DIAGNOSIS, TREATMENT, AND OUTCOME OF EASTERN BOX TURTLES (*TERRAPENE CAROLINA CAROLINA*) PRESENTED TO A WILDLIFE CLINIC IN TENNESSEE, USA, 1995–2007

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ABSTRACT: We performed a retrospective review of medical records for 574 eastern box turtles (*Terrapene carolina carolina*) that presented to the University of Tennessee College of Veterinary Medicine (UTCVM) wildlife clinic between 1995 and 2007. These cases were reviewed to document and investigate the presenting complaints, as identified by a good Samaritan, final diagnoses, treatment modalities, days in hospital, and final disposition. Presenting complaints were consistent with the final diagnoses in 81% of cases. Trauma was both the most common presentation and final diagnosis. Associations between final diagnosis and either environmental temperature or precipitation were not significant ($P > 0.05$). However, precipitation had a statistically significant positive association ($P < 0.05$) with aural abscessation. Approximately 5% (28/574) of the box turtles were evaluated for packed cell volume total protein and white blood cell count. No differences were observed between sick and healthy animals for any of these variables. Multiple treatment modalities were used in these cases and were often used in combination, rather than as individual therapies. The number of days spent in the hospital ranged from 0 to 66 days, with a median of 2 days. The wildlife clinic of UTCVM should continue to evaluate the health status of eastern box turtles. Our findings indicate that the high caseload, as well as case variety of eastern box turtles in eastern Tennessee, suggests potential use as a biomonitor for this species.

Key words: Aural abscessation, biomonitor, eastern box turtle, *Terrapene*, trauma.

INTRODUCTION

Free-ranging eastern box turtles are commonly presented annually to the University of Tennessee College of Veterinary Medicine (UTCVM) wildlife clinic through the Avian and Zoological Medicine service. However, no studies have examined the clinical characteristics and health status of these turtles or potential associations between changes in climatic conditions and the range of complaints or diagnoses observed. To evaluate the health of the box turtle population in east Tennessee, assessments of the morbidity and mortality need to be conducted.

A few investigations have evaluated trends in box turtle disease (Brown and Sleeman, 2002; Nazdrowicz et al., 2008). Infectious diseases of box turtles are an emerging concern in the southeastern US; aural abscesses and ranaviruses are increasingly identified in these animals (Brown et al., 2003, 2004; De Voe et al., 2004; Allender et al., 2006; Johnson et al., 2008). Additionally, current hypotheses suggest aural abscessation in eastern box turtles might be linked to pesticide use (Tangredi and Evans, 1997; Holladay et al., 2001; Brown and Sleeman, 2002; Sleeman, 2008). Therefore, an investigation into the general health status of this species could aid in designing studies that target the assessment of ecologic health of the region. The objectives of this study were to investigate and describe 1) the occurrence of presenting complaints and final diagnoses, 2) associations between environmental factors (temperature and precipitation) and presentation, and 3) usefulness of clinical pathology for the diagnosis of disease in the eastern box turtle.

MATERIALS AND METHODS

We evaluated 711 medical records for turtles that were presented to the UTCVM wildlife clinic by good Samaritans in eastern Tennessee 1 January 1995 to 31 December 2007. Patients were excluded if positive identification of the species could not be made on the basis of weight or medical notes, or if the patient was owned, leaving a total of 574 identified eastern box turtle patients. The age, sex, weight, presenting complaints, final diagnosis, treatment modalities, days in hospital, and final disposition of eastern box turtles were recorded (all parameters except presenting complaint were identified by clinicians and veterinary students). Outdoor monthly and yearly high, low, and average temperatures, as well as precipitation were recorded from meteorologic records from 1996 to 2007 (NOAA, 2008). Twenty-eight turtles had records for complete blood counts and plasma biochemistries. Results for packed cell volume (PCV), total solids (TS), white blood cell count (WBC), and biochemical parameters were recorded and compared between sick and healthy turtles.

