

CAUSE OF DEATH, PATHOLOGY, AND CHRONIC WASTING DISEASE STATUS OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) MORTALITIES IN WISCONSIN, USA

Marie L. J. Gilbertson,^{1,8} Ellen E. Brandell,¹ Marie E. Pinkerton,² Nicolette M. Meaux,¹ Matthew Hunsaker,^{1,3} Dana Jarosinski,^{3,4} Wesley Ellarson,³ Daniel P. Walsh,⁵ Daniel J. Storm,⁶ and Wendy C. Turner⁷

¹ Wisconsin Cooperative Wildlife Research Unit, Department of Forest and Wildlife Ecology, University of Wisconsin–Madison, 1630 Linden Dr., Madison, Wisconsin 53706, USA

² Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin–Madison, 2015 Linden Dr., Madison, Wisconsin 53706, USA

³ Wisconsin Department of Natural Resources, 1500 N Johns St., Dodgeville, Wisconsin 53533, USA

⁴ Warnell School of Forestry and Natural Resources, University of Georgia, 180 E Green St., Athens, Georgia 30602, USA

⁵ US Geological Survey, Montana Cooperative Wildlife Research Unit, University of Montana, 32 Campus Drive, NS205, Missoula, Montana 59812, USA

⁶ Wisconsin Department of Natural Resources, 1300 W Clairemont Ave., Eau Claire, Wisconsin 54701, USA

⁷ US Geological Survey, Wisconsin Cooperative Wildlife Research Unit, Department of Forest and Wildlife Ecology, University of Wisconsin–Madison, 1630 Linden Dr., Madison, Wisconsin 53706, USA

⁸ Corresponding author (email: mgilbertson5@wisc.edu)

ABSTRACT: White-tailed deer (WTD; *Odocoileus virginianus*) are a critical species for ecosystem function and wildlife management. As such, studies of cause-specific mortality among WTD have long been used to understand population dynamics. However, detailed pathological information is rarely documented for free-ranging WTD, especially in regions with a high prevalence of chronic wasting disease (CWD). This leaves a significant gap in understanding how CWD is associated with disease processes or comorbidities that may subsequently alter broader population dynamics. We investigated unknown mortalities among collared WTD in southwestern Wisconsin, USA, an area of high CWD prevalence. We tested for associations between CWD and other disease processes and used a network approach to test for co-occurring disease processes. Predation and infectious disease were leading suspected causes of death, with high prevalence of CWD (42.4%; of 245 evaluated) and pneumonia (51.2%; of 168 evaluated) in our sample. CWD prevalence increased with age, before decreasing among older individuals, with more older females than males in our sample. Females were more likely to be CWD positive, and although this was not statistically significant when accounting for age, females were significantly more likely to die with end-stage CWD than males and may consequently be an underrecognized source of CWD transmission. Presence of CWD was associated with emaciation, atrophy of marrow fat and hematopoietic cells, and ectoparasitism (lice and ticks). Occurrences of severe infectious disease processes clustered together (e.g., pneumonia, CWD), as compared to noninfectious or low-severity processes (e.g., sarcocystosis), although pneumonia cases were not fully explained by CWD status. With the prevalence of CWD increasing across North America, our results highlight the critical importance of understanding the potential role of CWD in favoring or maintaining disease processes of importance for deer population health and dynamics.

Key words: Comorbidity, co-occurrence, ectoparasitism, infectious disease, necropsy, nutritional condition, pneumonia.

INTRODUCTION

White-tailed deer (*Odocoileus virginianus*; WTD or deer hereafter) are a critical species for ecosystem dynamics (Rooney and Waller 2003), cultural practices (Holsman et al. 2010; Reo and Whyte 2012; Arnett and Southwick 2015), human subsistence (Reo and Whyte 2012), and funding for management and

conservation organizations (Arnett and Southwick 2015; Hewitt 2015). Given the ecological, cultural, and economic importance of WTD, understanding deer population dynamics is important to inform sustainable management practices. Of particular interest is the study of cause-specific mortality, which has been evaluated extensively (e.g., Whitlaw et al. 1998; DelGiudice et al. 2002; Carstensen et

al. 2009). Specific investigation of the underlying disease processes at the time of death in deer is relatively understudied, particularly for free-ranging deer. This creates a gap in knowledge regarding the specific disease processes contributing to mortality that could be mitigated or managed to promote healthier deer populations.

A few studies have used necropsy to characterize underlying disease processes and lesions among deer mortalities. In one such study of free-ranging WTD, bacterial infections, trauma, and nutritional deficits were the most common causes of death (Zhu et al. 2021). Among captive and free-ranging deer, pneumonia has been a leading infectious cause of death (Hattel et al. 2004; Haigh et al. 2005; Zhu et al. 2021), but without extensive tracking of individual deer (e.g., by GPS collaring), quickly recovering deceased animals for in-depth pathological assessment is extremely limited for free-ranging deer.

The gap in knowledge regarding deer pathological processes is particularly critical in light of the increasing prevalence of chronic wasting disease (CWD) in North American cervids. Pathological abnormalities of CWD-infected deer have been described, generally through observations of captive deer in research facilities (e.g., wasting, neurologic deficits; Williams et al. 2002). The rates and associations of CWD-associated abnormalities or lesions with other disease processes have not been explored. Because CWD weakens its hosts relatively slowly (clinical course in WTD typically 4 mo; Williams 2005), secondary disease processes—for example, aspiration pneumonia secondary to CWD-induced neurologic deficits (Williams and Young 1980, 1992; Williams and Miller 2002; Williams 2005)—may be expected to cause mortality in CWD-infected animals (i.e., proximate cause of death). Similarly, CWD-induced wasting, through alterations in host behavior or immune response, may make deer more susceptible to other infectious diseases such as transmissible respiratory or gastrointestinal (GI) disease (Sánchez et al. 2018). Reports are lacking on the relationship between CWD and other disease processes in free-ranging

deer populations: Zhu et al. 2021 reported no positive CWD cases; other reports are often from captive animals or are case reports of small numbers of free-ranging individuals (Williams and Young 1980; Wolfe et al. 2014; Benestad et al. 2016). In wild settings, disease co-occurrences may affect the nature and duration of the clinical phase of CWD-infected deer. Uncertainties regarding the comorbidities associated with CWD in free-ranging deer populations leave significant gaps in understanding population-level disease dynamics for both CWD and potentially associated disease processes.

We report on mortality, pathology, and disease process trends in free-ranging WTD in southwestern Wisconsin, USA, a region of high CWD prevalence (>40%; Wisconsin Department of Natural Resources 2020). Our objectives were to (1) determine common causes of death among deer by age class, sex, and time of year; (2) identify associations between deer demographics and CWD status and disease stage; and (3) test for associations between CWD and other disease processes. We hypothesized that CWD infection would be associated with poor nutritional condition and the presence of other infectious diseases.

