Blood flow detection in an Eastern chicken turtle (*Deirochelys reticularia reticularia*) oviductal egg

Collier C.M.[1]; Spaulding K.[2]; DeBolt R.[1]; Christian L.S.[3]; Lowe M.[1]; Lewbart G.A.[1]

Received: Aprıl 2024 Accepted: June 2024

Abstract

A gravid, previously-injured, female, wild Eastern chicken turtle (*Deirochelys reticularia reticularia*) was treated by the North Carolina State University College of Veterinary Medicine Turtle Rescue Team for prolonged anorexia and possible dystocia. Medical management included successful induction of oviposition with oxytocin. Radiographs and ultrasonography were used to confirm the presence of eggs within the oviducts prior to oxytocin therapy. Ultrasonography revealed Doppler blood flow within at least one egg and the presence of a developing embryo. It is hypothesized that these findings may be unique to Eastern chicken turtles. This case demonstrated that case management may be complicated by a patient's medical history and also unique life-history traits.

Keywords: Eastern chicken turtle, *Deirochelys reticularia reticularia*, Ovoposition, Oxytocin, life-history, Doppler ultrasonography

1-Department of Clinical Sciences, College of Veterinary Medicine Raleigh, North Carolina State University, 27607, USA
2-Department of Molecular and Biomedical Sciences, College of Veterinary Medicine Raleigh, North Carolina State University, 27607, USA
3- North Carolina Museum of Natural Sciences, Veterinary Sciences, Raleigh, North Carolina, 27601, USA
*Corresponding author's Email: galewbar@ncsu.edu

Introduction

One of the many unique natural history traits of chelonians is preovipositional embryonic arrest (Williamson *et al*., 2017). All turtles and tortoises display this trait, and in freshwater and marine species, arrest occurs at the mid or late gastrula stage (Ewert, 1985; Miller, 1985). The Eastern chicken turtle (*Deirochelys reticularia reticularia*) possesses unique reproductive strategies in contrast to other species, including a late-summer through late-winter egg-laying period (Gibbons, 1969; Gibbons and Greene, 1978; Jackson, 1988), prolonged egg retention in the oviduct (Cagle and Tihen, 1948; Congdon *et al*., 1983a; Buhlmann *et al*., 1995), limited food intake during periods of egg laying (Jackson, 1988), and an extended embryonic diapause after oviposition (Ewert, 1985; Jackson, 1988; Buhlmann, 1998). Recent work has found that at a maximum age of 21 years, the chicken turtle is the shortest lived of all chelonians (Congdon *et al.,* 2022).

Retained eggs in the oviducts can lead to grievous complications in turtles, including hind-limb paresis/paralysis, salpingitis, coelomitis, bladder rupture, and death (Glassford and Brown, 1977; Rosskopf and Woelrpel, 1983; Minter *et al.,* 2010; Mans and Sladky, 2012). As a clinician, the decision to medically-induce ovoposition is based upon several factors, including medical history, species, and time of year. Most freshwater turtle species do not retain shelled eggs for more than a few months under natural conditions (Behler and King, 1979; Buhlmann *et al.,* 1995). The clinician's decision to medically-induce ovoposition may be complicated by unique life-history traits of certain species, such as the Eastern chicken turtle. While published reports have highlighted the Eastern chicken turtle's ability to retain eggs over winter in laboratory and natural settings, the authors are unaware of a published report on the treatment of prolonged egg retention in an injured Eastern chicken turtle or of the use of Doppler ultrasonography to assess blood flow in oviductal eggs.

Methods

On February 10, 2003, an adult wild female Eastern chicken turtle was evaluated by the North Carolina State University College of Veterinary Medicine (NC State-CVM) Turtle Rescue Team (TRT) for prolonged anorexia of approximately 160 days. The turtle had been struck by a motor vehicle the previous fall and sustained a vehicular-induced forelimb amputation and multiple carapacial fractures. It was initially treated by a rehabilitator in Wilmington, NC, where Betadine® solution (Applicaire, Inc., Meriden, CT) and Triple Antibiotic Ointment® (Major Pharmaceuticals, Livonia, MO) were applied to the wounds that were allowed to heal by second intention. Initial evaluation by the TRT showed the turtle to be bright, alert, responsive (BAR), and in good body condition. At least four eggs were palpated via the prefemoral fossae. Radiographs revealed the presence of 13 eggs (Fig. 1).

