Serologic Evidence of Arthropod-Borne Virus Infections in Wild and Captive Ruminants in Ontario, Canada

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Abstract. Arthropod-borne viruses (arboviruses) are globally widespread, and their transmission cycles typically involve numerous vertebrate species. Serologic testing of animal hosts can provide a routine surveillance approach to monitoring animal disease systems, can provide a surveillance alternative to arthropod testing and human case reports, and may augment knowledge of epizootiology. Wild and captive ruminants represent good candidate sentinels to track geographic distribution and prevalence of select arboviruses. They often are geographically widespread and abundant, inhabit areas shared by humans and domestic animals, and are readily fed on by various hematophagous arthropod vectors. Ontario, Canada, is home to high densities of coexisting humans, livestock, and wild cervids, as well as growing numbers of arthropod vectors because of the effects of climate change. We collected blood samples from 349 livestock (cattle/sheep) and 217 cervids (wild/farmed/zoo) in Ontario (2016–2019) to assess for antibodies to zoonotic and agriculturally important arboviruses. Livestock sera were tested for antibodies to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV). Sera from cervids were tested for antibodies to BTV, EHDV, West Nile virus (WNV), eastern equine encephalitis virus (EEEV), Powassan virus (POWV), and heartland virus (HRTV). Fifteen (9.0%) cattle were seropositive for EHDV-serotype 2. Nine (4.2%) cervids were seropositive for arboviruses; three confirmed as WNV, three as EEEV, and one as POWV. All animals were seronegative for BTV and HRTV. These results reveal low seroprevalence of important agricultural, wildlife, and zoonotic pathogens and underline the need for continued surveillance in this and other regions in the face of changing environmental conditions.

INTRODUCTION

Arthropod-borne viruses (arboviruses) comprise a diverse group of pathogens that can infect and cause disease in a range of vertebrate hosts, including humans, livestock, and wildlife. The global geographic range of these viruses generally is closely aligned with the distribution of their associated vectors.^{1,2} Factors such as climate change, urbanization, and global trade can expand the geographic distribution of vectors and associated arboviruses, thus increasing the potential disease burden in susceptible hosts.^{1,3–5} Northern latitudes, such as Ontario, Canada, are especially vulnerable to the negative consequences of northward vector expansion,^{1,5–7} such as the change of vectorial capacity in already established vectors, and in many cases, these areas are also home to naive vertebrate hosts.^{1,5–7}

Despite the potential negative health effects of arboviruses on humans and other animals, the diversity and contributions of various host species to sylvatic transmission cycles in some cases remain poorly understood.^{8,9} There are many strategies regarding arbovirus surveillance that have been used, such as serologic testing of sentinel animals (i.e., chickens) and testing of the vectors themselves.¹⁰ Ruminants represent a diverse group of animals that can play a role in arbovirus life cycles and ecoepidemiology (e.g., as reservoir or maintenance hosts).^{11,12} They may also be useful for tracking the distribution of numerous arboviruses for a number of reasons, especially in light of climate and landscape changes that may contribute to shifts in geographic distribution of hosts and vectors. For instance, white-tailed deer (*Odocoileus virginianus*)

are widespread across much of North America, are fed on by numerous hematophagous arthropod vectors, and have relatively limited home range sizes.^{13,14} In addition, they use a variety of habitats, some of which are in close association with humans and livestock.^{13,14} Domestic livestock are also widely distributed and fed on by disease vectors and often remain on one or few farms for much of their lives, with their movements often traceable.

We aimed to assess the potential utility of ruminants (free-ranging, farmed, and zoo) as sentinels for select arboviruses of agricultural, wildlife, and public health significance. In particular, we sought to better understand the epidemiology of these potential arbovirus hosts across the landscape of Southern Ontario, Canada, where humans, livestock, and wildlife live in close proximity. Consequently, we identified the following objectives: 1) to determine the seroprevalence of bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV; family *Reoviridae*; genus *Orbivirus*) in ruminants (i.e., farmed cattle and sheep; wild, farmed, and zoo cervids) in Ontario and 2) to examine the geographic distribution of wild, farmed, and zoo cervids with antibodies to West Nile virus (WNV; family *Flaviviridae*; genus *Flavivirus*), eastern equine encephalitis virus (EEEV; family *Togaviridae*; genus *Alphavirus*), Powassan virus (POWV; family *Flaviviridae*; genus *Flavivirus*), and heartland virus (HRTV; family *Bunyaviridae*; genus *Phlebovirus*) in Ontario, Canada.

