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RESEARCH ARTICLE

Expanding CWD disease surveillance options using environmental contamination at deer signposts

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Abstract

1. Environmental surveillance can allow early detection of diseases, which increases management options and can improve disease trajectories. Chronic wasting disease (CWD) in cervids is a significant prion disease that has been spreading across North America since the 1960s, leading to cervid population declines and concern from hunters and state wildlife agencies. White-tailed deer have a unique breeding season behaviour called scraping, where they deposit urine and saliva at shared sites. Since both these fluids can contain CWD prions, scrape sites have the potential to serve as sentinel sites for environmental surveillance of CWD.
2. To examine this potential, we used camera traps to monitor deer behaviour and collected environmental samples from 105 scrape sites. The 48 km^2 study site was located at the centre of the CWD zone in southwestern Tennessee, where CWD prevalence is ~50%. We also sampled scrapes in northern Mississippi at the leading edge of the same CWD distribution to test the potential for early CWD detection using scrape sampling.
3. From camera data, we identified 218 unique bucks visiting 105 scrapes, with a mean of 12.2 ± 7.5 bucks per scrape (mean ± SD, range 1–39) and individual bucks visiting a mean of 5.9 ± 4.6 monitored scrapes each (range 1–23).
4. Using real-time quaking-induced conversion (RT-QuIC), we detected prion seeding activity in 20% of the soil and 41% of the licking branches of the scrape sites within the CWD study area, and in 25% of the soil and 11% of the licking branches of scrape sites sampled at the edge of the known CWD distribution.
5. Our data show there is environmental prion contamination at scrape sites. This supports the idea that scrapes could serve as early warning sentinel sites for

CWD surveillance through testing soil and licking branches for prion seeding activity, especially in areas with limited access to harvested deer samples.

KEYWORDS
chronic wasting disease, environmental contamination, environmental surveillance, prions, RT-QuIC, scraping behaviour, white-tailed deer

1 | INTRODUCTION

Monitoring and managing wildlife diseases is challenging. Mortalities may be difficult to find or removed from the landscape before they can be examined, sick animals may never be seen, and diagnostic options for wild animals can be limited (Sleeman et al., 2012). Due to these challenges, techniques that can improve surveillance of and increase response options to wildlife diseases are needed. One approach that can increase the efficiency of wildlife disease monitoring is using sentinels for surveillance (Nugent et al., 2002; VerCauteren et al., 2008). Historically, sentinels are animals monitored for disease to increase cost-efficiency, surveillance sensitivity, or detection speed by being more susceptible to the disease or easier to sample than a different species of interest (Sleeman et al., 2012).

However, as advances are made in laboratory capabilities, surveillance options can expand. Examples include the increasing use of environmental DNA (eDNA) to identify species present in an area (Bohmann et al., 2014) and testing wastewater for SARS-CoV-2 surveillance (Medema et al., 2020; Yao et al., 2021). These approaches can increase cost efficiency (Hart & Halden, 2020) and allow earlier detection of disease (Deshpande et al., 2003). Such advances also indicate potential for testing environmental samples from "sentinel sites" for wildlife disease surveillance in the future.

One notable wildlife disease currently is chronic wasting disease (CWD), a prion disease which affects 31 U.S. states and 4 Canadian provinces (USGS, 2023) with the potential to spread globally (Benestad et al., 2016; Kim et al., 2005). CWD has been spreading within cervid populations across North America since it was first identified in the 1960s (Escobar et al., 2020; Williams & Young, 1992). Billions of U.S. and Canadian dollars have been spent studying and managing this fatal, prion disease (Thompson & Mason, 2022). However, there is still much scientists do not know (Haley & Hoover, 2015) and surveillance is historically limited to post-mortem tests (Gillin & Mawdsley, 2018).

Indirect CWD transmission through exposure to environmental contamination is a recognised risk (Almberg et al., 2011; Miller et al., 2004). However, the extent and implications of environmental contamination with CWD prions are understudied, in part because, until recently, there were no viable methods for testing environmental sources for CWD prions (McNulty et al., 2019; Plummer et al., 2018). Infected cervids shed prions in their saliva, urine and faeces beginning as early as 3 months post-infection and continuing the duration of infection until death, generally 18–24 months later (Henderson et al., 2015). This interval creates ample opportunity for infected individuals to contaminate their environments, which is problematic for several reasons. Prions tend to bind tightly to soil particles and are therefore likely to remain near the soil surface where they are available to infect future animals (Jacobson et al., 2010). Further, prions do not readily degrade. CWD prions are still infectious at least 1.5 years after being deposited (Miller et al., 2004) and scrapie prions can persist least 16 years outside of a host (Georgsson et al., 2006).

