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Estimating Survival of Eastern Box Turtles (*Terrapene carolina carolina*) with *Mycoplasmopsis* sp., Adenovirus, and Herpesvirus Detection in Illinois, USA

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ABSTRACT: The impact of health and disease on wildlife population dynamics and individual survival is complex and poorly understood, especially in cryptic species such as chelonians. Eastern box turtles (*Terrapene carolina carolina*) are declining due to anthropogenic and natural factors, including disease, though the relative importance of these factors for individual survival is unknown. Determining survival rates in free-ranging chelonians is challenging because individuals are difficult to locate and recapture, deceased turtles can be quickly scavenged, and turtles can die underground during a brumation period. The purpose of this study was to estimate the apparent survival rate for wild eastern box turtles detected with common box turtle pathogens, including *Terrapene* herpesvirus 1, *Terrapene* adenovirus, and box turtle *Mycoplasmopsis* sp., using Cormack-Jolly-Seber models. We used mark-recapture data from 778 individuals from five box turtle populations collected over 7 yr (2016–22), paired with concurrently collected demographic and quantitative PCR pathogen detection data. Apparent survival estimates were different among the five sites, ranging from 71% to 88%, but similar between sexes. We found that pathogens modeled as a function of survival had a positive effect; turtles detected with a pathogen were two to six times more likely to survive than those without detected pathogens. However, this may be an artifact of high, unbiased pathogen prevalence paired with a relatively low probability of pathogen detection via intermittent testing. This analysis provides important estimates of apparent survival for the declining eastern box turtle and valuable information on the interaction between pathogen detection and estimates of individual survival, which can be used to better understand the drivers of population persistence in this species.

Key words: Apparent survival, disease ecology, eastern box turtle, *Terrapene carolina carolina*, wildlife health.

INTRODUCTION

Chelonian populations are declining worldwide, and understanding the factors that affect individual survival is important for conserving species because, as long-lived organisms, chelonian populations rely on low individual adult mortality to maintain stable populations (van Dijk 2011; Stanford et al. 2020; Rhodin et al. 2025). Determining survival rates in free-ranging chelonian populations is challenging for several reasons. Turtles can be difficult to locate without significant effort, and recapture rates are frequently low. For instance, a 15% recapture rate was reported in some years for free-ranging Florida box turtles (*Terrapene carolina bauri*; Dodd et al. 2006), whereas only a 9% recapture rate was reported in the closely related eastern box turtle (*Terrapene carolina carolina*; Brisbin et al. 2008). Also, it is often challenging to determine mortality, because animals are quickly scavenged, remains are easily obscured by ground foliage, and those that die underground during a brumation period are rarely recovered (Adamovicz et al. 2018; Rayl et al. 2020). Though challenging, characterizing survival is important for developing targeted and effective conservation strategies and can be crucial for protecting endangered chelonians.

Eastern box turtles are experiencing population declines across the USA due to anthropogenic factors and disease (Way Rose and Allender 2011; Adamovicz et al. 2018; Rayl

et al. 2020). Pathogen-related mortality events are usually associated with frog virus 3 (FV3; recently renamed *Ranavirus rana1*) outbreaks (De Voe et al. 2004; Johnson et al. 2008; Farnsworth and Seigel 2013; Sim et al. 2016; Kimble et al. 2017). Although FV3 represents a known cause of box turtle mortality, several other box turtle pathogens, including *Terrapene* herpesvirus 1 (TerHV1), *Terrapene* adenovirus (TerAdv), and a box turtle *Mycoplasmopsis* sp., have been identified as potential threats to box turtle health due to high morbidity and association with mortality in young or immunocompromised individuals, though the role in long-term survival is poorly characterized (Feldman et al. 2006; Farkas and Gál 2009; Jacobson et al. 2014; Sim et al. 2015; Palmer et al. 2016; Sim et al. 2016; Kolesnik et al. 2017). For example, TerHV1 was initially detected in a hatchling eastern box turtle with pneumonia that later died (Sim et al. 2015); however, subsequent surveillance in wild populations indicates that this virus is probably a host-adapted pathogen (Kane et al. 2017). The TerAdv is also considered host-adapted in eastern box turtles, although this virus was associated with gastrointestinal ulcerations and hepatic vascular necrosis in an ornate box turtle (*Terrapene ornata ornata*; Farkas and Gál 2009; Franzen-Klein et al. 2020). Box turtle *Mycoplasmopsis* sp. (BTMyco) has been associated with upper respiratory disease in free-ranging box turtles and is known to cause low mortality in immunocompromised individuals (Feldman et al. 2006; Palmer et al. 2016). Understanding the impact of these pathogens on survival in free-ranging eastern box turtles is important for shaping conservation efforts, particularly regarding the necessity for proactive or reactive intervention to mitigate disease risk.