Presenting complaints identified by good Samaritans were divided into the following categories: trauma, infectious, healthy, unknown, orphaned, and swellings (often misidentified as tumors or neoplasia by good Samaritans). For each turtle, only one complaint was identified on the basis of clinical signs. Final diagnoses were organized into the same categories, with the exception that orphaned animals were reclassified as healthy. For turtles with multiple final diagnoses, the turtle was considered once for its presenting complaint, as identified by the good Samaritans; therefore, final diagnoses outnumber cases presented. Several turtles presented more than once because of recheck appointments from the rehabilitators. Only the initial visit of these turtles was considered for statistical analysis. Final diagnoses were identified by UTCVM clinical and pathology teams for each case. Ancillary diagnostic tests, such as radiography, computed tomography, clinical pathology, bacterial culture and susceptibility, *Mycoplasma* PCR, *Ranavirus* PCR, herpesvirus PCR, and postmortem examination, were performed as necessary for each case. Neoplasia was diagnosed with histopathology.

All trauma cases were combined into one category and included motor vehicle accidents, garden equipment–related trauma, animal attack, and related injuries. The infectious etiology was further subdivided into aural abscess, upper respiratory tract infection, unknown systemic disease, skin disease or abscess, conjunctivitis, parasitic gastrointestinal disease, and lower respiratory tract disease. All abscesses were diagnosed by physical exam and confirmed at surgery. Bacterial culture and susceptibility were performed at the discretion of the clinician. Upper respiratory tract infections were defined and identified by nasal discharge and oral lesions. Skin diseases included granulomas, dermatitis, erosions, ulcerations, shell deficits, ectoparasites, and dysecdyis. Conjunctivitis was diagnosed by ocular discharge. Gastrointestinal diseases were defined by clinical signs such as diarrhea, regurgitation, cloacal prolapsed, and parasitism. Lower respiratory tract infections included pneumonia and tracheal obstructions. Unknown systemic disease encompassed the infectious cases in which a definitive diagnosis was not identified in the medical record and could not be placed into any other category.

Length of stay (days in hospital) was also recorded. Final disposition included released, euthanized, or died naturally (without euthanasia). Treatments recorded were divided into: surgery, euthanasia, fluid support, nutritional support, analgesia, antibiotics, antifungals, antivirals, steroids, vitamin injections, topical antiseptics, and antiparasiticides.

Data were assessed for normality with the Shapiro-Wilk test. Appropriate measures of central tendency and dispersion were computed for all continuous variables. Comparisons of differences between group medians were performed by the Mann-Whitney U-test (two groups) and the Kruskal-Wallis test (three or more groups). Fischer's exact test was used to assess categorical variable associations. All statistical tests were performed with STATA (STATA 9.1, Statacorp, College Station, Texas, USA). A value of $P=0.05$ was considered statistically significant.

RESULTS

A total of 574 eastern box turtles were identified that presented with a total of 596 visits, including recheck examinations from wildlife rehabilitators. Five turtles had multiple final diagnoses, for a total of 579 final diagnoses. Overall, total number of eastern box turtles that presented to UTCVM from 1995 to 2003 increased. However, from 2004 to 2007, numbers declined slightly (Fig. 1A). Eastern box turtles presented more often during the warmer months of April–October (Fig. 1B).
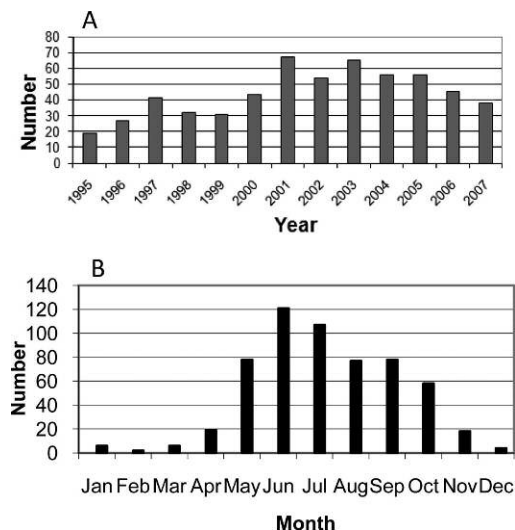
FIGURE 1. Distribution of eastern box turtles presented to the University of Tennessee, College of Veterinary Medicine (A) by year and (B) by month, 1995–2007.

During the study period, the highest precipitation was recorded in July (Fig. 2). Temperature maxima, minima, and means did not vary significantly by month or by year.

