

Emerging Infectious Diseases of Chelonians

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KEYWORDS

- Testudines • Chelonians • Adenovirus • *Iridovirus* • *Ranavirus* • Coccidiosis
- Cryptosporidiosis

KEY POINTS

- Intranuclear coccidiosis of Testudines is a newly emerging disease found in numerous chelonian species that should be on the differential list for all cases of systemic illness and cases with clinical signs involving multiple organ systems. Early diagnosis and treatment is essential, and PCR performed on conjunctival, oral and choanal mucosa, and cloacal tissue seems to be the most useful antemortem diagnostic tool.
- *Cryptosporidium* spp have been observed in numerous chelonians globally, and are sometimes associated with chronic diarrhea, anorexia, pica, decreased growth rate, weight loss, lethargy, or passing undigested feed. A consensus PCR can be performed on feces to identify the species of *Cryptosporidium*. No treatments have been shown to clear infection, but paromomycin did eliminate clinical signs of disease in a group of *Testudo hermanni*.
- Iridoviral infection is an emerging disease of chelonians in outdoor environments that usually presents with signs of upper respiratory disease, oral ulceration, cutaneous abscessation, subcutaneous edema, anorexia, and lethargy. The disease can be highly fatal in turtles, and can be screened for with PCR of oral and cloacal swabs, and whole blood.
- Adenoviral disease is newly recognized in chelonians and presents most commonly with signs of hepatitis, enteritis, esophagitis, splenitis, and encephalopathy. Death is common in affected chelonians. PCR of cloacal and plasma samples has shown promise for antemortem detection, although treatment has proved unsuccessful to date.

Several infectious diseases continue to be prevalent in captive and wild chelonians. Important diseases that have been well described include mycoplasmosis and herpesvirus in tortoises, and herpesvirus-associated fibropapillomas in sea turtles. Diseases that are currently emerging as important pathogens include intranuclear coccidiosis of Testudines, cryptosporidiosis in tortoises, *Iridovirus*, and adenovirus.

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This article describes each of these emerging pathogens by its biology, epidemiology, clinical signs, diagnosis, treatment, gross pathology, histopathologic changes, and any anticipated future trends.

INTRANUCLEAR COCCIDIOSIS OF TESTUDINES

Biology and Epidemiology

A disease-causing, primarily intranuclear, coccidian parasite of Testudines (TINC) was first identified in radiated tortoises in 1990.¹ Subsequently, the same pathogen (GenBank accession number AY728896), determined by sequencing of a coccidial 18S rRNA consensus polymerase chain reaction (qPCR) product, has caused systemic disease in impressed tortoises (*Manouria impressa*); leopard tortoises (*Stigmochelys [Geochelone] pardalis*); Forsten tortoises (*Indotestudo forstenii*); bowsprit tortoises (*Chersina angulata*); spider tortoises (*Pyxis arachnoides*); flat-tailed tortoises (*Pyxis planicauda*); Galapagos tortoises (*Chelonoidis nigra becki*); eastern box turtles (*Terrapene carolina carolina*); and Arakan forest turtles (*Heosemys depressa*).²⁻⁵ The authors have also confirmed the disease in several other chelonian species (Paul M. Gibbons, DVM, unpublished data, 2013) postmortem by histopathology usually after severe acute illness. Cases have been reported in Germany and the United States including New York, Louisiana, Florida, Georgia, Texas, California, and Hawaii, although no systematic prevalence study has been undertaken to date.¹⁻⁷ Most of these cases occurred in zoologic collections and a few had been recently imported from Indonesia.

The life cycle and method of transmission of the TINC organism are unknown and the organism has not been studied *in vitro*. Although it has not been assigned to a genus, the phylogenetic position of the organism has been characterized by sequencing an 18S small ribosomal unit. It is most closely related to *Eimeria arnyi*, a species identified by oocysts in the feces of the eastern ringneck snake (*Diadophis punctatus arnyi*).^{3,8} Life stages of the TINC organism including trophozoites, meronts, merozoites, macrogametocytes, microgametocytes, and nonsporulated oocysts have been found in the nucleus, cytoplasm, and extracellularly in tissues by histology and electron microscopy. Reports have failed to describe TINC oocysts in feces, and the authors have been unable to find oocysts on fecal flotation from several polymerase chain reaction (PCR) test-positive animals with clinical signs of illness. The TINC organism has not been identified outside of cells or tissues, and samples of various invertebrates and substrate from the enclosures of affected tortoises during an outbreak of TINC at a zoologic facility were negative when tested by quantitative PCR.⁴

