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LEPTOSPIRA, PARVOVIRUS, AND *TOXOPLASMA* IN THE NORTH AMERICAN RIVER OTTER (*LONTRA CANADENSIS*) IN NORTH CAROLINA, USA

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ABSTRACT: The North American river otter (*Lontra canadensis*) is the largest mustelid in North Carolina, US, and was once extirpated from the central and western portions of the state. Over time and after a successful reintroduction project, otters are now abundant and occur throughout North Carolina. However, there is a concern that diseases may have an impact on the otter population, as well as on other aquatic mammals, either through exposure to emerging diseases, contact with domestic animals such as domestic cats (*Felis catus*), or less robust condition of individuals through declines in water quality. We tested brain and kidney tissue from harvested otters for the pathogens that cause leptospirosis, parvovirus, and toxoplasmosis. Leptospirosis and toxoplasmosis are priority zoonoses and are maintained by domestic and wild mammals. Although parvovirus is not zoonotic, it does affect pets, causing mild to fatal symptoms. Across the 2014–15 and 2015–16 trapping seasons, we tested 220 otters (76 females, 144 males) using real-time PCR for *Leptospira interrogans*, parvovirus, and *Toxoplasma gondii*. Of the otters tested, 1% (3/220) were positive for *L. interrogans*, 19% (41/220) were positive for parvovirus, and 24% (53/220) were positive for *T. gondii*. Although the pathogens for parvovirus and toxoplasmosis are relatively common in North Carolina otters, the otter harvest has remained steady and the population appears to be abundant and self-sustaining. Therefore, parvovirus and toxoplasmosis do not currently appear to be negatively impacting the population. However, subsequent research should examine transmission parameters between domestic and wild species and the sublethal effects of infection.

Key words: Disease, leptospirosis, *Lontra canadensis*, North Carolina, otter, parvovirus, toxoplasmosis.

INTRODUCTION

The North American river otter (*Lontra canadensis*) is the largest mustelid inhabiting North Carolina, US. Otters were extirpated from the mountains and most of the Piedmont of North Carolina by the early 1900s, with small surviving pockets in some areas. Otters were successfully reintroduced to the western North Carolina mountains from the eastern North Carolina coastal plain during the 1990s (Spelman 1998). After the population recovered, an otter trapping season was opened in the mountains in November 2005, and bag limits (maximum number of animals allowed to be harvested by an individual) were removed in November 2009. Today, otter populations throughout North Carolina are believed to be abundant and self-sustaining. The International Union for Conservation of Nature Red List categorizes five of 13 otter species as endangered, with only *L. canadensis* listed as least concern and stable (Serfass et al. 2015). Studies of the North American river otter are important because they potentially provide information for vulnerable otter species (Kimber and Kollias 2000).

The International Union for Conservation of Nature and Natural Resources/Species Survival Commission Otter Specialist Group does not outline disease as a direct threat to global otter populations (Foster-Turley et al. 1990). However, it is vital to monitor diseases because they may regulate local populations (Kimber and Kollias 2000). Although some

diseases can have regulatory or even catastrophic effects on populations (Anderson and May 1978; May and Anderson 1978), they rarely cause extirpations or extinctions. Further, mustelids are regularly exposed to numerous diseases and their pathogens (Philippa et al. 2008; Barros et al. 2018; Akdesir et al. 2018), and it is possible for a disease to weaken local populations, making them vulnerable to stochastic events (Lafferty and Gerber 2002).

Leptospirosis is a bacterial zoonotic disease caused by an aerobic spirochete (*Leptospira interrogans*) and maintained globally by mammals, reptiles, and amphibians (Kimber and Kollias 2000; Plank and Dean 2000; Fouts et al. 2016). Infected animals shed leptospires in urine (Plank and Dean 2000), allowing humans and wildlife species to encounter leptospires through contaminated soil, water, animal tissue, or animal bites (Lecour et al. 1989; Everard et al. 1995; Faisal et al. 2012). Because otters are semiaquatic, infected water sources associated with urban-suburban areas may be detrimental (Gautam et al. 2010). Additionally, leptospirosis has been recorded in many mustelid species (Moinet et al. 2010), black bears (*Ursus americanus*) associated with urban areas (Sasmal et al. 2019) and is fatal to sea otters (*Enhydra lutris*; White et al. 2018).

