Epidemiologic Determinants of Aural Abscessation in Free-Living Eastern Box Turtles (Terrapene carolina) in Virginia

Authors: Brown, Justin D., Sleeman, Jonathan M., and Elvinger, François

Source: Journal of Wildlife Diseases, 39(4) : 918-921

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-39.4.918

Journal of Wildlife Diseases, 39(4), 2003, pp. 918–921

Epidemiologic Determinants of Aural Abscessation in Free-Living Eastern Box Turtles (*Terrapene carolina*) in Virginia

Justin D. Brown,[1] **Jonathan M. Sleeman,**[1,2,3] **and François Elvinger**[1] [1] Virginia–Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0442, USA; [2] Wildlife Center of Virginia, P.O. Box 1557, Waynesboro, Virginia 22980, USA; [3] Corresponding author (email: jsleeman@wildlifecenter.org)

ABSTRACT: Epidemiologic determinants of 46 cases of aural abscessation in free-living eastern box turtles (*Terrapene carolina*) admitted to the Wildlife Center of Virginia (Virginia, USA) from 1991 to 2000 were evaluated. County human population density, year and season of admission, weight, and sex did not affect the risk for box turtles to develop aural abscessation. Counties with cases of aural abscessation were not randomly distributed, but rather were clustered into two multi-county regions. Geographic location was the only risk factor associated with aural abscessation in box turtles found in this study. Possible etiologies could include chronic infectious disease, malnutrition, or chronic exposure to environmental contamination with organochlorine compounds.

Key words: Aural abscessation, box turtle, epidemiology, *Terrapene carolina,* Virginia.

Aural abscessation was the second highest diagnostic category in free-living eastern box turtles (*Terrapene carolina carolina*) admitted to the Wildlife Center of Virginia (WCV; Waynesboro, Virginia, USA) from 1991 to 2000 (Brown and Sleeman, 2002). Aural abscesses are accumulations of caseous debris in the tympanic cavity resulting from squamous metaplasia of the epithelium that lines the middle ear (Holladay et al., 2001). The lesion, which can be unilateral or bilateral, varies in size from subclinical enlargements to masses large enough to impede the animal from drawing its head back into the shell.

High body burdens of organochlorine (OC) compounds were associated with aural abscessation and squamous metaplasia of mucin secreting epithelial tissues of the upper and lower respiratory passages, eyes, and the middle ear in free-living eastern box turtles (Holladay et al., 2001). It was hypothesized that increased levels of OC compounds disrupted vitamin A metabolism, leading to hypovitaminosis A and accumulation of caseous debris in the middle ear of the turtles, visible grossly as aural abscesses. This is consistent with previous studies on humans, turtles, and rats, which showed that increased exposure to OC compounds led to a disturbance in vitamin A homeostasis (Coenraads et al., 1994; Poon et al., 1995) and upper respiratory infections (Tangredi et al., 1997).

A causal relationship between body burdens of OC compounds and aural abscesses in box turtles has not been established. However, should causality be determined, the geographic location of this condition in free-ranging eastern box turtles may be used as an indicator for the presence of OC compounds in the environment, particularly as box turtles have many of the attributes of an ideal bio-indicator species (Golden and Rattner, 2003).

To our knowledge there is no description of the epidemiology of aural abscessation in free-living box turtles or investigation of risk factors for development of this lesion. The objectives of this study were to describe the epidemiology of aural abscessation in free-living eastern box turtles admitted to the WCV during 1991 to 2000 and investigate the spatial and temporal distribution of these cases in relation to total box turtle caseload admitted to the WCV. The aim was to provide additional information on the potential etiology of this condition that would direct further study.

Submissions of 458 box turtles admitted to the WCV (38°02′N, 78°55′W) between 1991 and 2000 were reviewed. Case identification number, county of origin, admission date, sex, weight, and clinical diag-

nosis were extracted from case files and organized into a computerized database (Microsoft Excel 97, Redmond, Washington, USA). Spatial location data were entered into a geographic information system program (ArcView 3.2, ESRI, Redlands, California, USA) to generate maps showing the county-based distribution of box turtles with and without aural abscesses in Virginia.

Clinical diagnoses were determined by the attending WCV clinicians. Aural abscessation was defined as the presence of a grossly visible solid mass medial to the tympanum, either unilaterally or bilaterally (Brown and Sleeman, 2002). When possible, the diagnosis was confirmed by surgical exposure of the caseous mass within the abscess. All other box turtles admitted during the same time period without the presence of this lesion were classified as non-aural abscess cases. Sex was determined based on published sexual dimorphic characteristics (Mitchell, 1994). Sex could not be identified for a number of individuals due to immaturity. County of origin was grouped by human population density (five population density categories: $\leq$20 residents/km^2, 20–40 residents/km^2, 40–80 residents/km^2, 80–160 residents/km^2, $\geq$160 residents/km^2). County of origin was unknown for 32 submissions, of which three were aural abscess cases. Nine turtles were submitted from counties located in a state other than Virginia, of which one had aural abscessation. Admission date was categorized by year and season (winter: December through February, spring: March through May, summer: June through August, fall: September through November).