Clinical Signs

Although the organism is often reported in highest number in the kidney or pancreas, infection is frequently disseminated to an array of organs so clinical signs vary among cases and are not specific to TINC. Clinical signs range from mild chronic conjunctival or nasal erythema or discharge to severe gasping, subcutaneous edema, and ulceration of the cloacal mucosa. Additional clinical signs can include anorexia, lethargy, lack of normal diurnal behavior patterns, increased respiratory effort, mouth breathing, and rapid weight gain or loss. Rapid weight gain is common, and results from ascites or retention of urine in the urinary bladder (Paul M. Gibbons, DVM, personal communication, 2013). Death can occur within a few days of initial clinical signs, or after months of clinical management including improved husbandry and anticoccidial drug therapy (Paul M. Gibbons, DVM, personal communication, 2013). Stress and thermoregulatory challenges (eg, insufficient heat or evaporative heat loss) seem to

enhance progression of disease, probably because of reduced immune response. In groups housed together, morbidity and mortality vary from a few to all of the individuals showing signs of disease (Paul M. Gibbons, DVM, personal communication, 2013). Often a proportion of the animals in an affected group survives and apparently recovers (Paul M. Gibbons, DVM, personal communication, 2013).^{5,7} Some cases have survived after a positive diagnostic test, and some of these have become test-negative after treatment (Paul M. Gibbons, DVM, personal communication, 2013).⁵ Animals that recover from clinical signs of illness could become carriers; clinical signs can recrudescence, and recovered animals can become test-positive again (Paul M. Gibbons, DVM, personal communication, 2013). It is likely, but not proved, that test-positive animals shed infectious stages of the organism. No systematic study has been undertaken to determine the likelihood that recovered or exposed individuals become carriers or reservoirs. It is unknown whether the organism is directly transmitted or requires an intermediate host, but most of the animals in an affected group usually become test-positive (Paul M. Gibbons, DVM, personal communication, 2013).⁵

Diagnosis

Antemortem diagnosis is possible and TINC should be included in the differential diagnosis of all cases of Testudinoidea (Chelonia) with systemic signs of illness or clinical signs involving multiple organ systems. The organism can sometimes be identified on cytologic examination of nasal discharge stained by Wright-Giemsa, Fite acid-fast, or periodic acid-Schiff techniques (**Fig. 1**).³ Cytologic examination of nasal discharge also generally shows a mononuclear inflammatory response.³ Biopsy and histologic examination of affected tissues may also be diagnostic, particularly in cases with rhinitis or cloacitis, and renal or pancreatic biopsies may also be examined in cases with systemic signs of illness. At this time, however, the most useful clinical diagnostic tool is quantitative PCR performed on swabs from the conjunctiva, oral and choanal mucosa, and cloaca.⁴

Treatment

Treatment is focused on providing optimal husbandry and minimizing stress. Isolate affected individuals and remove fecal matter promptly to minimize the presence of potentially infectious material. Provide appropriate live plants (shelter and browse); natural substrates; surface duff; refuges; and an array of species-specific options for thermoregulation including diurnal temperature fluctuation and presence or absence of a basking lamp. Many species benefit from providing background temperatures in the upper one-third of the species-specific range during treatment, in addition to the usual basking site. Provide moisture as appropriate for the species, which may include regular misting, diurnal humidity fluctuation, morning dew, fog, and a humid refuge. Provide species-appropriate light spectrum, intensity, and diurnal cycles. Handle the animal infrequently, preferably only during administration of therapeutic agents. Nutritional therapy may not be indicated in many cases, because placing feed in the gastrointestinal tract could worsen disease in the presence of ileus or diffuse pancreatic, hepatic, or intestinal necrosis. It may be better to simply provide favorite feed items fresh daily and allow the tortoise to eat voluntarily when appetite returns. Consider fluid therapy only if not drinking or if more than 5% dehydrated. Administer broad-spectrum anthelmintic, antiprotozoal, and antibiotic therapy if animals exhibit signs of systemic illness including abnormal diurnal behavior patterns, lethargy, or anorexia. Toltrazuril (Baycox 5% oral suspension; Bayer Vital GmbH, Deutschland) 15 mg/kg by mouth (gavage) every 48 hours for 30 days might eliminate