Significantly more ($P<0.0001$) eastern box turtles presented for trauma than any other condition ($n=445$; 73% of cases) and trauma was the most common final diagnosis ($n=425$, $P<0.0001$; Table 1). Five turtles were categorized with multiple diagnoses. These cases had a diagnosis of infection coupled with either trauma ($n=4$) or neoplasia ($n=1$). The final diagnoses were consistent with the presenting complaints in 81% of cases. The
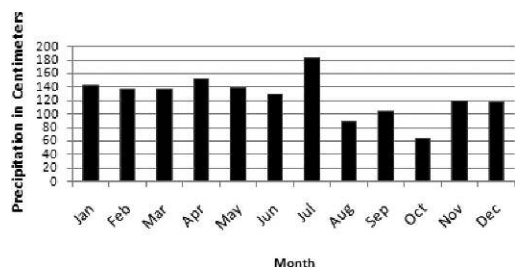
TABLE 1. Comparison of eastern box turtle presentations identified by good Samaritans and final diagnoses identified by fourth year clinical veterinary students and senior clinicians.

	Presenting complaint	Final diagnosis
Trauma	445	425
Infectious	82	107
Unknown	36	9
Orphaned	4	0
Healthy	4	37
Neoplasia	3	1

one neoplastic case was identified on necropsy and subsequent histopathology as a primary epidermal carcinoma.

Infectious etiologies were consistently the second most common primary diagnosis each year throughout the study period ($n=107$), with 18% of total cases. Aural abscesses were the most common presentation for infectious etiologies comprising 56% ($n=60$) of the 107 or 10% of the total patient population. Infectious diseases increased during the summer months (Fig. 3), with no significant trends by year. Aural abscess presentation showed a significant positive correlation with high precipitation ($P<0.05$).

Adults were significantly more likely to be presented ($n=425$), comprising 73% of cases ($P<0.0001$); 12% were juveniles ($n=71$), and 14% did not have a life stage recorded ($n=83$). Sex was not recorded in the majority of cases ($n=324$) and therefore was not analyzed. Median weight of animals was 0.318 kg (range, 0.006–0.594),

FIGURE 2. Total inches of rainfall by month in east Tennessee, 1996–2007.
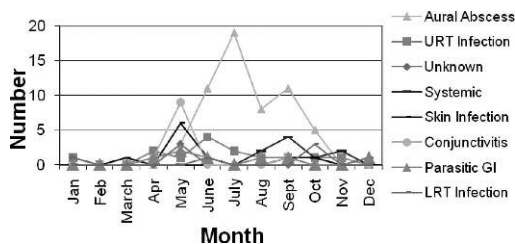

FIGURE 3. Distribution of final diagnoses of infectious diseases in eastern box turtles that presented to the University of Tennessee, College of Veterinary Medicine, 1995–2007.

TABLE 2.   Packed cell volume (PCV), total protein (TP), and white blood cell (WBC) count compared by average and range in sick and healthy eastern box turtles.

	PCV		TP		WBC	
	Sick	Healthy	Sick	Healthy	Sick	Healthy
Average	26.76±11.09	12.75±6.43	4.32±0.82	3.86±1.10	4.03±1.81	5.58±0.05
Range	15.67–37.85	6.32–19.18	3.50–5.14	2.76–4.96	1.60–10.00	5.50–5.65
No.	12	16	12	16	11	16

and there were no correlations with presentation or diagnosis.

Length of stay in the hospital was 0–66 days with a median of 2 days. The longest stay (66 days) was required for a turtle with a traumatic injury in 1997. However, patients with an infectious diagnosis tended to stay in the hospital longer (median=8 days) than other etiologies (trauma median=5 days; unknown diagnosis=2 days, healthy=5 days).

Treatments used at UTCVM were often multimodal and included antibiotics or antifungals ($n=254$), euthanasia ($n=241$), topical antiseptics ($n=91$), fluid support ($n=86$), analgesia ($n=86$), surgery ($n=78$), vitamin injections ($n=24$), antiparasiticides ($n=14$), steroids ($n=10$), nutritional support ($n=4$), and antivirals ($n=2$). Surgery, nutritional support, and antiparasiticides were never used as sole treatments. For 130 cases, no treatments were either recorded or administered.