MATERIALS AND METHODS

Farm site selection and sample collection. Blood was collected from cattle and sheep at 12 sites (representing 11 farms) in Southern Ontario for two consecutive field seasons (i.e., January 2017–December 2017; and January 2018–February 2019). One of the participating farms changed

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locations by approximately 6 km between the first and second field seasons. Farms were selected based on the following criteria: cattle or sheep focused, with primarily outdoor housing and geography (i.e., sites close to the Canadian/American border based on recent orbiviral activity detected in the United States). Among the 11 participating farms, six primarily raised cattle (five beef and one dairy cattle operation) and five were sheep farms (Figure 1). Two blood collection efforts occurred on each farm site, the first in 2017 and again in 2018–2019, and were opportunistic based on available animals. Venipuncture sites were the coccygeal vein and jugular vein in cattle and sheep, respectively, and 5 mL of whole blood was collected into 6-mL red-top vacutainer tubes (BD Vacutainer, Mississauga, Canada) from each animal. Only animals older than 6 months and born in Ontario with no history of travel outside the province were included. All animals were apparently healthy at the time of sampling.

Blood samples were opportunistically collected across mainly Southern Ontario from wild, farmed, and zoo cervids from December 2016 to January 2019. The cervid species sampled included white-tailed deer, red deer (*Cervus elaphus*), elk (i.e., wapiti; *Cervus canadensis*), elk–red deer hybrids (*C. canadensis* × *C. elaphus*), moose (*Alces alces*), and woodland caribou (*Rangifer tarandus caribou*). Blood was collected from hunter-harvested and road-killed white-tailed deer carcasses during routine surveillance efforts by the Ontario Ministry of Natural Resources and Forestry, by licensed hunters; from members of the public; and from diagnostic cases submitted to the Canadian Wildlife Health Cooperative, Ontario/Nunavut node. In addition, blood was also collected from farmed cervid carcasses (i.e., red deer and elk–red deer hybrids) at Ontario abattoirs, and captive elk, moose, and woodland caribou at a zoological park. The blood

samples were absorbed into Nobuto blood filter strips (Advantec MFS, Inc., Dublin, CA; hereafter, filter strips) either by immersing the filter strip into pooled fresh blood or by placing whole blood onto filter strips via syringe. The blood samples that were opportunistically collected from cervid species at the zoological park occurred during routine handling, as per the same methods as those described earlier under livestock. Live animal handling and sample collection methods were conducted under institutional animal care and use committee approval (protocol AUP#3529; University of Guelph, Guelph, Canada).

Sample storage and serologic testing. Whole blood samples from cattle and sheep were stored at 4°C for ≤ 24 hours and centrifuged at 2,200 × *g* for 15 minutes for separation of serum. Sera samples were aliquoted and stored at –20°C until testing. The filter strips were dried completely (≥ 24 hours at room temperature), then stored in paper envelopes at room temperature for 1 month, and then stored at –20°C until testing. For BTV and EHDV testing (from cattle and sheep), frozen serum samples were shipped overnight on blue ice packs and filter strips (from captive and wild cervids) at ambient temperature to the National Centre for Foreign Animal Disease (NCFAD) in Winnipeg, Manitoba, Canada, for sample elution (filter strips) and serologic testing. For WNV, EEEV, POWV, and HRTV testing, filter strips were shipped at ambient temperature to the Southeastern Cooperative Wildlife Disease Study at the University of Georgia and to the Animal Disease Laboratory at Colorado State University. In all cases, sera were eluted from filter strips as previously described,¹⁵ resulting in a 1:10 serum dilution.