MATERIALS AND METHODS

Sampling and necropsies

In Wisconsin, CWD was first detected in the southwest in 2001 (Joly et al. 2003); the subsequent two decades of research have focused on understanding CWD transmission dynamics, surveillance, and control. As part of these research efforts, the Wisconsin Department of Natural Resources, in collaboration with more than 300 landowners, captured 1,157 individual WTD from 2017 to 2020. Of these, 763 (452 female, 311 male) deer >8 mo old at capture were fitted with GPS collars; 323 (168 female, 155 male; 21 later recaptured as adults) neonate deer were fitted with VHF collars. Captures occurred in the CWD-endemic areas of Iowa, Grant, and Dane counties in southwestern Wisconsin from December to March each year. Deer were captured using a combination of clover traps, drop nets, box traps, and darting, and chemically immobilized with intramuscular injections of BAM (27.3 mg/mL butorphanol + 9.1 mg/mL azaperone + 10.09 mg/mL medetomidine; ZooPharm, Laramie, Wy-

oming, USA; 1–2 mL based on deer age and body weight; Miller et al. 2009). During captures, deer were monitored via rectal temperature, respiratory and heart rates, and capillary refill time. Deer were subsequently partially reversed with atipamezole (25 mg/mL, ZooPharm, with 25 mg atipamezole administered per 10 mg of medetomidine). Deer capture and handling protocols were approved under Wisconsin Department of Natural Resources's Animal Care and Use Committee (Protocol 16-Storm-01).

Biological samples were collected (e.g., ear punches, rectal lymphoid tissue) and individual data recorded (e.g., sex, age, standard body measurements) during captures. Collared deer were monitored until death or collar failure, with collars providing a mortality signal based on lack of collar motion. When a mortality signal was detected, field teams attempted to locate the carcass and recorded the conditions of the mortality as soon as possible, usually within 24 h of detection of the mortality signal. Neonate VHF collars were monitored daily through August each capture year, then weekly; detection of mortality signals from VHF collars could therefore potentially be delayed, compared to GPS collars. Generally, intact carcasses for which the cause of death was uncertain were submitted for a full laboratory necropsy at the UW-Madison School of Veterinary Medicine (UW-SVM). Partially intact carcasses (e.g., moderate decomposition, scavenging) underwent a field necropsy. Limited evaluation or no analysis was performed in instances in which the cause of death was immediately evident (e.g., some vehicle strikes) or when too little carcass material was available for further assessment (e.g., extensive scavenging). Hunter harvested animals were not included in necropsy assessment, although unrecovered kills were evaluated when identified.

Laboratory necropsies at the UW-SVM were performed or supervised by board-certified veterinary anatomic pathologists with protocol development and necropsy oversight by a single pathologist (M.E.P.). They included a full gross examination of all body systems, including evaluation of nutritional condition (based on muscle atrophy and overall body fat including subcutaneous, epicardial/pericardial, perirenal, and bone marrow), and evaluation of ecto- and endoparasites; collection and formalin fixation of a complete tissue set including gross lesions; and histologic evaluation of major organs and gross lesions. For histologic examination, tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μ m, and stained with H&E; tissue sections with suspected bacterial infection were also stained with Brown-Hopps Gram stain. When a bacterial infectious agent was suspected, aerobic cultures were performed to

attempt to identify etiologic agents. All findings were compiled in formal diagnostic necropsy reports.

Field necropsies were performed by the field team and, although less extensive than laboratory necropsies, were still able to identify gross abnormalities or indications of infection or trauma; nutritional condition based on presence or absence of fat including epicardial and pericardial, perirenal, and bone marrow; and presence or absence of readily apparent parasites (e.g., nasal bots, ticks, lice). Limited evaluations, depending on availability of tissues and assessment of the scene of the mortality, included suggested causes of death.

All mortalities with available lymphoid or brain tissues underwent post-mortem CWD testing via immunohistochemistry. Ages of deceased individuals were estimated based on age estimates at capture and the estimated date of death. At capture, adult deer ages were estimated by tooth wear ($n=18$; conservatively assumed age of 2 yr at capture) or incisor cementum annuli ($n=96$; Storm et al. 2014); ages at capture were apparent for neonates and most juveniles and yearlings based on body size and tooth wear and emergence (one juvenile and 19 yearlings had ages verified by cementum annuli).

Laboratory, field, and limited necropsy results were digitized in a Microsoft Access database. These data (Gilbertson et al. 2022) included individual nutritional condition, presence, or apparent absence of pathological processes (e.g., GI lesions, infectious diseases), and characteristics of detected lesions or disease processes. The suspected cause of death for each mortality was categorized broadly as infectious, predation, trauma (e.g., vehicle strike, hay cutter injury, capture-associated), nutritional, unrecovered kill, mixed (unclear mixture of multiple probable causes of death), or unknown, based on the predominant process suspected of leading to death. While capture-associated injuries should not be considered a population-level mortality, as fresh carcasses these individuals provide important pathological information. The “nutritional” cause of death represented distinct starvation processes (i.e., as may be experienced by neonates) and was therefore not intended to represent CWD-induced emaciation.

Statistical analyses

We conducted several analyses to examine CWD trends and disease process associations; all analyses were conducted in R v3.6.3 (R Core Team 2018). Analyses evaluated only individuals with relevant data for a specific analysis, unless otherwise specified; all analyses, hypotheses, and sample sizes per test are given in Supplementary

Material Table S1. To examine demographic effects on CWD status, we performed logistic regression for CWD status by deer sex and age. For this analysis, we focused on the subset of CWD-tested individuals with age estimates based on initial capture as neonates, juveniles, or yearlings, or based on tooth cementum annuli ($n=227$), and rounded ages at death to the nearest year. We treated age as a continuous variable, which was modeled using a second order polynomial.

To identify potential relationships between CWD status and the presence or apparent absence of individual disease processes, we used Fisher exact tests with simulated P values (5000 replicates) and a Bonferroni correction for multiple comparisons. To ensure consistency across necropsy types, we kept individual disease process classifications broad (e.g., “any pneumonia”), but screened for associations with CWD among top relevant subclassifications (e.g., “bronchopneumonia”). All Fisher tests were performed on the subset of individuals greater than 1 yr old at death, because individuals under 1 yr old were considered least likely to experience even sub-clinical effects of CWD. In addition, we tested for differences in nutritional condition (determined based on presence or absence of body fat during necropsy) by CWD and pneumonia status. To account for potential variation in classification among different pathologists or field team observers, we condensed nutritional condition as recorded in necropsies into three general categories: good (“excellent” or “good”), fair (“fair-good,” “fair,” or “poor-fair”), and poor (“poor” or “severe-poor”). We performed ordinal logistic regression for nutritional condition as a function of CWD and pneumonia status, including an interaction between the two disease processes. We used logistic regression to test for differences in the stage of CWD clinical progression between infected male and female deer, accounting for age. We defined CWD-positive deaths as end stage (severe or poor nutritional condition with moderate to severe bone marrow fat atrophy) or non-end stage (fair to excellent body condition with zero to moderate bone marrow fat atrophy). Due to limited sample sizes, here we broadly classified deer ages as “subadult” (age estimates under 2 yr), “adult” (2–7 yr), and “senior” (≥ 8 yr).