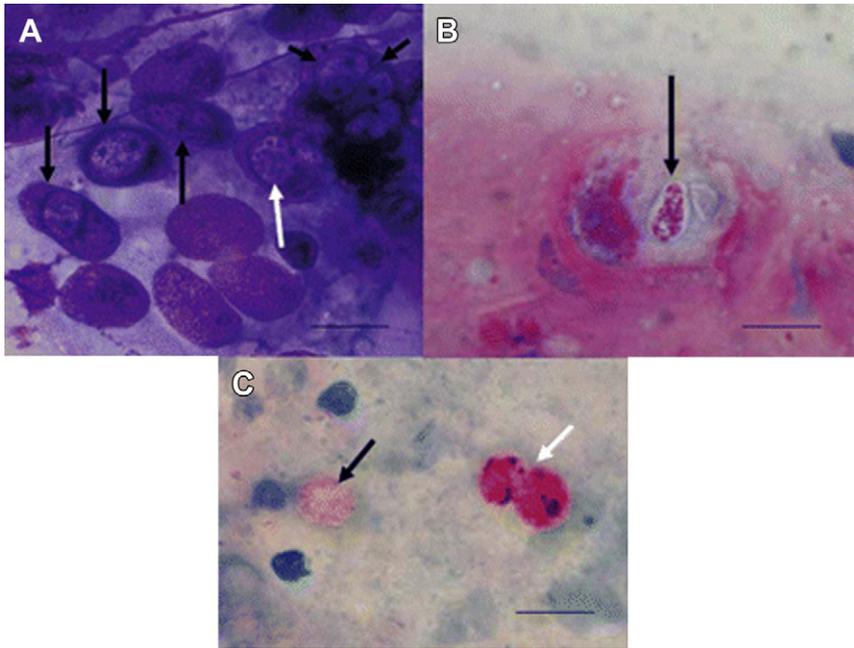


Fig. 1. Nasal mucosal smear, Sulawesi tortoise, Case no. 4. (A) Cluster of respiratory epithelial cells, many of which contain single intranuclear gametes (*long black arrows*), one that contains multiple gametes (*short black arrows*). A meront (*white arrow*) is noted in the nucleus of one cell. Modified Wright-Giemsa. (B) Intranuclear gamete (*arrow*) stains positive for the periodic acid-Schiff reaction. (C) Single intranuclear acid-fast gamete (*black arrow*) and two extracellular unsporulated acid-fast oocysts (*white arrow*). Fite acid-fast. Bar = 8 μ m. (From Innis CJ, Garner MM, Johnson AJ, et al. Antemortem diagnosis and characterization of nasal intranuclear coccidiosis in Sulawesi tortoises (*Indotestudo forsteni*). J Vet Diagn Invest 2007;19(6):660-7; with permission.)

infection, and shorter durations seem to reduce clinical signs without clearing the organism (Paul M. Gibbons, DVM, personal communication, 2013). Ponazuril, the active metabolite of toltrazuril, has also been used and may be equally effective at a similar dosage for similar duration.⁵ Chronic TINC-associated conjunctivitis has been observed in some cases (Paul M. Gibbons, DVM, personal communication, 2013). Abbreviated treatments (2 weeks) with toltrazuril have been followed by chronic infection (particularly brain) and death many months later (Paul M. Gibbons, DVM, personal communication, 2013). After the organism was apparently eliminated by treatment (absent on histopathology), one of the authors (PMG) has observed several cases with organ damage caused by TINC (present on histopathology) that was sufficient to cause chronic maldigestion, weight loss, subcutaneous edema, urine retention (urinary bladder distended with urine and urates), and death many months later. Taken together, this case information suggests that delayed or abbreviated treatment is less likely to be effective than early prolonged treatment.

Gross Pathology and Histopathologic Changes

Death occurs in many cases. Gross pathologic abnormalities may include thick oral mucus, severely distended urinary bladder, firm gray kidneys, pericloacal erythema,

red lungs, epicardial petechiae, gastrointestinal mucosal pseudomembrane formation, erythematous and edematous intestinal serosa and submucosa, and voluminous pale yellow to red translucent coelomic fluid.⁵ Histopathologic changes in all body tissues generally include necrosis and lymphocytic or lymphoplasmacytic inflammation associated with various stages of coccidia in the nucleus and rarely in cytoplasm or extracellular spaces.² Lesions suggestive of coccidiosis (necrosis and inflammation) are sometimes detected in the heart and central nervous system without coccidia.² Coccidia are best observed in sections stained with hematoxylin and eosin (H&E), and the organism is not observed in sections stained by periodic acid–Schiff or Fite acid-fast techniques.² Cases that die after treatment often have fibrosis that is distributed in a pattern matching the distribution of lesions in cases with coccidia present. This suggests that treatment can eliminate the organism, but tissue sclerosis can have a long-term effect on organ function, which emphasizes the importance of early diagnosis and treatment (Paul M. Gibbons, DVM, personal communication, 2013).

CRYPTOSPORIDIOSIS

Biology and Epidemiology

Cryptosporidiosis has been recognized in snakes and lizards for many years.^{9–11} Cryptosporidia are now being reported with increasing frequency in chelonians including Indian star tortoise (*G elegans*); pancake tortoise (*Malacochersus tornieri*); Russian tortoise (*Agrionemys horsfieldii*); radiated tortoise (*Astrochelys* [*Geochelone*] *radiata*); gopher tortoise (*Gopherus polyphemus*); an *Indotestudo*-like tortoise (probably *I forsterii*); and several species of tortoise in the genus *Testudo*.^{12–18} Cases have been reported in Italy; Germany; Spain; Switzerland; the Czech Republic; Ghana; Australia; and the United States including North Carolina, Georgia, Missouri, Kentucky, Washington DC, Maryland, and Kansas. At least two distinct novel species have recently been identified in tortoises, one with intestinal tropism and a proposed name of “*C ducismarci*” (GenBank accession numbers EF519704 and EF547155), and another with gastric tropism that is yet unnamed, but is referred to as “*Cryptosporidium* sp. tortoise 750” (GenBank accession number AY120914).^{13,15,19}

Life cycles and methods of transmission for these novel *Cryptosporidium* sp have not been described, but direct, fecal-oral transmission is likely because oocysts can be identified in feces and in the gastrointestinal tract on histopathology, and this is the usual mode of transmission for cryptosporidia. Several surveys have been reported, and cryptosporidia were found in a proportion of the sample group in each report.^{13,15,17,18}

Clinical Signs

Clinical signs of gastrointestinal disease occur in some cases in which cryptosporidial organisms are found. Clinical signs, when they occur, can include chronic diarrhea, decreased appetite, pica, decreased growth rate, weight loss, lethargy, and passing undigested feed.^{12,15,20} Concurrent infection with additional pathogens has been reported, and concomitant infections may enhance disease progression.

