Inclusion Body Disease in a Columbian Red-tailed Boa Constrictor (*Boa imperator*)

Introduction

Inclusion body disease (IBD) was first discovered in the 1970s in boa constrictors in the United States and is sometimes referred to as "Boid inclusion body disease" (BIBD).[1,3] Since then it has become a commonly found disease in captive boas and pythons worldwide. Recently, there have been reports of IBD in wild boa constrictors in Costa Rica and Brazil, and rare incidences of this disease reported in colubrids and viperids.[1,2] This is an immunosuppressive disease which was named for the pathognomonic eosinophilic or amphophilic, electron-dense intracytoplasmic inclusion bodies that can be found in almost all cell types throughout the body of infected animals.[1,2,3] The etiology was unknown until the early 2010s when reptarenaviruses were shown to be the causative agent for IBD.[1,3] These enveloped single-stranded RNA viruses are in the arenavirus family, with multiple strains having been identified including the California reptarenavirus, Gissen reptarenavirus, Golden reparenavirus, Ordinary reptarenavirus, and Rotterdam reptarenavirus.

Clinical signs of IBD often start nonspecific; such as inappetence and/or weight loss. As the disease progresses, there can be a variety of multisystemic changes, including respiratory, hepatic, gastrointestinal, and dermatological, but it almost always ends with severe progressive neurological abnormalities including incoordination, opisthotonus ("star-gazing"), torticollis, lack of righting reflex, flaccid paralysis, disorientation, and head tremors.[2,3] Chronic regurgitation frequently occurs in boas, but less often in pythons. Due to immunosuppression from the virus, secondary illnesses including pneumonia, stomatitis, osteomyelitis, and lymphoproliferative diseases, such as leukemia and round cell tumors, are also common.[3] An

additional concern is that some snakes can be asymptomatic carriers and can shed viral RNA in urates, feces, and during ecdysis.[3] Differential diagnoses for snakes with neurologic symptoms include: head trauma, toxins, bacterial sepsis, parasitic migration, metabolic disorders like hypocalcemia or hypoglycemia, hyperthermia/hypothermia, or IBD and other viral diseases such as ferlavirus, sunshine virus, or reovirus.

Although no changes on complete blood count or serum chemistry are pathognomonic for IBD, intracytoplasmic inclusions may be seen on blood smears. Leukocytosis, lymphocytosis, low total protein and globulins, and elevated AST have been reported in acute cases. Definitive diagnosis can be made by reverse transcription polymerase chain reaction (RT-PCR), immunohistochemistry (IHC), cytology of blood smears, histopathology, electron microscopy, or virus isolation. RT-PCR to detect reptarenavirus RNA can be performed on oral or cloacal swabs, whole blood, and/or liver or esophageal tonsil biopsies. The specificity for this test is low and false negatives can occur if the snake is not actively shedding the virus. If IBD is suspected, but RT-PCR is negative, it is recommended to confirm with a secondary testing method such as histopathology or virus isolation. IHC can be performed on liver, esophageal tonsils, pancreas, kidney, and/or brain tissue. On cytology and histopathologic examination, intracytoplasmic inclusions can be seen in neurons and in epithelial cells of the organs. For unclear reasons, intracytoplasmic inclusions are more commonly found in tissues throughout the body in boas, but are often limited to just the central nervous system tissues in pythons. Protein electrophoresis has also been investigated as a diagnostic tool, but has yet to be correlated with the progressive clinical signs of IBD.

Treatment/management/prognosis

Currently there is no effective treatment for IBD. When there is more than one snake in a household or in large collections, euthanasia is often the best choice when a snake tests positive, (even if they are asymptomatic), to attempt to decrease transmission to others. If any snakes in a collection test positive, the whole collection should be tested to screen for asymptomatic carriers. In single-snake households, supportive care and antibiotics for secondary infections can be provided until symptoms worsen. These snakes can maintain an acceptable quality of life for an indeterminate amount of time, but most of these patients are euthanized once they become clinically affected due to severe neurologic symptoms.

Reparenavirus is considered to be highly infectious, and can be transmitted through snake mites (*Ophionissus natricis*), aerosol, and vertical transmission.[3] Since has been discovered in wild boas, there is also concern for possible cross-species transmission from prey animals.[1] Because of the virus' highly infective nature, the best preventative measures for IBD include good biosecurity and quarantine strategies, proper cleaning, and strict testing protocols in large collections as subclinical cases are common. Any new snakes brought into a collection should always be quarantined for at least 6 months (at minimum in a separate room, ideally in a separate building). All boas or pythons should be tested at the beginning and end of the quarantine period. Sodium hypochlorite (bleach) has been shown to be the best disinfectant for reptarenavirus.