To test for co-occurrences of disease processes beyond pairwise associations, we also generated co-occurrence networks among 10 key disease processes or lesions: CWD; bronchopneumonia and pneumonia; mixed or other pneumonias (e.g., interstitial; mutually exclusive from bronchopneumonia and pneumonia); emaciation; GI lesions; cardiac lesions; ectoparasites; pulmonary nematodes; skeletal muscle sarcocystosis; and pulmo-

nary abscess (Fountain-Jones et al. 2019; see Supplementary Material for additional details). To determine if particular disease processes or lesions in the network were more likely to co-occur, we performed a community or cluster detection analysis using a random walk algorithm with three steps (‘cluster_walktrap’ function in *igraph* package in R v3.6.3; Csardi and Nepusz 2006; R Core Team 2018). To maintain the maximum number of recorded lesions, we retained all deer in our dataset ($n=433$), and missing data were set to zero (acted as “no detected co-occurrence”), but we repeated this analysis with the subset of deer with full data ($n=51$) to ensure our results were not biased by missing data.

RESULTS

Suspected causes of death and lesions

In total, 1,065 unique WTD were captured and collared; of these, at the time of analysis, 645 (60.5%) had died, 278 (26.1%) were lost to follow-up (e.g., collar failed), and 142 (13.3%) were still alive. We evaluated 433 WTD mortalities (424 collared individuals); of the remaining known mortalities ($n=221$), 199 (90.0%) were known to be harvest mortalities. Recovery of deceased collared individuals that were candidates for necropsy was therefore very high (95.1%). Of the mortalities evaluated (Table 1), 141 received full laboratory necropsies, 116 field necropsies, and 176 received limited evaluation.

Predation and infectious disease were the top suspected causes of death, followed by trauma (Figs. 1, Supplementary Material Fig. S1); note that harvest-associated mortalities are not represented in our results. The most common cause of mortality among deer <1 yr of age was predation (Fig. 1). Mortalities peaked in May–July, with an additional smaller peak in February–March. The majority of May–July mortalities were among individuals <1 yr old; February–March mortalities were more broadly distributed among age classes (Fig. 1).

Among mortalities with post-mortem CWD tests ($n=245$), 42.4% were CWD-positive. We found CWD-positive deer in all age classes, including <1 yr. There was a trend toward increasing CWD prevalence with increasing age that peaked at approximately 6 yr old,

TABLE 1. Overview of white-tailed deer (*Odocoileus virginianus*) necropsies performed in southwest Wisconsin, 2017–21, by age, sex chronic wasting disease (CWD) status, year, and type of necropsy.^a

Characteristic	Necropsy			Total
	Laboratory	Field	Limited	
Sex				
Female	85	62	93	240
Male	56	54	79	189
Age class at death				
<1	47	46	101	194
1–2	25	19	32	76
>2	65	51	43	159
CWD status				
post-mortem				
Negative	62	47	32	141
Positive	52	30	22	104
Year				
2017	29	9	19	57
2018	34	17	52	103
2019	44	50	76	170
2020	28	31	26	85
2021	2	9	3	14

^a Some mortalities were missing data, so totals do not align across all strata. In addition, not all individuals were evaluated in each analysis; see Supplementary Table S1 for an overview of sample size per analysis.

then declined at older ages (Table S2 and Fig. S2). In addition, there were more older females than males in our sample (Fig. 2), which is consistent with previously observed sex and age distributions in this region (Storm et al. 2014). Hence, female deer were 1.60 times more likely to be CWD-positive than males among our sample (95% confidence interval [CI], 0.85–3.03; $P=0.14$), though this difference was not statistically significant when accounting for age (Table S2).

Of the 168 mortalities in which lung pathology could be evaluated, 86 (51.2%) were diagnosed with some form of pneumonia; the majority of these (59.3%) were classed as moderate to severe in grade. Bronchopneumonia was the most common type of pneumonia identified, and lesions were most often multifocal and/or cranioventral (Fig. S3). The etiologic agents for pneumonia cases were typically unclear. In only 15 cases were etiologic agents identified to bacterial genus

or species; most were mixed infections, but in all 15 cases *Trueperella pyogenes* was identified. *Bibersteinia trehalosi*, *Pasteurella multocida* subsp. *multocida*, *Escherichia coli*, an α -hemolytic *Streptococcus* sp., and *Serratia marcescens* were also identified, although the last three were most likely contaminants. Among etiologic agents confirmed histologically but not detected via bacterial culture, coccobacilli were most commonly identified (12 cases).

In addition to CWD and pneumonia cases, necropsies identified a range of additional pathological processes (Table S3). Pulmonary abscesses were identified in 18.4% of individuals evaluated (of $n=147$ evaluated). Hematopoietic atrophy was generally evaluated when expected to be abnormal based on the gross appearance of bone marrow ($n=49$) and was therefore identified in 49% of examined individuals. Cardiac abnormalities were identified in 43.8% of evaluated deer ($n=169$). Of these, 41.9% included a description of hemorrhage, which is a nonspecific and often nonclinical finding, but 29.7% included myocardial degeneration or necrosis. Gastrointestinal lesions were noted in 35.9% of individuals ($n=153$), and these cases were typically inflammatory lesions (e.g., enterocolitis). A parasitic origin for inflammatory GI lesions was present in at least one case (haemonchosis), and GI nematodiasis was independently present (i.e., not specifically associated with inflammatory lesions) in at least seven cases. Ectoparasitism, in the form of lice and/or ticks, was noted in 22.5% of evaluated individuals ($n=138$). Pulmonary nematodiasis and skeletal muscle sarcocystosis were identified histologically in 45.5% and 65.2% of evaluated individuals, respectively ($n=121$ and $n=115$); both were typically classed as mild or minimal.