Laboratory testing. All serum samples and filter eluates were heat-inactivated at 56°C for 30 minutes before testing. At the NCFAD, the serum and filter eluates were tested for

FIGURE 1. Distribution of farm sites ($N = 12$; R number indicates specific site) from which livestock serum samples were tested for antibodies to bluetongue virus and epizootic hemorrhagic disease virus in Southern Ontario, Canada (2017–2019).

antibodies to BTV and EHDV by virus-specific blocking and competitive ELISA as previously described.¹⁶ Positive samples were subsequently tested for BTV- and EHDV-neutralizing antibodies by serum neutralization assay for BTV-2, 8, 10, 11, 13, and 17, and EHDV-1 and 2 as previously described.¹⁷ All cervid samples with sufficient remaining volume were screened for antibodies to WNV, EEEV, POWV, and HRTV. For WNV serologic testing, eluates were screened for WNV-neutralizing antibodies at a 1:20 dilution by the plaque reduction neutralization test (PRNT). Samples with $\geq 90\%$ neutralization were further tested to determine the causative virus as WNV or St. Louis encephalitis virus (SLEV) by a 4-fold or greater PRNT₉₀ titer. If a 4-fold difference could not be demonstrated, the sample was considered anti-flavivirus antibody positive.^{18–20} For EEEV PRNT, a Sindbis virus–EEEV chimera with structural proteins derived from EEEV prM/E was used as previously described.²¹ Eluates were screened for EEEV-neutralizing antibodies at a 1:20 dilution and were considered positive at $\geq 80\%$ neutralization. For POWV PRNT, a deer tick virus (DTV)–WNV chimera with structural proteins derived from DTV was used to screen for POWV as previously described.¹¹ Eluates were screened for POWV-neutralizing antibodies at a 1:20 dilution and were considered positive at $\geq 80\%$ neutralization. For both EEEV and POWV samples that screened antibody positive, serial 2-fold dilutions were performed (from 1:20 to 1:320) to determine PRNT₈₀ titers, and the positive result was confirmed at a PRNT₈₀ titer of $\geq 1:20$. Eluates were also screened for antibodies to HRTV by the serum microneutralization test.²² The samples were challenged with $\sim 1,000$ tissue culture infectious dose of HRTV suspension; eluates were incubated with virus (at a 1:20 serum dilution) for 1 hour at 37°C and 5% CO₂, and wells were subsequently seeded with Vero E6 cells and incubated at

37°C for 8 days. The samples were considered positive at $\geq 50\%$ neutralization.¹²

Statistical analysis. Prevalence and exact CIs were estimated using STATA14 Intercooled (StataCorp, College Station, TX). A 95% CI was used for positive test results, and a 97.5% CI was used when no positive results occurred.

RESULTS

Blood was collected from a total of 349 farmed cattle and sheep from 12 farm sites; 167 animals were sampled in 2017 and 182 animals in 2018–2019. For both sampling seasons, beef cattle made up the largest sample group (229/349; 65.6%), followed by sheep (108/349; 30.9%) and then dairy cattle (12/349; 3.4%). For 2017, initial screening by ELISA detected antibodies to EHDV in 8.9% (15/167) of the samples, which was confirmed as EHDV-serotype 2 by the serum neutralization test in all cases (Figure 1). In 2018–2019, no antibodies to EHDV or BTV were detected in any livestock sample.

Samples were collected from a total of 217 cervids across Ontario (Figure 2), of which the wild cervids comprised the majority (167/217; 77.0%), followed by farmed (40/217; 18.4%) and zoo animals (10/217; 4.6%). Most samples were from white-tailed deer, followed by elk–red deer hybrids, elk, moose, woodland caribou, and red deer (Table 1). Among samples tested for antibodies to WNV ($N = 213$), 4.2% (9/213; 95% CI: 1.9–7.8; woodland caribou [$N = 2$], white-tailed deer [$N = 7$]) had anti-flavivirus antibodies, three of which were determined to be WNV; WNV was not distinguishable from SLEV for six samples presumably because of virus cross-reactivity.²³ In addition, antibodies to POWV and EEEV ($N = 217$, each) were identified in 0.5% (1/217; 95% CI: 0.0–2.5; elk

FIGURE 2. Distribution of cervids (wild, farmed, and zoo) that were sampled and tested for antibodies to bluetongue virus, epizootic hemorrhagic disease virus, *Flavivirus*/West Nile virus (WNV), Powassan virus (POWV), eastern equine encephalitis virus (EEEV), and heartland virus in Ontario, Canada (2016–2019). Seropositive samples based on counties/districts: *Flavivirus*/WNV (York County, $N = 3$; Elgin County, $N = 1$; Muskoka district, $N = 1$; Oxford County, $N = 1$; Welland County, $N = 1$; Wentworth County, $N = 1$; Stormont, Dundas, and Glengarry counties, $N = 1$), EEEV (Lincoln County, $N = 3$), and POWV (York County, $N = 1$).

