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Source: Journal of Zoo and Wildlife Medicine, 54(4) : 785-795

Published By: American Association of Zoo Veterinarians

URL: https://doi.org/10.1638/2022-0173

DOI: 10.1638/2022-0173 *Journal of Zoo and Wildlife Medicine* 54(4): 785–795, 2023

CUTANEOUS MYIASIS AND ITS RELATIONSHIP TO WELLNESS IN EASTERN BOX TURTLES (*TERRAPENE CAROLINA CAROLINA*) IN CAPE COD, MASSACHUSETTS

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Abstract: Eastern box turtles (*Terrapene carolina carolina*) face a variety of anthropogenic, infectious, and environmental threats and have been affected by high morbidity and mortality disease events. Wellness parameters in free-ranging eastern box turtles with a high prevalence of myiasis on Cape Cod, MA, were documented to identify epidemiologic trends or associations with several health parameters. There were 109 samples collected from 59 individual box turtles over the course of 4 mon. Six turtles died over the course of this study. Fly larvae infestations varied in severity and were observed in the cutaneous and subcutaneous tissue (n = 18; 30.5%). Animals with myiasis had fewer plastron abnormalities than those without ($P = 0.034$), and all turtles found in bogs had evidence of fly larvae infections ($P < 0.0001$). Individuals with myiasis also had lower body condition index ($P = 0.014$), lower total white blood cells ($P = 0.031$), lower PCV ($P < 0.0001$), lower total solids ($P < 0.0001$), higher erythrocyte sedimentation rate ($P < 0.0001$), lower calcium ($P = 0.018$), and lower phosphorus ($P = 0.017$). Three turtles tested positive for terrapene herpesvirus 1, but presence was not associated with myiasis. Heavy metal analysis revealed no significant differences between turtles with and without myiasis. This study examined the health of a population of eastern box turtles, and continued health assessments will be beneficial in determining the impact of myiasis on future conservation plans.

INTRODUCTION

Eastern box turtles (EBT) located in Cape Cod, MA, face an array of potential threats to their conservation.[23,33] In MA, these turtles are listed as a species of special concern under the Massachusetts Endangered Species Act; thus, conservation is a priority for land managers and natural resource personnel.[23] Many threats to EBT conservation are due to anthropogenic causes, such as habitat fragmentation,[50] vehicle strikes,[14] farming, illegal collection, and prescribed burns.[23] There are also multiple documented infectious diseases that may pose a threat to EBT conservation, including ranavirus,[3,6,59] herpesviruses,[31,60,67] *Mycoplasmopsis* sp.,[25] and adenovirus.[8,27] Infectious diseases such as adenovirus, herpesvirus, and *Mycoplasmopsis* sp. can be host adapted in the population and produce little to no clinical signs except in incidences of severe stress.[8,27] However, ranaviral disease can cause devastating outbreaks and acutely debilitate a population of turtles.[3,56,59]

Cape Cod has the largest density of EBTs in MA with over 42% of the state population.[23] Camp Edwards, a secure military base, is a training site for the Massachusetts Army National Guard and covers almost 15,000 acres (6,070 hectares) in Cape Cod. This area is within the Atlantic Coastal Pine Barrens Ecoregion that consists of mid to late successional forest, pitch pine–scrub oak barrens, and various wetlands.[33] The home ranges and habitat preferences of EBTs at this site have been assessed for several years to influence management decisions; however, little information exists on disease prevalence and mortality rates.[23,33] The Camp Edwards Natural Resources Office manages this population of turtles via radiotelemetry and educates military personnel on how to conserve and protect these animals.

Cutaneous fly larvae infections (myiasis) have become increasingly common in this population, but the extent to which they affect the health and wellness of the turtles has not been investigated. Myiasis is not a new threat to box turtles and has been previously documented in turtles from IL, KS, MS, GA, NC, FL, MD, NJ, CT, MA, and NY.[1,16,21,28,35,41,47,51,53,55,57,58] In affected turtles,

