

Thomas M. Donnelly, DVM, Column Editor

## Opisthotonus, torticollis and mortality in a breeding colony of *Anolis* sp. lizards

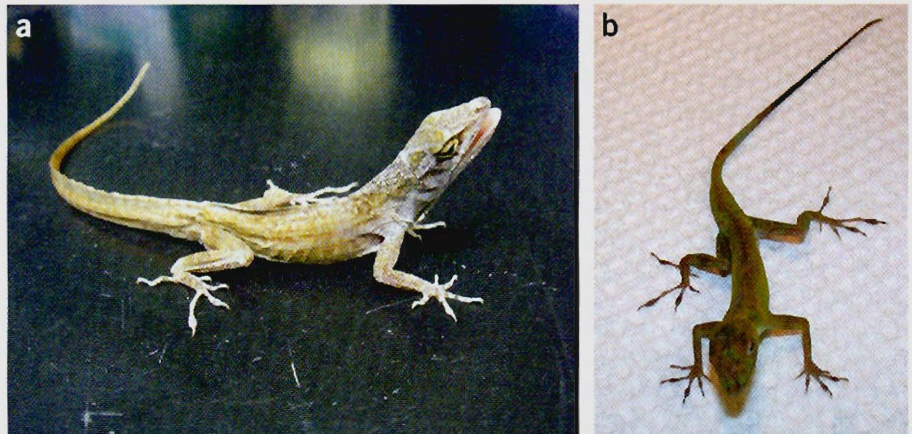
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Anoles are typically small (3–7 in long), arboreal, insectivorous lizards found throughout the southeastern US, the Caribbean and various other regions of the western world. Approximately 300 species have been described, half of which dwell on Caribbean islands. The word 'anole' comes from the French *anolis*, from Arawak, an indigenous language of the Lesser Antilles.

Several adult anoles over 1 y of age representing the species *Anolis sagrei*, *A. cristatellus*, *A. grahami*, *A. lineatopus* and *A. evermanni* presented with abnormal body postures that included torticollis and opisthotonus. Affected anoles often died within 24 h.

The anoles had been wild-caught in the Greater Antilles and Bahamas by the investigators who were researching the evolution of genetic covariance structure in repeated radiations of Caribbean *Anolis*<sup>1</sup>. The repeated evolution of similar ecomorphs of Caribbean *Anolis* provided an opportunity to investigate the extent to which evolutionary change is dictated by genetic constraints versus the extent to which selection can modify genetic architecture. The project involved captive breeding of at least eight species of *Anolis* representing three common ecomorphs from three different lineages.

Captured anoles were maintained for at least 4 months in a breeding colony under an approved animal protocol at the University of Virginia. The investigators had not introduced any new animals into the colony for the 4 months preceding our observations of neurological signs and ensuing death in some lizards. The animals were maintained



**FIGURE 1** | Abnormal body postures in anole lizards. (a) Opisthotonus in *Anolis sagrei*. (b) Torticollis in *Anolis evermanni*.

in an environment of high humidity (~80% in cage, 55–65% ambient room humidity), with a room temperature of 82 °F during the light phase and 75 °F during the dark phase, and a 12-h:12-h light:dark cycle. Animals were caged in polycarbonate plastic boxes or containers lined with removable all-weather carpet. Each cage contained a wooden perch, a plant (genus *Coleus*) and a hammock for basking, as well as an ultraviolet light mounted across the top of the cage. Daily misting of the interior of the cages with deionized water provided droplets of drinking water and helped raise the cage humidity. The lizards were fed daily with either crickets raised on T-Rex Calcium Plus Food for Crickets (T-Rex Products, Chula Vista, CA) or other insects. All live insect food had been dusted with Herptivite Multivitamin and Rep-Cal Calcium with Vitamin D3, phosphorus-free (T-Rex Products).

On clinical examination, the affected anoles presented with a reluctance to move, tremors of the distal appendages and postural distortion of the axial skeleton, including hyperextension (Fig. 1a) or twisting and serpentine distortion (Fig. 1b). Because of the acute onset of these presentations in multiple individuals, the differential diagnosis included infectious disease, metabolic disease, nutritional deficiency and toxicosis. Gross necropsy of affected lizards was unremarkable with the exception of gout affecting multiple joints. Microscopic and bacteriologic evaluations were not done.

What do you think is the cause of the problems? Is it infectious, metabolic, toxic or nutritional? How would you investigate? How would you treat it?

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**What's your diagnosis?**

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## Diagnosis | Thiamine deficiency

Typical husbandry conditions for anoles have been previously described<sup>2,3</sup>. Our housing conditions were consistent with these recommendations for atmospheric humidity and temperature. Our lizards' diet was also consistent with these recommendations, as they were fed insects dusted with a vitamin and calcium preparation. Given the unusual presentation of torticollis and opisthotonus, however, a tentative diagnosis of thiamine (vitamin B1) deficiency was presumed.

In reptiles, clinical signs of hypothiaminosis or hypovitaminosis B1 are generally nonspecific and include muscle twitching, incoordination, blindness, seizure activity, torticollis, abnormal posture, spiral locomotion, jaw gaping, dysphagia and, potentially, death<sup>4</sup>. Affected snakes may be unable to strike prey accurately; in affected chelonians, the most notable clinical sign is a sinking of the eye within the bony orbit (enophthalmos)<sup>4</sup>. At necropsy, usually no gross lesions are seen. Histologically, leukoencephalopathy consisting of cerebral cortical necrosis with peripheral neuritis and cardiomyopathy is frequently encountered<sup>5</sup>. Histologic lesions also include a diffuse eosinophilia with severe demyelination and axon sheath fragmentation; generally inflammatory cells are absent<sup>6</sup>.

We administered 0.1 ml of vitamin B complex (Butler-Schein Animal Health, Melville, NY) intraperitoneally once per day on four consecutive days to an affected anole; clinical symptoms resolved after the second day of injection. We interpreted this response as confirmation of our initial diagnosis. Because the treated lizard responded so rapidly, we began to administer vitamin B complex to any anoles presenting with neurologic symptoms until the cause of the thiamine deficiency was ascertained.

In conversation with the laboratory personnel caring for the anoles, we learned that the Herptivite supplement and Calcium Plus had been purchased in bulk more than 2 y before we began observing symptoms in the lizards and had been stored in the dark at room temperature. Thiamine is a highly labile vitamin (as are vitamins A and C) that undergoes deterioration under normal storage conditions; this is one reason why laboratory animal diets should be used within 6 months of their milling date<sup>7</sup>. We recommended that laboratory personnel purchase smaller quantities of the vitamin mix, store the mix in a refrigerator at 4 °C and discard any remaining vitamin supplement 6 months after its manufacture date.

Thiamine deficiency was originally reported in silver foxes farmed for fur; the resulting neurological disease was known as Chastek paralysis<sup>8,9</sup>. Investigations determined that thiamine was inactivated in diets containing fishmeal<sup>10</sup>. Calcium Plus contains some fishmeal. Thiaminase, a naturally occurring enzyme that destroys thiamine in a diet, is present in large amounts in fish meal<sup>11,12</sup>. Manufacturers often correct for potential thiamine breakdown by adding 1 mg of thiamine per kg of fish meal<sup>13</sup>. Another potential but less common cause of opisthotonus in lizards is cerebral xanthomatosis<sup>14</sup>.

In conclusion, we report a common nutritional cause of a neurological problem of lizards in a laboratory setting. This case emphasizes the need to pay particular attention to the composition of the diet of unusual laboratory species and the need

to extend good husbandry practices to all species used in the research environment.

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