Diagnosis

Several clinical diagnostic tests are available. Fecal flotation techniques may be useful to prepare samples for microscopy and Ziehl-Neelsen or Fite acid-fast staining, carbol-fuchsin staining, and immunoassay.^{15–18,20} Oocysts are round and range from 3.4- to 6.3- μ m diameter on fecal examination or cytologic examination of mucosal

smears.^{15,16} On histopathology, oocysts range from 1- to 5- μ m diameter.¹² The most specific, readily available fecal test consists of DNA extraction and consensus PCR with sequencing of the *Cryptosporidium* 18S rRNA gene to identify the species.^{13,17}

Treatment

Treatment is focused on providing optimal husbandry as described previously for TINC. A few anticoccidial drugs have been used, but none is proved to eliminate infection. Suggested chemotherapeutics include paromomycin, toltrazuril, ponazuril, and possibly halofuginone or spiramycin.^{9,20,21} Paromomycin, 100 mg/kg by mouth every 24 hours for 7 days, eliminated clinical signs and led to negative test results for 9 months in a group of Herman tortoises (*Testudo hermanni*).²⁰ Supportive care can include fluid therapy for dehydrated tortoises, and some authors suggest immunostimulants.²⁰ Nutritional supplementation is important for tortoises with chronic anorexia.

In general, cryptosporidia are resistant to most disinfectants and survive well in the environment for many months. Equipment should be discarded when no longer needed for infected individuals. Temperatures greater than 65°C (149°F) can be applied with steam or a flame thrower. Formalin (10%), glutaraldehyde (2.65%), and possibly 5% to 10% ammonia solution may be effective on clean, smooth, impermeable surfaces, but must be used with care to prevent toxicity to humans or animals in the vicinity.

Gross Pathology and Histopathologic Changes

The pathologic changes associated with cryptosporidiosis in tortoises have been described in few cases.¹² Few, if any, gross changes occur. The stomach-associated species (*Cryptosporidium* sp tortoise 750) is found in gastric mucosal cells and may be associated with mild lymphocytic and heterophilic inflammation of the lamina propria of the mucosa.¹² The species with a proposed name of *Cryptosporidium ducismarci* is associated with intestinal mucosal cells and may elicit a mixed inflammatory response in the lamina propria of the mucosa or in the submucosa.¹² Cryptosporidia appear on H&E-stained histopathologic preparations as 1- to 5- μ m diameter amphiphilic round organisms with an eccentrically located dense basophilic internal structure.¹²

IRIDOVIRUS

Biology and Epidemiology

Iridoviruses are rapidly gaining notoriety as a serious emerging pathogen causing morbidity and mortality in turtle and tortoise populations. Iridoviruses of the genus *Ranavirus*, well known for causing mass mortality events in fish and amphibians, have increasingly been determined to be the cause of disease and mass mortality events in turtle and tortoise populations worldwide.²² *Ranavirus* causes high mortality in exposed turtle populations.^{23,24}

Iridoviruses are large, double-stranded, enveloped DNA viruses with a diameter of 120 to 200 nm, and an icosahedral nucleocapsid.^{25–27} Replication initially occurs in the nucleus, and is followed by replication in the cytoplasm.²⁶ In vertebrates, eosinophilic and basophilic intracytoplasmic inclusions may be seen in H&E-stained tissue sections²⁶ and have also been visualized in circulating leukocytes²⁸ and circulating red blood cells.^{29,30} The family Iridoviridae consists of four genera: *Chloriridovirus* and *Iridovirus*, which both infect insects; *Lymphocystivirus*, which infects fish; and

Ranavirus, which has been shown to be capable of infecting fish, amphibians, and reptiles.²⁶

Before 2003 there were few cases of iridoviral infection noted in chelonians worldwide. In 1982, a Hermann tortoise (*T hermanni*) that died after 2 days of anorexia was found to have cytoplasmic inclusions in hepatocytes consistent with iridoviral infection.³¹ In 1996, a free-living gopher tortoise (*G polyphemus*) presented with clinical signs associated with upper respiratory disease and was subsequently euthanized because of poor response to therapy. It was shown to have iridoviral particles in epithelial cells of the trachea, lung, and necrotic cells of the tracheal lumen.³² In 1999, an *Iridovirus* was shown through experimental viral infection studies to be the cause of "red-neck disease" in soft-shelled turtles (*Trionyx sinensis*)³³ and two Hermann tortoises (*T hermanni*) that died of systemic disease.²⁷ In 2002, five eastern box turtles (*T carolina carolina*) died after showing acute signs of cutaneous abcessation, oral ulceration or abcessation, respiratory distress, anorexia, and lethargy, with an *Iridovirus* being isolated in two of the five turtles.²⁵