Pathogen dynamics in wildlife are complex, especially in cryptic chelonian species, and repeated testing of the same individuals over time is rarely reported due to logistical challenges or financial constraints or both (Johnson et al. 2008; Adamovicz et al. 2015, 2018; Archer et al. 2017; Aplasca et al. 2019). Due to the lack of longitudinal data from natural settings, the clinical effect of pathogens, and impacts on long-term morbidity and mortality in free-ranging populations, is often unknown or poorly characterized. The effect of pathogens on host survival can be estimated from mark-recapture models to determine the relative importance of pathogens for the conservation of free-ranging populations. The Cormack-Jolly-Seber (CJS) model is a framework in wildlife ecology for estimating individual survival rates and capture probabilities (Cormack 1964; Jolly 1965; Seber 1965). This model is often applied in population ecology studies to estimate how different factors, such as environmental conditions, influence the survival and detection of individuals in the population (Kéry and Schaub 2012). This model can also be adapted to study how pathogens affect host survival by incorporating pathogen-related variables as covariates within the model. This extension enables the investigation of whether individuals detected with a particular pathogen have different survival rates compared with those not detected with a pathogen. Therefore, the objective of our study was to estimate the apparent survival of eastern box turtles in five Illinois populations and to determine whether these estimates are influenced by the presence of three commonly detected box turtle pathogens.

MATERIALS AND METHODS

Study sites and sample collection

The Wildlife Epidemiology Laboratory at the University of Illinois College of Veterinary Medicine and the Illinois Natural History Survey have performed demographic mark-recapture surveys and multipathogen surveillance for 7 yr (2016–22) in five central Illinois, USA populations of eastern box turtles. These include four populations in Vermillion County (sites A–D) and a single population in Marion County (site E; Fig. 1). Teams of three to five dogs were used to capture eastern box turtles during a 2- to 4-h search period, one to three times during the active season (May–September) each year (Adamovicz et al. 2018). The same track was searched at each visit for the Vermillion County sites, but multiple areas within a large preserve were searched intermittently at the Marion County site. Each

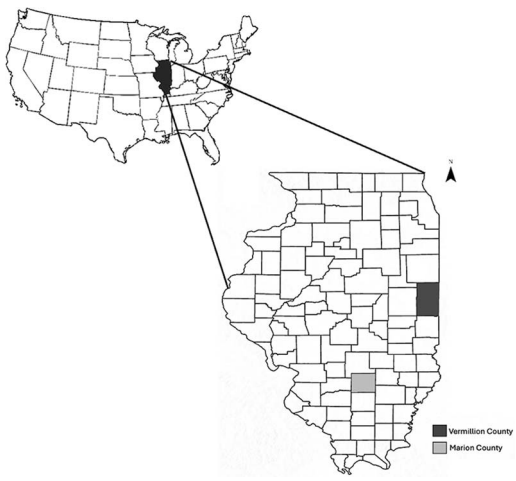

FIGURE 1. County map of Illinois, USA, highlighting Vermillion and Marion counties, where five eastern box turtle (*Terrapene carolina carolina*) populations were sampled annually using canine search teams during the active season (May–September) 2016–22.

site was representative of Illinois open woodlands, consisting of 30–80% canopy cover composed primarily of fire-tolerant oak and hickory trees and poorly developed understories, promoting a diverse herbaceous layer of forbs, grasses, and sedges covering 50–100% of the ground (Illinois Department of Natural Resources 2023).