Effects of explanatory variables including county human population density, year and season of admission and sex, on presence of aural abscesses were evaluated first for each independent variable by univariate analysis with Proc FREQ of SAS® (SAS Inc., Cary, North Carolina, USA), followed by multivariable logistic regression, with forward selection of explanatory variables if $P<0.20$ in the univariate analysis, using Proc LOGISTIC. The Cochran-Armitage trend test (Armitage, 1955) in Proc FREQ was used for county population density, year and season of admission. Relationship of aural abscessation, corrected for sex and season of admission, including 2- and 3-way interactions, with turtle weight were evaluated by analysis of variance of ranked weight data using Proc GLM (Conover and Iman, 1981), as weight had been determined to be nonnormally distributed across abscess, sex and admission season categories by the Anderson-Darling test in Proc UNIVARIATE.

Box turtle submissions were evaluated for spatial clustering of abscesses using Grimson's method (Grimson, 1991; Stat!, BioMedware, Ann Arbor, Michigan, USA), testing the null hypothesis that counties with aural abscess cases were randomly distributed among the counties from which a box turtle case had been admitted during the study period. This method detects spatial clustering of labeled objects (counties) based on adjacency, which is present when two objects share a common border. Counties were determined to be elevated risk if they had an aural abscess case during the study period. All counties from which box turtles had been admitted, but without cases of aural abscessation were designated low risk.

Of the 458 submissions, 50 turtles had aural abscesses. Complete data on origin and weight were available for 355 turtles, with 46 aural abscess cases. Sex did not influence presence of abscesses in turtles (males: 21 [17.1%] of 123, females: 12 [14%] of 85; $P=0.57$), but the 147 turtles for which sex was not determined were less likely to have aural abscesses (sex determined: 33 [15.9%] of 208, sex not determined: 13 [8.8%] of 147; $P=0.05$). Turtles for which sex was not determined were lighter (male, mean weight ± SD: 340±89 g; female: 312±122 g; sex not determined: 306±114 g; $P=0.015$), but no effect of weight on presence of aural ab-

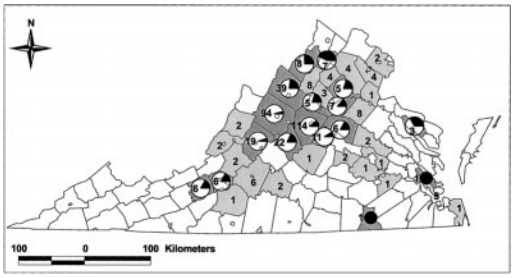

FIGURE 1. Numbers of eastern box turtles (*Terrapene carolina*) submitted from counties of Virginia during 1991 to 2000. Counties from which eastern box turtles were received are shaded gray (dark gray for counties with aural abscess cases, light gray for counties from which none of the submitted turtles had aural abscesses). Pie charts in counties with aural abscess cases indicate county proportion of turtles with aural abscesses in black, and turtles with no aural abscess in white.

scess was detected (turtles with aural abscess: 308±112 g; turtles without abscess: 321±109 g; $P=0.60$).

Yearly proportions of aural abscesses varied from 4% of 28 turtles submitted in 1993 to 18% of 40 turtles submitted in 1995 ($\chi^2_{9df}=13.8$, $P=0.13$), with no trend detected in prevalence of diagnosed aural abscesses from 1991–2000 ($P=0.52$). The proportion of aural abscessation cases was not affected by submission season (winter: two of nine (22%), spring: three of 42 (7%), summer: 28 of 188 (14.9%), fall: 13 of 116 (11.2%); $P=0.54$) or month ($P=0.31$). Human population density by county also did not affect the proportion of aural abscessation cases ($P=0.93$).

Turtles with aural abscesses were admitted from 17 of the 41 counties in Virginia from which box turtle cases were admitted during the study period. Counties with cases of aural abscessation were located throughout the state (Fig. 1). Among the counties that had a box turtle case during the study, spatial clustering was observed indicating that the location of aural abscess cases was not randomly distributed ($P<0.001$) in relation to total box turtle admissions. The counties with aural abscessation formed two clusters, consisting of 12 and two counties.

County human population density, year and season of admission, weight, and sex were not associated with an increased risk for box turtles developing aural abscesses. However, turtles in which sex was not determined were lower in weight and less likely to develop aural abscesses indicating that younger animals were less likely to develop this lesion.

