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Frequency of Virus Coinfection in Raccoons (*Procyon lotor*) and Striped Skunks (*Mephitis mephitis*) During a Concurrent Rabies and Canine Distemper Outbreak

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ABSTRACT: Rabies and canine distemper virus infections in wildlife share similar presenting signs. Canine distemper virus was detected using real-time PCR of conjunctival swabs in rabies positive raccoons (22/32) and skunks (7/34) during a concurrent rabies and canine distemper outbreak in Ontario, Canada in 2015–16. Coinfections with both viruses should be considered, particularly in distemper endemic areas that are at risk of rabies incursion.

In eastern North America, rabies and canine distemper virus (CDV) are important causes of mortality in wild carnivores, including raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*). Rabies and CDV infections in wildlife share similar presenting signs, including abnormal behavior and death (Deem et al. 2000). Animals that are diagnosed with distemper might not be tested for rabies, particularly in areas where rabies is considered low risk; however, the implications of a rabies diagnosis for human and animal health and wildlife management make accurate identification of all rabies cases an important priority. Coinfections with CDV and rabies virus in raccoons occur (Hamir and Rupprecht 1990; Hamir et al. 1998); however, the frequency of occurrence of rabies and distemper virus coinfections in raccoons and skunks is not known.

Raccoon rabies virus variant recently reemerged in Ontario after a 10-yr absence (Stevenson et al. 2016). This outbreak, centered in the Hamilton area of Ontario, is occurring against the backdrop of an ongoing distemper outbreak. We assessed the frequency of rabies and distemper virus coinfections in raccoons and skunks during concurrent outbreaks.

Samples used in our study were collected between December 2015 and August 2016 from a subsample of raccoons and skunks that were acting strangely or found dead and tested as part of the Ontario Ministry of Natural Resources and Forestry's ongoing rabies surveillance and control efforts in southern Ontario (43°14′N, 79°52′W; Stevenson et al. 2016; Fig. 1). Initial rabies testing was done using direct rapid immunohistochemistry test of brain tissue (Niezgoda and Rupprecht 2006) and confirmed with the fluorescent antibody test at the Canadian Food Inspection Agency, Ottawa Laboratory, Nepean, Ontario (Dean et al. 1996).

Conjunctival swabs for distemper testing by real-time PCR (Elia et al. 2006) were opportunistically collected from 32 rabies-positive and 60 rabies-negative raccoons and 34 rabies-positive and 35 rabies-negative skunks. Swabs were stored at 4 C in TriPure isolation reagent (Roche Applied Science, Mannheim, Germany) for a minimum of 24 h prior to sample processing. Total nucleic acids were extracted from 50 µL aliquots and CDV RNA was detected using a real-time PCR assay as described previously (Ethier et al. 2017). Pearson chi-squared/Fisher exact tests (α=0.05) were used to determine if CDV status differed for rabies-positive and -negative raccoons and skunks.

Canine distemper virus PCR threshold cycle (Ct) values ranged from 17–37 (Table 1). Using a Ct cut-off value of 37, we found 98% of animals (158/161) tested positive for CDV RNA. In our laboratory, a Ct cut-off