TABLE 1
Seroprevalence of numerous arthropod-borne viruses of public health or agriculture importance in cervids in Ontario, Canada, from 2016 to 2019*

Species†	2016–2017			2018–2019		
	WNV‡, no. positive/N (%); 95% CI§	EEEV, no. positive/N (%); 95% CI	POWV, no. positive/N (%); 95% CI	WNV, no. positive/N (%); 95% CI	EEEV, no. positive/N (%); 95% CI	POWV, no. positive/N (%); 95% CI
White-tailed deer (<i>O. virginianus</i>)	0/83 (0.0); 0.0–4.3	0/86 (0.0); 0.0–4.2	0/86 (0.0); 0.0–4.2	7/81 (8.6); 3.5–17.0	3/82 (3.7); 0.7–10.3	0/82 (0.0); 0.0–4.4
Red deer (<i>C. elaphus</i>)	0/2 (0.0); 0.0–84.1	0/2 (0.0); 0.0–84.1	0/2 (0.0); 0.0–84.1	NT	NT	NT
Elk-red deer hybrid (<i>C. canadensis</i> × <i>C. elaphus</i>)	0/17 (0.0); 0.0–19.5	0/17 (0.0); 0.0–19.5	0/17 (0.0); 0.0–19.5	0/20 (0.0); 0.0–16.8	0/20 (0.0); 0.0–16.8	0/20 (0.0); 0.0–16.8
Elk (<i>C. canadensis</i>)	0/4 (0.0); 0.0–60.2	0/4 (0.0); 0.0–60.2	1/4 (25.0); 0.6–80.5	NT	NT	NT
Woodland caribou (<i>R. tarandus caribou</i>)	2/3 (66.7); 9.4–99.1	0/3 (0.0); 0.0–70.7	0/3 (0.0); 0.0–70.7	NT	NT	NT
Moose (<i>A. alces</i>)	0/3 (0.0); 0.0–70.7	0/3 (0.0); 0.0–70.7	0/3 (0.0); 0.0–70.7	NT	NT	NT
Total	2/112 (1.8); 0.2–6.3	0/115 (0.0); 0.0–3.1	1/115 (0.9); 0.0–4.7	7/101 (6.9); 2.8–13.8	3/102 (2.9); 0.6–8.3	0/102 (0.0); 0.0–3.6

A. alces = *Alces alces*; C. canadensis = *Cervus canadensis*; C. elaphus = *Cervus elaphus*; EEEV = eastern equine encephalitis virus; NT = not tested; O. virginianus = *Odocoileus virginianus*; POWV = Powassan virus; R. tarandus caribou = *Rangifer tarandus caribou*; WNV = West Nile virus.

*None of the serum samples from cervids had evidence of antibodies to heartland virus, bluetongue virus, or epizootic hemorrhagic disease virus.

†White-tailed deer (*O. virginianus*; N = 167 wild; N = 1 farmed), red deer (*C. elaphus*; farmed), elk-red deer hybrid (*C. canadensis* × *C. elaphus*; farmed), elk (*C. canadensis*; zoo), woodland caribou (*R. tarandus caribou*; zoo), and moose (*A. alces*; zoo).

‡Nine samples had anti-flavivirus antibodies, three of which were determined to be WNV; WNV was not distinguishable from St. Louis encephalitis virus for six samples because of presumed virus cross-reactivity.²³

§95% CI was used for positive test results, and 97.5% CI was used when no positive results occurred.

[N = 1]) and 1.4% (3/217; 95% CI: 0.2–3.9; white-tailed deer [N = 3]) of ungulates, respectively (Table 1). None of the cervid samples tested had evidence of antibodies to HRTV (N = 212), EHDV (N = 217), or BTV (N = 217; Table 1).