Mineral licks, a site of deer congregation, have been shown to be contaminated with CWD prions in endemic areas (Plummer et al., 2018), but there are likely other hotspots across the landscape. One area that could be at increased risk for CWD contamination due to deer congregation is white-tailed deer (*Odocoileus virginianus*; WTD) scrapes sites. Scrapes are a form of visual and olfactory communication in which WTD create and mark a patch of bare ground with urine and glandular secretions and mark an overhanging branch (i.e. licking branch) with saliva and glandular secretions (DeYoung & Miller, 2011), all of which may contain CWD prions (Henderson et al., 2015; Ness et al., 2022).

This potential for prion accumulation suggests that scrapes could serve not only as hotspots for CWD contamination but also aid in environmental surveillance for CWD. This could permit earlier detection of CWD because it is easier and cheaper to acquire environmental samples. Early detection increases management options, especially if detected before CWD becomes endemic (Gillin & Mawdsley, 2018). Further, given that many individual WTD visit scrapes, there is potential for scraping to contribute to CWD spread (Egan et al., 2023; Hearst et al., 2021; Kinsell, 2010).

The objectives of this study were to examine the potential role of scraping behaviour in the ecology of CWD and to determine if scrapes can be used for CWD surveillance through monitoring deer behaviour and testing environmental samples for evidence of prions. Given the role of urine and saliva in scraping behaviour and the high prevalence of CWD at our primary study site, we expected to find evidence of prions in the soil and on the licking branches at scrape sites.

2 | MATERIALS AND METHODS

2.1 | Study area

We conducted this study at the University of Tennessee's Ames Research and Education Center, a 74 km^2 property located in Fayette and Hardeman counties in southwestern Tennessee (Figure 1). The landscape is rolling and predominated with Grenada-Loring-Memphis

soil types (Longwell et al., 1963), occupied primarily by forests (loblolly pine, upland hardwoods and bottomland hardwoods), along with horse and cow pastures and commodity row crops (cotton, soybean, wheat and corn). CWD was first detected at Ames in late 2018; the following hunting seasons (2019–20, 2020–21) saw an apparent CWD prevalence of 37% both years and prevalence among bucks of 62% and 66%, respectively (Turner et al., 2022).

To test the viability of using scrapes for CWD surveillance, we also tested scrapes at the leading edge of the CWD distribution, where it expanded into northern Mississippi (Benton and Marshall counties). The county-level apparent prevalence was 8% and 4%, respectively, decreasing along a gradient from north (50%) to south (0%). In these two counties, we sampled six properties where CWD had not yet been detected and two properties where CWD was detected for the first time during that hunting season (Fall 2021; Figure 1). Of those properties where CWD had not been detected, the distance to the nearest harvested WTD that tested positive for CWD was approximately 1–6 km (mean ± standard deviation: 2.7 ± 1.8) from the edge of the property. No permits were required for this fieldwork.

2.2 | Field sampling

We searched for scrapes around areas of past scraping activity, field edges and forest paths (Alexy et al., 2001; Kile & Marchinton, 1977) within a 48 km^2 study area at Ames. We monitored deer visitation at scrapes using camera traps (Exodus Lift II, Exodus Outdoor Gear, Warren, OH, USA) set to take 3-photo bursts when movement was detected, as often as every 5 s. Every 2 weeks we visited camera traps for maintenance and photo recovery. If a site ceased having scraping behaviour (i.e. no WTD visitation for >7 days), we moved the camera to a new scrape site. We monitored 105 scrapes with camera traps starting 24 September 2021 and ending 20 January 2022. We chose this timeframe because peak breeding in the study area is the first week of December (MDWFP, 2023) and scraping activity generally peaks 2–3 weeks before peak breeding (DeYoung & Miller, 2011; Ozoga, 1989).

From camera trap images, we evaluated deer demographics, scraping behaviours, and visit timing for each scrape interaction (Hearst et al., 2021). Many scrapes were located near deer travel corridors, so we defined scrape interactions as deer stopping and engaging with the scrape. Scrape behaviours included urination, interactions with the licking branch, and pawing at the soil (DeYoung & Miller, 2011). When possible, we identified unique bucks by body characteristics such as antlers and facial markings. This allowed us to track buck behaviour across monitored scrapes and over time. Given that some bucks were harvested and tested, this also allowed us to determine CWD status of several individual bucks (13 CWD-positive, 8 CWD-not-detected).