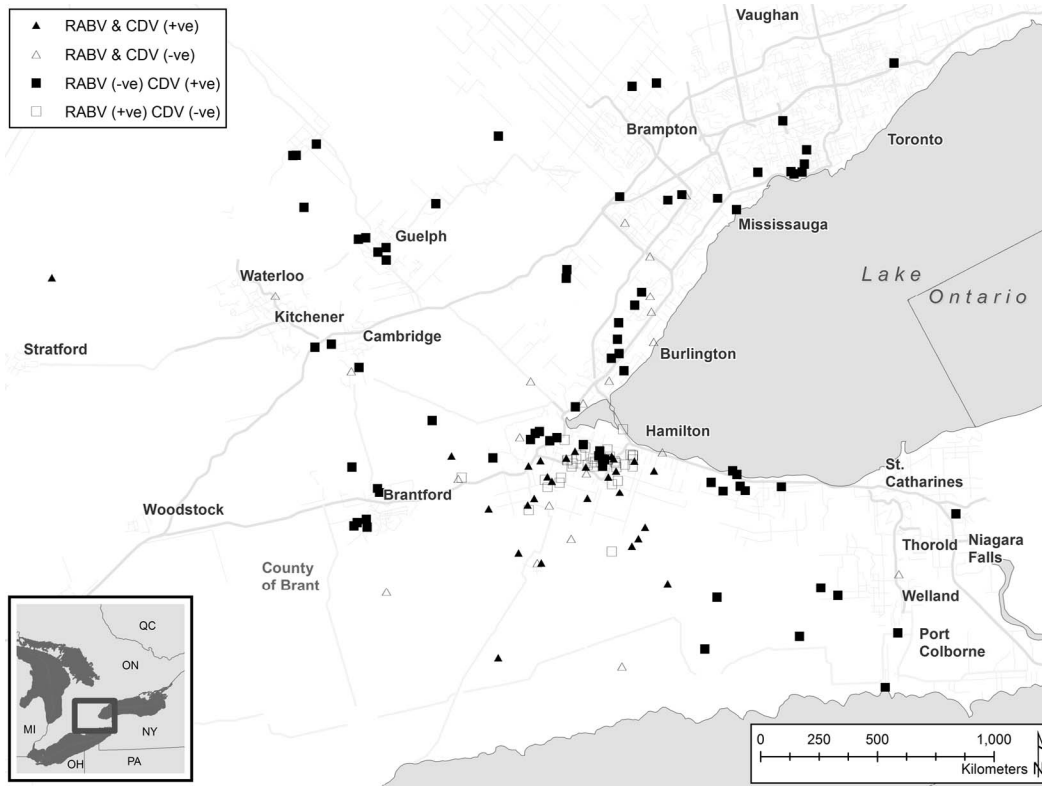

FIGURE 1. Map of southern Ontario, Canada, showing the location of rabies and distemper positive (PCR threshold cycle value ≤30) and negative raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*) sampled between December 2015 to August 2016 during a concurrent rabies and canine distemper outbreak. RABV=rabies virus; CDV=canine distemper virus.

value of ≤30 is consistently associated with obvious pathological evidence of CDV infection and positive CDV immunohistochemistry in lung or lymph node in raccoons (Nemeth et al. 2018). Using a cut-off value of ≤30, 36% (25/69) of skunks and 85% (78/92) of raccoons were positive for CDV (Table 1).

Median Ct values for CDV were lower in rabies-negative animals compared to rabies-positive animals (Table 1). Using a Ct cut-off of ≤30, rabies-negative raccoons and skunks were significantly more likely to be positive for CDV than were rabies-positive animals ($P<0.001$). In total, 78% (74/95) of rabies-

TABLE 1. Median threshold cycle (Ct) values for canine distemper real-time PCR using conjunctival swabs and percentage of animals with Ct values ≤30 for raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*) that tested positive and negative for rabies during a concurrent rabies and canine distemper outbreak in Ontario, Canada in December 2015–August 2016.

Species	Rabies status	No. sampled	Median distemper virus Ct value (range)	Percent of animals with distemper Ct value ≤30 (95% confidence interval)
Raccoon	Positive	32	29 (18–36)	69 (50–53)
	Negative	60	20 (17–32)	93 (83–98)
Skunk	Positive	34	31 (27–36)	21 (9–38)
	Negative	35	29 (18–37)	51 (34–68)

negative animals were CDV-positive (18/35 skunks and 56/60 raccoons), indicating that CDV is an important differential diagnosis for raccoons and skunks suspected of being rabid.

A total of 69% (22/32) of rabies-positive raccoons and 21% (7/34) of rabies-positive skunks were positive for CDV, indicating that concurrent disease can occur commonly, particularly in raccoons. We did not determine if the CDV infection in these raccoons and skunks was acute or chronic. Future studies examining tissues from coinfected animals could help to determine if coinfections occur at all stages of distemper infection; however, experimental infections will be required to get a better understanding of the potential impact of distemper virus infection on the pathogenesis of rabies and vice versa. The high frequency of coinfection in our area, particularly in raccoons, was likely a consequence of the co-occurrence of both a rabies and distemper outbreaks during the study. It will be interesting to see how the frequency of coinfections change as the prevalence of each of these diseases change over time.

Our findings could be useful for researchers who are developing models of the epidemiological dynamics of rabies and CDV transmission (e.g., Nouvellet et al. 2013) and also have important public and animal health implications. Although wildlife surveillance for rabies in cases with human or domestic animal contact was maintained in Ontario during a 10-yr period of absence of raccoon rabies, noncontact raccoons and skunks that tested positive for distemper using PCR were not routinely examined for evidence of rabies infection. The potential implications of missing a rabies diagnosis are serious, and diagnosticians need to be aware of how commonly these viruses can co-occur. This is relevant in areas that are endemic for both rabies and CDV, but could be particularly important in areas that are considered rabies-free (as our study area was prior to this outbreak). Rabies can emerge in these areas (Stevenson et al. 2016) and, particularly if disease in wildlife associated with CDV infections is occurring in the area, rabies cases can be missed, allowing the outbreak to develop unchecked. Our findings illustrate the importance of maintaining vigilance and support for ongoing and comprehensive wildlife disease surveillance activities.

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