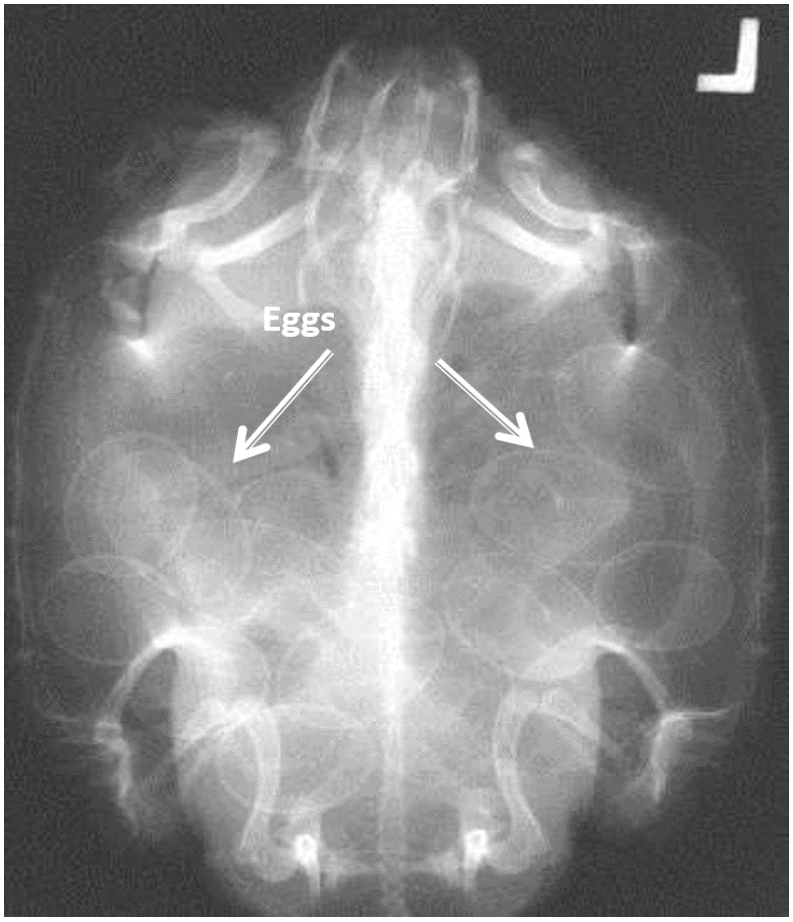

Figure 1: Dorsoventral radiograph. Thirteen well-formed eggs are noted in this adult female chicken turtle (*Deirochelys reticularia reticularia*).

Follow-up ultrasonography was performed and no eggs were found in the urinary bladder (Fig. 2A). Doppler blood flow was observed within at least one egg (Fig. 2B-C), and a developing embryo could be visualized within one egg (Fig. 2D).

Initially, 10 IU/ kg oxytocin (Oxoject, 20 IU/mL, IVX Animal Health Inc., St. Joseph, MO) was administered IM, with two additional doses administered 1.5 and 4 hours after the initial dose. Approximately 1.5 hours after the initial oxytocin dose, 100 mg/kg of calcium gluconate (calcium gluconate injection solution, 94 mg/ml, APP Pharmaceuticals LLC., Schaumberg, IL) was administered IM. The turtle was monitored continuously for egg-laying activity. Six hours after the first oxytocin dose was administered, 12 eggs were laid within a one-hour interval. The turtle was returned to water 22 hours later, and the 13th egg was found within 2 hours. Due to the turtle's continued anorexia, 0.1 ml/kg Vitamin B complex (Vitamin B complex, Sparhawk Lab Inc., Lenexa, KS) was administered subcutaneously, and the turtle was assist-fed. The turtle remained BAR, continued to urinate and defecate normally, and was returned to the rehabilitator 3 weeks later.