To test scrape sites for the presence of prions, we collected soil samples and licking branch tips. We sampled scrape sites from 11

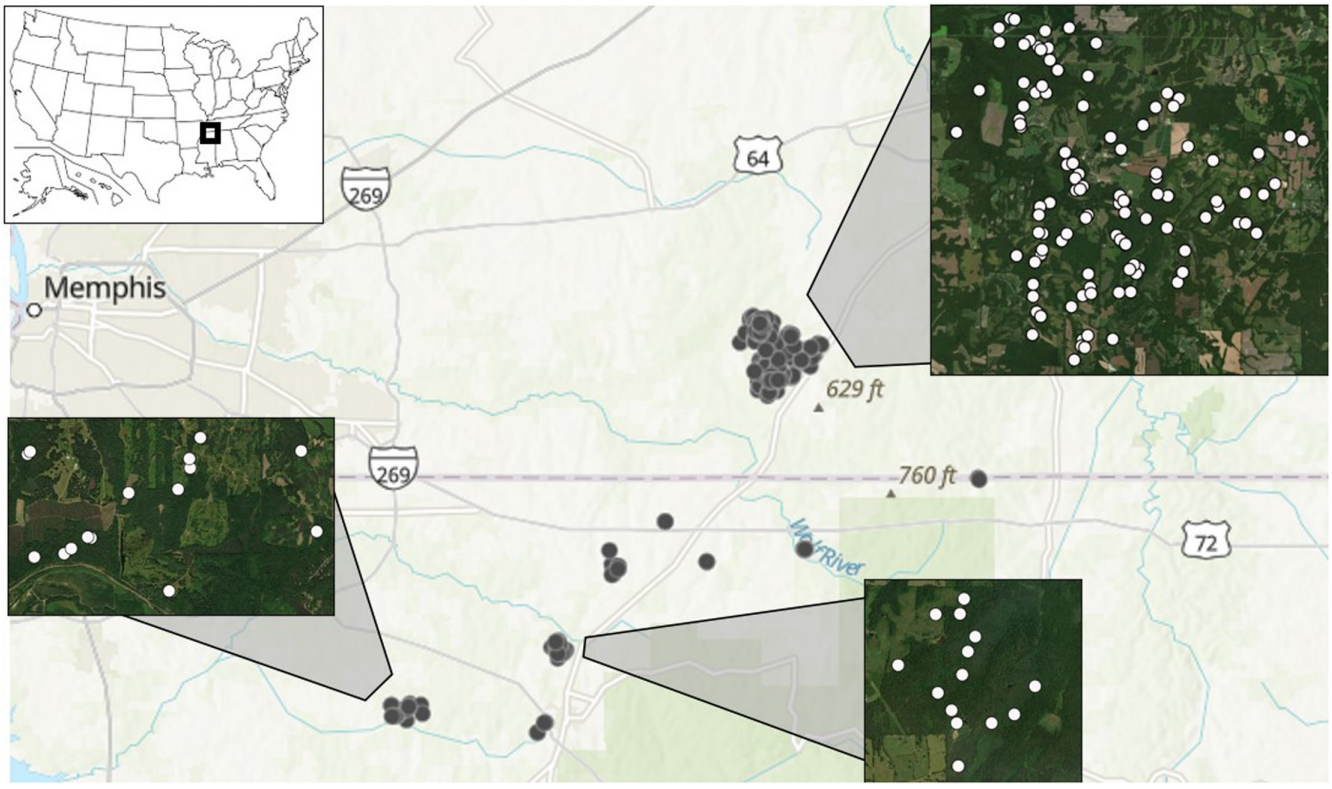

FIGURE 1 Site map of Ames Research and Education Center in southwestern Tennessee and the eight properties in northern Mississippi where white-tailed deer scrapes were sampled in January 2022. Pop-out images show the distribution of sampled scrapes at Ames and the two Mississippi properties with the most scrapes.

to 20 January 2022, which was near the end of both hunting and breeding seasons. Soil samples consisted of 700 cm^3 of soil collected from five subsamples of the scrape's patch of bare ground, which represented approximately 250 cm^2 of surface area. We collected soil from the surface level to a depth of 2–4 cm using a new, sterile scoop for each site. Licking branch samples consisted of the terminal 1–3 centimetres of each branch that had evidence of interaction (e.g. missing bark, broken). We collected branch tips by breaking them off using gloved hands and we wore fresh boot covers at each site to avoid cross contamination. All samples were frozen until testing.

We recorded the age and sex of all deer harvested at Ames and extracted retropharyngeal lymph nodes (RPLN) for CWD testing using enzyme-linked immunosorbent assay (ELISA). RPLN portions of 0.20 ± 0.02 g from at least two different areas were macerated using the TeSeE® Process (BioRad, Hercules, CA, USA). Prion purification and ELISA detection were carried out using TeSeE® Purification Kit and the TeSeE® Detection Kit (BioRad, Hercules, CA, USA), respectively.

