 | Mink, n = 8 | Raccoon, n = 31 | Skunk, n = 3 |
|---------------------------|---------------|---------------------------|-------------|-----------------|--------------|
| Ascarid                   | –             | –                         | –           | 12.9            | –            |
| *Baylisascaris procyonis* | –             | –                         | –           | 35.5            | –            |
| Cestode                   | 33.3          | 50.0                      | 16.7        | 25.8            | 66.7         |
| Coccidia                 | 66.7          | 33.3                      | –           | 22.6            | 33.3         |
| Cystoisospora spp.        | –             | –                         | –           | 3.2             | –            |
| Giardia spp.              | –             | –                         | –           | –               | –            |
| Hookworm                  | –             | –                         | –           | 12.9            | 33.3         |
| Strongylid                | –             | 16.7                      | 33.3        | 12.9            | 33.3         |
| Trichuris spp.            | –             | –                         | 33.3        | 22.6            | –            |
| Other†                    | 66.7          | 33.3                      | 50.0        | 3.2             | –            |

*=, not present.
†Other parasites were unidentified metastrongyles, nematodes, and protozoans.
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Table 2. Parasites identified by DNA sequencing of the cox1 gene in samples of wild carnivore feces collected on the grounds of and near the Milwaukee County Zoo, Milwaukee, Wisconsin, USA, 2014*.

| Host              | GenBank accession no.† | Most similar to         | % Similarity |
|-------------------|------------------------|-------------------------|--------------|
| Mink              | KT223036               | Alaria alata            | 91.2 (HM022221) |
| Raccoon           | KT223037               | Baylisascaris procyonis | 100.0 (KC172104) |
| Long-tailed weasel| KT223038               | Parafilaroides normani  | 89.6 (KJ801815) |
| Skunk             | KT223039               | Taenia crassiceps       | 86.9 (EU445459) |
| Mink              | KT223040               | Toxascaris leonina      | 93.1 (JF780951) |

*Only samples with parasite eggs resembling those of cestodes were tested; mink samples were from a local wildlife rehabilitation center. Sequences matching noncestode parasites indicate nonspecificity of PCR primers; results do not exclude the possibility of mixed infections. cox1, cytochrome c oxidase subunit 1.
†For parasite sequences newly generated during this study.
‡Nucleotide similarity to the most similar sequence in GenBank (accession no. in parentheses) as of June 26, 2015.

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Ms. Lee is a veterinary student who participated in this research as part of the University of Wisconsin-Madison School of Veterinary Medicine’s Summer Scholars Program. Her interests include wildlife medicine, small animal medicine, and infectious disease.

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