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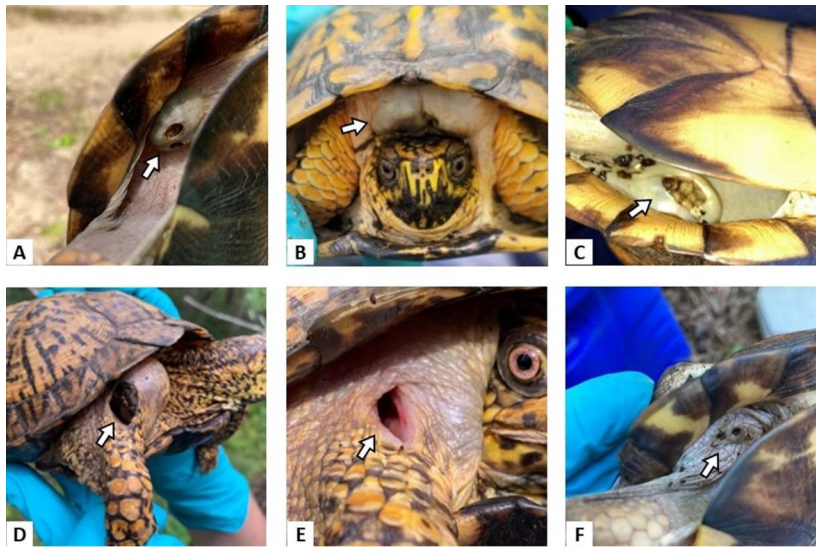

Figure 1. Appearance of cutaneous myiasis in free-living eastern box turtles (*Terrapene carolina carolina*). Subdermal cavities containing fly larvae (**A–C**), cutaneous plug of necrotic debris obstructing the pore following larval egress before (**D**) and after (**E**) plug removal, and postinfection scar (**F**).

larvae are found in subdermal cavities with associated breathing pores typically on or around the limbs. Individuals eventually form plugs of necrotic debris that seal the pores following larval egress, and the areas become scar tissue once healed.[53]

The goal of this study was to conduct health assessments and targeted pathogen surveillance in eastern box turtles in a population with high prevalence of myiasis at Camp Edwards. Specific biologic hypotheses were cutaneous myiasis causes systemic changes in EBT health parameters and concurrent infections make EBT more prone to myiasis.

MATERIALS AND METHODS

Ethics statement

Live animal use in this study was approved through the University of Illinois Institutional Animal Care and Use Committee (protocol 20258) and Massachusetts Division of Fisheries and Wildlife (permit 075.21SCRA).

Study sites

Turtles were sampled from May 2021 through August 2021 on Cape Cod, MA, within Camp Edwards, a secure military base and at a frequent nesting location in Sandwich, MA. The latitude and longitude of each turtle were obtained using global positioning software via handheld Garmin Oregon 450t device (Garmin International Inc, Olathe, Kansas, 66062, USA). Environmental conditions, including habitat (forest, field, or edge), substrate (leaves, grass, brambles, soil, road, or moist area) and sky conditions (sunny, partly sunny, partly cloudy, cloudy, or raining), were also documented. Turtles in habitats classified as edge were located within 15 m of the forest edge.[10]

Physical examination and sample collection

Turtles were found incidentally and tracked via radiotelemetry. Each turtle was assigned a permanent identification, and mass, sex, and age status were recorded. Straight carapace length, straight carapace height, carapace width, anterior plastron length, and posterior plastron length were measured. Body condition index (BCI) as a measure of fat percentage was calculated for EBT based on a previous study.[20] Physical examinations were performed, noting visual appearance of the eyes, nose, oral cavity, ears, legs, digits, shell, integument, and cloaca. Turtles less than or equal to 150 g were classified as juveniles. Sex was assigned based on plastron concavity and position of the cloacal opening.[9,12] Turtles were categorized as having cutaneous myiasis if a subdermal cavity containing fly larvae was present or a cutaneous plug of necrotic debris was visualized (Fig. 1). Heart rate (beats per minute) was taken with a Sonotrax Vascular Doppler with 8-MHz probe (Edan USA, San Diego, CA 92117, USA). Venipuncture was performed using a 22-ga, 1.5-inch needle from the subcarapacial

sinus (no more than 0.8% body weight), placed in lithium heparin microtainers (Becton, Dickinson and Company, Franklin Lakes, NJ 07417, USA) and lithium heparin plasma separator tubes (Becton, Dickinson and Company), and stored in a cooler with ice packs until processing (<8 h). Combined oral–cloacal swabs were collected and placed in individually labeled Eppendorf tubes. All swab samples were stored at −20°C until analysis. Individual turtles were marked with a unique shell notch code and were released at coordinates of capture. For turtles that were sampled multiple times over the study period, serial samples were conducted more than 4 wk apart.