Three outcomes were identified: released ($n=302$), euthanized ($n=241$), and died naturally without euthanasia ($n=36$). Trauma patients were more likely to be euthanized (53%, $n=227$) than released (41%, $n=173$), whereas patients with infectious conditions were more likely to be released (80%, $n=86$) than euthanized (9%, $n=10$). Patients with aural abscesses ($n=60$) were more likely to be released (92%, $n=55$) than euthanized (7%, $n=4$) or die (2%, $n=1$).

Differences ($P<0.05$) between sick and healthy turtles for PCV, total protein (TP), or WBC (Table 2) were not significant. There were not enough results from plasma chemistries to report.

DISCUSSION

Eastern box turtles presented with a variety of conditions, predominately trauma and infection. Numerous factors were likely driving the presentation of these animals, including activity period, environmental contaminants, changes in public awareness, and potential changes in population status (Mitchell, 1994; Ultsch, 2006). As expected, the majority of patients were presented during the warmer months. This relates to the natural history of eastern box turtles overwintering during the winter and emerging once the weather turns warm (Mitchell, 1994; Ultsch, 2006). The few winter presentations could be explained by early emergence during warm spells, which makes these animals susceptible to infection and injury and subsequent presentation to a rehabilitation center (Ultsch, 2006).

High prevalence of traumatic cases corresponds with findings of another report (Brown and Sleeman, 2002). Urbanization and encroachment into undeveloped areas decreases natural habitats, thereby increasing probability of vehicular encounters and human and pet interactions (Budischak et al., 2006) and likely led to the high incidence. We did not differentiate between types of trauma resulting from human activity (motor vehicles, garden equipment, dog attacks, etc.). Box turtles have been recognized as especially susceptible to road mortality (Budischak et al., 2006). There was no correlation between trauma and temporal or environmental factors such as month, temperature, or precipitation.

Infectious diseases were the second

most prevalent diagnoses. However, the true prevalence of infectious etiologies was difficult to ascertain in this study because, in most trauma cases, only a brief physical exam was completed before euthanasia. Twenty turtles were presented for traumatic injuries that were subsequently diagnosed with a primary infectious etiology. We postulate that if more thorough evaluations were performed in trauma cases before euthanasia, an increase of patients simultaneously diagnosed with infectious disease is likely.

Aural abscesses were the most common infectious cause of morbidity. Joyner et al. (2006) showed no common etiologic agent that caused aural abscess and concluded that secondary infection occurred as a result of an altered environment. Turtles that presented to the University of Tennessee with aural abscesses were treated with surgical lancing of the abscess. An etiologic agent was not identified in most of these patients, and antibiotic treatment was given empirically. Turtles were also treated with vitamin A injections to treat suspected hypovitaminosis A, because this disease process is suspected to play a role in the pathogenesis of aural abscesses (Brown et al., 2003). The outcome of these patients was excellent; 55 of the 60 turtles with aural abscesses were alive at the time of discharge. Four turtles were euthanized and one died without euthanasia.

Precipitation was positively correlated with aural abscesses. Precipitation changes have been documented to be associated with changes in disease prevalence in chelonians. In free-ranging desert tortoises (*Gopherus agassizzii*), an increase in positive *M. agassizii* nasal cultures and upper respiratory tract disease occurred in years with decreased precipitation (Christopher et al., 2003). The current study cannot factor in the delayed temporal effect inherent in animals presenting to a rehabilitation center; therefore, definitive conclusions should not be made. However, it should provide a mechanism for further

studies to determine whether a similar association between precipitation and disease pathogenesis exists in chelonians. Alternatively, changes in precipitation might not lead to an increase in aural abscesses but rather alter behavior leading to increased interactions with good Samaritans.

There were no temporal associations with any final diagnosis. However, we have found a nonsignificant trend toward aural abscess presentation in July. Previous studies have not suggested a temporal risk factor for manifestation of aural abscesses (Brown et al., 2003), but future investigations should include date in their analyses.