2.3 | Soil prion extraction

From each bulk soil sample, 500 mg wet soil subsamples were massed and collected in 1.5 mL microcentrifuge tubes. Disposable spatulas and weigh boats were used with each sample to reduce the probability of cross contamination. Each subsample was then extracted with 1 mL MSB buffer (0.6 mM myristyl sulfobetaine [Sigma-Aldrich T7763, St. Louis, MO, USA], 75.4 mM dibasic sodium phosphate, 24.6 mM monobasic sodium phosphate). Subsamples were briefly vortexed and allowed to incubate at room temperature for 1 h, with rotation. Subsamples were then centrifuged at 8000×*g* for 10 min. A portion (~750 μL) of the supernatant was drawn off and retained in separate, clean 1.5 mL microcentrifuge tubes. An additional 250 μL of MSB buffer was added to the original soil subsample, vortexed briefly, and incubated again, as previously described. Subsamples were centrifuged again at 8000×*g* for 10 min, and 250 μL of supernatant was drawn off and added to the original retained supernatant. The consolidated supernatant was then centrifuged again at 8000×*g* for 10 min. Finally, 950 μL was drawn off the consolidated supernatant and placed in a clean 1.5 mL microcentrifuge tube, with care to not disturb any pellet which may have accumulated. To each clarified supernatant, 80 μL of sodium phosphotungstate stock (6.8% sodium phosphotungstate [Sigma-Aldrich 496,626, Burlington, MA, USA], 170 mM magnesium chloride) was added and incubated overnight at 4°C. Supernatants were then centrifuged at 16,000×*g* at 4°C for 30 min. The aqueous supernatant was then carefully removed and discarded. The resultant pellet was gently rinsed with 200 μL 18 MΩ distilled water and centrifuged again at 16,000×*g* at 4°C for 30 min. Then, the supernatant was again removed, and the pellet was resuspended in 0.1% sodium dodecyl sulfate in 1X phosphate buffered saline/N2 supplement (Gibco 17502, Grand Island, NY, USA). Resuspended pellets were then subject to RT-QuIC analysis as described below or stored at −20°C.

2.4 | Real-time quaking induced conversion assays

Real-time quaking induced conversion (RT-QuIC) was conducted according to the protocol of Orrù et al. (2017), with the following modifications. In lieu of sodium chloride in the assay master mix, we used sodium iodide, as this has been demonstrated to improve detection efficiency, particularly at low prion concentrations (Metrick et al., 2019). The substrate utilised was truncated recombinant Syrian golden hamster PrP (HarPrP 90-231), generated as previously described (Orrù et al., 2017). Assays were run in BMG Labtech FLUOstar instruments (BMG Labtech, Cary, NC, USA) at 42°C for 48 h. During the assay, double-orbital agitation was performed at 700 rpm, with 1 min on, 1 min off cycles. Fluorescence measurements were taken in 15-min intervals, with a manual gain setting of 1600. Eight technical replicates were analysed per sample. Each plate was run with positive/negative controls of known CWD-positive/negative WTD obex diluted to 10^{-4} for quality assurance purposes.

2.5 | RT-QuIC data analysis

To reduce the possibility of misinterpretation of a negative sample as a false positive due to background fluorescence, a baseline fluorescence was calculated for each sample by taking the mean of fluorescence measurements 3 through 14 and adding 10 times the standard deviation of the same samples. Time to threshold was calculated for each plate based upon the time at which a given sample fluorescence crossed the baseline fluorescence.

To establish a baseline time cut-off for negative soil, 11 putative negative soils sourced from the vicinity of the Ames site with no external indication of deer activity were extracted as described above, and analysed by RT-QuIC, with eight technical replicates analysed per soil sample, giving a total of 88 data points used in analysis of putative negative soil. A mean and sample standard deviation of the time to threshold was calculated for any negative soil sample wells experiencing seeding activity. The mean minus half of the sample standard deviation was used as the negative cut-off time. To reduce variability and the possibility of false positive due to spontaneous seeding activity, a sample was considered as positive for prion seeding activity if 50% or more of the technical replicates (4/8 or more) for a given sample crossed the baseline fluorescence threshold before the negative cut-off time, similar to the practice used in tissue RT-QuIC.