CWD associations and disease process clustering

Fisher exact tests identified significant associations between CWD status and the presence of emaciation and atrophy of bone marrow fat, with tentative associations ($P<0.05$, but not significant with Bonferroni

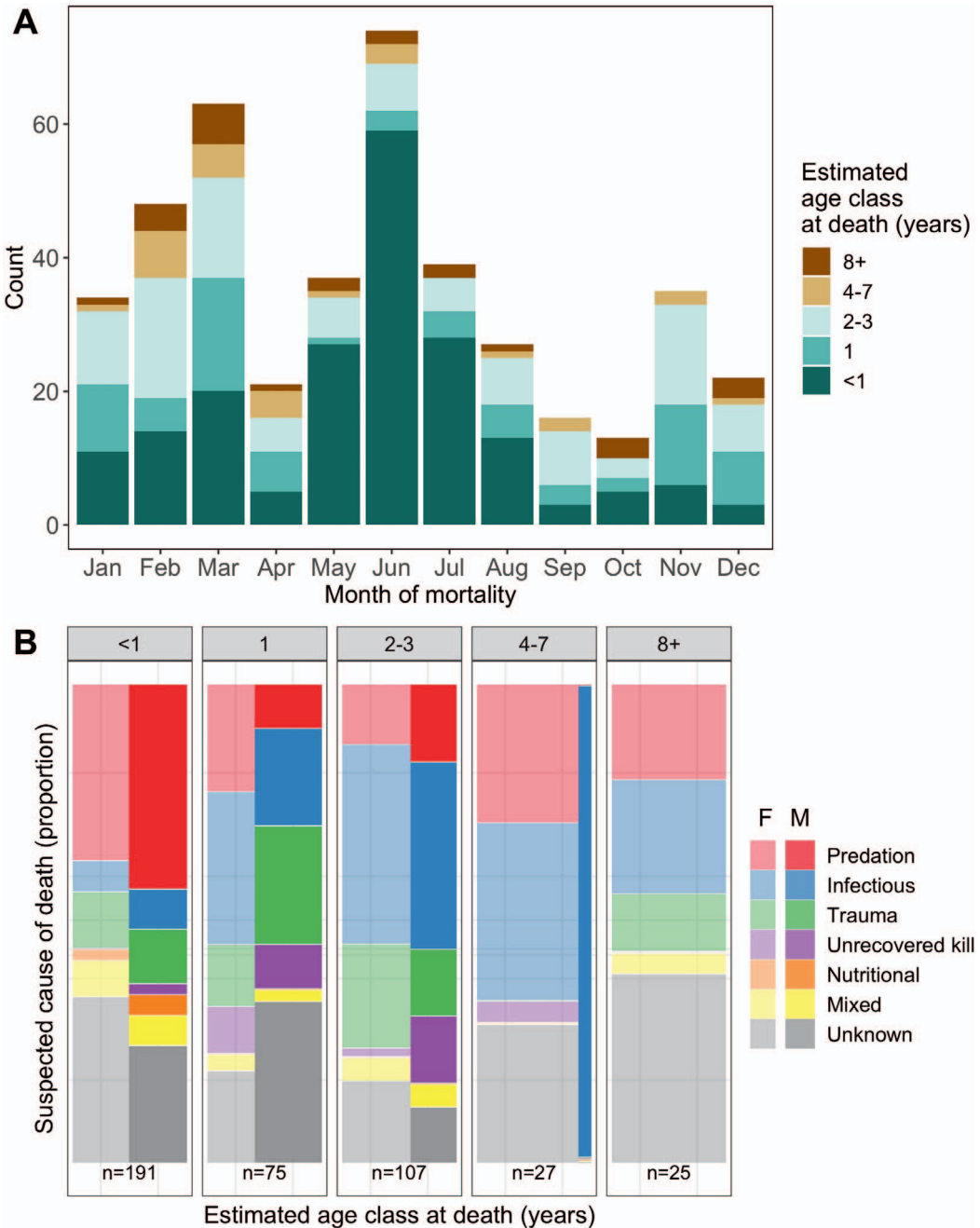


FIGURE 1. Counts of (A) month of Wisconsin white-tailed deer (*Odocoileus virginianus*) mortality by estimated age class and (B) mosaic plot showing cause of death (COD) conditional on age class, with shading by sex. In (B), major causes of death are shown as colors where the height of the color bar represents the proportion of that age and sex class that died from a given cause. The width of the shaded bars within each age class shows the sex proportions (female = light, male = dark). Sample size is denoted at the bottom of each age class column. Ages were binned for visualization purposes. Note that “Predation” = mortality due to predation event; “Infectious” = mortality due to infectious disease; “Trauma” = mortality due to trauma (e.g., vehicle strike, haycutter injury, capture associated); “Unrecovered kill” = unrecovered harvest-associated mortality; “Nutritional” = mortality due to starvation, especially among neonates; “Mixed” = unclear mixture of multiple probable causes of death; “Unknown” = cause of death unknown.

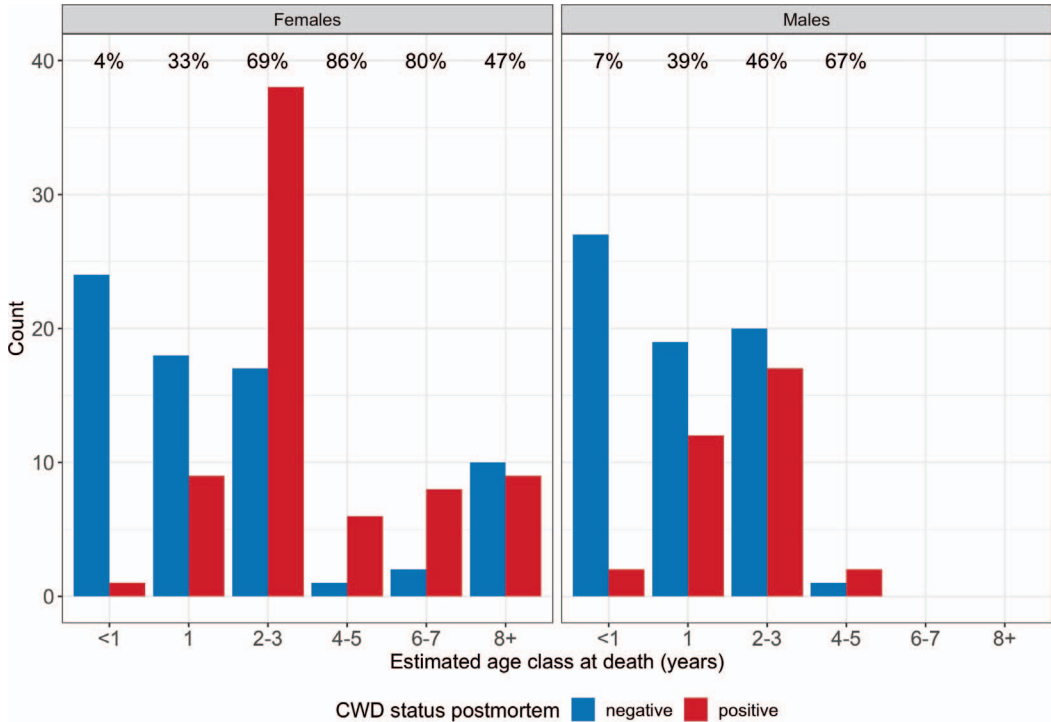


FIGURE 2. Age distributions of Wisconsin white-tailed deer (*Odocoileus virginianus*) that were chronic wasting disease (CWD)-positive (red) versus CWD-negative (blue) by sex and estimated age class at time of death. Percent values represent the proportion of individuals testing CWD positive per age class by sex.

correction) with ectoparasitism (presence of lice and/or ticks) and hematopoietic atrophy (Table 2 and Fig. S4). While a broad classification of “any pneumonia” was not associated with CWD status, evaluating only cases specified as bronchopneumonias found a stronger relationship with CWD (odds ratio, OR=3.22, $P=0.032$, though not statistically significant with a Bonferroni correction). Top GI and cardiac lesion subclassifications (inflammatory lesions, myocardial degeneration/necrosis, respectively) did not show an association with CWD status (GI inflammation: OR=0.90, $P=1$; myocardial degeneration/necrosis: OR=0.37, $P=0.14$).