Since 2003, additional outbreaks and infections have been diagnosed in free-ranging and captive chelonians. In 2006, a free-ranging eastern box turtle was presented for suspected trauma and blindness, and subsequently became anorexic, lost weight, had increased clear ocular and nasal discharges, became progressively depressed, and died 6 days postpresentation. Iridoviral intracytoplasmic inclusions were discovered in circulating leukocytes.²⁸ In 2008, *Iridovirus* was described in a gopher tortoise, numerous eastern box turtles, a Florida box turtle (*Terrepene carolina bauri*), and a Burmese star tortoise (*Geochelone platynota*).²⁴ A free-ranging gopher tortoise was euthanized after presenting for palpebral swelling and ocular and nasal discharge, and was positive for *Ranavirus* by PCR and viral isolation.²⁴ A captive female Burmese star tortoise died 3 days after presenting with nasal discharge, conjunctivitis, severe subcutaneous edema of the neck, and yellow-white cutaneous plaques observed on the tongue, and was positive for *Ranavirus* by PCR, virus isolation, and transmission electron microscopy (TEM).²⁴ Numerous eastern box turtles died or were euthanized after presenting from various locations with palpebral edema, ocular discharge, fluid draining from the mouth, with or without caseous plaques in the oral cavity, and were *Ranavirus* positive by PCR, virus isolation, and TEM in the cases where those tests were performed.²⁴ A free-living Florida box turtle was euthanized after not responding to therapy on exhibiting palpebral edema, nasal and ocular discharge, and yellow-white caseous plaques in the oral cavity, and was positive for *Ranavirus* by PCR and viral isolation.²⁴

Clinical Signs

The most common clinical signs associated with *Ranavirus* infection include upper respiratory tract disease, including respiratory distress and nasal discharge, oral ulceration, cutaneous abcessation, subcutaneous edema, anorexia, and lethargy.^{23,29,31,32} Less common clinical signs include red skin lesions on the neck in moribund 4- to 6-g soft-shelled turtles,³³ and severe unilateral conjunctivitis and cellulitis of the head and neck.²⁵

Few clinical pathology reports exist from turtles and tortoises with confirmed iridoviral infection, but consistently elevated values from box turtles in one study included urea (three of five), aspartate aminotransferase (three of five), creatine kinase (four of five), lactate dehydrogenase (five of five), and anemia,²⁸ and toxic changes to the heterophils were noted in all turtles.²⁵ Bloodwork abnormalities are likely the result of moderate to severe dehydration and tissue damage associated with infection.

Histopathologic Changes

Histopathologic lesions have been described for many of the confirmed cases of *Ranavirus* infection. Lesions include fibrinoid vasculitis of the integument, mucous membranes, liver, and lungs²⁵; severe necrotizing conjunctivitis with cytoplasmic inclusions; severe acute necrotizing glossitis; esophagitis; tracheitis; multifocal acute necrotizing pneumonia with cytoplasmic inclusions; moderate multifocal random acute hepatocellular necrosis with cytoplasmic inclusions; moderate multifocal acute necrotizing enteritis and glomerulonephritis; mild focal acute necrotizing pancreatitis; mild multifocal acute necrotizing cystitis; and intramural gastric nematodes of undetermined species.²⁸ Multifocal necrosis and heterophilic infiltration is commonly found in the mucosa of the oral cavity, tongue, pharynx, esophagus, small and large intestine, and cloaca.²⁷ Additional findings can include multicentric fibrinoid vasculitis and formation of fibrin thrombi in small blood vessels in numerous tissues²³; focal necrotizing hepatitis, focal necrotizing enteritis, and confluent necrotizing splenitis³¹; and necrotizing and ulcerative stomatitis or esophagitis, fibrinous and necrotizing splenitis, and multicentric fibrinoid vasculitis.²⁴

Viral cytoplasmic inclusions consistent with *Ranavirus* infection appear most commonly in hepatocytes,^{27,31} trachea,²⁴ lung,²⁷ tongue,²⁴ esophagus,²⁴ spleen,²⁴ endothelial cells,²⁴ stomach,²⁴ and leukocytes.²⁸ There are cases of experimentally induced infection^{27,32} and case reports of naturally occurring infection^{24,25} where no viral inclusions were noted in tissues with light microscopy. Because inclusion bodies do seem to be an inconsistent finding, their lack of discovery in tissues should not be used to rule out possible *Ranavirus* infection.^{23,25} TEM examination of tissues revealing no viral inclusions with light microscopy did reveal icosahedral particles 150 to 190 nm in diameter consistent with *Iridovirus* virions in two cases.^{23,25} TEM evaluation of tissues in cases suggestive of *Ranavirus* infection based on clinical signs is recommended, because it does seem to be able to detect viral particles even in the absence of visible inclusions on histologic section.