It has been suggested that environmental contaminants such as organochlorines alter retinol metabolism, subsequently leading to aural abscesses (Tangredi and Evans, 1997; Holladay et al., 2001). The prevalence of aural abscesses ($n=60$) was high in this study. Organochlorine pesticides exceed acceptable tissue concentrations in game birds in eastern Tennessee (Blevins, 1979). Blevins' report indicated the risk of accumulation and magnification in wildlife and human populations. A biomonitor for organochlorine toxicity would be valuable because these chemicals have been identified as potential toxic agents in humans as well (Howsam et al., 2004; Bhatia et al., 2005). Because of the risk for human exposure to organochlorines, future investigations should evaluate the exposure level of eastern box turtles to such contaminants in the east Tennessee population. This will allow the evaluation of the utility of this species as a biomonitor.

We found no significant differences in PCV, TP, or WBC between sick and healthy turtles. This is consistent with the current knowledge that hematologic values in chelonians are affected by numerous nondisease factors such as age, sex, environment, season, and nutritional status (Christopher et al., 2003; Campbell, 2006). This implies that evaluating hematologic parameters of a single sample does

not aid in diagnosis of disease. However, these tests could have value in monitoring response to treatment and disease progression with serial sampling as cases progress.

Wildlife health monitoring and disease investigations can give valuable insight into the ecologic health of a particular region. Long-term studies evaluating population health trends are rare because of longevity of the projects and financial limitations. Retrospective evaluations of wildlife medical records provide a snapshot of such trends. The use of wildlife clinics and rehabilitation facilities to monitor wildlife health could lead toward bias to sick or injured individuals. However, this bias enables these facilities to serve as a source to monitor and identify patterns in ecosystem health and emerging diseases in certain bioindicator species.

A limitation of this study was the inability to run spatial analyses associated with specific disease occurrences. It highlights a deficiency commonly encountered in studies using retrospective data from wildlife health centers, which rely on community members that are often unprepared or unwilling to disclose the exact location of capture. Future geographic assessments that assess the locations of frequent box turtle traumatic occurrences would be useful in the design of management plans that promote creating safe passageways across roads in those areas. Furthermore, if traumatic injuries occur more frequently in turtles with concurrent infectious diseases, similar management plans can include creating off-road basking sites in these areas.

In summary, eastern box turtles that presented to the UTCVM wildlife clinic primarily had traumatic injuries. However, infectious diagnoses—specifically aural abscesses—were also commonly encountered. Disease patterns only followed climatic changes concerning increased precipitation and aural abscesses. Hematologic parameters were not beneficial in the diagnosis of disease in the eastern box turtle in cases at UTCVM. Because of the small sample size of cases in which bloodwork was evaluated, as well as varied diagnostic technique, conclusions cannot be drawn about the utility of these values in diagnosis. Collecting a minimum database in more cases will aid in identifying infectious or metabolic disease and will provide valuable information about the population. Wildlife clinics should consider performing a complete blood count (hematocrit or PCV, mean corpuscular volume, thrombocyte count, leukocyte count and differential, including heterophils, lymphocytes, eosinophils, basophils, and monocytes) and chemistry (calcium, phosphorus, glucose, cholesterol, total protein, uric acid, sodium, creatinine, potassium, aspartate aminotransferase, creatine phosphokinase, lactate dehydrogenase, and alkaline phosphatase). *Mycoplasma* and *Ranavirus* PCR should be performed in every case as a part of full patient evaluation. In addition, to monitor further and better assess the health status of the population, it would be valuable for the wildlife clinic to record approximate age, weight, sex, shell length, and precise location the animal was found.

This study was a useful tool to evaluate the health of eastern box turtles in the east Tennessee population. The eastern box turtle (*Terrapene carolina carolina*) has been proposed as an excellent indicator for biological ecosystems because of their wide distribution, small home range, and longevity (Sleeman, 2008), as well as their judicious use of climate to dictate overwintering patterns (Ultsch, 2006). Many box turtle populations are in decline as a result of anthropogenic effects on habitats (Nazdrowicz et al., 2008), and the effects of these factors and others on prevalence of disease is unknown. In this study, a potential link with aural abscess occurrence and precipitation might exist and further emphasizes the importance of retrospective studies in defining prospective evaluations for ecologic implications of this clinical finding. Future investiga-

tions should assess the role of infectious disease as a predisposing factor for increased risk of vehicular trauma, as well as the potential role of environmental contaminants in the etiopathogenesis of aural abscesses.

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