2.6 | Statistical analysis

2.6.1 | Response variables

All statistical analyses were conducted using RStudio version 4.1.1 (R Core Team, 2021). To identify factors affecting the risk of CWD prions in WTD bucks and scrapes, we used logistic regressions

(package: stats) with response variables that were binary for if CWD prion seeding activity was detected (1) or not (0). The response variables were: (1) detection in soil at scrape sites, (2) detection on licking branches at scrape sites, (3) detection in either soil or licking branches at scrape sites and (4) detection in bucks. The first three models examine factors associated with scrapes becoming contaminated and the data for their response variables were collected in January when scrapes were sampled. The fourth model examines the potential relationship between scraping behaviour and a buck testing positive for CWD and the data for its response variables were collected September–January by camera traps and as each buck was harvested.

2.6.2 | Explanatory variables

Most potential explanatory variables described WTD behaviour observed by camera traps. For the buck model, the variables included were number of: visits to all scrapes, visits to contaminated scrapes, different scrapes visited, different contaminated scrapes visited, scrape interactions and branch-specific interactions. We also included age, both as a continuous variable and as a category of young (≤2.5 years) and old bucks (3.5+ years), and the total duration of visits to monitored scrapes.

For the three scrape models behavioural variables consisted of number of: interactions by all WTD, interactions by known-positive bucks, urinations, urinations by known-positive bucks, branch interactions, branch interactions by known-positive bucks and unique bucks that visited (both in total and specifically known-positive bucks). Scrape models also included total duration of scrape interactions occurring at each site and distance to the nearest contaminated scrape.

To further delve into the role of behaviour in CWD ecology around WTD scrapes, especially indirect transmission, we generated two types of directed, weighted social networks (Craft, 2015; Silk et al., 2017). The buck networks focused on dynamics within deer populations using bucks as nodes and shared scrape use as edges (Egan et al., 2023; Hearst et al., 2021). We generated three of these networks using different sets of data: all identified bucks, only the bucks that were harvested and tested for CWD, and only the bucks that tested positive for CWD. We then used a scrape network using scrapes as nodes and the bucks moving between them as edges.

Social network structure can be described using a variety of variables (Craft, 2015; Silk et al., 2017). In this manuscript, we focus on degree, in-degree, out-degree, eigenvector centrality, betweenness and authority. Degree is the total number of connections entering and leaving the focal node and is the sum of the in-degree (connections entering) and out-degree (connections leaving; Wey et al., 2008). Eigenvector centrality summarises both the strength of connections from the focal node (i.e. number of indirect contacts from the buck of interest to other bucks) and the connections to significant nodes within the network (Kasper & Voelkl, 2009). Betweenness describes how many connections pass through a focal node if random paths are created between pairs of all individuals other than the focal individual (Newman, 2005). Finally, authority identifies individuals that are significant in the network by examining both incoming connections and the tendency to receive connections from individuals sending connections to many significant individuals (Kleinberg, 1999).

2.6.3 | Model selection

Candidate variables were screened in a correlation analysis with a cut off at |0.5|. When two variables were correlated, the one with a lower Akaike information criterion (AIC) in a univariate model was chosen for inclusion in multivariate model selection. Next, we conducted forward step selection (package: stats; R Core Team, 2021) using AIC change to identify the best model. Variables that improved AIC by at least two points were included in the model. The WTD model only included data from harvested bucks that could be matched to bucks documented visiting scrape sites since this allowed us to know both the CWD status and specific scraping behaviours of individuals.

2.6.4 | Absolute goodness of fit

We evaluated the absolute goodness of fit of top models using the area under the curve receiver operating characteristics in the R package pROC (Robin et al., 2011).

3 | RESULTS

We monitored 105 unique scrapes for 7482 camera days from 24 September 2021 to 20 January 2022. This allowed us to detect 3063 scrape interactions performed by does ($n = 702$ interactions), fawns ($n = 138$), and bucks ($n = 2223$). We identified 218 unique bucks visiting monitored scrape sites. On average, individual bucks visited 5.9 ± 4.6 scrapes (mean ± SD, range: 1–23) and scrapes were visited by 12.2 ± 7.5 unique bucks (mean ± SD, range: 1–39).

Among the 97 deer harvested on the study site, CWD prevalence was 49% overall (53% of males, 48% of females; Table 1). We matched 21 of 29 adult, harvested bucks to bucks identified in camera trap photos (13 CWD-positive, 8 CWD not-detected; 61% prevalence). We used these 21 bucks in the model predicting CWD status of WTD using data collected after harvest and from camera traps at scrapes. The best model included buck age ($p = 0.10$) and the out degree within a social network of the known, harvested bucks ($p = 0.05$; Table 2). The model showed that, in young bucks (≤2.5 years), as out degree increased from 1 to 3, the probability of being positive for CWD increased from 12% to 60% (Figure 2). Buck age was not statistically significant due to the small sample size but there was a strong trend of higher infection probability in older deer.

TABLE 1 Chronic wasting disease results for harvested deer by sex and age class at Ames Research and Education Center, in southwestern Tennessee, during the 2021–2022 hunting season.