Parvovirus is a highly contagious genus of viruses identified in the 20th century that spreads in many vertebrates, including mammals, through direct contact with an infected animal or by indirect contact with contaminated objects or feces (Parrish 1990; Goddard and Leisewitz 2010). Although parvovirus is not zoonotic, it can cause mild to fatal symptoms in pets, may affect reproduction (Parrish 1990; Kostro et al. 2014), and is capable of infecting other species (Allison et al. 2014; Nituch et al. 2015). Interestingly, canine parvovirus has had devastating effects on gray wolf populations (Mech and Goyal 1995; Mech et al. 2008) and is lethal to Asian small-clawed otters (*Aonyx cinerea*; Gjeltema et al. 2015).

Toxoplasma gondii is a eukaryote parasite that causes the zoonotic disease toxoplasmosis (Dubey 2008). Toxoplasmosis is globally distributed, but most hosts are asymptomatic. Outdoor cats serve as the definitive host, but many species (e.g., crayfish, fish, geese, mice, mussels, oysters, pigs) are intermediate hosts (Dubey 1996; Massie et al. 2010; Cenci-Goga et al. 2011; Sandfoss et al. 2011). *Toxoplasma gondii* moves from its feline host to other species most commonly through ingestion of meat or water contaminated by cat feces (Vanwormer et al. 2013) and may be vertically transmitted from mother to offspring (Parameswaran et al. 2009; Gontijo da Silva et al. 2015; Vargas-Villavicencio et al. 2016). Sea otter exposure to *T. gondii* may be at least partially influenced by freshwater runoff (Miller et al. 2002; Conrad et al. 2005), and Shapiro et al. (2012) determined that *T. gondii* was the cause of death in 14% of sea otters tested in central California. Additionally, human population density has been connected to *T. gondii* rates in sea otters (Gaydos et al. 2007) and southern river otters (*Lontra provocax*; Barros et al. 2018).

Detection of *L. interrogans*, parvovirus, and *T. gondii* in otters may reveal a possible transmission risk between wildlife, domestic species, and humans; may be indicative of exposure to aquatic mammals (e.g., muskrat [*Ondatra zibethicus*], American beaver [*Castor canadensis*], American mink [*Neovison vison*]); and may highlight the impacts of humans and domestic species on wild populations. Therefore, our objective was to survey the otter population to determine the prevalence of *L. interrogans*, parvovirus, and *T. gondii* across the three furbearer management units (FMUs; i.e., Mountain, Piedmont, and Coastal Plain) and 14 river basins of North Carolina. Additionally, we determined if sex or age were important covariates for determining the probability of infection.

MATERIALS AND METHODS

Study area

We conducted our study across the entire state of North Carolina. The North Carolina Wildlife Resources Commission divided the state into three FMUs (i.e., Mountain, Piedmont, and Coastal Plain). The FMUs followed physiographic regions and county boundaries (Fig. 1). However, because otters are semiaquatic, their territories