Captured turtles were marked with a unique notch code on the marginal scutes, enabling identification of previously captured turtles (Cagle 1939). Turtles used in this analysis were identified as males and females on the basis of cloaca location and the concavity of the plastron (Dodd 1997; Dodd 2002). When individuals were sexed differently between years (n=23) due to differences in observers or errors in reporting, we defaulted to a male classification for analysis. Adult turtles, identified by size and secondary sex characteristics (Dodd 2002), were used in this analysis. Swabs of the oral cavity and cloaca (COS) were collected using cotton-tipped plastic applicators (Fisher Scientific, Pittsburgh, Pennsylvania, USA). These were placed in a 2-mL polypropylene microcentrifuge tube (Fisher Scientific), stored on ice packs in the field, and maintained at −20 °C until DNA extraction. Each turtle was released to its original capture site within 6 h of initial contact.

The DNA was extracted from frozen swabs 2–6 wk after collection using a commercial kit (QIAamp DNA Blood Mini Kit and DNeasy kit, Valencia, California, USA). The DNA concentrations were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Turtle COS DNA samples were assayed for TerAdv, BTMyco, and TerHV1 using previously described TaqMan quantitative PCR (qPCR) assays (Kane et al. 2016; Adamovicz et al. 2018; Franzen-Klein et al. 2020). Quantitative PCR was performed using a 7500 ABI Real-Time PCR System (Applied Biosystems, Carlsbad, California, USA), and data were analyzed using the associated software (Sequence Detection Software, version 2.05). Serial dilutions of positive controls for BTMyco, TerHV1, and TerAdv were prepared from 10^7 to 10^1 copies per reaction. A nontemplate control (sterile water) was included on each plate. The COS DNA samples were considered positive if they had a mean quantification cycle value less than the lowest detected standard dilution on the plate.

Survival analysis

Apparent survival (ϕ) and detection probability (p) in this eastern box turtle cohort were modeled using CJS models (Cormack 1964; Jolly 1965; Seber 1965) in Program MARK (White and Burnham 1999). We investigated the effects of BTMyco, TerHV1, and TerAdv separately by creating a suite of 12 models for each pathogen and comparing the relative fit of each model. Covariates for modeling turtle detection included pathogen presence at the time of capture; covariates for modeling apparent survival included study site, sex, pathogen presence, and their combinations. The presence of clinical signs was not used as a covariate in this analysis, due to a minimal number of turtles (<2%) with external signs of illness (ocular discharge, nasal discharge, ocular swelling, or aural abscesses). Finally, we used an information-theoretic approach to compare model performance within Program MARK, specifically relying on Akaike information criterion corrected for small sample size (Hurvich and Tsai 1989).

TABLE 1. *Terrapene* adenovirus (TerAdv), *Terrapene* herpesvirus 1 (TerHV1), and box turtle *Mycoplasmopsis* sp. (BTMyco) prevalence estimates with 95% confidence intervals (CI) from five eastern box turtle (*Terrapene carolina carolina*) populations in two counties in Illinois, USA, 2016–22.

		Prevalence		
Illinois county	Site (population)	TerAdv	TerHV1	BTMyco
Vermillion	A	18% 95% CI, 13–4%	5.0% 95% CI, 2.0–9.0%	11% 95% CI, 7.0–16%
	B	16% 95% CI, 9.4–23%	12% 95% CI, 6.8–19%	5.0% 95% CI, 2.0–11%
	C	4.3% 95% CI, 1.0–11%	10% 95% CI, 5.0–17%	5.0% 95% CI, 2.0–11%
	D	10% 95% CI, 4.0–19%	0% 95% CI, 0–5.0%	4.0% 95% CI, 1.0–12%
Marion	E	13% 95% CI, 9.0–17%	13% 95% CI, 9.2–17%	6.0% 95% CI, 4.0–10%

RESULTS

Study population

Data from 778 turtles collected during 2016–22 were included in this analysis. Most turtles were only caught once (75%). Most turtles were evaluated from site E (n=289), followed by site A (n=208), site B (n=116), site C (n=94), and site D (n=71). The greatest proportion of recaptured turtles was encountered at site B (n=38; 33%), followed by site E (n=77; 27%), site C (n=23; 25%), site A (n=47; 23%), and site D (n=13; 18%). Site B also had the most years in which turtles were serially recaptured, including four turtles recaptured annually for 4 yr and five turtles recaptured annually for 3 yr.