Diagnosis

Diagnosis is initially based on suggestive clinical signs, and intracytoplasmic viral particles of appropriate diameter (120–200 nm) observed with TEM. Virus isolation has been used to successfully confirm *Ranavirus* infection.^{23–25,27,28} Virus was cultured from 4- to 6-g soft-shelled turtles and identified with TEM. Cultured virus was then used to experimentally challenge unexposed soft-shelled turtles.³³ An enzyme-linked immunosorbent assay has shown the ability to detect IgM and IgY anti-*Iridovirus* antibodies in gopher tortoises and eastern box turtles.²⁹ The enzyme-linked immunosorbent assay seems to be able to detect recent infections (IgM) and longer standing infections (IgY), but it is unknown how long anti-*Iridovirus* antibodies remain at detectable levels in chelonians or if all animals mount an immune response.²⁹ PCR analysis has been used successfully in numerous studies to detect *Iridovirus* infections.^{22–25,27,28} PCR was used to detect *Ranavirus* infection from oral, cloacal, and urine samples in ornate box turtles (*Terrepepe ornata ornata*) and red-eared sliders (*Trachemys scripta elegans*) with success.²³ Recently, PCR analysis was performed on whole blood samples and oral swabs in free ranging eastern box turtles, with both samples showing the ability to detect the presence of virus.²² Oral and cloacal swabs and whole blood may be reasonable samples to submit for PCR until further information is obtained.

The mechanism of transmission of iridoviruses is still unknown. Because iridoviral particles were observed in circulating leukocytes it has been postulated that

transmission by blood-feeding parasites or biting insects may be possible.^{23,28} Other thoughts include cannibalism of infected animals²⁴; common environmental sources of virus (eg, bodies of water)²³; fomites²⁸; and amphibian and fish reservoirs.²⁸ In one study ornate box turtles and red-eared sliders were inoculated with Burmese star tortoise *Ranavirus* orally and intramuscularly. All intramuscularly inoculated turtles in this study showed clinical signs, and three died as a result of infection. No orally inoculated turtles showed signs of infection or died. Based on these findings the authors hypothesized that turtles may not become exposed to *Ranavirus* through ingestion of infected animals or water sources, and that abrasions naturally acquired from ingesting bones or other material may be necessary for the virus to be introduced systemically. It is also possible that the amount of virus provided orally needs to be in greater quantity, or repeated exposure may be necessary.²³ One of the intramuscularly inoculated turtles did seem to recover from disease, and this makes the possibility of asymptomatic carriers in turtle populations as vectors.²³ Reports of disease outbreak in turtles have noted the presence of frogs in or near the enclosures,^{24,25} but at this time it is not clear whether they are the source of the virus, or just also susceptible to disease and coincidentally noted.

Treatment

Most case reports show limited success with treatment of turtles and tortoises with *Ranavirus* infection. General treatment with systemic antibiotics (ceftazidime, enrofloxacin, clindamycin),^{24,25,28,32} warm water soaks,²⁴ intracoelomic fluid therapy,²⁴ parenteral fluid therapy,²⁵ nutritional support,^{25,32} analgesics,^{24,25} topical antibiotics (triple antibiotic ointment),²⁵ antiviral therapy (acyclovir, interferon),^{24,25} and vitamin A and D supplementation²⁸ has been attempted. The only report showing any treatment success involved Burmese star tortoises where antiviral therapy and intracoelomic fluids were administered.²⁴ Based on cases in the literature it seems that antiviral therapy and fluid therapy are indicated, and it is recommended to provide nutritional and fluid support. Systemic antibiotics, analgesics, topical antibiotics, and vitamin supplementation did not seem to alter the course of the disease, but because there are still a small number of proved cases in the literature these treatments should be used on a case by case basis.

Anticipated Future Trends

Numerous cases of *Ranavirus* infection are being reported, and chelonian species not previously documented are being shown susceptible to infection.²⁴ *Ranavirus* seems to be a fatal viral disease capable of causing high mortality in wild chelonian populations. Infection has been observed in wild populations in Florida, Tennessee, Georgia, New York, Pennsylvania, and Texas. Because more cases of *Ranavirus* infection in wild populations are being encountered, it is important to pay close attention to how affected turtles are handled, and eventually released. Current studies show the prevalence of *Ranavirus* infection in the wild is low, but because the disease has high mortality, many that have succumbed to disease in the wild likely were probably not found.²² The methods of transmission are not yet clear, and questions remain about whether the disease is arthropod-borne, acquired by ingestion of infected materials, or acquired by direct contact. Because diagnostic evaluation performed on collected turtles could miss infection in asymptomatic carriers of disease, it is especially important for rehabilitators to release turtles as close to the site of capture as possible even if *Iridovirus* is not detected. Because the possibility remains that chelonians may be capable of serving as asymptomatic carriers,²³ *Iridovirus*-positive animals should not be released into the wild until more is learned about carrier status

and disease transmission. Infection in chelonians, and more specifically box turtles, in the United States seems to be a significant threat to populations, particularly those undergoing environmental stress, such as that expected with climate change, and this disease needs to be monitored closely in wild populations moving forward.