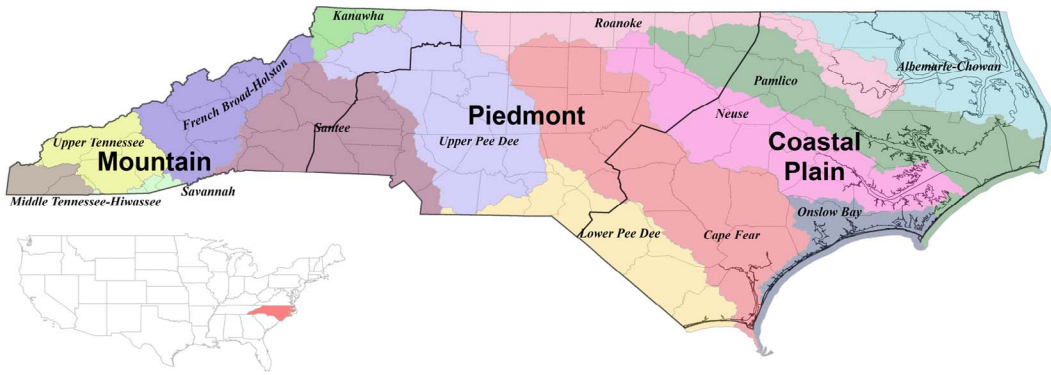

FIGURE 1. River basins and furbearer management units in North Carolina, USA, including the Outer Banks, during the 2014–15 and 2015–16 trapping seasons.

are linear and tend to correspond with river basin geographic features (Melquist and Dronkert 1987; Reid et al. 1994; Sauer et al. 1999), we also focused our study on the 14 river basins that occur throughout North Carolina (Fig. 1). Several river basins occur in multiple FMUs, including the Santee and Upper Pee Dee basins, which span the Mountain and Piedmont FMUs, and the Lower Pee Dee, Cape Fear, Neuse, Pamlico, and Roanoke basins, which span the Piedmont and Coastal Plain FMUs (Fig. 1).

Data collection

During the 2014–15 and 2015–16 regulated trapping seasons, we collected otter carcasses from licensed trappers across North Carolina, mostly during January and February. We recorded the date and location trapped, including specific coordinates, addresses, or a general description of the trap site location. General descriptions included the county, locality, roads, and any prominent landmarks.

Trappers froze the carcasses after pelting and we kept all carcasses frozen prior to necropsy. We extracted a lower canine tooth, which was sent to Matson's Laboratory (Manhattan, Montana, USA) for cementum annuli aging (Stephenson 1977). Brain and kidney tissue were chosen because *L. interrogans*, parvovirus, and *T. gondii* appear readily in these tissues (Parrish 1990; Cenci-Goga et al. 2011; Adler 2015). We removed 5 g of brain tissue and 2 g of kidney tissue; tissue samples were frozen until analysis.

We used IDEXX Laboratories (Columbia, Missouri, USA) for real-time PCR testing for *L. interrogans,* parvovirus, and *T. gondii*. We extracted total nucleic acids from brains and kidneys with standard protocols using a commercially available platform (One-For-All Vet Kit, Qiagen, Valencia, California, USA). The canine parvovirus 2 and *T. gondii* PCR assays were based on the IDEXX BioResearch proprietary service platform (IDEXX Laboratories, Inc., Westbrook, Maine, USA) and used a FAM/TAMRA-labeled hydrolysis probe.

Assays passed analytical validation by being tested in triplicate against dilutions of a known positive control and a known positive clinical case sample with the following criteria being met and reproduced on different run days: amplification efficiency of 95–105%, linearity over five points, calculated coefficient of variation of crossing points equal to or smaller than 3%, r^2 value equal to or larger than 0.993, signal to noise ratio of fluorescent signal $\geq$10, and analytical sensitivity of 10 molecules or less per PCR reaction. Assays passed clinical validation by being tested against well-characterized clinical samples. Sequence analyses were performed on select positive samples during assay validation to confirm the amplification of the intended target.

We used a hydrolysis probe–based real-time PCR targeting a housekeeping gene (18S ribosomal RNA) to determine the amount of genomic DNA present in the test sample, confirm DNA integrity, and ensure the absence of PCR inhibitors. We performed diagnostic real-time PCR with a standard primer and probe concentrations using the LC480 ProbesMaster (Roche Applied Science, Indianapolis, Indiana, USA) on the Roche LightCycler 480 (Roche Applied Science). Because brain and kidney tissues are suited for individual diseases and often used in the literature for disease evaluation, we considered specimens positive for a disease if either tissue sample was positive.