The overall prevalence of TerAdv among all five sites throughout the 7 yr was 13% (n=102; 95% confidence interval [CI], 11–16%). Site A had the highest prevalence of TerAdv (18%; 95% CI, 13–24%), followed by site B (16%; 95% CI, 9.4–23%), site E (13%; 95% CI, 9.0–17%), site D (10%; 95% CI, 4.0–19%), and site C (4.3%; 95% CI, 1.0–11%). The prevalence of TerHV1 among all five sites throughout the 7 yr was 8.7% (n=68; 95% CI, 6.8–11%). The TerHV1 prevalence was highest at site E (13%; 95% CI, 9.2–17%) and site B (12%; 95% CI, 6.8–19%) but was lower at site C (10%; 95% CI, 5.0–17%) and site A (5.0%; 95% CI, 2.0–9.0%) and was absent at site D (0%; 95% CI, 0–5.0%). The overall prevalence of BTMyco across all sites was 7.8% (n=61; 95% CI, 6.0–10%). Similar to TerAdv, site A also had the greatest BTMyco prevalence (11%; 95% CI, 7.0–16%), followed by site E (6.0%; 95% CI, 4.0–10%), site C (5.0%; 95% CI, 2.0–11%), site B (5.0%; 95% CI, 2.0–11%), and site D (4.0%; 95% CI, 1.0–12%; Table 1).

Survival analysis

The best-performing models indicated that apparent survival differed considerably among the five sites. Specifically, apparent survival estimates ranged from 71% to 88%, with the sites with the lowest apparent survival estimates being sites E, D, and A, whereas sites B and C had the highest apparent survival estimates (Table 2). The apparent survival estimate from the constant model was 81% (95% CI, 74–86%). Apparent survival was similar between the sexes, although males (ϕ=87%; 95% CI, 72–94%) had slightly higher apparent survival estimates than females (ϕ=80%; 95% CI, 74–86%). The most parsimonious models

TABLE 2. Apparent survival estimates from a Cormack-Jolly-Seber model in Program MARK with 95% confidence interval (CI) between five eastern box turtle (*Terrapene carolina carolina*) populations in two counties in Illinois, USA, 2016–22.

Illinois county	Site (population)	Survival
Vermillion	B	88% CI, 72–96%
	C	86% CI, 69–95%
	A	74% CI, 61–83%
	D	74% CI, 56–86%
Marion	E	71% CI, 61–80%

TABLE 3. Model ranking on the basis of Akaike information criterion corrected (AICc) for small sample size from Program MARK considering the presence of *Terrapene* adenovirus (TerAdv), site, and sex modeled as covariates for functions of survival (ϕ) and recapture probability (*p*) for 778 eastern box turtles (*Terrapene carolina carolina*) in Vermillion and Marion counties, Illinois, USA, 2016–22. Model rankings also include the number of parameters used in each model and deviance. Dots represent constant variables.

Model	AICc	Delta AICc	AICc weight	No. parameters	Deviance
ϕ(Sex+Site+TerAdv)*p*(.)	1562.5405	0.000	0.3918	8	1546.3742
ϕ(Site+TerAdv)*p*(.)	1563.2263	0.686	0.2781	7	1549.0971
ϕ(Sex+TerAdv)*p*(.)	1564.7483	2.208	0.1299	4	1556.7023
ϕ(Sex+Site)*p*(.)	1566.8987	4.358	0.0443	7	1552.7695
ϕ(Sex+Site)*p*(TerAdv)	1567.2555	4.715	0.0371	8	1551.0892
ϕ(Site)*p*(.)	1567.2829	4.742	0.0366	6	1555.1861
ϕ(Site)*p*(TerAdv)	1567.7732	5.233	0.0286	7	1553.6440
ϕ(TerAdv)*p*(.)	1568.1251	5.585	0.0240	3	1562.0975
ϕ(Sex)*p*(.)	1569.1483	6.608	0.0144	3	1563.1207
ϕ(Sex)*p*(TerAdv)	1569.5774	7.037	0.0116	4	1561.5314
ϕ(.)*p*(.)	1573.1440	10.60	0.0020	2	1569.1302
ϕ(.)*p*(TerAdv)	1573.5595	11.02	0.0016	3	1567.5319

for each of the pathogens included turtle detection probability as a constant variable that was not influenced by pathogen presence, site, or sex. The constant recapture probability of turtles was 14% (95% CI, 11–17%).