ADENOVIRUS

Biology and Epidemiology

Adenoviruses have received increasing attention in tortoises since 2009. Before 2009, the only reported case of adenovirus in a chelonian was from a leopard tortoise (*Geochelone pardalis*) with biliverdinuria, wasting, and episodes of hemorrhage.³⁴ Since 2009, there have been reports of adenovirus infection in an ornate box turtle (*T ornata ornata*),³⁵ Sulawesi tortoises (*I forstenii*),³⁶ impressed tortoises (*M impressa*),³⁷ and a Burmese star tortoise (*G platynota*).³⁷

Adenoviruses are double-stranded, linear, nonenveloped DNA viruses with a diameter of 80 to 110 nm, a 26- to 45-kbp genome and an icosahedral nucleocapsid. Replication of the virus is within the nucleus of host cells, and intranuclear inclusions can be visualized during stages of reproduction in H&E-stained tissue sections. Viral inclusions are typically basophilic with H&E stain, but eosinophilic inclusions have also been reported.^{26,35} Adenoviruses have been described in all tetrapod classes and a sturgeon, and are classified into five genera: (1) *Siadenovirus*, (2) *Mastadenovirus*, (3) *Aviadenovirus*, (4) *Atadenovirus*, and (5) *Icthadenovirus*.³⁷ There are numerous reports of adenovirus-related disease in reptiles^{38–48} but it currently seems to be an emerging pathogen in chelonians.

Adenoviruses are often host specific and transmitted by the fecal-oral route or direct contact by oronasal secretions.⁴³ Adenovirus-related disease most often occurs in young and immunocompromised animals.⁴³ The wild-caught Sulawesi tortoises had recently been confiscated, the result of illegal importation. Tortoises intended for the pet trade are often exposed to other animals and pathogens not in their normal environment, and being exposed to such conditions as overcrowding, poor sanitation, poor nutrition, and other physiologic stresses.³⁷ The ornate box turtle was an adult; age and exposure status to conspecifics was not reported.³⁵ One impressed tortoise was a wild-caught adult that had been healthy for the 2.5 previous years in the collection. The other impressed tortoise was a previously healthy, 5-year-old captive hatched tortoise. The Burmese star tortoise was a previously healthy, 4-year-old captive bred tortoise.³⁷

It does seem that Sulawesi tortoise adenovirus-1 can be horizontally transmitted between tortoise species because the virus was found in three previously healthy tortoises with known exposure to infected tortoises.³⁷ Vertical transmission has not been described in tortoises. It is suspected in the case of the impressed tortoises and Burmese star tortoise that the virus was spread by fomites, aerosols, or animal caretakers.³⁷ Adenoviruses are very environmentally persistent, and disinfection of adenovirus contamination is challenging.³⁷ Because this does seem to be an emerging and important pathogen of tortoises it should be considered when introducing newly acquired tortoises to collections. Spatial separation of quarantined tortoises from established collections, ideally in separate buildings with separate caretakers, should be considered especially critical in captive assurance colonies of endangered species.³⁷

Concurrent disease may also make tortoises more susceptible to severe systemic disease the result of adenoviral infection.³⁶ Coinfections with intranuclear coccidiosis,³⁷ amoeba,³⁶ nematodes,³⁶ *Escherichia coli*,³⁶ *Aeromonas hydrophila*,³⁶

Chlamydophila sp.,³⁶ and a *Mycoplasma* sp were observed.³⁵ Some of the diseases noted as coinfections can cause morbidity and mortality in tortoises alone, and the relationship between adenoviral infection and concurrent infections remains to be better described.

The first brief report of adenoviral-related disease involved a leopard tortoise in 2004.³⁴ In 2009, a case of systemic *Siadenovirus* infection in a group of 105 illegally imported Sulawesi tortoises was reported. Thirty of the tortoises died before veterinary examination, and 62 of the 75 remaining tortoises died despite aggressive therapy at various institutions.³⁶ In 2009, an ornate box turtle was reported to have died the result of a mixed adenoviral and mycoplasmal infection.³⁵ In 2012, two impressed tortoises and one Burmese star tortoise were determined to have died with Sulawesi tortoise adenovirus-1 infections after being in the same facility as confiscated Sulawesi tortoises.³⁷

Clinical Signs

The most common lesions in reptiles presenting with adenoviral infections include hepatitis, enteritis, esophagitis, splenitis, and encephalopathy.⁴⁸ The ornate box turtle had no premonitory signs of disease described and was found deceased³⁵; the Sulawesi tortoises presented with anorexia, lethargy, mucosal ulcerations and palatine erosions of the oral cavity, nasal and ocular discharge, and diarrhea³⁶; the impressed tortoises both died without premonitory signs³⁷; and the Burmese star tortoise died after a 2-week period of lethargy and anorexia.³⁷

Diagnosis

Clinical pathology was only reported for the Sulawesi tortoises (N = 19), with the most common complete blood count results being anemia (33% of affected animals), leukopenia (21%), leukocytosis (21%), heteropenia (21%), heterophilia (31%), lymphopenia (37%), lymphocytosis (10%), eosinophilia (5%), and azurophilic monocytosis (11%), and were consistent with abnormalities expected with a chronic inflammatory response.³⁶ Plasma biochemical abnormalities were elevated aspartate aminotransferase activity (28%), elevated creatine phosphokinase activity (5%), hypoglycemia (21%), hyperglycemia (58%), elevated blood urea nitrogen (92%), hyperkalemia (22%), and elevated uric acid concentration (21%), and were suspected to be secondary to muscle and possibly liver damage, and moderate to severe dehydration.³⁶