Nutritional condition was consistently poor among deer diagnosed with CWD, regardless of pneumonia status (Fig. 3). Pneumonia cases that were negative for CWD were frequently in good body condition (Fig. 3), often despite severe lung lesions. These differences were supported by ordinal logistic regression: the effect of pneumonia infection alone (i.e.,

CWD-negative and pneumonia-positive) was not a significant predictor of body condition; however, among pneumonia-negative individuals, those that were CWD-positive were eight times more likely to be in poor body condition than those that were CWD-negative (CWD-positive OR=8.02; 95% CI, 2.31–37.80; $P=0.003$; Tables S4, S5). In addition, among CWD-positive individuals with adequate documentation of condition for staging clinical progression ($n=58$; Fig. S5), females were more likely to be classified as “end stage” than males (OR=5.16; 95% CI, 1.11–25.72; $P=0.04$; Table S6). Note that this difference was based on small sample sizes for non-end-stage individuals (total of 12 non-end-stage individuals, five of which were males).

We identified two distinct communities in the pathology co-occurrence network (modularity=0.037) where disease processes or lesions within the same community were more likely to co-occur: CWD, bronchopneu-

TABLE 2. Prevalence of lesions in Wisconsin white-tailed deer (*Odocoileus virginianus*) by chronic wasting disease (CWD) status, 2017–21.^a

Lesion	Lesion detection	CWD status		Fisher exact test	
		Not detected (%)	Detected (%)	Odds ratio	P value
Emaciation	Not detected	36 (27.7)	15 (11.5)	9.25	< 0.001 ***
	Detected	16 (12.3)	63 (48.5)		
Atrophy of marrow fat	Not detected	21 (17.5)	14 (11.7)	3.37	< 0.01 **
	Detected	26 (21.7)	59 (49.2)		
Pneumonia	Not detected	20 (18.9)	22 (20.8)	1.41	0.43
	Detected	25 (23.6)	39 (36.8)		
Pulmonary abscess	Not detected	29 (32.2)	38 (42.2)	1.43	0.62
	Detected	8 (8.9)	15 (16.7)		
Hematopoietic atrophy	Not detected	6 (17.1)	11 (31.4)	8.73	0.041*
	Detected	1 (2.9)	17 (48.6)		
Cardiac lesions	Not detected	19 (18.1)	36 (34.3)	0.45	0.051
	Detected	27 (25.7)	23 (21.9)		
Gastrointestinal lesions	Not detected	33 (34.0)	35 (36.1)	1.78	0.27
	Detected	10 (10.3)	19 (19.6)		
Ectoparasitism	Not detected	29 (34.5)	31 (36.9)	4.60	0.012*
	Detected	4 (4.8)	20 (23.8)		
Pulmonary nematodiasis	Not detected	13 (17.3)	19 (25.3)	1.15	0.81
	Detected	16 (21.3)	27 (36.0)		
Skeletal muscle Sarcocystosis	Not detected	8 (10.8)	14 (18.9)	0.85	0.80
	Detected	21 (28.3)	31 (41.9)		

^a The “CWD status” columns give the number of individuals in which CWD was detected or not detected among individuals evaluated for a given lesion. Numbers in parentheses give this number as a proportion of the total number of individuals evaluated for a given lesion and CWD. For example, 36 individuals were classified as “not emaciated” and CWD “not detected” in their post-mortem testing; this equates to 27.7% of the individuals evaluated for both emaciation and CWD. Statistically significant *P* values for Fisher exact tests are indicated by asterisks; only those that were statistically significant after a Bonferroni correction for multiple comparisons are highlighted in bold.

monia and pneumonia, emaciation, ectoparasites, and pulmonary abscess (purple nodes in Fig. 4); and GI lesions, cardiac lesions, pulmonary nematodes, skeletal muscle sarcocystosis, and other/mixed pneumonias (turquoise nodes in Fig. 4). Community detection and classification were robust to changes in the number of steps (2–5) and whether missing data were included or removed. Thus, these communities were distinct and stable.

DISCUSSION

We found a range of pathological processes to be present and co-occurring in WTD in a region of high CWD prevalence. Our finding that CWD prevalence increased with age before decreasing among older classes aligns with previous findings in this region (Osnas et

al. 2009; Heisey et al. 2010). Our documentation of cases of CWD in individuals under 1 yr old is consistent with previous findings of CWD-positive fawns in this study region (Gear et al. 2006). Notably, older males were largely missing from our sample, probably because we did not include hunter-harvested mortalities in this study and because hunter harvest is a significant source of mortality for older males in this system. Females, with their older age distribution, were consequently more likely than males to be CWD-positive, in contrast to trends in previous studies in Wisconsin and other regions of the US (e.g., Miller and Conner 2005; Gear et al. 2006; Jennelle et al. 2014; Samuel and Storm 2016; Smolko et al. 2021). This sex-based difference was not statistically significant when we accounted for age of individuals. Given the

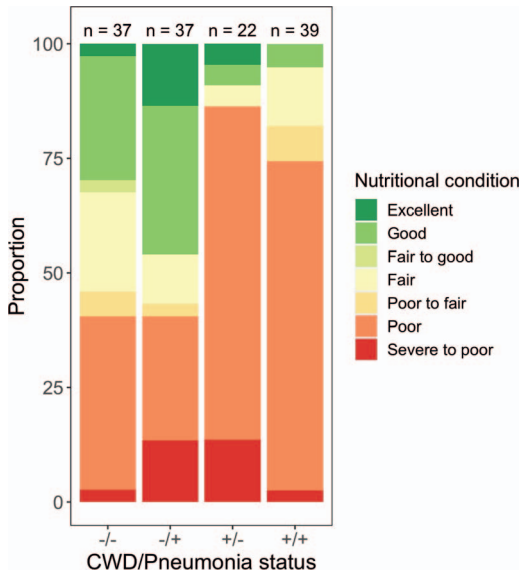


FIGURE 3. Prevalence of body condition classes by chronic wasting disease (CWD) and pneumonia status among Wisconsin white-tailed deer (*Odocoileus virginianus*), 2017–21. The x-axis gives postmortem status for CWD/pneumonia (e.g., -/+ corresponds to CWD-negative and pneumonia-positive individuals). Numbers above bars give sample sizes per CWD/pneumonia classification.

significant role of age in explaining CWD status and the older age distribution for females, it is likely that the age distribution among CWD-positive males versus females shapes the relative role of each sex in transmission. Our assessment of the clinical stage of CWD among males versus females provides evidence that females were more likely to die in end-stage disease than males. This finding suggests that males may be removed from the population in earlier disease stages than females, which is supported by evidence that CWD-positive individuals may be more susceptible to hunter harvest (Conner et al. 2000; Edmunds et al. 2016). If female deer live longer during the terminal stages of CWD disease progression—which corresponds with higher rates of prion shedding (Davenport et al. 2018)—they may consequently have an underrecognized role in CWD transmission.