Clinical pathology

The WBC, PCV, total solids (TS), and fixed and stained blood smears were performed within 24 h of sample collection as previously described.[2] Erythrocyte sedimentation rate (ESR) was performed using heparinized plastic hematocrit tubes (Drummond Scientific Company, Broomall, PA 19008, USA) using a previously described technique.[4,49] Lithium-heparinized blood smears were made the same day and stained with a modified Wright–Geimsa stain (Hema 3, Fisher Scientific), and 100 white blood cell differential counts were performed by a single observer (LA). Biochemistry profiles were performed on plasma by the Veterinary Diagnostic Lab of the University of Illinois (Urbana, IL 61802, USA) to quantify the following: bile acids, calcium (Ca), uric acid, phosphorous (P), aspartate aminotransferase (AST) and creatine kinase. The Ca:P ratio was calculated by dividing Ca values by P values.

DNA extraction

DNA was extracted from oral–cloacal swabs using Qiagen DNA Blood Mini Kits (Qiagen, Valencia, CA 91355, USA), according to the manufacturer protocol for buccal swabs. DNA concentration and purity were assessed spectrophotometrically (NanoDrop 1000, Thermo Fisher Scientific, Waltham, MA 02451, USA), and DNA samples were stored at −20°C prior to qPCR.

Pathogen detection and amplicon sequencing

The qPCR was performed in a multiplex format to evaluate 21 pathogens simultaneously using published or in-house primer–probe assays (Table 1). Initially, specific target amplification was performed on each sample with pooled pathogen TaqMan assays and PreAmp Master Mix (Thermo Fisher Scientific). Each reaction was performed under the following cycling program on an MJ Tetrad thermocycler (Marshall Scientific, Hampton, NH 03842 USA): 95°C (10 min); 14 cycles of 95°C (15 s); and 60°C (4 min). The qPCR assay was then performed in triplicate using 2.25 μl of amplified DNA from the first reaction on a Fluidigm 96.96 Gene Expression IFC and amplified on the Fluidigm Biomark HD Real-Time PCR thermocycler (Fluidigm, South San Francisco, CA 94080, USA) using the following cycling protocol: 70°C (30 min); 25°C (10 min); and 95°C (1 min), followed by 35 cycles at 96°C (5 s) and 60°C (20 s). Serial dilutions of positive controls for FV3-like ranavirus (FV3), pan-ranavirus, *Emydomyces testavorans*, box turtle *Mycoplasmopsis* sp., *Mycoplasmopsis agassizii*, *Mycoplasmopsis testudineum*, terrapene herpesvirus 1, terrapene herpesvirus 2, emydid herpesvirus 1, and terrapene adenovirus 1 were prepared from 10^7 to 10^1 copies per reaction. A nontemplate control was included on each plate. All reactions were then analyzed using Fluidigm Real-Time PCR Analysis Software (Version 1.0.2; Fluidigm). Following Fluidigm analysis, all positive samples were verified in a simplex reaction. Samples were considered positive if all three replicates had a lower cycle threshold value than the lowest detected standard dilution.

Table 1. Pathogens and copathogens tested in eastern box turtles (*Terrapene carolina carolina*) in MA and source for qPCR/PCR primers.

Pathogen	Source
Frog virus 3–ranavirus qPCR	6
Ambystoma tigrinum virus–ranavirus qPCR	54
Bohle iridovirus–ranavirus qPCR	52
Epizootic hematopoietic necrosis virus–ranavirus qPCR	52
Pan-ranavirus qPCR	63
Mycoplasmopsis agassizii qPCR	13
Mycoplasmopsis testudineum qPCR	13
Box turtle *Mycoplasma* sp. qPCR	2
Salmonella tymphimurium qPCR	42
Salmonella enteritidis qPCR	44
Intranuclear coccidia of Testudines qPCR	7
Human-pathogenic *Leptospira* spp. qPCR	62
Coxiella burnetii IS1111 qPCR	37
C. burnetii ICD qPCR	37
Emydomyces testavorans qPCR	66
Terrapene herpesvirus 1 qPCR	31
Terrapene herpesvirus 2 qPCR	22
Testudinid herpesvirus 2 qPCR	13
Emydid herpesvirus 1 qPCR	Internal
Emydoidea blandingii herpesvirus 1 qPCR	43
Terrapene adenovirus 1 qPCR	27

Plasma protein electrophoresis

Plasma samples were analyzed according to the procedure provided by the Helena SPIFE 3000

system with the use of Split Beta gels (Helena Laboratories, Inc, Beaumont, TX 77703, USA) at the University of Miami (Miami, FL 33146, USA). Results were produced after gel scanning and analysis by Helena software (QuickScan Touch Plus Version 2.3.2.0). Fraction delimits were placed as previously demonstrated for other reptiles. Percentages for each fraction were determined by this software, and absolute values (grams per deciliter) for each fraction were obtained by multiplying the percentage by the TS concentration. The albumin:globulin ratio was calculated by dividing albumin by the sum of the globulin fractions.