Data management and modeling

We used the SAS GENMOD procedure to predict the maximum likelihood of an otter being positive for the disease using logistic regression in

TABLE 1. PCR test results of *Leptospira interrogans*, parvovirus, and *Toxoplasma gondii* for 220 river otters (*Lontra canadensis*) collected from trappers in North Carolina, USA, during the 2014–15 and 2015–16 trapping seasons.

	% PCR-positive tissue samples			No. PCR-positive otters by tissue			
Pathogen	Total	Brain	Kidney	Total	Brain	Kidney	Both tissues
L. interrogans	1.4	0	1.4	3	0	3	0
Parvovirus	18.6	6.4	16.8	41	14	37	10
T. gondii	24.1	24.1	0	53	53	0	0

a generalized linear model (SAS Institute, Inc., Cary, North Carolina, USA). There was no effect of year on prevalence rates for any pathogens (*L. interrogans*: t=–1.75, df=87, P=0.0832; parvovirus: t=0.14, df=218, P=0.8882; *T. gondii*: t=–0.90, df=218, P=0.3698), so we combined years for all analyses. We treated age (0–13 yr old based on cementum annuli) as a numeric variable with sex, river basin, and FMU as classification variables. All ages were assigned based on a date of birth of 1 April annually. Age classifications were assigned by year up to year 4, after which all otters age 4 or greater were combined into a single age class because of low sample sizes. We limited our candidate model set to four a priori covariates to avoid including spurious effects. We used Akaike's information criterion (AIC) to assess model weights and rank candidate models (Burnham and Anderson 2002). We developed relative support for the models by using Akaike weights and then calculated the unconditional variance estimates with their associated 95% confidence interval (Burnham and Anderson 2002; Anderson 2008). We ignored noninformative parameters within 2 change in AIC (ΔAIC) units of the top model (Arnold 2010).

We used indicator kriging to predict the probability of testing positive for parvovirus and *T. gondii* throughout North Carolina. We created the kriging models in ArcGIS 10.3 with the Geostatistical Analyst Wizard (Esri, Redlands, California, USA). We set our threshold value to zero and optimized the semivariogram. We used the standard neighbor type with eight sectors. For parvovirus, we used a maximum of 10 neighbors and a minimum of three neighbors, and for *T. gondii* we used a maximum of five neighbors and a minimum of two.

RESULTS

We tested 132 otters (49 females, 83 males) from the 2014–15 trapping season and 88 otters (27 females, 61 males) from the 2015–16 trapping season, collected from over 50 trappers and fur dealers. Of those, three (1%) were positive for *L. interrogans,* 41 (19%) were positive for parvovirus, and 53 (24%) were positive for *T. gondii* (Table 1). Because of low overall prevalence (1%) we did not model *L. interrogans* further. Parvovirus prevalence was highest in yearling otters (age class=1, 22%; Table 2), highest in the Coastal Plain FMU (24%), and not detected in the Mountain FMU (0%; Table 2). The Lower Pee Dee (35%; Table 3) had the highest prevalence of *Parvovirus* ssp., and the lowest was in the French Broad–Holston and Roanoke river basins (0%; Table 3). *Toxoplasma gondii* prevalence ranged from 17% to 43% among FMUs and was highest in females (34%) and individuals 4 yr old or older (33%; Table 2). The Upper Pee Dee (40%) had the highest prevalence of *T. gondii* among river basins (Table 3).

We documented significant influences of age and river basin on the occurrence of parvovirus (Tables 4, 5), and of age, sex, and FMU on *T. gondii* among the otter population in North Carolina. Of the 15 models we ran for parvovirus, four were within 2 ΔAIC and explained only ~50% of the variation; all models included age, river basin, and/or sex (Table 4). Therefore, we model averaged, which indicated the Albemarle, Cape Fear, Neuse, Pamlico, and Upper Pee Dee river basins were significant predictors, whereas age and sex were not significant predictors (Table 5). The best model for *T. gondii* positive otters included FMU, sex, and age and held 79.4% of the model weight.

TABLE 2. Prevalence of *Leptospira interrogans*, parvovirus, and *Toxoplasma gondii* in 220 river otters (*Lontra canadensis*) collected from trappers in North Carolina, USA, during the 2014–15 and 2015–16 trapping seasons.