The prognosis for IBD is grave. It is considered to be fatal once a snake is clinical as they typically succumb to the disease within a period of weeks to months.[1,3] Pythons seem to deteriorate faster than boas. Future research is being concentrated on developing a vaccine for this fatal disease.[2]

Case history and presentation

A captive-bred 10-year-old female Columbian red tailed boa (*Boa imperator*) was presented for a two week history of a suspected respiratory infection and a four month history of inappetence. The owner reported the snake would sometimes go on "hunger strikes", but they scheduled the appointment when it started wheezing and blowing bubbles. The patient was kept in a large aquarium with a ceramic heat emitter, but temperatures and humidity were not routinely measured. A large frozen/thawed rat was offered once a month. The owners had adopted her when she was a hatchling, and since then she had not displayed any medical problems other than the occasional periods of inappetence. There were no other reptiles in the household.

On physical examination, the patient was quiet, alert, and responsive. She was underweight, weighed 3.8 kg, and was not neurologically appropriate; she appeared disoriented, was occasionally "star-gazing", and had a delayed righting reflex. There was excess mucus in the oral cavity, but the remainder of the examination was unremarkable.

Case management and outcome

Blood was drawn from the ventral coccygeal vein. An in-house complete blood count (Table 1) and serum chemistry (Table 2) on an Abaxis VetScan2 [a] were run, but were unremarkable overall. Due to the respiratory symptoms, a tracheal wash was performed using an 8 Fr/Ch red rubber catheter [b] and sterile saline [c]. The sample was submitted for culture and susceptibility. Based on the species, history, and clinical signs, inclusion body disease (IBD) was a concern in this patient so samples (oral swab, cloacal swab, and whole blood) were collected

for a reptarenavirus PCR. Radiographs were recommended to further assess the lungs and look for any other concurrent disease, but were declined by the owner.

The patient was hospitalized in an incubator [d] at 88° F, isolated from other reptiles. She was started on ceftazidime [e] (30 mg/kg IM q72h) for the respiratory infection and was tube-fed (30 ml/kg Critical Care [f]) for the inappetence. Since no regurgitation was noted after the tube-feeding, the patient was sent home the following morning. The ceftazidime was continued pending the culture and PCR results. The isolation incubator was cleaned with sodium hypochlorite following the patient's hospitalization.

The culture results (Table 3) came back with a light mixed growth of *Staph. intermedius* and *E. coli*. Both of these are not typical bacteria cultured from the respiratory tract in snakes, and were more likely to be contaminants, but as the patient still had clinical signs of a respiratory infection and both cultured strains of bacteria were susceptible to ceftazidime the course of this medication was continued. The patient returned one week after the initial presentation for another tube-feeding. She had remained stable, but without any clinical improvements.

The following week the reparenavirus PCR results (Table 4) came back positive. The grave prognosis was discussed with the owner, but they elected to continue supportive care of antibiotics and weekly tube-feedings since they felt that the patient was maintaining a decent quality of life and there were no other reptiles in the household.

Each successive week when the patient returned for tube-feedings, her neurologic signs had worsened. Her respiratory infection also progressed into suspected pneumonia, likely due to immunosuppression from the IBD. The owner still declined any imaging. Four weeks later, she had completely lost her righting reflex, and was occasionally open-mouth breathing. Due to concerns for quality of life, humane euthanasia was elected. The snake was premedicated with

dexmedetomidine [g] (0.1 mg/kg IM), ketamine [h] (20 mg/kg IM), and midazolam [i] (1 mg/kg IM) and was euthanized with pentobarbital solution [j] (220 mg/kg IC). Necropsy with histopathology was declined by the owner.

Endnotes

a. VetScan2 a product of Abaxis; Zoetis, Parsipanny, New Jersey 07054

b. 8 Fr/Ch Kendall Feeding Tube and Urethral Catheter; Convidien llc, Mansfield Massachusetts 02048

c. UtiGene Terminally Sterilized Normal Saline Solution; VEDCO, INC., St. Joseph, Missouri, 04507

d. Brinsea TLC Series II ECO; Brinsea Products Inc., Titusville, FL 32796

e. Ceftazidime; SAGENT Pharmaceuticals, Schaumburg, Illinois 60195

f. Critical Care - Carnivore; Oxbow Animal Health, Omaha, Nebraska 68138

g. Dexdomitor (dexmedetomidine hydrochloride); Orion Corporation, Espoo, Finland

h. KetaVed (ketamine hydrochloride injection); Vedco Inc., St. Joseph, Missouri 64507