We further found that CWD was associated with poor nutritional condition and tentatively

with ectoparasitism in our sample. The association with poor nutritional condition was expected, and infected animal emaciation was severe enough to be the proximate cause of death in many CWD cases. We found that pneumonia was not significantly associated with nutritional condition, with or without coinfection with CWD. Coinfected individuals do not die in better condition; pneumonia does not, therefore, appear to significantly shorten the time to death among CWD cases.

The tentative association we observed between ectoparasitism and CWD has not been reported previously. Recording bias cannot be ruled out here, because recorders may be more likely to screen for and observe ectoparasites present on poor-condition animals. Nevertheless, body condition is generally (although not always) expected to be negatively associated with ectoparasitism in endotherms, with mechanisms such as direct tissue damage and the energetic costs of immunity underlying these observed relationships (Sánchez et al. 2018). The tentative relationship we observed between CWD and ectoparasitism might result from CWD-induced wasting affecting immune responses to ectoparasitism (e.g., Trager 1939; Allen and Kemp 1982; Kamath et al. 2014). Alternatively or additionally, severe CWD probably contributes to changes in grooming behavior in affected animals (e.g., via lack of awareness or due to the neurological degeneration that causes excess salivation and head tremors; Williams 2005). Severely ill animals may also have reduced contact with conspecifics (as in Tasmanian devils, *Sarcophilus harrisii*; Hamilton et al. 2020), and/or conspecifics may be less likely to participate in allogrooming of severely ill animals through changes in social cohesion or dominance rank (Hirth 1977; Forand and Marchinton 1989) or through behavioral avoidance (Oaten et al. 2009; Weinstein et al. 2018). Given that behavioral avoidance may subsequently alter transmission dynamics (Croft et al. 2011), the potential relationship between ectoparasitism, CWD, and disease-induced social or behavioral changes merits further study.

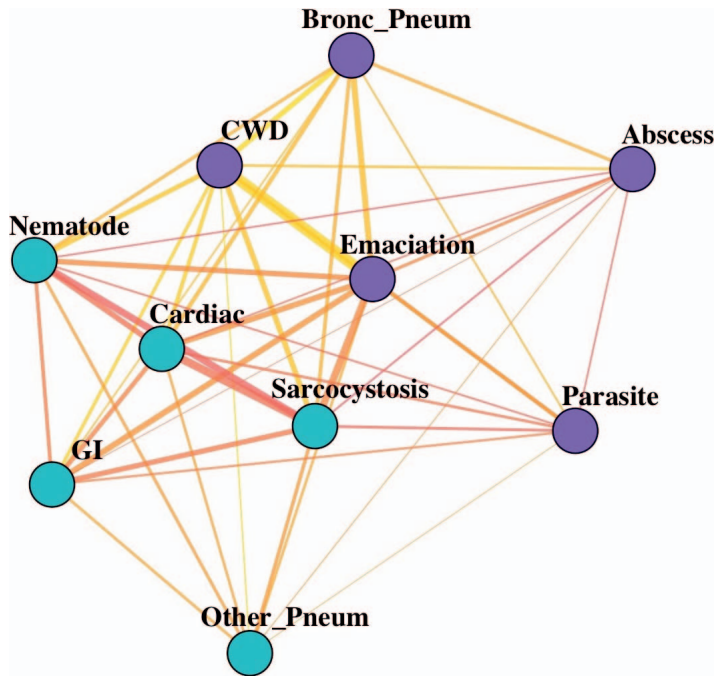


FIGURE 4. Pathology co-occurrence network among necropsied Wisconsin white-tailed deer (*Odocoileus virginianus*), 2017–21. The width and color (yellow to red) of the edges represent the frequency of co-occurrence (i.e., edge weight). Node color is the community in which the node was classified. The network is displayed as force-directed (i.e., Fruchterman-Reingold algorithm; igraph, Csardi and Nepusz 2006). Note that “Bronch_Pneum” = bronchopneumonia or pneumonia; “Abscess” = pulmonary abscess; “Parasite” = ectoparasitism; “Other_Pneum” = pneumonia other than bronchopneumonia or pneumonia (e.g., interstitial pneumonia); “GI” = gastrointestinal lesions; “Nematode” = pulmonary nematodiasis; “CWD” = chronic wasting disease; “Emaciation” = emaciated body condition; “Sarcocystosis” = skeletal muscle sarcocystosis; “Cardiac” = cardiac lesions.

Other studies of WTD pathology trends have reported pneumonia as a common cause of morbidity and mortality, with pneumonia given as the primary cause of death or illness in about 8–24% of cases (Hattel et al. 2004; Haigh et al. 2005; Magle et al. 2012; Zhu et al. 2021). Although our finding of high (52.1%) prevalence of pneumonia among our sample of deer mortalities reflects any pneumonia lesions, not just pneumonia as the primary cause of death, the prevalence of pneumonia lesions here is at least consistent with other studies, if not suggestive of higher rates of serious pneumonia. The high prevalence of pneumonia could certainly be explained by the high prevalence of CWD in our region. The observed pneumonias were frequently cranioventral bronchopneumonia, which is consistent with bacterial etiologic agents and/

or aspiration pneumonia (Zachary and McGavin 2007). Aspiration pneumonia is expected to be associated with late-stage CWD as motor function declines (Williams and Young 1980, 1992; Williams et al. 2002), which could explain the relationship we observed between specifically bronchopneumonia and CWD.

Pneumonias more generally were not associated with CWD, and pneumonia-associated deaths among deer in otherwise good body condition remain unexplained (Fig. 3). The most common agent identified, *Trueperella pyogenes*, has been observed as a common pneumonia agent in deer previously (Hattel et al. 2004; Haigh et al. 2005; Zhu et al. 2021). A limitation is that etiologic agents were not identified for most pneumonia cases. Anaerobic bacteria, such as *Fusobacterium necrophorum*, which has also been reported in

cases of WTD pneumonia (Hattel et al. 2004; Haigh et al. 2005; Leighton 2008; Zhu et al. 2021), would not be detected with the aerobic cultures used here. In addition, more complex pneumonias (e.g., as in *Mycoplasma pneumonia* in bighorn sheep, *Ovis canadensis*; Besser et al. 2008) cannot be ruled out. Deer with CWD could act as reservoirs or maintenance hosts for transmissible pneumonias (Haydon et al. 2002), allowing pneumonia to persist at higher prevalence than in healthy, CWD-free herds, and resulting in higher than normal rates of pneumonia in non-CWD-infected individuals. Investigating potential etiologic agents and the broader population transmission dynamics of pneumonia in the context of high CWD prevalence may be important future lines of inquiry.