	% Prevalence by sex (sample size)		% Prevalence by age class (%)					% Prevalence by furbearer management unit (%)		
Pathogen	Male (144)	Female (76)	0 (49)	1 (95)	2 (36)	3 (16)	4 (24)	Mountain (7)	Piedmont (88)	Coastal Plain (125)
L. interrogans	2.1	0	0	2.1	0	6.3	0	0	1.1	1.6
Parvovirus	18.1	19.7	14.3	22.1	16.7	18.8	16.7	0	12.5	24.0
T. gondii	18.8	34.2	18.4	22.1	27.8	31.3	33.3	42.9	33.0	16.8

For the indicator kriging analyses, parvovirus and *T. gondii* overlapping points were averaged together, resulting in sample sizes of 97 for each disease analyzed. The standardized mean±SE and the standardized root mean square for parvovirus (0.0018±0.3824 and 1.0373, respectively) and *T. gondii* (–0.0176±0.5012 and 0.9880, respectively) demonstrated the indicator kriging had a high degree of model performance. Parvovirus appeared to be ubiquitous and at low levels across North Carolina, with the lowest prevalence in the Mountain FMU but with a primary probability of occurrence of 19% across North Carolina (Table 3 and Fig. 2). *Toxoplasma gondii* was present at relatively high levels throughout North Carolina, with high prevalence areas in the southeast Coastal Plain FMU and eastern part of the Mountain FMU and with a primary probability of occurrence of 24% for all of North Carolina (Table 3 and Fig. 3).

DISCUSSION

Our study examined pathogens in otters in North Carolina. We determined that *L. interrogans* occurred at low levels in North Carolina. Because *L. interrogans* can spread through contaminated soil or water and remains infective in the soil for months or longer (Thibeaux et al. 2017), the potential of zoonotic exposure and impact on aquatic ecosystems is a primary concern. Aquatic and semiaquatic species such as Caspian seals (*Pusa caspica*), American mink, and nutria (*Myocastor coypu*) have tested positive on multiple continents (Aviat et al. 2009; Barros et al. 2014; Vein et al. 2014; Namroodi et al. 2018), and leptospirosis is lethal to sea otters (White et al. 2018). Although our low prevalence is encouraging, it may be explained by the difficulty of isolating *L. interrogans* because carrier animals often shed leptospires intermittently (Faine 1999). However, Shearer et al. (2014) detected higher prevalence rates using similar methods. We suggest

TABLE 3. Prevalence (%) of *Leptospira interrogans*, parvovirus, and *Toxoplasma gondii* in 220 river otters (*Lontra canadensis*) collected from trappers in North Carolina, USA, during the 2014–15 and 2015–16 trapping seasons. Sixteen specimens were unable to be assigned to a river basin because of a lack of precision in the location data.

	% Prevalence by river basin (sample size)[a]								
Pathogens	AB-CH (34)	CF (54)	FB (6)	LPD (23)	NE (20)	PAM (14)	ROA (9)	SAN (10)	UPD (35)
L. interrogans	0	1.9	0	0	10.0	0	0	0	0
Parvovirus	11.8	18.5	0	34.8	15.0	14.3	0	30.0	8.6
T. gondii	11.8	22.2	33.3	21.7	30.0	14.3	11.1	30.0	40.0

[a] AB-CH = Albemarle-Chowan; CF = Cape Fear; FB = French Broad; LPD = Lower Pee Dee; NE = Neuse; PAM = Pamlico; ROA = Roanoke; SAN = Santee; UPD = Upper Pee Dee.

TABLE 4. Model selection results using Akaike's information criterion (AIC) for the effect of sex, age, river basin, and region on whether river otters (*Lontra canadensis*) tested positive for parvovirus by PCR in North Carolina, USA, in November–February 2014–16. Model weight$=\frac{exp(-0.5^* \times \Delta AIC)}{\Sigma exp(-0.5^* \times \Delta AIC)}$.