The best-performing models for TerAdv (ϕ=71%; 95% CI, 52–85%), TerHV1 (ϕ=87%; 95% CI, 44–98%), and BTMyco (ϕ=79%; 95% CI, 45–94%), incorporated each pathogen, sex, and site as factors of survival (Tables 3–5). The TerAdv-positive turtles were two times more likely to survive than turtles that tested negative for this pathogen, and turtles detected with TerHV were six times more likely to survive than TerHV1-negative individuals. Turtles detected with BTMyco were three times more likely to survive than turtles that had not been detected with this pathogen.

TABLE 4. Model ranking on the basis of the corrected Akaike information criterion (AICc) from Program MARK considering the presence of *Terrapene* herpesvirus 1 (TerHV1), site, and sex modeled as covariates for functions of survival (ϕ) and recapture probability (*p*) for 778 eastern box turtles (*Terrapene carolina carolina*) in Vermillion and Marion counties, Illinois, USA, 2016–22. Model rankings also include the number of parameters used in each model and deviance. Dots represent constant variables.

Model	AICc	Delta AICc	AICc weight	No. parameters	Deviance
ϕ(Site+Sex+TerHV1)*p*(.)	1556.8860	0.000	0.31776	8	1540.7197
ϕ(Site+TerHV1)*p*(.)	1557.0705	0.185	0.28976	7	1542.9413
ϕ(Sex+TerHV1)*p*(.)	1560.2650	3.379	0.05866	4	1552.2190
ϕ(TerHV1)*p*(.)	1563.8917	7.006	0.00957	3	1557.8641
ϕ(Sex+Site)*p*(.)	1566.8987	10.02	0.00213	7	1552.7695
ϕ(Site)*p*(.)	1567.2829	10.36	0.00176	6	1555.1861
ϕ(Site+Sex)*p*(TerHV1)	1568.8261	11.94	0.00081	8	1552.6598
ϕ(Sex)*p*(.)	1569.1483	12.26	0.00069	3	1563.1207
ϕ(Site)*p*(TerHV1)	1569.1495	12.26	0.00069	7	1555.0203
ϕ(Sex)*p*(TerHV1)	1571.0276	14.14	0.00027	4	1562.9816
ϕ(.)*p*(.)	1573.1440	16.26	0.00009	2	1569.1302
ϕ(.)*p*(TerHV1)	1574.9839	18.10	0.00004	3	1568.9563

TABLE 5. Model ranking on the basis of the corrected Akaike information criterion (AICc) from Program MARK considering the presence of box turtle *Mycoplasmopsis* sp. site, and sex modeled as covariates for functions of survival (ϕ) and recapture probability (p) for 778 eastern box turtles (*Terrapene carolina carolina*) in Vermillion and Marion counties, Illinois, USA, 2016–22. Model rankings also include the number of parameters used in each model and deviance. Dots represent constant variables.

Model	AICc	Delta AICc	AICc weight	No. parameters	Deviance
ϕ(Sex+Site+BTMyco)p(.)	1562.6077	0.000	0.366	8	1546.4414
ϕ(Site+BTMyco)p(.)	1563.3289	0.721	0.255	7	1549.1997
ϕ(Sex+BTMyco)p(.)	1565.3672	2.760	0.092	4	1557.3212
ϕ(Sex+Site)p(BTMyco)	1565.6366	3.029	0.080	8	1549.4703
ϕ(Site)p(BTMyco)	1565.8098	3.202	0.073	7	1551.6806
ϕ(Sex+Site)p(.)	1566.8987	4.291	0.043	7	1552.7695
ϕ(Site)p(.)	1567.2829	4.675	0.035	6	1555.1861
ϕ(Sex)p(BTMyco)	1568.3647	5.757	0.021	4	1560.3187
ϕ(BTMyco)p(.)	1568.8951	6.287	0.016	3	1562.8675
ϕ(Sex)p(.)	1569.1483	6.541	0.014	3	1563.1207
ϕ(.)p(BTMyco)	1572.4489	9.841	0.003	3	1566.4213
ϕ(.)p(.)	1573.1440	10.53	0.002	2	1569.1302