i. Midazolam injection, USP; Almaject, Inc., Morristown, New Jersey 07960

j. Euthasol (euthanasia solution); Virbac AH Inc., Fort Worth, Texas 76161

References

1. Alfaro-Alarcon A, Hetzel U, Smura T, et al. Boid inclusion body disease is also a disease of wild boa constrictors. *Microbiol Spectr.* 2022; 10(5):1-17
2. Dietz J, Kolesnik E, Heckers KO, et al. Detection of an arenavirus in a group of captive Wagler's pit vipers (*Tropidolaemus wagleri*). *J Zoo Wildl Med.* 2020; 51(1):236-240
3. Hetzel U, Sironen T, Laurinmaki P, et al. Isolation, identification, and characterization of novel arenaviruses, the etiological agents of boid inclusion body disease. *J Virol*. 2013; 87(20):10918-10935

Lab data/imaging

Test	Result	Reference Range
WBC (10^3/uL)	10.8	7.37 (1.47-19.6)
Heterophils (10^3/uL)	7.98	1.93 (0.20-6.50)
Lymphocytes (10^3/uL)	2.7	2.89 (0.34-11.9)
Monocytes (10^3/uL)	0.06	0.27 (0.03-2.38)
Azurophils (10^3/uL)	0	0.84 (0-4.74)
Eosinophils (10^3/uL)	0	0.13 (0-0.60)
Basophils (10^3/uL)	0.06	0.21 (0.03-1.01)
PCV (%)	21	29 (12-40)

Table 1: In-house complete blood count results. Reference ranges from Sladky KK, Klaphake E, DiGirolamo, et al. Reptiles. In: Carpenter JW, Harms CA, eds. Carpenter's exotic animal formulary. 6th ed. St. Louis (MO): Elsevier; 101-221.

Test	Result	Reference Range
Albumin (g/dL)	2.8	2.9 (1.6-4.3)
AST (U/L)	23	15 (2-64)
Calcium (mg/dL)	> 16.0	15.3 (10-20)
Glucose (mg/dL)	26	34 (7-74)
Phosphorus (mg/dL)	5.8	4.3 (2.4-8.6)
Potassium (mmol/L)	**> 8.5**	**4.7 (3.1-7.3)**
Protein, total (g/dL)	6.0	7.0 (4.0-10.3)
Sodium (mmol/L)	159	159 (143-173)
Uric acid (mg/dL)	5.0	4.0 (0.3-15.0)

Globulin (g/dL)	3.2	3.9 (2.0-6.8)
BA (umol/L)	< 35	N/A
Creatine kinase (U/L)	625	489 (57-2099)

Table 2: Serum chemistry results from Abaxis Vetscan. Reference ranges from Sladky KK, Klaphake E, DiGirolamo, et al. Reptiles. In: Carpenter JW, Harms CA, eds. Carpenter's exotic animal formulary. 6th ed. St. Louis (MO): Elsevier; 101-221.

Antibiotic	***Staph. intermedius***	***E. coli***
Ceftazidime	S	S
Flagyl	-	-
Penicillin	R	R
Amikacin	S	S
Ampicillin	R	R
Cephalothin	R	R
Azithromycin	S	R
Clindamycin	S	R
Chloramphenicol	S	S
Doxytetra/Tetracycline	S	R
Sulfa/Trimethoprim	S	R
Sulfisoxazole	-	-
Polymyxin B	R	S
Nitrofurantoin	-	-
Gentamicin	S	S
Neomycin	-	-
Clavamox	S	R

Baytril	S	R
Oxacillin	R	R
Cefpodoxime	R	S
Orbifloxacin	S	R
Marbofloxacin	S	R
Ciprofloxacin	S	R
Cefovecin	R	S
Florfenicol	S	S
Zosyn	S	S
Timentin	S	R

Table 3: Culture and susceptibility results from Microbiological Laboratories Inc. KEY: R = Resistant, S = Sensitive, M = Moderate, (-) = No Test

Test	**Result**
Reptarenavirus PCR	**Positive** - PCR produced an amplicon (band) of the correct size consistent with the target agent. Direct sequencing confirmed the presence of *Giessen reptarenavirus* in the submitted sample

Table 4: Reptaranavirus PCR results from the University of Florida Zoological Medicine and Wildlife Disease Laboratory.