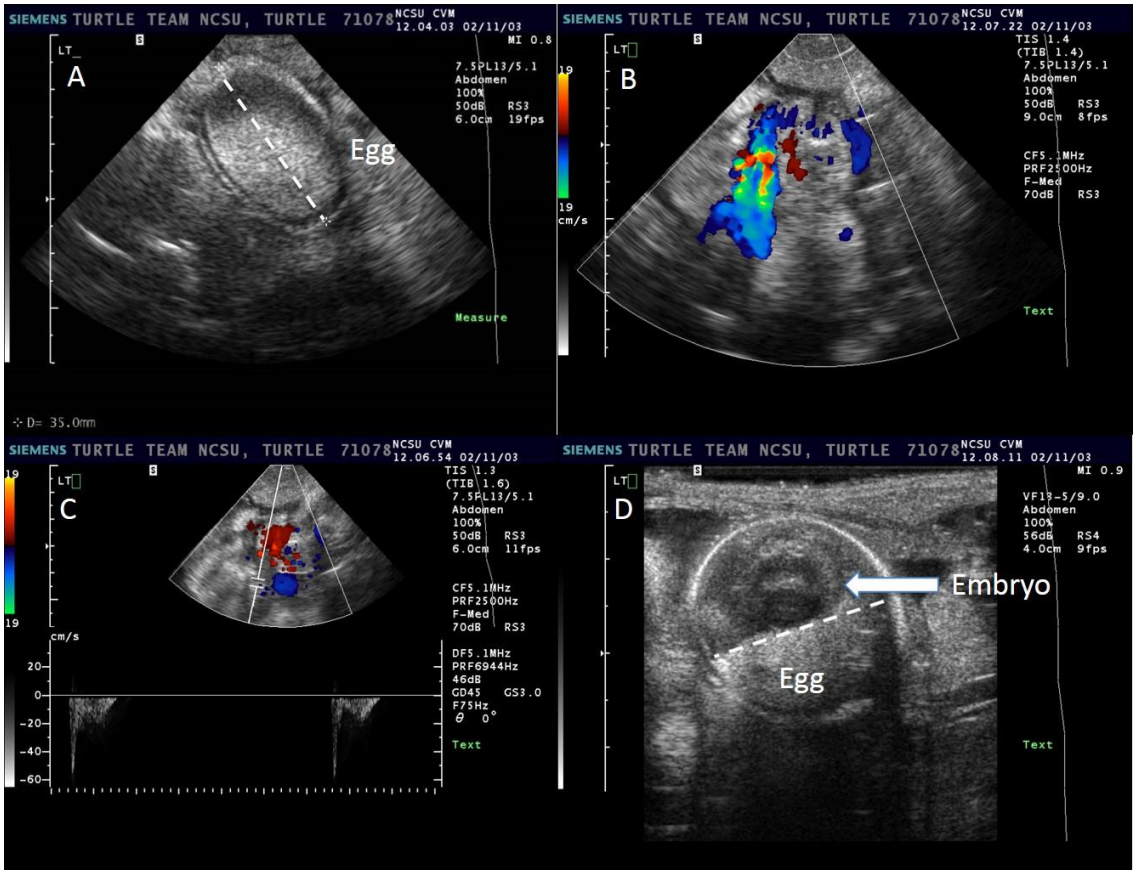

Figure 2: Ultrasonographic images, mature female chicken turtle (*Deirochelys reticularia reticularia*). A. One of 13 eggs visible in the oviduct contains a hyperechoic rim. B. Doppler blood flow is observed within an egg. C. Velocity of blood flow within the egg is monitored. D. Image of embryo is visualized within the egg.

The eggs were placed in a plastic container filled with approximately 15 cm (6 inches) of coconut fiber soil and fully covered with an additional 12-15 cm (4-6 inches) of soil. The container was covered with a corresponding lid with holes to ensure oxygen circulation. The egg container was kept in an incubator repurposed from a refurbished refrigerator. The incubator was kept around 29 degrees C (85 degrees Fahrenheit), and 130 days post oviposition, one egg hatched. The hatchling was monitored for 2.5 weeks before being released in Wilmington, NC close to where the mother was found.

Discussion

Doppler blood flow and ultrasonographic imaging

The authors are unaware of published reports documenting Doppler blood flow within egg(s) and visualization of embryo(s) on ultrasonographic imaging in chelonians. These unique findings may suggest that embryonic development within the chicken turtle's oviduct is more complex than originally thought. In other chelonians, embryos arrest in the oviduct after reaching the gastrula stage. Arrest may be due to the limited exchange of oxygen and other resources between the mother and embryo (Andrews and Mathies, 2000;

Rafferty *et al*., 2013). Embryo development continues after ovoposition in response to environmental triggers, such as normal oxygen levels and temperature (Rafferty *et al*., 2013). Blood flow within oviductal eggs may contribute to the Eastern chicken turtle's unique ability to retain viable eggs for prolonged periods of time.

Clinical complications of dystocia

While the authors acknowledge ex post facto that intervention may not have been warranted in this case due to the Eastern chicken turtle's life-history traits, the decision to induce ovoposition was based upon the turtle's medical history. Injury, stress, and unideal husbandry conditions (i.e. substrate, temperature/humidity, and nutrition) may predispose turtles to dystocia (Holt, 1979; Jackson, 1988; Lloyd, 1990; Denardo, 2006; Minter *et al*., 2010). It is possible that if offered appropriate conditions the turtle would have laid the eggs on her own in time.