Toxicology

Plasma samples were submitted frozen to the Savannah River Ecology Lab (Aiken, SC 29802, USA). Samples were analyzed using inductively coupled plasma mass spectrometry (Perkin Elmer NexION 300, Waltham, MA 02451, USA) for cobalt, nickel, copper (Cu), zinc (Zn), strontium, molybdenum, silver, cadmium, lead (Pb), arsenic (As), and selenium (Se).

Bone marrow sampling

EBT shells were found and collected between June 2021 and November 2021. Bone marrow from these shells was extracted and processed according to the methods outlined previously.[15] The qPCR was performed on the samples to detect FV3, as described previously in the simplex format.

Statistical analysis

Clinical pathology: Descriptive statistics were tabulated for all continuous variables (hematologic, plasma biochemical, and protein electrophoretic analytes). Normality was assessed using the Shapiro–Wilk test, Q–Q plots, skewness, and kurtosis. Normally distributed data were compared between sexes, sites, and ectoparasite presence using an independent samples *t* test. When data were not normally distributed, a Mann–Whitney *U*-test or a Kruskal–Wallis one-way ANOVA test was used. Repeated measures ANOVA was performed on all analytes sampled in individuals with multiple samples (less than three samples). A single summary table was then calculated when there was no significant difference in continuous variables based on sex, site, month, or ectoparasite presence. The qPCR detection status for each pathogen (positive or negative) was tested for an association with month, sex, and location using the Fisher exact test. Descriptive statistics were performed using SPSS (Version 28, IBM Statistics, IBM Corporation, Armonk, NY 10504, USA), and α levels were set at $P = 0.05$.

RESULTS

Sampling effort

There were 109 total samples collected in 2021, from 59 unique individuals. Sixteen adults were sampled twice, and 17 adults were sampled three times. Forty-eight turtles were sampled on Camp Edwards, and 11 animals were sampled at Sandwich, MA. Fifty-eight turtles were adults (32 females and 26 males), and 1 was a juvenile (unknown sex). Samples were collected in May (n = 25), June (n = 24), July (n = 8), and August (n = 2). Turtles were found in a range of habitats at initial capture, including bog (n = 4), edge (n = 11), forest (n = 35), and roads (n = 9). Microhabitats at initial capture varied and included leaves (n = 38), brambles (n = 4), pavement (n = 8), soil (n = 4), and moist areas (n = 5).

Physical examination

The most notable clinical sign was the presence of fly larvae infestation into the cutaneous and subcutaneous tissue of individual turtles (n = 18 of 59; 30.5%) and overall turtle encounters (n = 29 of 109; 26.6%). For individuals with myiasis that were sampled multiple times (n = 17), lesions were typically first observed as subdermal cavities filled with larvae (n = 16), then cutaneous plugs (n = 7), and finally postinfection scars (n = 13; Fig. 1). Infections resolved in several of the serially sampled animals by the time of the last exam (approximately 4 to 8 wk; n = 9 of 21; 42.9%). The number of cutaneous myiasis lesions per turtle encounter varied from one (n = 11), two (n = 15), three (n = 4), four (n = 2), or five (n = 1). Lesions were located in the prefemoral fossae (n = 30), prescapular fossae (n = 20), appendages (n = 7), neck (n = 5), or near the cloaca (n = 4). Individuals found in bogs had the highest prevalence of fly larvae (n = 4; 100%; $P < 0.0001$) compared with turtles on roads (56%), forest (26%), and edge habitats (0%). Besides fly larvae, no other ectoparasites were observed on these animals. No other associations were observed between ectoparasites and demographic factors, clinical signs, or presence of a pathogen ($P > 0.05$). In turtles that were sampled more than once, cutaneous myiasis was more common in May (42.9%) than either June or July ($P = 0.007$).

Aside from myiasis, the most common physical exam abnormalities were plastron or carapace lesions (n = 52, 88%). These lesions included flaking (n = 15), erosions (n = 32), and predator injuries (n = 17). Interestingly, animals with myiasis had fewer plastron abnormalities (50%) compared with animals without (78%; $P = 0.034$).