In addition to the pairwise associations we observed for CWD, we also identified two main communities among the suite of pathological processes we examined. Although this is not a causative analysis, it may act as a springboard for future areas of research. The first community we identified represented disease processes that were associated with CWD independently (emaciation and ectoparasitism), together with pulmonary abscess, pneumonia, and bronchopneumonia. The link between the pneumonias and pulmonary abscesses is unsurprising, particularly given our findings in support of bacterial agents of pneumonia, and supports pulmonary abscesses typically being of bacterial origin (rather than parasitic). Together, the disease processes in this CWD-associated community appear to represent a cluster of interrelated processes that correspond with severe infectious disease and wasting. In contrast, the non-CWD-associated community probably represents less severe or less complex disease processes (pulmonary nematodiasis, skeletal sarcocystosis, GI lesions, other/mixed pneumonias, some cardiac lesions) clustering with disease processes more typically associated with noninfectious disease (e.g., cardiac myonecrosis). Given that infectious diseases—especially CWD and pneumonia—were a top cause of death in our sample, these results suggest that the CWD-associated community of processes

has a notable role in nonharvest deer mortalities in our study area.

In addition to limitations due to excluding hunter-harvested animals and areas of potential recording bias highlighted above, our study could be affected by other sampling limitations. For example, conditions in which too little carcass could be recovered for necropsy (due to predation or scavenging) might otherwise have been associated with specific disease processes (e.g., severe CWD). Further, although staff performing laboratory and field necropsies remained as objective as possible, some subjectivity is unavoidable, including in attempting to assign a final cause of death. Future work may benefit from incorporating hunter-harvested deer and aiming for a demographically representative sample. Nevertheless, this work represents a novel examination of disease processes and comorbidities present in a high CWD-prevalence deer population, which may be used as a catalyst for future research and management.

ACKNOWLEDGMENTS

We thank the many field technicians who contributed to collaring and mortality investigation efforts, including S. Bundick, L. Hahn, T. Johannes, T. Klein, K. Luukkonen, H. Manninen, and M. Watt. In addition, we thank the Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin Veterinary Care, the UW-SVM Anatomic Pathology Service, and the UW-SVM Histology Laboratory. Funding was provided by the Federal Aid in Wildlife Restoration Act, administered through the Wisconsin Department of Natural Resources and the University of Wisconsin–Madison. Additional funding was provided by the US Geological Survey Biological Threats program (G20AC00353). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at <http://dx.doi.org/10.7589/JWD-D-21-00202>.

LITERATURE CITED

Allen JR, Kemp DH. 1982. Observations on the behaviour of *Dermacentor andersoni* larvae infesting normal

- and tick resistant guinea-pigs. *Parasitology* 84:195–204.
- Arnett EB, Southwick R. 2015. Economic and social benefits of hunting in North America. *Int J Environ Stud* 72:734–745.
- Benestad SL, Mitchell G, Simmons M, Ytrehus B, Vikøren T. 2016. First case of chronic wasting disease in Europe in a Norwegian free-ranging reindeer. *Vet Res* 47:88.
- Besser TE, Cassirer EF, Potter KA, VanderSchalie J, Fischer A, Knowles DP, Herndon DR, Rurangirwa FR, Weiser GC, Srikumaran S. 2008. Association of *Mycoplasma ovipneumoniae* infection with population-limiting respiratory disease in free-ranging Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*). *J Clin Microbiol* 46:423–430.
- Carstensen M, DelGiudice GD, Sampson BA, Kuehn DW. 2009. Survival, birth characteristics, and cause-specific mortality of white-tailed deer neonates. *J Wildl Manage* 73:175–183.
- Conner MM, McCarty CW, Miller MW. 2000. Detection of bias in harvest-based estimates of chronic wasting disease prevalence in mule deer. *J Wildl Dis* 36:691–699.
- Croft DP, Edenbrow M, Darden SK, Ramnarine IW, van Oosterhout C, Cable J. 2011. Effect of gyrodactylid ectoparasites on host behaviour and social network structure in guppies *Poecilia reticulata*. *Behav Ecol Sociobiol* 65:2219–2227.
- Csardi G, Nepusz T. 2006. The igraph software package for complex network research. *Int J Complex Syst* 1695:1–9.
- Davenport KA, Mosher BA, Brost BM, Henderson DM, Denkers ND, Nalls AV, McNulty E, Mathiason CK, Hoover EA. 2018. Assessment of chronic wasting disease prion shedding in deer saliva with occupancy modeling. *J Clin Microbiol* 56:e01243–17.
- DelGiudice GD, Riggs MR, Joly P, Pan W. 2002. Winter severity, survival, and cause-specific mortality of female white-tailed deer in north-central Minnesota. *J Wildl Manage* 66:698–717.
- Edmunds DR, Kauffman MJ, Schumaker BA, Lindzey FG, Cook WE, Kreeger TJ, Grogan RG, Cornish TE. 2016. Chronic wasting disease drives population decline of white-tailed deer. *PLoS One* 11:e0161127.
- Forand KJ, Marchinton RL. 1989. Patterns of social grooming in adult white-tailed deer. *Am Midl Nat* 122:357–364.
- Fountain-Jones NM, Packer C, Jacquot M, Blanchet FG, Terio K, Craft ME. 2019. Endemic infection can shape exposure to novel pathogens: Pathogen co-occurrence networks in the Serengeti lions. *Ecol Lett* 22:904–913.
- Gilbertson MLJ, Turner WC, Storm D. 2022. *White-tailed deer necropsy data from Wisconsin, 2017–2021*. US Geological Survey data release. <https://www.sciencebase.gov/catalog/item/622b98c9d34ec9f19eea432b>.
- Grear DA, Samuel MD, Langenberg JA, Keane D. 2006. Demographic patterns and harvest vulnerability of chronic wasting disease infected white-tailed deer in Wisconsin. *J Wildl Manage* 70:546–553.
- Haigh J, Berezowski J, Woodbury MR. 2005. A cross-sectional study of the causes of morbidity and mortality in farmed white-tailed deer. *Can Vet J* 46:507–512.
- Hamilton DG, Jones ME, Cameron EZ, Kerlin DH, McCallum H, Storfer A, Hohenlohe PA, Hamede RK. 2020. Infectious disease and sickness behaviour: Tumour progression affects interaction patterns and social network structure in wild Tasmanian devils. *Proc Biol Sci* 287:20202454.
- Hattel AL, Shaw DP, Love BC, Wagner DC, Drake TR, Brooks JW. 2004. A retrospective study of mortality in Pennsylvania captive white-tailed deer (*Odocoileus virginianus*): 2000–2003. *J Vet Diagn Invest* 16:515–521.
- Haydon DT, Cleaveland S, Taylor LH, Laurenson MK. 2002. Identifying reservoirs of infection: A conceptual and practical challenge. *Emerg Infect Dis* 8:1468–1473.
- Heisey DM, Osnas EE, Cross PC, Joly DO, Langenberg JA, Miller MW. 2010. Linking process to pattern: Estimating spatiotemporal dynamics of a wildlife epidemic from cross-sectional data. *Ecol Monogr* 80:221–240.
- Hewitt DG. 2015. Hunters and the conservation and management of white-tailed deer (*Odocoileus virginianus*). *Int J Environ Stud* 72:839–849.
- Hirth DH. 1977. Social behavior of white-tailed deer in relation to habitat. *Wildl Monogr* 53:3–55.
- Holsman RH, Petchenik J, Cooney EE. 2010. CWD After “the fire”: Six reasons why hunters resisted Wisconsin’s eradication effort. *Hum Dimensions Wildl* 15:180–193.
- Jennelle CS, Henaux V, Wasserberg G, Thiagarajan B, Rolley RE, Samuel MD. 2014. Transmission of chronic wasting disease in Wisconsin white-tailed deer: Implications for disease spread and management. *PLoS One* 9:e91043.
- Joly DO, Ribic CA, Langenberg JA, Beheler K, Batha CA, Dhuey BJ, Rolley RE, Bartelt G, Van Deelen TR, Samuel MD. 2003. Chronic wasting disease in free-ranging Wisconsin white-tailed deer. *Emerg Infect Dis* 9:599–601.
- Kamath PL, Turner WC, Küsters M, Getz WM. 2014. Parasite-mediated selection drives an immunogenetic trade-off in plains zebras (*Equus quagga*). *Proc Biol Sci* 281:20140077.
- Leighton FA. 2008. *Fusobacterium necrophorum* infection. In: *Infectious diseases of wild mammals*, 3rd Ed., Williams ES, Barker IK, editors. Iowa State University Press, Ames, Iowa, pp. 493–496.
- Magle SB, Chamberlin JC, Mathews NE. 2012. Survival of white-tailed deer in Wisconsin’s chronic wasting disease zone. *Northeast Nat* 19:67–76.
- Miller BF, Osborn DA, Lance WR, Howze MB, Warren RJ, Miller KV. 2009. Butorphanol-azaperone-medetomidine for immobilization of captive white-tailed deer. *J Wildl Dis* 45:457–467.