While aural abscess cases were admitted from counties throughout the state, they were not randomly distributed among the counties from which box turtles had been admitted, but rather were clustered into two distinct foci (Fig. 1). Clustering of elevated risk counties as detected by the Grimson's method may indicate a common source epidemiologic entity, such as food or environmental contamination (Jacquez, 1994). A 12 county cluster surrounding the WCV and extending to the northeast represented the majority of aural abscess cases admitted during the study period. Within this cluster Warren, Shenandoah, Rockingham, Greene, and Culpepper had the highest proportion of aural abscess cases, representing 20% or more of the total box turtle caseload in each of these counties. A smaller two-county cluster was observed composed of Roanoke and Montgomery counties.

Turtles in this study were not randomly sampled and spatial analysis conducted at the county level may lack sensitivity due to the small home range of box turtles consisting only of a few hectares (Mitchell, 1994). Future studies should be performed at a geographic scale more sensitive than county, in order to more precisely determine the geographic distribution and potential risk factors for this condition.

In conclusion, geographic location of the turtle was the only risk factor associated with aural abscessation in box turtles found in this study. Possible etiologies could include chronic infectious disease, malnutrition, or chronic exposure to environmental contamination with OC compounds. The increased risk for development of these lesions in known sex, and

thus potentially older animals, is consistent with chronic exposure and accumulation of environmental contaminants throughout an animal's life (Friend and Franson, 1999). The results of this study are consistent with the hypothesis proposed by Holladay et al. (2001). Should OC exposure be the cause of this lesion then possible sources for environmental contamination with OC compounds include agricultural pesticides (herbicides, fungicides, rodenticides, insecticides), industrial products, and moisture proof coating for wood, brick, and other materials (Friend and Franson, 1999). These compounds are known to persist in the environment for years (Beyer et al., 1996). Further studies should be conducted to evaluate this and alternative hypotheses. Research is currently being conducted at the WCV to determine the relationship between OC compounds and aural abscessation, including whether a dose response exists between body burdens of OC compounds in box turtles and observed lesions, and correlating concentrations of OC compounds in the soil and food items with the presence of this lesion, as well as radiographic and microbiologic evaluation of the aural abscesses.

We thank the Geraldine R. Dodge Foundation Frontiers for Veterinary Medicine for funding this study. We also thank the staff of the Wildlife Center of Virginia who were responsible for the medical care of the cases analyzed in this study, and S. Snead for assistance in record retrieval.

LITERATURE CITED

ARMITAGE, P. 1955. Tests for linear trends in proportions and frequencies. Biometrics 11: 375–386.

BEYER, W. N., G. H. HEINZ, AND A. W. REDMON-NORWOOD. 1996. Environmental contaminants in wildlife. CRC Press Inc., Boca Raton, Florida, 512 pp.

BROWN, J. D., AND J. M. SLEEMAN. 2002. Morbidity and mortality of reptiles admitted to the Wildlife Center of Virginia, 1991–2000. Journal of Wildlife Diseases 38: 699–705.

COENRAADS, P. J., A. BROUWER, K. OLIE, AND N. TANG. 1994. Chloracne: Some recent issues. Dermatologic Clinics 12: 569–576.

CONOVER, W. J., AND R. L. IMAN. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. The American Statistician 35: 124–129.

FRIEND, M., AND J. C. FRANSON. 1999. Field manual of wildlife diseases: General field procedures and diseases of birds. US Geological Service, Biological Resources Division, Washington, D.C., pp. 295–308.

GOLDEN, N. H., AND B. A. RATTNER. 2003. Ranking terrestrial vertebrate species for utility in biomonitoring and vulnerability to environmental contaminants. Reviews in Environmental Contamination and Toxicology 176: 67–136.

GRIMSON, R. C. 1991. A versatile test for clustering and a proximity analysis of neurons. Methods of Information in Medicine 30: 299–303.

HOLLADAY, S. D., J. C. WOLF, S. A. SMITH, D. E. JONES, AND J. L. ROBERTSON. 2001. Aural abscesses in wild-caught box turtles (*Terrapene carolina*): Possible role of organochlorine-induced hypovitaminosis A. Ecotoxicology and Environmental Safety 48: 99–106.

JACQUEZ, G. M. 1994. Usermanual for Stat!: Statistical software for the clustering of health events. Biomedware, Ann Arbor, Michigan, pp. 103–128.

MITCHELL, J. C. 1994. The reptiles of Virginia. Smithsonian Institution Press, Washington, D.C., 352 pp.

POON, R., P. LECAVALIER, P. CHAN, AND C. VIAU. 1995. Subchronic toxicity of medium-chained chlorinated paraffin in the rat. Journal of Applied Toxicology 15: 455–463.

TANGREDI, B. P., AND R. H. EVANS. 1997. Organochlorine pesticides associated with ocular, nasal, or otic infection in the eastern box turtle (*Terrapene carolina carolina*). Journal of Zoo and Wildlife Medicine 28: 97–100.

Received for publication 23 July 2002.