Clinical pathology

Descriptive statistics for clinical pathology parameters were tabulated for all analytes from apparently healthy individuals and by the presence of ectoparasites (Table 2). Apparently healthy individuals had no ocular or nasal discharge, oral plaques, or evidence of myiasis. Females had lower lymphocytes (L) and higher Ca, P, and $\alpha 1$, $\alpha 2$, and β globulins than males. Several differences were observed in individuals with cutaneous myiasis, including lower BCI, WBC, PCV, TS, Ca, P, AST, total protein, prealbumin, albumin, and $\alpha 1$, $\alpha 2$, β, and γ globulins, and higher ESR and Ca:P. In animals sampled multiple times, absolute heterophils (H) were higher in May (median: 3,872 cells/μL), followed by July (median: 2,871 cells/μL), and were lowest in June (median: 1,456 cells/μL; $P = 0.002$). The same trend was observed with H:L ratio ($P = 0.038$). Bile acids were significantly higher in June (median: 7.6 μmol/L) compared with May (median: 2.3 μmol/L; $P < 0.001$), but no other differences were observed.

Pathogen surveillance

One hundred and nine combined oral–cloacal swabs were assayed for pathogens.[24,39,46] Samples were collected between one and three times per individual. The only pathogen detected was terrapene herpesvirus 1, occurring in 3 of the 59 turtles (5.1%; 95% CI: 1.1–14.2%) sampled in June (n = 1), July (n = 1), and August (n = 1). These detections occurred in males (n = 2) and a female (n = 1), two at Cape Cod and one at Sandwich, MA, but statistical significance was not reached for either sex ($P = 0.085$) or site ($P = 0.724$). One of these animals tested negative before and after the positive result, one was negative twice before, and the last was only sampled once. No physical exam abnormalities were observed for any of these turtles, including the presence of myiasis. Bone marrow was extracted from seven individual EBT shells. These shells were collected in June (n = 2), July (n = 2), August (n = 1), September (n = 1), and November (n = 1). All shells were negative for FV3.

Toxicology

Twenty-seven individual turtles (17 females and 10 males) were sampled for 11 heavy metals in plasma, with 16 individuals being classified as apparently healthy and 11 animals with myiasis at the time of sampling. Sampling took place in June (n = 3), July (n = 13), and August (n = 11). For turtles sampled multiple times, the latest sample was submitted to detect the highest heavy metal concentration from bioaccumulation. No differences in heavy metal concentrations were observed between sexes, ectoparasite presence (Table 2), habitat, or microhabitat ($P > 0.05$). Animals with abnormalities of the plastron had significantly higher plasma Zn (median: 6.89 ppm; $P = 0.035$) and Se (0.366 ppm; $P = 0.007$) concentrations than animals with normal plastrons (median Zn: 5.17 ppm; median Se: 0.198 ppm). In addition, there was a positive correlation between PCV and plasma Zn concentrations ($P < 0.0001$).

Survival

Six animals died during the activity season of 2021 (6.8%; 95% CI: 1.9–16.5%). One of these (turtle 3) had shell erosions at three points prior to being found dead on 11 October. Another (turtle 13) was apparently healthy at three time points prior to being killed by a lawn mower on 1 November. Turtle 18 had shell erosions at three time points prior to being found dead on 1 September. Turtle 68 was sampled once on 9 June with an abundance of fly larva and was found dead the next day. This turtle had multiple cutaneous myiasis lesions in the prefemoral fossae bilaterally and caudodorsal to the head and a large subdermal cavity with at least 30 fly larvae in the left prescapular fossa. Turtle 74 was sampled in June and July with cutaneous myiasis lesions in the prefemoral fossae bilaterally during each sampling and was found dead on 27 August. Turtle 121 was sampled once in August with a cutaneous plug in the left prescapular fossa and a myiasis scar in the right prescapular fossa and was found dead on 7 October from a vehicle strike while in a road puddle. There were no associations between any demographic, clinical sign, or pathogen detection parameter and outcome (survived or died; $P > 0.05$).

DISCUSSION

The goal of this research was to perform population health analysis and disease surveillance of a population EBT with high prevalence of myiasis

Table 2. Descriptive statistics and comparison for body condition index (BCI), clinical pathology, and protein electrophoresis between 40 apparently healthy and 18 eastern box turtles (*Terrapene carolina carolina*) with myiasis on Cape Cod, MA, in 2021.