PCR was effective at detecting adenoviral nucleic acid in most turtles and tortoises from which samples were submitted. Adenovirus PCR amplicons were detected from 97.6% of Sulawesi tortoise samples submitted during a multi-institutional disease outbreak (N = 42)³⁶; all samples submitted from two impressed tortoises and one Burmese star tortoise that had been exposed to adenovirus-infected Sulawesi tortoises³⁷; and from the adult ornate box turtle in Hungary.³⁵ All nasal flush, nasal mucosa, choanal and choanal-cloacal swab, colon, liver, lung, kidney, and spleen samples were PCR positive, and 19 of 20 plasma samples were PCR positive.³⁶ Sample collection for PCR is generally noninvasive and practical in conscious animals, and thus seems to be useful for antemortem diagnosis of active adenoviral infection. Until more is known about adenovirus infection in chelonians, cloacal and plasma samples should be submitted for testing by PCR.³⁶ In situ hybridization using a riboprobe specific for Sulawesi tortoise adenovirus-1 did detect hybridization signals using a fluorescein-labeled riboprobe (RNA probes).³⁷ As more sequence data become available, development of additional probes may improve the ability to develop sensitive and specific diagnostic in situ hybridization techniques that can be used to

complement PCR, TEM, and histopathologic findings when evaluating for the presence of adenovirus.³⁷

Treatment

Sulawesi tortoise adenovirus-1 caused severe systemic disease with a mortality rate of 87.6% (92 of 105 animals).³⁵ Medical therapy varied based on the institution where the Sulawesi tortoises were treated, but all tortoises were treated with systemic antibiotics, antiparasitics, fluid therapy, and nutritional support.³⁶ Most of the other cases were found deceased so no treatment was attempted, although the Burmese star tortoise was treated for 2 weeks before dying, without success. Based on previous cases, owners should be warned of the poor prognosis of adenoviral disease in chelonians. As of now supportive therapy with fluids, nutritional support, antibiotics for secondary infections, thermal support, and antiparasitics (because parasites were commonly noted in the previous tortoises) can be attempted.³⁶

Histopathologic Changes

Histopathologic lesions were described for most of the turtles and tortoises that died as the result of adenoviral infection. Most commonly affected organ systems were liver,^{35–37} intestinal tract,^{35–37} bone marrow,^{36,37} and spleen.^{36,37} Less common findings were interstitial pneumonia, myocarditis, renal tubular necrosis, focal ulcerative stomatitis, facial dermatitis, and nonsuppurative meningoencephalitis.³⁷

Viral intranuclear inclusions consistent with adenovirus infection in tortoises seem to be most commonly found in the liver,^{35–37} but also occurred in bone marrow,³⁶ reticuloendothelial cells of the spleen,^{36,37} biliary epithelium,³⁷ pancreas,³⁶ testis,³⁶ ovary,³⁶ respiratory epithelium,³⁶ renal epithelium,³⁶ vascular and cardiac epithelium,³⁶ cerebral glia and choroid plexus,³⁶ and colon.³⁷ The intranuclear inclusions were evaluated by TEM, which confirmed the presence of nonenveloped, 70- to 90-nm diameter, viral particles in nuclei with marginated chromatin and abnormal nuclear sizes and shapes, which are consistent with an adenovirus.^{36,37}

Anticipated Future Trends

The mortality of the initial outbreak in Sulawesi tortoises (87.6%), along with the pathology seen in the cases of adenoviral infections, indicates that this virus has the potential to affect tortoise populations.^{35–37} Wild-imported tortoises were the first reported to show evidence of adenoviral infection.³⁶ It is not known if these animals had adenoviral disease before collection for the pet trade, or were exposed during the importation process. The origin of the disease is not known, but it may be present in wild animals. Adenoviral-related disease in tortoises is still not completely understood. When considering release of animals back into a wild population a decision tree analysis has been previously developed.⁴⁹ Until more is known about this disease, adenovirus-positive tortoises should not be considered for release into wild populations, and should be kept separate from other chelonians.

SUMMARY

Numerous pathogens have been described in chelonians and of these TINC, cryptosporidiosis, *Ranavirus*, and adenovirus are emerging as important diseases. Much remains to be learned about the biology and epidemiology of these organisms, and continuing research is needed to fully characterize them. In general, disease caused by these organisms is associated with stressors that can include changing environmental conditions, inappropriate care in captivity, shipping, and translocation.

Chelonians that exhibit signs of systemic disease must be isolated to reduce the risk of transmission to additional individuals. Diagnostic tests are available, and together with quarantine are essential to prevent movement of pathogens into new populations. Collections must be managed with strict biosecurity, and conservation introductions must include thorough diagnostic testing, health evaluation, and quarantine before movement to the release site. Fortunately, these tools can be effective and sufficient to prevent outbreaks if applied in the context of appropriate environmental conditions.

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