Model[a]	AIC	ΔAIC[b]	Model weight	K[c]	Log likelihood
River basin+age	206.09	0.00	0.247	11	184.09
FMU+river basin+age	206.96	0.87	0.160	13	180.96
River basin	207.91	1.82	0.100	10	187.91
River basin+sex+age	208.06	1.97	0.092	12	184.06
FMU+river basin	208.37	2.28	0.079	12	184.37
FMU+age	208.39	2.30	0.078	4	200.39
FMU+river basin+sex+age	208.80	2.71	0.064	14	180.80
River basin+sex	209.90	3.81	0.037	11	187.90
FMU	210.08	3.99	0.034	3	204.08
FMU+river basin+sex	210.33	4.24	0.030	13	184.33
FMU+sex+age	210.38	4.29	0.029	5	200.38
Age	210.96	4.87	0.022	2	206.96
FMU+sex	212.02	5.93	0.013	4	204.02
Sex+age	212.95	6.86	0.008	3	206.95
Null	213.60	7.51	0.006	1	211.60
Sex	215.51	9.42	0.002	2	211.51

[a] FMU = furbearer management unit.
[b] ΔAIC = change in AIC.
[c] K = number of model parameters.

continued monitoring of prevalence rates in aquatic mammal species in North Carolina along with the further study of the transmission routes and effects on various wild aquatic species.

We documented parvovirus in 19% of the samples tested. Although no otter mortality attributed to parvovirus has been documented in North Carolina, there have been fatalities for North American river otters (Famini et al.

TABLE 5. Model-averaged coefficients for the effects of age (per year), sex, furbearer management unit (FMU), and river basin on whether a river otter tested positive for parvovirus by PCR in North Carolina, USA, during 2014–16.

Variable	Estimate	Unconditional variance SE	Unconditional 95% confidence interval
Age	0.007	0.134	−0.256, 0.270
Sex (female)	−0.056	0.398	−0.836, 0.724
FMU (Coastal Plain)	0.845	0.529	−0.192, 1.883
FMU (Mountain)	−23.307	167,431.461	−328,188.971, 328,142.357
River basin (Albemarle)	−2.245	0.763	−3.740, −0.750
River basin (Cape Fear)	−1.592	0.630	−2.827, −0.356
River basin (French Broad)	−15.063	174,071.435	−341,195.075, 341,164.949
River basin (Lower Pee Dee)	−0.827	0.709	−2.217, 0.563
River basin (Neuse)	−1.844	0.817	−3.446, −0.242
River basin (Pamlico)	−1.976	0.940	−3.819, −0.133
River basin (Roanoke)	−24.928	92,123.913	−180,587.798, 180,537.941
River basin (Santee)	−0.644	1.011	−2.626, 1.338
River basin (Upper Pee Dee)	−2.236	0.902	−4.003, −0.469

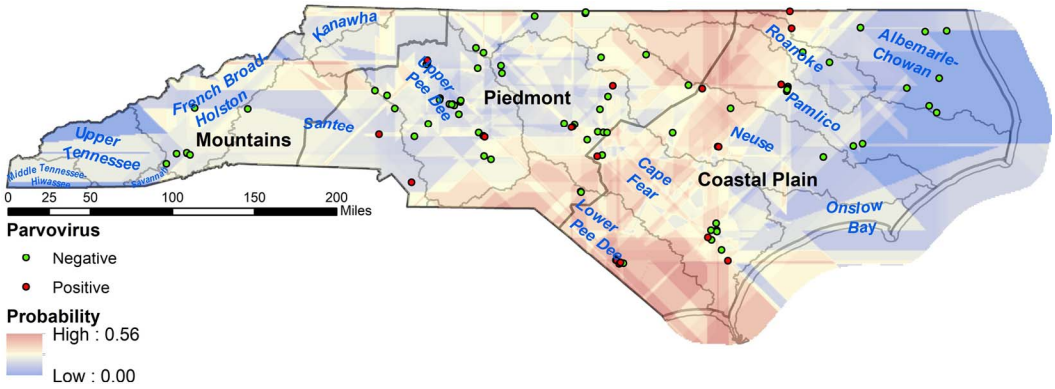

FIGURE 2. Positive and negative results and probabilities of parvovirus infection in harvested North American river otters (*Lontra canadensis*) in North Carolina, USA, during the 2014–15 and 2015–16 trapping seasons.