DISCUSSION

Apparent survival estimates were different between Illinois eastern box turtle populations, with turtles at sites E, D, and A having the lowest apparent survival estimates. Each of the five sites shares similar habitat characteristics, consisting of the same native and nonnative plant species. Unfortunately, Illinois forest habitats are degraded due to changes in natural disturbances (e.g., fires, tornadoes), inappropriate timber harvesting, and altered flooding patterns (Illinois Department of Natural Resources 2023). These changes can alter plant and animal communities, potentially negatively affecting the survival of eastern box turtles. The Marion County site (site E) is the largest of the five surveyed sites, and only portions of this site were surveyed, sometimes inconsistently between years, so the low survival estimates could be due to emigration of captured turtles outside the study areas. Our estimates of survival of eastern box turtles are consistent with population estimates (Adamovicz et al. 2018), indicating a high likelihood of local extirpation in the next 20–50 yr; however, previous population viability analyses have indicated that site E is the most stable population overall and least likely to be affected by mortality events (Adamovicz et al. 2018).

Apparent survival estimates from all five populations were lower than the 96% for populations from the south-central region of Indiana, USA (Currylow et al. 2011). However, that estimate was based on radiotelemetry-tracked turtles, for which the fate was known, unlike in our study, where the survival was estimated despite most mortality events being unobserved. Other radiotelemetry and mark-recapture studies of free-ranging and translocated *Terrapene* spp. have found survival estimates ranging from 89% to 93%, with a high number of transient individuals (Williams and Parker 1987; Cook 2004). The CJS model encompasses both survival and emigration, so our low apparent survival estimates might similarly be showing high dispersal rates of individuals. Our eastern box turtle survival estimates were also lower than recent ornate box turtle survival estimates in Illinois, which were 84 and 97% for two sites and were determined to be unsustainable for both ornate populations (Edmonds et al. 2024). If our estimates are accurate, this could indicate that all the populations that we assessed are decreasing, although the CIs for our survival estimates are rather large.

Previous studies have, as in our study, found similarities in survival estimates between sexes, in eastern and Florida box turtles (*Terrapene carolina bauri*; Cook 2004; Dodd et al. 2006). When using radiotelemetry, survival probabilities for eastern box turtles in South Carolina were also similar between sexes, at 94 and 92% for males and females, respectively (Brisbin et al. 2008). The apparent higher survival of males than females in our dataset might be an artifact of our choice to default to male if there were discrepancies in sex identification of an individual turtle between years, leading to a greater number of male recaptures within the dataset, which the model then associated with higher survival.

The survival estimates we determined may appear relatively low compared with previous survival analyses of box turtle populations because of the methods we used. Specifically, eastern box turtle survival studies using the Kaplan-Meier estimator or other "known fate" programs require known fate data and rely on smaller sample sizes (Cook 2004; Currylow et al. 2011). As a result, known fate programs can be influenced by observing the death of a few monitored individuals. Our estimates of apparent survival encompass both true survival and permanent emigration; the inability to account for permanent emigration probably leads to differences in apparent survival estimates in recapture studies, compared with known fate analyses. When comparing mark-recapture methods to radiotelemetry, the estimates of survival were higher using the mark-recapture method (95%) compared with using radiotelemetry (93%) in free-ranging eastern box turtles, although only 86 captured turtles and 23 radiotelemetry turtles were used in the analysis (Brisbin et al. 2008). We chose to implement a model that accounts for imperfect detection, given the cryptic nature of box turtles, to estimate survival rates using a large and robust dataset spanning multiple years and study sites.