The turtle was injured during a critical point in the nesting season (September). Eastern chicken turtles frequently nest in the late summer/autumn (August/September) when resources are limited in the wetlands; this time period coincides with the Eastern chicken turtle's natural reduction in food intake (Jackson, 1988; Buhlmann *et al*., 2009). When autumnal temperatures drop rapidly, Eastern chicken turtles may delay nesting until brief warm periods occur from October to January or overwinter the eggs (Buhlmann, 1998; Buhlmann *et al*., 2009). Most Eastern chicken turtles that retain eggs in the wild lay clutches from February through mid-March (Buhlmann, 1998).

Oxytocin therapy

Oxytocin therapy stimulates oviductal contractions and is a common treatment for chelonians experiencing dystocia (Glassford and Brown, 1977; Lloyd, 1990; Denardo, 2006; Minter *et al*., 2010). One potential complication of oxytocin therapy is retrograde passage of ectopic egg(s) into the urinary bladder (Holt, 1979; Thomas *et al*., 2002; Wilkinson *et al*., 2004; Denardo, 2006; Knotek *et al*., 2009; Minter *et al*., 2010). Turtles possess a shared urethral opening between the urodeum and bladder. When an egg passes down the oviduct to the urodeum, it may retrograde through the shared urethral opening into the urinary bladder. Prolonged presence of an egg(s) within the urinary bladder may result in cystitis, necessitating surgical or cytoscopic removal as soon as possible (Thomas *et al*., 2002; Wilkinson *et al*., 2004; Denardo, 2006; Minter *et al*., 2010).

Oxytocin therapy is not the only factor that may contribute to ectopic eggs passing into the urinary bladder. Trauma has been suggested as another likely cause (Thomas *et al*., 2002; Minter *et al*., 2010). Due to history of trauma, an ultrasound of the turtle was performed to confirm that ectopic eggs were not present in the urinary bladder prior to oxytocin therapy.

Low hatching success rate

Low hatching success rate of the turtle's clutch may be due to a number of factors, such as incidence of trauma, compromised health, unfertilized eggs, prolonged egg retention, and unideal laboratory incubation methods. Previous studies showed that clutches retained overwinter have less hatching success than clutches ovoposited directly. The authors acknowledge ex post facto that chicken turtles have unique incubation conditions that should be considered for hatch-rate success in the laboratory. Eastern chicken turtle embryos require a minimum 30-day chilling period at 10°C (50°F) to 20°C (68°F) (Buhlmann, 1998; Buhlmann *et al*., 2009). In laboratory settings, poor hatching success has been noted in clutches that were collected in the autumn and immediately incubated at normal developmental temperatures (Ewert, 1985; Jackson, 1988; Buhlmann *et al*., 2009; Ewert *et al*., 2006). In nature, chilling time varies depending on whether eggs were laid in the previous late-summer/fall or in the recent late-winter. During the chilling period, ovoposited embryos remain in diapause until late-April when nest temperatures are above the 25°C (77°F) needed for development (Ewert, 1985; Buhlmann *et al*., 2009). Hatching occurs in August-September after an 80 to 100 day incubation period between 26°C (78.8°F) and 29°C (84.2°F) (Buhlmann, 1998; Buhlmann *et al*., 2009).

Acknowledgements

The authors would like to thank Mr. Jeff Beane of the NC Museum of Natural Sciences, and Ms. Beth Howard of Wilmington, North Carolina, for their input into this case. The authors would like to acknowledge the NC State-CVM TRT for their efforts and contributions.

References

Andrews, R.M. and Mathies, T., 2000. Natural history of reptilian development: constraints on the evolution of viviparity. *BioScience*, 50(**3**),227–232. DOI:10.1641/0006-3568(2000)050[0227:NHORDC]2.3.CO;2

Behler, J.L. and King, F.W., 1979. Turtles. *In* Behler JL, King FW (eds), The National Audubon Society Field Guide to Reptiles and Amphibians. Alfred A. Knopf, New York, NY:433-474.

Buhlmann, K.A., Lynch, T.K., Gibbons, J.W. and Greene, J.L., 1995. Prolonged Egg Retention in the turtle *Deirochelys reticularia* in South Carolina. *Herpetologica*, 51(**4**),457-462.

Buhlmann, K.A., 1998. Ecology, terrestrial habitat use, and conservation of a freshwater turtle assemblage inhabiting a seasonally fluctuating wetland with emphasis on the life history of *Deirochelys reticularia*. [Ph.D. dissertation]. Athens, Georgia: University of Georgia. 165P.