DISCUSSION

This study revealed a small percentage of sampled cervids with circulating antibodies to WNV, EEEV, and POWV in Ontario, Canada. In addition to the detection of antibodies to zoonotic, vector-borne pathogens in cervids, resident livestock had antibodies to EHDV-serotype 2. Sample collection in the present study serendipitously and temporally encompassed a small outbreak of EHDV-serotype 2 in white-tailed deer in the fall of 2017 in Southern Ontario.²⁴ The EHDV-serotype 2-seropositive beef cattle were sampled in regions adjacent to and within the vicinity of this outbreak. This EHDV-serotype 2 activity in Ontario wildlife and livestock represents a thus far rarely documented event at this latitude in North America. However, reported arbovirus infections in northern latitudes, including hemorrhagic disease-causing orbiviruses, have increased in recent years, suggesting a northern expansion.^{5,25} The expanding geographic range of these agriculturally relevant, vector-borne viruses in part has been attributed to changing climate and landscape conditions that favor vector survival and establishment.⁵

Globally, the geographic ranges of arboviruses are changing based on human activities (e.g., human travel via air and other means, and commerce involving livestock movement) and climate change.^{1,2,5} In Canada, WNV is currently the leading cause of mosquito-borne diseases in humans, after being first identified in Ontario in 2001 and subsequently spreading throughout most of the province.^{26–28} Conversely, EEEV activity generally is restricted to southeastern Canada (e.g., Ontario and Québec), despite the earliest documented evidence in Ontario in moribund horses in 1938 and recently documented incursions into Southern and Eastern Ontario.²⁹ Among the arboviruses assessed in the present study, only WNV and EEEV activities are tracked consistently in Ontario; both mosquito pool testing and veterinary (equine) cases are used.^{30,31} In the case of EEEV, it appears that the results differ between the two sample types (pools and equine cases) year to year (2002–2018).³¹ Human cases of POWV have been documented in the United States, Canada, and Russia; in Canada, POWV is endemic in Ontario, mainly across the northern and southern portions, with evidence of transmission in Quebec, Alberta, and British Columbia.³² Heartland virus was first identified in 2009 in Missouri, with serological evidence of a broader distribution throughout the Midwestern United States.^{33,34} Although there is no evidence of HRTV circulation in Ontario to date, the province is home to a number of mammalian species that could potentially act as reservoir hosts, and the primary tick vector (*Amblyomma americanum*) has sporadically been identified in the province through passive surveillance.³⁵ Finally, BTV and EHDV are undergoing northward geographic expansion into novel environments with abundant immunologically naive hosts on multiple continents.^{36,37} There have been recent outbreaks of BTV and EHDV serotypes among livestock and wildlife in Europe, the United States, the Middle East, and more recently in Ontario, Canada.^{2,24,38,39} In Canada, EHDV has been sporadically detected in the southern portions of British Columbia, Alberta,

and occasionally Saskatchewan.^{25,40,41} Evidence of BTV transmission is thought to be limited to the Okanagan Valley, British Columbia,^{40,42,43} and Ontario is mostly considered BTV and EHDV free, with rare serologic detections in dairy cattle (BTV-serotype 13) and clinical disease in wild white-tailed deer (EHDV-serotype 2).^{24,44} Continued surveillance is important to tracking any future changes in orbivirus activity in Southern Canada.

The role that ruminants play in the transmission and ecology of arboviruses varies and is poorly understood. However, ruminants, especially local livestock (i.e., not transported out of home region), and wildlife with relatively defined home ranges, may serve as useful arbovirus sentinels and, as such, aid in tracking virus spread.¹⁴ Although birds are the primary maintenance hosts for WNV and EEEV, spillover infections occur in mammals, including cervids,⁴⁵ which generally are dead-end hosts.^{14,45–47} In Eastern Canada, *Culex pipiens* and *Culex restuans* are considered important WNV vectors, and for EEEV, *Culiseta melanura* is an important vector.^{5,46,48} Although *Cx. pipiens* and *Cs. melanura* can feed on white-tailed deer, these species are generally ornithophilic.^{49,50} In the case of tick-borne viruses, such as POWV and HRTV, cervids tend to primarily serve as a food source and are a means for transporting ticks to new geographic areas.^{12,51,52} Powassan virus is primarily transmitted by the black-legged tick (*Ixodes scapularis*) and groundhog tick (*Ixodes cookei*), and HRTV primarily by the lone star tick (*A. americanum*).^{1,34,53} White-tailed deer have been identified as a host for adult *I. scapularis* and all *A. americanum* life stages.^{1,34,53} For some *Culicoides*-borne viruses, such as BTV and EHDV, it is thought that wild ruminants may play a role as amplifying hosts and viral reservoirs.^{54,55}