		Healthy individuals				Myiasis					
Variable	Units	n	Measure of central tendency	Min	Max	n	Measure of central tendency	Min	Max	Sig.	Comparison to healthy individuals
BCI	%	41	13.49	5.66	18.04	18	11.55	6.2	16.34	0.014	Decreased
WBC	cells/µl	40	9,223.37	6,453.33	39,977.14	17	13,535.73	4,877.71	24,369.23	0.031	Decreased
Absolute heterophils (H)[a]	cells/µl	40	2,096.48	154	16,443.43	17	3,183.27	186.12	11,612.86	0.688	
Absolute lymphocytes (L)[a]	cells/µl	41	7,405.2	18.76	2,7561.6	17	8,130.19	2,585.19	28,783.54	0.5	
Absolute monocytes[a]	cells/µl	40	214.82	0	2,091.29	17	378.15	0	2,554.83	0.511	
Absolute eosinophils[a]	cells/µl	40	2,037.75	422.4	4,767.4	17	2,868.17	536.55	8,371.2	0.312	
Absolute basophils[a]	cells/µl	40	1,218.78	0	4,305.16	17	1,176.36	379.31	10,464	0.565	
H:L ratio[a]	—	40	0.233	0.01	2.16	17	0.45	0.05	3.57	0.116	
PCV	%	40	21.8	10	33	17	12.7	4	22	<0.0001	Decreased
Total solids	g/dl	40	4.17	2	8.2	18	2.9	1	5.4	<0.0001	Decreased
Erythrocyte sedimentation rate	mm	39	6.47	2.6	12.1	17	16.52	2.6	12.1	<0.0001	Increased
Ca	mg/dl	40	9.29	3.5	25.5	18	6.73	3.1	13.1	0.018	Decreased
P	mg/dl	40	2.36	0.7	5.4	18	1.36	0.6	3.5	0.017	Decreased
Ca:P ratio	—	40	5.82	2.34	55.45	18	5.35	3.42	7.33	0.049	Increased
Uric acid	mg/dl	40	0.35	0	3	18	0.51	0	3.3	0.89	
Bile acids	µmol/L	40	5.54	2	14.3	18	4.58	0	18.8	0.057	
Glutamate dehydrogenase	U/L	39	4.48	0.1	31.1	18	43.4	6	320	0.015	Decreased
Aspartate aminotransferase	U/L	40	40.9	7	98	18	147	0	684	0.078	
Creatine kinase	U/L	40	381.78	0	4,695	17	3.26	0.1	14.8	0.09	
Total protein	g/dl	40	4.17	2	8.2	18	2.86	1	5.4	<0.0001	Decreased
Prealbumin	g/dl	40	0.07	0.48	2.09	18	0.05	0.48	2.09	<0.0001	Decreased
Albumin[a]	g/dl	40	0.06	0	0.18	18	0.06	0	0.18	<0.0001	Decreased
Albumin:globulin ratio	—	40	0.98	0.2	0.51	18	0.64	0.2	0.51	0.957	
α1 Globulin	g/dl	40	0.27	0.12	0.48	18	0.21	0.12	0.48	0.002	Decreased
α2 Globulin	g/dl	40	1.19	0.46	2.73	18	0.75	0.46	2.73	<0.0001	Decreased
β Globulin	g/dl	40	1.13	0.44	1.92	18	0.78	0.15	1.57	0.001	Decreased
γ Globulin	g/dl	40	0.52	0.31	0.95	18	0.42	0.17	0.8	0.019	Decreased
Cobalt[a]	ppm	16	0.000	0.000	0.009	11	0	0.000	0.009	0.904	
Nickel[a]	ppm	16	0.018	0.000	0.422	11	0.28	0.000	0.081	0.577	
Cu[a]	ppm	16	0.807	0.328	1.411	11	0.667	0.333	1.165	0.645	
Zn	ppm	16	6.536	3.114	10.587	11	5.631	2.258	8.190	0.272	
Strontium[a]	ppm	16	0.150	0.080	0.251	11	0.161	0.121	0.283	0.318	
Molybdenum[a]	ppm	16	0.000	0.000	0.010	11	0	0.000	0.000	0.610	
Silver[a]	ppm	16	0.000	0.000	0.000	11	0	0.000	0.009	0.716	
Cadmium[a]	ppm	16	0.000	0.000	0.000	11	0	0.000	0.110	0.716	
Pb[a]	ppm	16	0.024	0.000	0.149	11	0.015	0.000	0.072	0.272	
As[a]	ppm	16	0.000	0.000	0.000	11	0	0.000	0.000	1.000	
Se	ppm	16	0.373	0.095	0.727	11	0.319	0.000	0.845	0.272	