2013) and Asian small-clawed otters (*A. cinerea*; Gjeltema et al. 2015) recorded. Although there are no overarching relationships between anthropomorphic development and disease prevalence (Brearley et al. 2013), the dispersion of some diseases that are spread by direct contact may be aided by the disturbance associated with higher human density, development, agriculture, domestic animals, and pest populations (Gaydos et al. 2007). Specifically, canine parvovirus 2 is more common in rural areas, often because of the lower likelihood of domestic dogs (*Canis lupus domesticus*) being vaccinated (Sepúlveda et al. 2014; Zourkas et al. 2015; Curi et al. 2016). The Santee and Lower Pee Dee river basins are largely agricultural, which may explain the higher prevalence detected in our study and how the model showed significantly lower prevalence in other basins. Interestingly, adult dogs are less affected by parvovirus due to environmental exposure, and weaned puppies less than 6 mo old are usually the most at-risk group (Goddard and Leisewitz 2010). In our study, yearling otters had the highest prevalence, possibly because of greater rates of dispersal, encountering multiple latrine sites, and coming into contact with more otters and other species (Boyle 2006). Because of the 41 specimens that tested positive only 10 were positive for parvovirus in both samples, we suggest continuing to test both kidney and brain tissue for parvovirus and recommend

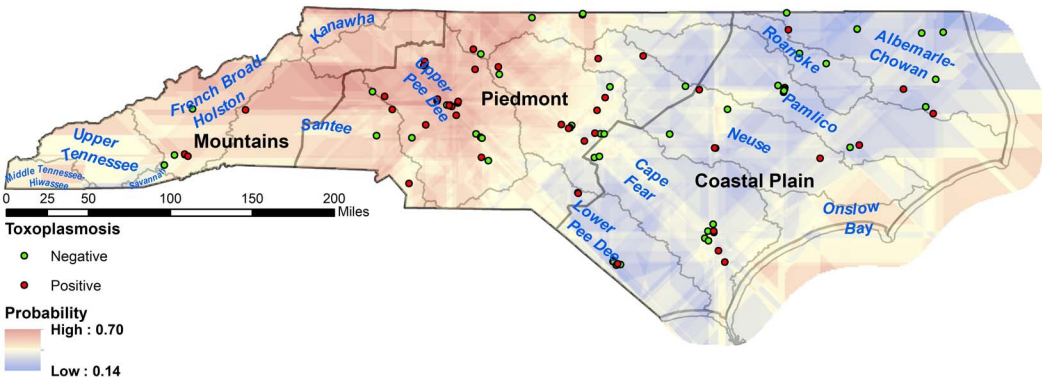

FIGURE 3. Positive and negative results and probabilities of *Toxoplasma gondii* infection in harvested North American river otters (*Lontra canadensis*) in North Carolina, USA, during the 2014–15 and 2015–16 trapping seasons.

molecular characterization of the virus to help determine the source of origin.

We documented *T. gondii* in 24% of the specimens tested, and, consistent with clinical toxoplasmosis, we only located it in brain tissue samples (Dubey 1996). We determined that FMU, sex, and age were significant predictors of *T. gondii* in North Carolina otters. The Mountain FMU had the highest prevalence of *T. gondii* at 43%, which was similar to the 45% in Coastal Plain FMU otters during the relocation project in 1996 (Tocidlowski et al. 1997). Although the Coastal Plain FMU otters formed the base of the Mountain FMU population and may have contributed to the high prevalence we observed, some otters from Louisiana were released in Great Smoky Mountain National Park by the National Park Service. It is unknown whether the otters imported from Louisiana were infected with *T. gondii*. It is possible that environmental factors (i.e., temperature, climate, humidity, rainfall) may have contributed, but our study was not designed to detect those differences. The prevalence in the heavily populated Piedmont FMU and Upper Pee Dee river basin was significantly higher, which was not unexpected because of the established link between anthropomorphic development and toxoplasmosis (Miller et al. 2002, 2008; Barros et al. 2018).