Buhlmann, K.A., Congdon, J.D., Gibbons, J.W. and Greene, J.L., 2009. Ecology of chicken turtles (*Deirochelys reticularia*) in a

seasonal wetland ecosystem: exploiting resource and refuge environments. *Herpetologica*, 65(**1**),39-53. DOI:10.1655/08-028R1.1

Cagle, F.R. and Tihen, J., 1948. Retention of eggs by the turtle *Deirochelys reticularia*. *Copeia*, 1:66. doi.org/10.2307/1438801.

Congdon, J.D., Buhlmann, K.A. and Gibbons, J.W., 2022. Comparing life histories of the shortest-lived turtle known (chicken turtle, *Deirochelys reticularia*) with long-lived Blanding's turtles (*Emydoidea blandingii*). *Chelonian Conservation and Biology*, 21(**1**),28-36. DOI:10.2744/CCB-1521.1

Denardo, D., 2006. Dystocia. *In* Mader DR (ed), Reptile Medicine and Surgery. WB Saunders Co, Philadelphia, PA:727-792.

Ewert, M.A., 1985. Embryology of Turtles. *In* Gans C, Billett F, Maderson PFA (eds), Biology of the Reptilia 14 (Development A). Academic Press, New York, NY:76-267.

Ewert, M.A., Jackson, D.R. and Buhlmann, K.A., 2006. *Deirochelys reticularia*- Chicken Turtle. *In* Meylan PA (ed), Biology and Conservation of Florida Turtles. Chelonian Research Monographs 3. Chelonian Research Foundation, Lunenburg, MA:249-259.

Gibbons, J.W., 1969. Ecology and population dynamics of the chicken turtle, *Deirochelys reticularia*. *Copeia*, 669-676.

Gibbons, J.W. and Greene, J.L., 1978. Selected aspects of the ecology of the chicken turtle, *Deirochelys reticularia* (Latreille) (Reptilia, Testudines, Emydidae). *Journal of Herpetology*, 12, 237-241. doi.org/10.2307/1563412

Glassford, J.F. and Brown, K., 1977. Treatment of egg retention in a turtle. *Veterinary Medicine, Small Animal Clinician*, 72, 1641-1645.

Holt, P.E., 1979. Obstetrical problems in two tortoises. *Journal of Small Animal Practice*, 20:353-359.

Jackson, D.R., 1988. Reproductive strategies of sympatric freshwater emydid turtles in northern peninsular Florida. *Bulletin of the Florida State Museum Biological Science*, 33, 15-152.

Knotek, Z., Jekl, V., Knotkova, Z. and Grabensteiner, E., 2009. Eggs in chelonian urinary bladder: is celiotomy necessary? *Proceedings of the Association of Reptile and Amphibian Vetetinarians*, 12-121.

Lloyd, M.L., 1990. Reptilian dystocias review – causes, prevention, management, and comments on synthetic hormone vasotocin. *Proceedings of the Association of Reptile and Amphibian Vetetinarians* , 290-296.

Mans, C. and Sladky, K., 2012. Diagnosis and management of oviductal disease in three red-eared slider turtles (*Trachemys scripta elegans*). *Journal of Small Animal Practice*, 53(**4**),195-251.

Miller, J.D., 1985. Embryology of Marine Turtles. In C. Gans and F. Billett, eds. Biology of the Reptilia.

Vol. 14. Wiley, New York. pp. 269–328.

Minter, L.J., Wood, M.S., Hill, T.L. and Lewbart, G.A., 2010. Cystoscopic guided removal of ectopic eggs from the urinary bladder of the Florida Cooter turtle (*Pseudemys floridana floridana*). *Journal of Zoo and Wildlife Medicine*, 41(**3**), 503-509.

Rafferty, A.R., Evans, R.G., Scheelings, T.R. and Reina, R.D., 2013. Limited oxygen availability in utero may constrain the evolution of live birth in reptiles. *The American Naturalist*, 121(**2**),245-253.

Rosskopf, W.J. and Woelrpel, R., 1983a. Treatment of an egg-bound turtle. *Modern Veterinary Practice*, 64, 644-645.

Thomas, H.L., Willer, C.J., Wosar, M.A., Spaulding, K.A. and Lewbart, G.A., 2002. Egg-retention in the urinary bladder of a Florida cooter turtle, *Pseudemys floridana floridana*. *Journal of Herpetological Medicine and Surgery*, 12, 4-6.

Wilkinson, R., Hernandez-Diver, S., Lafortune, M., Calvert, I., Gumpenberger, M. and McArthur, S., 2004. Diagnostic imaging techniques. *In* McArthur S, Wilkinson R, Meyer J (eds.), Medicine and Surgery of Tortoises and Turtles. Blackwell Publishing, Oxford, United Kingdom:127–232.