[a] Indicates values that were not normally distributed.

on Cape Cod, MA, in 2021. This initial investigation sought to identify epidemiologic trends or associations with multiple health parameters. The presence of cutaneous myiasis in EBT appeared to have a significant impact on individual health based on physical examination, blood work findings, and death in at least one heavily affected individual. The species of fly causing myiasis in these turtles was not determined because proper specimen preservation and either molecular characterization or knowledge of specific larval morphologic characteristics is necessary for speciation.[26] Many studies have identified *Cistudinomyia cistudinis*, a species of fly in the family Sarcophagidae, to cause myiasis in box turtles.[11,16,21,30,35,38,41,47,51,53,55,57,64] These reports describe varying degrees of myiasis from single to multiple lesions and report lesions in similar anatomic locations to the EBT in this study. Myiasis due to species in Oestridae[65] and Calliphoridae[1] has also been reported and cannot be entirely excluded without speciation attempts. Sarcophagidae species cannot penetrate the skin to lay eggs, and it is suggested that ticks may play a role in facilitating deposition of eggs via the wounds created after they detach from the skin.[38,53] No ticks were identified on any of the turtles during physical examination; however, this does not completely rule out the possibility of previous tick bites causing irritation to the skin. Larvae could also be deposited by flies in wounds from trauma, but this is considered less likely, given the location of the lesions. Most turtles had subdermal cavities in the prescapular or prefemoral fossae, both of which are well-protected areas on the body that experience low friction and are not routinely in contact with the environment. Individuals with myiasis had fewer plastron lesions than those without, and the exact cause for this correlation remains unknown. It is possible that older animals have acquired more shell lesions over their lifetimes and have also developed a more robust immune response against fly larvae compared with younger animals. In addition, myiasis has shown to have an impact on the mobility of smaller mammals, and because many EBT had pockets of larvae on or around the legs, this could cause difficulty with ambulation.[61] Decreased movement could also explain the decrease in plastron defects seen in EBT with myiasis, but further studies are needed to assess if mobility is affected by these infections. All EBT found near the periphery of a wetland had cutaneous myiasis present upon physical examination. This could be due to an increased concentration of flies in this area and thus increased chance of interaction or due to the high prevalence of coparasites (namely mosquitos) that inhabit these wetlands. Many families of flies reach highest quantities of individuals around wetland environments, and some species in Sarcophagidae feed on the liquid and dead insects in bog-inhabiting plants.[34] Mosquitoes were subjectively more common near wetlands, suggesting that mosquitoes may play a role in these infections, as some species of bot flies lay eggs on blood-sucking insects.[48] The eggs mature into larvae, and when the mosquitoes land on a host to take a blood meal, the larvae are deposited around the site of the wound, facilitating entry into the skin.[40,48] This suggests that mosquito removal strategies may decrease the occurrence of cutaneous myiasis in EBTs, but this warrants further study.

The clinicopathologic findings of EBT with myiasis are consistent with an inflammatory response, as evidenced by decreased albumin, increased ESR, and decreased PCV.[45] Fly larvae in mammals have been documented to feed on the host interstitial fluid to grow, and this could explain the combined decrease in both albumin and globulins seen in EBT with myiasis compared with healthy individuals.[19,61] This diversion of host nutrients can weaken these turtles both physically and immunologically, which might explain the observed decrease in body condition. As the larvae grow, they initiate an inflammatory response in the tissue causing the formation of a capsule.[61] There was no apparent active blood loss in MA EBT due to wounds, but myiasis may cause an anemia of chronic disease. Interestingly, fly larvae infections were more common in May, which may be due to recent inactivity, timing of the fly life cycle, or transient finding from sampling in only a single year. As the summer progressed, many of these infections resolved, which may explain the decrease in prevalence, and this could be due to the maturation of larvae and subsequent exit from the wounds. Because many of these infections resolved, this may not be a significant conservation concern at the population level. However, individual turtles may succumb to substantial larval loads if infestations are severe, larvae migrate to internal tissues, myiasis occurs in turtles with comorbidities, or if environmental conditions that favor infestation persist over multiple years.

