Pathology in Practice

History

A female Home’s hingeback tortoise (Kinixys homeana) of unknown age that was part of a captive population at the Knoxville Zoological Gardens was euthanized after a week of anorexia and lethargy that failed to respond to symptomatic treatment. This tortoise was 1 of 8 in the population of 11 that had died or been euthanized over a 3-year period. The other tortoises included both males and females, with ages ranging from 12 days to 21 years.

Gross Findings

At necropsy, the liver was enlarged (95.8 g; 6.5% of body weight) and yellow. On cut section, there was an irregular 3-cm-diameter area of brown and red discoloration in a reticular pattern (Figure 1).

Figure 1—Photograph of the liver from an adult Home’s hingeback tortoise (Kinixys homeana) of unknown age that was part of a captive population and was euthanized after a week of anorexia and lethargy.

Histopathologic and Diagnostic Findings

All hepatocytes were expanded by cytoplasmic lipid droplets. Some sinusoids were dilated and filled with blood (corresponding to the red reticular pattern visible grossly). Some hepatocyte nuclei had membrane-bound clear to stippled structures that compressed the chromatin to the periphery. These same intranuclear structures were seen in the renal tubular epithelium, exocrine pancreas, thyroid gland, adrenal gland, urinary bladder urothelium, ovary, spleen, esophageal epithelium, stomach, duodenum, colon, lung, and lining of the inner ear. There were infiltrates of lymphocytes and heterophils in the kidney (Figure 2), pancreas (Figure 3), thyroid gland, adrenal gland, urinary bladder, spleen, esophagus, duodenum, ovary, lung, and inner ear. There were plasma cells with a few Mott cells, lymphocytes, and macrophages in the meninges. The exocrine pancreas was markedly atrophic.

A sample of frozen liver was submitted for a PCR assay to test for coccidia. The PCR target was a 350-bp segment of the small ribosomal RNA gene of Toxoplasma gondii. Amplification products were sequenced and analyzed with an automated DNA sequencer, and a diagnosis of intranuclear coccidia of tortoises was confirmed.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis: lymphocytic and heterophilic interstitial nephritis, pancreatitis, thyroiditis, adrenalitis, cystitis, splenitis, esophagitis, enteritis, oophoritis, pneumonia, and otitis interna with intranuclear coccidia; diffuse hepatocellular lipidosis with intranuclear coccidia; moderate multifocal hepatic congestion; and moderate chronic meningitis.

Case summary: tortoise intranuclear coccidiosis (TINC) in a captive Home’s hingeback tortoise.

Comments

There are > 30 species of coccidia known to affect chelonians, with Eimeria spp being the most common.1-5 TINC was first reported in 1990 and is now considered to likely be present worldwide in captive chelonians;1,5,6 however, there are no data on
The specific coccidia that cause TINC are unknown, but the 18S ribosomal RNA sequence appears most similar to that of Eimeria arnyi. There have also been reports of coccidia belonging to the Eimeria, Isospora, and Cyclospora genera that exhibit intranuclear stages. There is a possible association between TINC and coinfection with organisms such as Mycoplasma spp and Aspergillus spp, but it is unclear whether that association reflects the fact that coinfected individuals were already immuno-compromised for other reasons or is due to the fact that TINC weakens the immune system. No other infectious organisms were found in the tortoise of the present report. Formalin-fixed, paraffin-embedded tissues (kidney, liver, or both) from the other 7 tortoises in this collection that had died were retrospectively submitted for testing with the PCR assay for TINC. One other tortoise was also positive and had intranuclear coccidia present microscopically. The lack of additional positive cases could have been due to the low sensitivity of the assay on formalin-fixed, paraffin-embedded tissue or to low transmission between tortoises.

Clinical signs associated with TINC are typically nonspecific, such as anorexia, lethargy, and weight loss, and important differential diagnoses include infection with Mycoplasma spp, herpesvirus, and ranavirus. Other reported clinical signs include subcutaneous swelling, ocular or nasal discharge, dyspnea, and ascites. There is a report of 3 tortoises with chronic rhinosinusitis and oronasal fistulae, but it was not clear whether the fistulae were the result of TINC or other comorbidities. The diagnosis in those tortoises was made antemortem by recognition of the intranuclear coccidia during cytologic examination of nasal smears. In many cases, tortoises either die despite appropriate symptomatic treatment or are euthanized for failure to respond to treatment, as was the case here.