	Detected	Not detected	Total	Prevalence
Females	30	33	63	48%
Fawns	3	5	8	–
1 year	2	4	6	–
2–3 years	22	21	43	51%
4+ years	3	3	6	–
Males	18	16	34	53%
Fawns	1	4	5	–
1 year	0	2	2	–
2–3 years	15	9	24	62%
4+ years	2	1	3	–
Total	48	49	97	49%

Note: Prevalence is only calculated for groups with >20 samples.

TABLE 2 Best binomial regression models for predicting chronic wasting disease status of white-tailed deer (*Odocoileus virginianus*) and scrape sites (soil contamination, branch contamination or contamination in either sample type).

	Estimate	SE	*p*
Buck Model, AUC = 0.85			
Intercept	−11.4	7.0	0.10
Buck age	3.2	2.0	0.10
Out degree	1.2	0.6	>0.05
Soil Model, AUC = 0.72			
Intercept	−2.2	1.0	0.02
Authority	11.9	4.8	0.01
Eigenvector centrality	−1.7	0.8	<0.05
Branch Model, AUC = 0.67			
Intercept	0.7	0.4	0.13
Eigenvector centrality	−2.1	0.7	0.004
Any Scrape Contamination Model, AUC = 0.72			
Intercept	1.7	0.5	0.001
Number of branch interactions by positive bucks	−0.4	0.3	0.12
Eigenvector centrality	−2.3	0.8	0.003

Of the monitored scrapes at Ames, we tested soil samples from 99 scrapes and branch samples from 98 scrapes (one sample was lost). Prion seeding activity was detected in 20 of the soil samples (20% prevalence) and 40 of the branch samples (41% prevalence; Figure 3). There were 6 scrapes for which both the soil and branch were contaminated, 34 scrapes with only positive branch samples, and 14 scrapes with only positive soil samples. In total, 54 scrapes had CWD prion seeding activity in at least one sample type (Figure 3).

To test the potential for scrapes to serve as environmental sentinels, we visited sites in northern Mississippi along the leading edge of the same CWD distribution that Ames was in the centre of. We sampled 34 scrapes at 6 properties that had submitted harvested deer for CWD testing but had not detected CWD. Prion seeding activity was detected in soil from 10 of those scrapes (29%) and licking branches from three scrapes (9%), for a total of 13 (38%) contaminated scrapes. We also sampled two properties that harvested their first CWD-positive deer during the 2021–2022 hunting season. Of the 19 scrapes sampled at these properties, CWD prion seeding activity was detected in soil from three scrapes (16%) and licking branches from three different scrapes (16%). This meant that 32% of scrapes had detectable levels of prions on either the branch or in the soil. No Mississippi scrape with CWD seeding activity in the soil had seeding activity on the licking branch and vice versa.

Models for predicting scrape contamination only included data from Ames, where camera traps were used to monitor WTD use. The best model for predicting scrape contamination varied across the three scrape response variables (Table 2). The best model for soil contamination included authority ($p < 0.02$) and eigenvector centrality ($p < 0.05$). For branch contamination, the best model included only eigenvector centrality ($p < 0.01$). Finally, the best model for prion seeding activity in any sample at a scrape included the number of branch interactions by known positive bucks ($p = 0.12$) and eigenvector centrality ($p < 0.01$). All candidate models within 2 ΔAICs of the four models presented above are included in Appendix S1. Plots of predictive relationships between CWD status and model variables are included as Appendix S2.

4 | DISCUSSION

We detail the first recovery of prions from cervid scrapes, in both soil and licking branch samples. Past research found CWD prions in the soil and water at mineral lick sites (Plummer et al., 2018), which can be used to support bans of artificial attractants, a common management intervention for CWD (Gillin & Mawdsley, 2018). Though there is no equivalent intervention for scrapes, these sites could be leveraged to aid in CWD surveillance and management. For example, research suggests that enzyme treatment may be able to decontaminate small sites, so targeting these hot spots where deer may gather year after year could lessen CWD spread within a population (Kuznetsova et al., 2018; Saunders et al., 2011; Sohn et al., 2019).