Serologic studies suggest subclinical WNV, POWV, EEEV, and HRTV infections in various wild cervids may occur; however, the potential health effects of these viral infections are not well understood.^{47,56,57} The cervid serologic results from the present study appear to be relatively consistent with that of the neighboring state of Michigan, the United States but vary with other northeastern states.^{14,21,58} Fatalities and clinical disease resulting from infection with WNV or EEEV have been documented in white-tailed deer^{56,57,59,60}; however, they are rare and tend to result in neurological disease (e.g., tremors and ataxia). Evidence of WNV infection, without known clinical disease, has been reported in white-tailed deer from a number of U.S. states and a captive woodland caribou herd from Alberta, Canada.^{14,45,61} Eastern equine encephalitis virus infection in white-tailed deer and moose in a number of U.S. states and Quebec, Canada, has been reported.^{14,21,58,62–64} Cervids, especially wild cervids, are not commonly assessed for anti-POWV antibodies, and thus, the present data serve as a baseline from which to make future comparisons.¹⁴ Although there have been a number of recent white-tailed deer serosurveys for HRTV, morbidity and mortality in cervids have not yet been documented.^{12,34,65}

Both BTV and EHDV infect a wide variety of wild and domestic ruminants and cause a range of health effects.^{2,66} In general, BTV can cause severe morbidity and/or mortality in sheep and deer, but clinical disease is rare in other species such as cattle.^{67–69} Acute infections can lead to lethargy, pyrexia, multiorgan hemorrhage, and edema.^{2,70,71} For EHDV, white-tailed deer are the most severely affected species, although outbreaks have been reported in a variety of other wild

ruminant species (e.g., pronghorn [*Antilocapra americana*] and mule deer [*Odocoileus hemionus*]).^{2,25} Clinical signs in wild cervids with EHDV and BTV are similar to those of BTV in sheep and can result in large-scale outbreaks, causing severe morbidity and/or mortality in deer populations across North America.^{2,25} Thus, BTV and EHDV have the potential to cause high levels of morbidity and/or mortality in livestock and/or wildlife. The identification of antibodies to EHDV-2 in Ontario livestock was limited spatially and was believed to have been an extension, likely representing the northern range, of a concurrent outbreak in the United States in 2017.²⁴ No antibodies to BTV were identified among deer or livestock in the present study and have only been documented once previously in Ontario in three dairy cattle.⁴⁴ Previous studies in cattle in non-endemic BTV areas in the United States revealed a low (< 1%) seropositive rate.^{2,72}

In the present study, we recruited biologists, hunters, and conservationists to help with blood sample collection from wild and captive cervids onto filter strips. This allowed for opportunistic sample collection from higher numbers of deer and from a broader region than would have otherwise been possible. Although the use of these strips has been documented in numerous wildlife species for the purpose of serologic testing for a variety of infectious agents,⁶¹ there remain numerous challenges. For example, sensitivity may be lowered for some serologic assays.¹⁵ In addition, sample quality can be affected by varying postmortem conditions, collection methods, and storage conditions.^{73,74} Finally, because of presumed cross-reactivity while testing for anti-flavivirus antibodies in the present study (i.e., WNV versus SLEV), some samples were not able to be confirmed as WNV-induced, although they were suspected as such because of evidence of WNV circulation in Southern Ontario during the study period^{30,31} and the lack of SLEV activity reported in Southern Ontario since 1976.⁷⁵

This study identified rare occurrences of WNV, EEEV, POWV, and EHDV-serotype 2 in farmed, wild, and zoo ruminants in Southern Ontario, Canada. Because of the likely increasing risk of vector-borne zoonotic, wildlife, and agricultural arboviruses in Ontario, Canada, as well as other in northern latitudes globally, continued surveillance and monitoring are imperative. Such studies may allow for early or otherwise undetected evidence of pathogen transmission and, thus, provide opportunities to mitigate risk of infections to humans, wildlife, and livestock. This serologic testing, including opportunistic, is an important option, especially in scenarios in which arthropod testing may not provide accurate predictions of vertebrate cases. Future serosurveillance and targeted research that encompass vectors, wildlife, and domestic animals will continue to increase our understanding of the risk of outbreaks and epidemiologic patterns over diverse and dynamic landscapes, as the virus and vector relationships continue to evolve.

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