To the best of our knowledge, pathogen data have not previously been incorporated into apparent survival estimates for free-ranging eastern box turtles. Pathogen presence was included in the top-ranked models, and interestingly, there was a positive correlation between pathogen presence and apparent survival. This finding could theoretically indicate that the presence of these pathogens had a protective effect on the host, possibly through competition with other pathogens or with other deleterious factors. They may also establish a symbiotic relationship with their host, potentially supporting the host's survival. However, these hypotheses are unlikely, due to the diseases associated with these pathogens (Feldman et al. 2006; Farkas and Gál 2009; Sim et al. 2015). Furthermore, it is possible that only turtles with a competent immune system successfully combat the infection with these agents and survive the infection, becoming chronic carriers that sporadically shed pathogen DNA, whereas animals that die from acute infections are lost between sampling periods. The time from exposure to infection of those pathogens is unknown because challenge studies have not been performed for these pathogens in this species; thus, the experimental mortality rate is also unknown, but future research should consider investigating these relationships. In addition, many factors contribute to an individual's survival; although these pathogens were positively associated with pathogen presence, this association does not imply a causal relationship.

Alternatively, and potentially more parsimoniously, the positive association of these viruses and bacteria with survival could be an artifact of imperfect detection of the pathogens themselves. If pathogen DNA is absent during sampling, qPCR cannot detect the pathogen within a particular host; however, the host may still harbor a quiescent or active infection (Marschang et al. 2021). If the true prevalence of each of these pathogens is much higher than previously indicated by raw prevalence estimates (Archer et al. 2017; Kane et al. 2017; Adamovicz et al. 2018; Franzen-Klein et al. 2020; Daleo et al. 2025), then we may be capturing many individuals who are infected but were undetected using qPCR tests. Therefore, the more an individual is recaptured, the more likely the individual will

eventually test positive for a pathogen (i.e., longer lived individuals will be more likely to be detected with a pathogen), although there is a limited number of recaptured turtles in this dataset.

The pathogens that we investigated may be more prevalent than we are able to detect; low detection has been observed in captive turtles (Daleo et al. 2025). Both TerHV1 and TerAdv are considered host adapted in free-ranging eastern box turtle populations, given the high prevalence and lack of clinical signs of illness from other free-ranging surveillance studies (Kane et al. 2017; Franzen-Klein et al. 2020). Consequently, these pathogens are believed not to cause disease in eastern box turtles unless the turtles are infected with another pathogenic organism, or the host is young, old, or immunocompromised (Farkas and Gál 2009; Rivera et al. 2009; Sim et al. 2015; Adamovicz et al. 2018). The unbiased prevalence of these pathogens in this study's populations is probably much higher than the prevalence estimates presented, suggesting that many false negatives occur during qPCR surveillance, due to a lack of coordination between pathogen shedding and sample timing, quiescent pathogens, or undetectably low pathogen loads being shed. In addition, BTMyco has only been intermittently associated with morbidity in eastern and ornate box turtles (Feldman et al. 2006; Farkas and Gál 2009). This bacterial infection may cause disease in box turtle hosts, but free-ranging individuals may be able to overcome the infection through natural immune responses; therefore, it may be present but undetected.

Although this is a useful dataset for understanding eastern box turtle survival and the impact of three commonly detected pathogens on survival, it is important to consider additional pathogens not tested for in this study. The prevalence of coinfections between these three pathogens was below 2% in this study, so we could not investigate the apparent survival of coinfected individuals. However, the three pathogens detected in this study have been associated with disease when coinfected within box turtle hosts (Farkas and Gál 2009; Sim et al. 2015, 2016). In addition, although this study used highly sensitive and specific assays for pathogen surveillance, there is a possibility that closely related pathogens, such as *Terrapene* alphaherpesvirus 3, may affect the health of free-ranging turtles. The pathogen surveillance used incorporated results from qPCR, but no sequencing was performed to confirm or exclude the presence of closely related viruses that share conserved gene segments. Similarly, the TerAdv assay detects a highly conserved gene segment between two box turtle adenoviruses (Franzen-Klien et al. 2020). As these viruses continue to be explored, future studies may find differing clinical impacts between the closely related viruses.

This study provides a framework for evaluating pathogen dynamics in cryptic wildlife species, where direct observation of individuals and mortality is challenging. By integrating pathogen detection and individual survival, these findings highlight the importance of accounting for imperfect detection when assessing pathogenic threats in chelonians. Future studies should continue to explore the complexities between pathogen dynamics and strains to better inform conservation efforts for free-ranging turtles facing diverse infectious disease challenges.

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