Scrape sites can also improve understanding of social drivers of CWD spread within and among deer populations (Egan et al., 2023; Hearst et al., 2021). By using social network analysis on camera trapping data at scrapes, we could examine the potential influence of social dynamics of individual WTD on the likelihood of testing positive for CWD. The social network variables included in our final models (out-degree, eigenvector centrality and authority) measure different facets of individuals' level of social interaction (Kasper & Voelkl, 2009; Kleinberg, 1999; Newman, 2005; Wey et al., 2008). The five-fold increase in probability of CWD infection

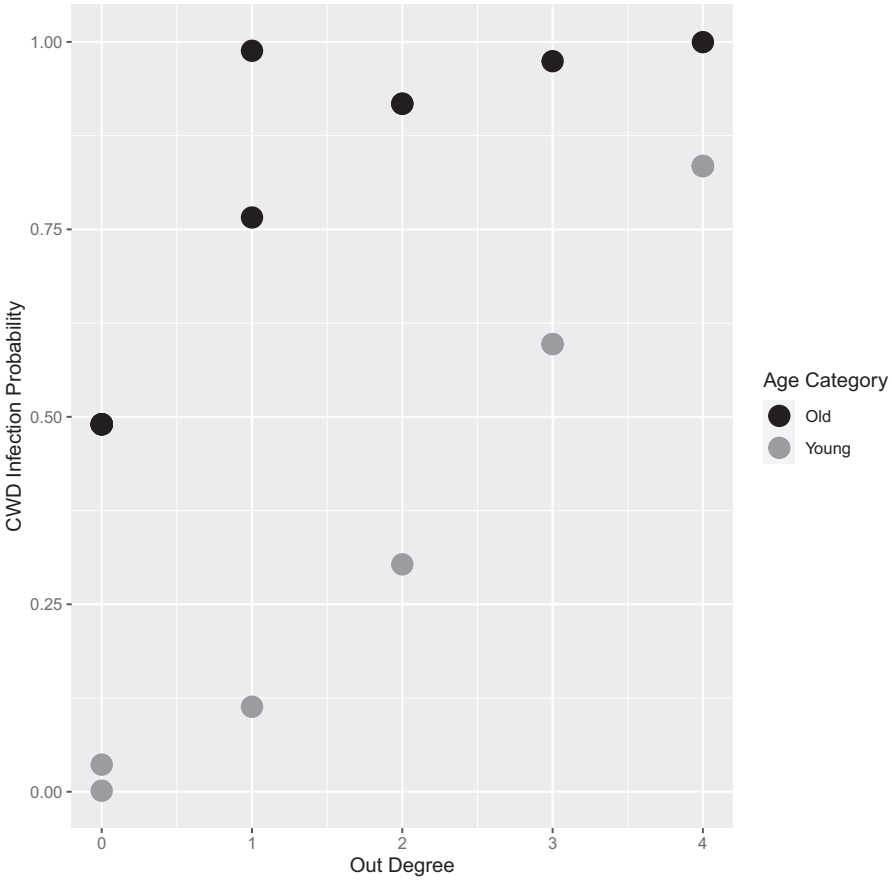

FIGURE 2 Plot of predicted chronic wasting disease infection probability by out-degree of harvested bucks, colour coded by buck age category (Old: 3.5+ years, Young: ≤2.5 years). Infection probability increases with out-degree for both old and young bucks.