Gross abnormalities in tortoises with TINC, if any, are usually limited to an enlarged and yellow liver (due to hepatic lipidosis, not the intranuclear coccidia), poor body condition, and, rarely, subcutaneous swelling of the extremities. The organisms are typically in the nuclei of various organs, with the most abundant organisms in the kidney, pancreas, and intestines. Hepatic lipidosis and pancreatic atrophy are common findings in anorexic chelonians; however, detection of inflammation in the pancreas and kidney should initiate a thorough search for intranuclear coccidia. There is a single case report of a radiated tortoise with systemic coccidiosis in which the organisms were within the cytoplasm of lymphocytes and plasma cells rather than the nuclei, but the clinical history was very similar to other reported cases of TINC. This could represent a different species of coccidia or a different point in the disease progression. TINC is usually associated with predominantly lymphocytic to lymphoplasmacytic inflammation of various degrees in affected organs, and this likely contributes to the severity and spectrum of clinical signs. Of particular note, coccidia are usually not present in the CNS, but inflammation there is often substantial, as was the case in this tortoise. In addition, the exocrine pancreas is consistently severely affected, either by marked atrophy, infiltration of inflammatory aggregates, or both, and this likely plays a role in the anorexia and weight loss exhibited by most affected tortoises. The organisms in this tortoise were in every organ examined, except the brain, heart, and oviduct. The pancreas and kidney were the most severely affected organs. All organisms in this case were within the nucleus with none in the cytoplasm, and although no organisms were present in the meninges, there was inflammation present there.

Transmission of TINC occurs through the fecal-oral route, which has been confirmed by experimental infection. Oocysts are shed in the feces, and sporulation occurs over 3 to 4 days. The oocysts are...
difficult to find on routine sugar flotation because they degrade rapidly.\(^2\) However, an antemortem diagnosis can be made through cytologic examination of nasal or cloacal swab samples or by performing a PCR assay on feces, tissue biopsy specimens, or swabs of the conjunctiva, nasal or ocular discharge, or cloaca.\(^5\) Alvarez et al.\(^7\) developed a quantitative PCR assay specific for TINC that allows for faster diagnosis as compared with the previous method, which involved a pan-coccidia PCR assay that required several days for results. The organisms that cause TINC can also potentially be shed in other secretions, but whether this is a viable route of transmission is unknown. Because clinical signs are nonspecific, treatment is often symptomatic and involves fluid administration and nutritional support to tortoises that are not eating, appropriate antimicrobials for concurrent infections, and appropriate husbandry to minimize stress caused by changes in thermoregulation and light cycles.\(^1\) Toltrazuril or ponazuril, the active metabolite of toltrazuril, has been used in suspected cases; treatment is recommended to continue for at least a month, as abbreviated courses can lead to temporary alleviation of clinical signs without clearance of infection.\(^7\) The surviving tortoises in this collection were treated with 15% ponazuril (0.3 mL, PO, q 24 h for 3 d); no additional diagnostic testing was performed.

Intranuclear coccidiosis has been described in many other species of reptiles, as well as farm animals, small mammals, and birds, and this condition is most often caused by coccidia of the genus *Eimeria* or *Cyclospora*.\(^9\)\(^-\)\(^11\) Intranuclear coccidiosis is believed to most often be asymptomatic or cause minimal clinical signs in most species, but there have been reports\(^9\)\(^-\)\(^10\) of substantial morbidity in calves. Clinical signs typically include anorexia, growth retardation, and watery diarrhea, and most animals either die or are euthanized after failure to respond to treatment.\(^9\)\(^-\)\(^10\) Case reports\(^9\)\(^-\)\(^11\) from both calves and geese demonstrate fecal-oral transmission, and oocysts appear to also be difficult to detect on routine fecal flotation.\(^9\) Gross abnormalities in calves range from none to enlarged mesenteric lymph nodes and thickening of the small intestinal mucosa.\(^9\)\(^-\)\(^10\) Interestingly, Yamada et al.\(^10\) described absence or atrophy of the thymus in 2 cases. In both calves and geese, intranuclear coccidia are present throughout the gastrointestinal tract.\(^9\)\(^-\)\(^11\) but in calves, the highest concentration is in the jejunal villi with atrophy of the villi a common histologic finding in this species.\(^9\)\(^-\)\(^10\) Intranuclear coccidia in calves can lead to major economic loss because the organisms are difficult to detect on routine fecal examination and affected individuals typically show nonspecific clinical signs.

Because many captive populations of chelonians include species that are threatened or endangered, TINC is considered an important emerging infectious disease of captive chelonians\(^1\)\(^-\)\(^2\) and should be considered a differential diagnosis in any captive chelonian with signs of systemic illness. Further study is warranted to investigate the life cycle and modes of transmission. In addition, captive collections should consider performing quantitative PCR assays on nasal and cloacal swab specimens to screen new additions while in quarantine to help prevent spread to existing collections.

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### References