We observed higher probabilities of female and older otters contracting *T. gondii* across all three FMUs of North Carolina. Additionally, females and older otters were more likely to be vulnerable to symptoms of toxoplasmosis, which is supported by research indicating that immunocompromised individuals were at greater risk (Dubey 1996). Although there have been no recorded toxoplasmosis-related otter mortalities in North Carolina, sea otter mortalities have been linked to *T. gondii* in California (Cole et al. 2000; Shapiro et al. 2012; White et al. 2018). Although direct mortalities are important, there may be sublethal effects of toxoplasmosis, such as litter failure, that are difficult to document (Cenci-Goga et al. 2013; Formenti et al. 2015). We encourage future research to focus on the sublethal effects of toxoplasmosis on wild otter populations.

The link between felines and *T. gondii* is well documented (Suzán and Ceballos 2005; Vanwormer et al. 2013; Barros et al. 2018), and the size and spatial distribution of outdoor cat populations are closely tied to local human populations (Ferreira et al. 2011; Finkler et al. 2015). Unmanaged outdoor cat populations are known to shed large numbers of oocysts that are distributed across the landscape via stormwater runoff (Miller et al. 2002, 2008) and infect native wild species (e.g., otters; Vanwormer et al. 2013).

Aquatic ecosystems offer a plethora of opportunities for pathogens to spread and thrive (Johnson and Paull 2011). Although river otters seem to be robust to pathogens such as *L. interrogans*, parvovirus, and *T. gondii*, other species may not be. Semiaquatic furbearers in particular, such as American mink, muskrats, and American beaver, remain at risk (Smith and Frenkel 1995; Jordan et al. 2005) because landscape drainage directs pathogen exposure toward rivers, streams, and wetlands (Shapiro et al. 2012; Ahlers et al. 2015). As apex predators, otters are exposed to pathogens not only through the environment but also through their diet including fish and invertebrates (Krusor et al. 2015; Barros et al. 2018). The generalist diet of otters makes them an ideal sentinel species and suggests that when otter populations are exposed to these pathogens it is likely that other aquatic species will also be exposed, particularly in areas influenced by the human population.

As human encroachment expands across the landscape, development brings activities, domestic animals, and invasive species (e.g., outdoor cats) that enhance the exposure of wild populations to pathogens (Hess 1994; McCallum and Dobson 2002; Brearley et al. 2013). Our research established baselines that can be used for comparisons to future surveys to monitor the spread of leptospirosis, parvovirus, and toxoplasmosis. Although traditional research concludes that zoonoses pass from wild populations to domestic animals and

humans (Bengis et al. 2004; Shearer et al. 2014), other studies have determined that wild populations away from human development have lower or no prevalence of the same diseases (Gaydos et al. 2007; Plowright et al. 2008; Brearley et al. 2013; Becker et al. 2015). As the human-wildlife interface continues to expand, diseases being passed from humans and domestic animals (e.g., outdoor cats) to wild populations and vice versa are of increasing concern for all three groups.

Disease transmission is often complex and difficult to determine, requiring new methods and approaches such as molecular characterization, Web-based surveillance, and infectious disease modeling (McCallum and Dobson 1995; Plowright et al. 2008; Langwig et al. 2015). Although aquatic ecosystems provide a hub for pathogens to be encountered and spread (Gortázar et al. 2007; Johnson and Paull 2011), otters may provide crucial data for the management and conservation of other species. As development and habitat loss increase and force more human-wildlife interactions, subsequent research should examine transmission parameters between domestic and wild species and the sublethal effects of infection. Additionally, future surveys should further elucidate the role of agricultural development and human densities in pathogen prevalence in river otters across different regions and climes.

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