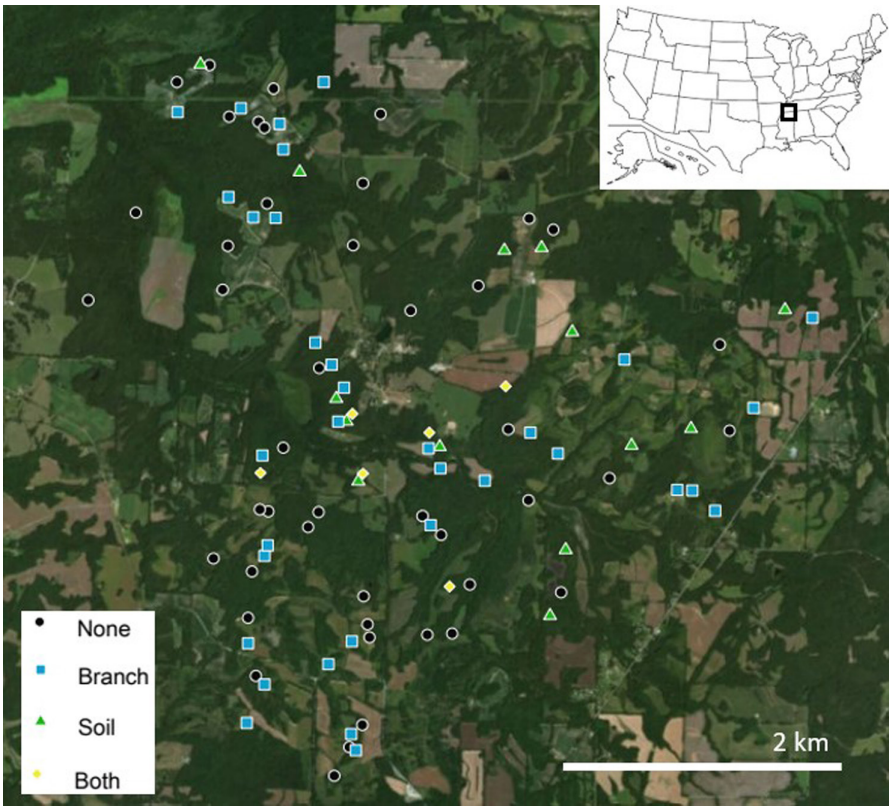

FIGURE 3 Map of scrapes sampled at Ames Research and Education Center, in southwestern Tennessee, during January 2022. Black dots represent scrapes where prions were not detected. Scrapes where prion seeding activity occurred in samples are represented by blue squares (branch only), green triangles (soil only) and yellow diamonds (activity in both branch and soil samples).

in young bucks as out-degree increased from 1 to 3 indicates the importance of social traits in disease risk, as previous studies have also shown (Craft, 2015; Drewe, 2010). Further study of WTD social networks built around scrapes sites may reveal other relevant variables, improve models of CWD spread, and, potentially, inform management options.

Recovery of prions by environmental surveillance has applications in early detection of the spatial extent of CWD. As of this writing, there is not a widely available and approved antemortem test for CWD (USDA APHIS, 2020), so monitoring the spread of CWD requires testing cervids post-mortem, through hunter-harvested, road killed, or otherwise dead cervids (Gillin & Mawdsley, 2018). However, this can be difficult in areas where hunters are resistant to submitting samples or when a heavy burden is placed on state wildlife agency staff for CWD surveillance and management (Texas Legislative Budget Board Staff, 2019). In the future, CWD surveillance could be performed through environmental sampling of scrape sites on public lands or over the course of routine, private-land visits. Though more research is needed before best management practices could be developed for using scrapes for CWD surveillance, our detection of CWD prions in 54% of scrapes where CWD prevalence is 49% in WTD and 36% of scrapes at the leading edge of a CWD distribution suggests that scrape surveillance might not require overly extensive sampling.

From camera trap images, we were only able to uniquely identify individual bucks, meaning doe and fawn data were less detailed. However, mature males are the primary group performing scraping behaviours (Alexy et al., 2001; Hearst et al., 2021) and tend to be at higher risk for CWD infection (Miller et al., 2008; Samuel & Storm, 2016). Therefore, this group is the most likely to contribute to CWD ecology at scrapes (Egan et al., 2023). Another limitation in interpretation of the data is that we did not monitor every single scrape in the Ames' study area, so data on scrape visitation rate per individual buck and overall network connectivity are likely underestimated.

There are many potential routes of future research such as examining the potential for scrapes to spread CWD among visiting deer. Scrape sampling in diverse areas could permit the creation of models that allow managers to estimate prevalence within a deer population based on prevalence of contaminated scrapes. Finally, sampling of scrapes at various distances from known edges of CWD distributions would help determine the geographic range where environmental surveillance might be effective for finding CWD in a novel area.

As lab capacities expand, the options for environmental surveillance increase. Environmental surveillance has already been leveraged by using eDNA to detect rare or difficult to sample species (Bohmann et al., 2014) and testing wastewater for SARS-CoV-2 surveillance (Medema et al., 2020; Yao et al., 2021). These and other systems have proven the potential for environmental surveillance to serve as early-warning systems (Deshpande et al., 2003; Medema et al., 2020) and to increase cost-efficiency (Hart & Halden, 2020). Our results demonstrate the potential for environmental surveillance to be used CWD too.

AUTHOR CONTRIBUTIONS

Steve Demarais, Bronson K. Strickland, Scoty Hearst, Allan Houston, Miranda H. J. Huang and Kurt C. VerCauteren conceived the ideas and designed the field methodology. Stuart Lichtenberg designed the soil prion extraction protocol. Alejandro Banda, Anna Grace Welch and Stuart Lichtenberg implemented the soil extraction protocol. Miranda H. J. Huang, Alejandro Banda, Stuart Lichtenberg and Anna Grace Welch collected the data. Miranda H. J. Huang, Kim M. Pepin, Bronson K. Strickland, Scoty Hearst, Alejandro Banda and Steve Demarais contributed to data analysis. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data available in Scholars Junction, Mississippi State University Institutional Repository after a 1-year embargo (15/11/2024): https://scholarsjunction.msstate.edu/cfr-publications/25/ (Huang et al., 2023).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1: Candidate models for all logistic regressions within 2 delta AICs of the models presented in the text.

Appendix S2: Graphs of predictive relationships for variables included in the logistic regressions.

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