- Miller MW, Conner MM. 2005. Epidemiology of chronic wasting disease in free-ranging mule deer: Spatial, temporal, and demographic influences on observed prevalence patterns. *J Wildl Dis* 41:275–290.
- Oaten M, Stevenson RJ, Case TI. 2009. Disgust as a disease-avoidance mechanism. *Psychol Bull* 135:303–321.
- Osnas EE, Heisey DM, Rolley RE, Samuel MD. 2009. Spatial and temporal patterns of chronic wasting disease: Fine-scale mapping of a wildlife epidemic in Wisconsin. *Ecol Appl* 19:1311–1322.
- R Core Team. 2018. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. Accessed November 2021.
- Reo NJ, Whyte KP. 2012. Hunting and morality as elements of traditional ecological knowledge. *Hum Ecol* 40:15–27.
- Rooney TP, Waller DM. 2003. Direct and indirect effects of white-tailed deer in forest ecosystems. *For Ecol Manage* 181:165–176.
- Samuel MD, Storm DJ. 2016. Chronic wasting disease in white-tailed deer: Infection, mortality, and implications for heterogeneous transmission. *Ecology* 97:3195–3205.
- Sánchez CA, Becker DJ, Teitelbaum CS, Barriga P, Brown LM, Majewska AA, Hall RJ, Altizer S. 2018. On the relationship between body condition and parasite infection in wildlife: A review and meta-analysis. *Ecol Lett* 21:1869–1884.
- Smolko P, Seidel D, Pybus M, Hubbs A, Ball M, Merrill E. 2021. Spatio-temporal changes in chronic wasting disease risk in wild deer during 14 years of surveillance in Alberta, Canada. *Prev Vet Med* 197:105512.
- Storm DJ, Samuel MD, Rolley RE, Beissel T, Richards BJ, Van Deelen TR. 2014. Estimating ages of white-tailed deer: Age and sex patterns of error using tooth wear-and-replacement and consistency of cementum annuli. *Wildl Soc Bull* 38:849–856.
- Trager W. 1939. Acquired immunity to ticks. *J Parasitol* 25:57–81.
- Weinstein SB, Buck JC, Young HS. 2018. A landscape of disgust. *Science* 359:1213–1214.
- Whitlaw HA, Ballard WB, Sabine DL, Young SJ, Jenkins RA, Forbes GJ. 1998. Survival and cause-specific mortality rates of adult white-tailed deer in New Brunswick. *J Wildl Manage* 62:1335–1341.
- Williams ES. 2005. Chronic wasting disease. *Vet Pathol* 42:530–549.
- Williams ES, Miller MW. 2002. Chronic wasting disease in deer and elk in North America. *Rev Sci Tech* 21:305–316.
- Williams ES, Miller MW, Kreeger TJ, Kahn RH, Thorne ET. 2002. Chronic wasting disease of deer and elk: A review with recommendations for management. *J Wildl Manage* 66:551–563.
- Williams ES, Young S. 1980. Chronic wasting disease of captive mule deer: A spongiform encephalopathy. *J Wildl Dis* 16:89–98.
- Williams ES, Young S. 1992. Spongiform encephalopathies in Cervidae. *Rev Sci Tech* 11:551–567.
- Wisconsin Department of Natural Resources. 2020. CWD prevalence in Wisconsin. Wisconsin Department of Natural Resources, Madison, Wisconsin. <https://dnr.wisconsin.gov/topic/WildlifeHabitat/prevalence.html>. Accessed December 2021.
- Wolfe LL, Fox KA, Miller MW. 2014. “Atypical” chronic wasting disease in PRNP genotype 225FF mule deer. *J Wildl Dis* 50:660–665.
- Zachary JF, McGavin MD. 2007. *Pathologic basis of veterinary disease*. 4th Ed. Elsevier, St Louis, Missouri, 1322 pp.
- Zhu S, Buckles E, Bunting E, Hynes K, Schuler K. 2021. Diagnostic evaluation of unknown white-tailed deer morbidity and mortality in New York State: 2011–2017. *Wildl Biol* 2021:wlb.00860.

Submitted for publication 22 December 2021.

Accepted 30 June 2022.