In this exploratory analysis, several health factors were studied that may explain the high prevalence of myiasis, including presence of heavy metals or pathogens. In this cross-sectional study, no direct associations were observed with any of these factors and myiasis, but it is possible that there are temporal factors leading to this result, and future studies would identify relationships

not observed in a single study. The heavy metal ranges were similar to previously published data on serum from apparently healthy Herman's tortoises (*Testudo hermanni*).[36] Compared with EBT populations in IL and TN, MA turtles have higher Cu, Zn, Se, and Pb values but lower concentrations of As.[5] Exposure of these turtles to heavy metals is complex because concentrations in the soil are influenced by many natural and anthropogenic sources.[68] In this population, EBT with plastron lesions were specifically observed to have higher Zn and Se levels in the plasma. Elevated levels of Se can cause damage to keratinized tissues and have experimentally been proven to cause epidermal lesions at a cellular level in yellow-bellied sliders (*Trachemys scripta scripta*),[29] which may predispose these turtles to skin lesions and subsequent myiasis. Interestingly, elevated Zn concentrations were associated with an increase in PCV, and this is the opposite of the expected correlation for these variables because elevated Zn typically causes hemolysis. However, previous studies in rats (*Rattus norvegicus*) and carp (*Cyprinus carpio*) have demonstrated an erythropoietic property of Zn, resulting in an increased production of red blood cells.[17,18] This property may account for the increased PCV seen in turtles with higher Zn concentrations in this study.

The only pathogen present in this population was terrapene herpesvirus 1 (TerHV1), and it was only detected in three animals. No clinical signs, including presence of myiasis, were associated with animals testing positive for TerHV1 in this study, which is consistent with other published data on this virus.[8,31] Significant changes in EBT pathogen prevalence have been observed in other states between different years and seasons.[8] Thus, it is possible that EBT sampled had latent infections not detectable with the sampling strategy or that there were pathogens present not included on the panel.[60] Many wildlife pathogens are host adapted and only present clinically with accompanying detectable shedding in periods of severe stress or in extremely young or old animals.[8] Outbreaks of pathogens such as ranavirus are devastating in EBT and can quickly overwhelm a population; however, prevalence in nonoutbreak conditions is typically very low.[3,56,59] At present, it does not appear that presence of pathogens is associated with presence of myiasis. Several turtles died during the summer, including some with myiasis earlier in the year, but due to carcass decomposition, were unavailable for necropsy for internal assessment. Two of these turtles died due to suspect trauma; however, without histopathologic analysis of the tissue, it is unclear whether the trauma was secondary to systemic illness or if these were previously healthy animals. Bone marrow sampling can be extremely helpful in cases in which the internal organs are missing, but detection of blood-borne pathogens, such as ranavirus, is most appropriate, and it is not considered useful for detecting localized infections, such as myiasis, herpesviruses, or *Mycoplasmopsis* sp. The exact cause of death in the animals in this study is unknown, but as new threats emerge, targeted necropsy and pathogen testing should be performed.

This study provides insight into the effects of myiasis in Cape Cod EBT populations. Continued health assessments would offer enhanced understanding of morbidity and mortality events in this population by identifying trends, so informed management strategies can be enacted in the face of a disease outbreak.[3,8,32,56] Disease outbreak interventions are inherently complex, but intense medical management of critical animals has proven to be a successful option if clinically indicated.[59] Future investigations to speciate the fly causing myiasis are warranted, as well as following affected turtles longitudinally to determine the rates of myiasis-associated morbidity and mortality. Also, evaluating the fine-scale daily movements and nesting rates of turtles with myiasis would help establish subclinical effects of fly larva on fitness and fecundity. Bacterial and fungal cultures of wounds from fly larvae would identify if there is a consistent secondary pathogen associated with myiasis or if there is a primary infection eroding epithelial surfaces to allow deposition of eggs beneath the skin. Advanced imaging would be helpful in determining if internal lesions due to myiasis or other pathogens are present.[51] These recommendations will aid in creating a more complete picture of the health of these turtles and how they interact with the environment to guide conservation efforts.

Acknowledgments: The authors thank the Camp Edwards Natural Resources and Integrated Training Area Management Office for the use of the facilities and the field technicians for assistance locating turtles. The authors also thank Eversource and Oxbow Associates for the ability to sample turtles from their study and Matt Penella for assistance with developing the study design. This project was funded by the Massachusetts Army National Guard.

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Accepted for publication 7 September 2023