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Inclusion Body Disease: pathogenesis, routes of transmission, husbandry procedures and diagnostic flow

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Summary

IBD inclusion body disease, also more specifically defined as boid inclusion body disease (BIBD), is a transmissible and progressive viral disease; caused by *Reptarenavirus* infection may remain dormant (without overt signs) over several years; but leading then towards severe losses in snakes' collections (population) such as Boid and Pythonidae.

Reservoir host of the virus is still unknown, but connections with arthropods and pathologic cases in wild have been found.

For the first time recognized in the U.S. in 1970, IBD has effects on several species of snakes, most commonly in Burmese pythons, 1970-till mid 80 in private and zoological collections.

Other diagnoses occurred in 1998, in several species:

-Morelia spilota variegata (Australia)

-Boa constrictor (Canary Islands); together with Green anaconda, Yellow anaconda, Rainbow boa, Haitian boa, Indian python, Reticulated, and Ball python.

In early 1990s to unknown causes of epidemiology shift, more boa constrictors than pythons have been affected by IBD. Later on, the causative agent was confirmed to be *Reptarenvirus* infection, often coinfection with other viruses were found in large collections. Novel hypotheses indicate boid snakes as the natural reservoir of *Reptarenaviruses*, ordinarily found in captive snakes. Snakes affected by this pathology present several clinical signs, often neurological, and a further diagnosis, based on microscopic examination will show the existence of eosinophilic intracytoplasmic inclusion (defined as inclusion body), composed by an uncommon protein.

To delineate the geographical position, Boas and pythons are non-venomous constrictor snakes populating tropics habitats. Boas are located in Central and South America and Madagascar, i.e., in the New World, whereas pythons occupy habitats in Africa, Asia, and Australia, i.e., in the Old World. Thus, the natural habitats of boas and pythons do not overlap much. [1] Several among the more than hundreds of boas and pythons known species, are susceptible to IBD. Three species of boas: Ringed or Annulated Tree boa, Common and Rainbow shown to be susceptible to BIBD; they are differently distributed but partly share habitats.

Introduction

Always depicted as the villain in every story and myth, snakes have often divided public opinion in half, between fear and admiration. Essential components of the ecosystem, despite their double nature of prey and predator. Nowadays, they are playing a role in a growing market as pets. Chiefly those belonging to Boide family, which are collectively listed in Cites' Appendix II with few of them listed in Appendix I, are now among the 1.366.000 pet reptiles kept in Italy. Often result of unregulated international trade and frequently sold online; this species is subjected to worldwide transportation in inadequate conditions. All the previous, supported by poor husbandry, lack of knowledge or interest of owners and sellers, ease the pathogens and infections transmission, which may lead to the uncontrolled spreading of diseases such as Inclusion Body Disease (IBD).

The growing interest in this disease also drove the pathology group of the BCA department to deepen the diagnostic aspects of post-mortem examination performed on captive snakes. In this scenario as part of this work, the present study aim is to review the literature on several aspects such as the etiological agent, the clinical development of the disease, the clinical signs, and the pathology findings; and recreate the pathogenesis comparing the test results.

Etiology

Albeit IBD-positive snake's clinical signs were first described in early 1990's publications, the correct etiology has continued to be unknown for approximately 20 years. Previous studies [2] conducted on inclusion body disease, has revealed a wrong connection with Retroviruses making them potential candidate as etiological agents; since they were isolated from several boas IBD affected. In a study performed in 2010, [2] they isolated and sequenced Retrovirus from a Brumese which was positive for IBD, without having its clinical history; the IBD-positive snake revealed intracytoplasmic inclusions; therefore, it was concluded that there was a link between this virus and IBD and the connection wasn't casual. [3] Until recently a causal relationship with novel divergent arenaviruses could have been demonstrated.

Ample old and novel research, [2][4][5] confirming the causative agents focused on the origin of IBDP (inclusion body disease protein). However, to understand the actual nature of that protein it needs to be partially or entirely sequenced; this process may result useful to improve immunodiagnostic tests, introducing peptides effective as antigens. A unique 68KDa protein (IBDP) was identified in an electrophoretogram of IBD-infected tissues. Some inclusion bodies' configurations appeared to resemble a viral particle, although further findings indicate that inclusions mainly consisted of IBDP. The first hypothesis on protein origin, and consequently on IBD genesis, was that IBD might present a protein-storage disease (LSDs) induced by viral infection. Caused by an inborn error of metabolism, the resulting dysfunction led to accumulation of protein that should be broken down by lysosomes. The lack of those enzymes, lead to an intracellular accumulation of endogenous substance; perceived as inclusion bodies. Inflammation and oxidative stress induced by poor husbandry or other pathologies can be crucial players in LSDs.

The identification of *Reptarenaviruses* as etiological agents in past studies has been confirmed through virological examination; rejecting the first wrong connection with Retroviruses. In 2012, thanks to NGS (next-generation sequencing); the main cause of IBD was found, and a new virus genus in the *Arenaviridae* family was discovered: *Reptarenavirus*; The strict linkage was proved firstly by the virus behavior in cell culture, developing inclusion similar to the ones noticed within snakes; but also, because the immunoglobulins, produced to resist against, in cell culture, bind directly towards inclusions within the affected animals' cells.

Reptarenaviruses belong to *Arenaviridae* family; part of the same family are 4 genera:
-*Antennavirus* (natural host fish)
-*Hartmanivirus* (reptiles)
-Mammarenavirus (rodents and bats borne).
-*Reptarenavirus* was identified in the early 2010s and included in *Arenaviridae* family, enlarging arenaviruses host range. *Hartmanvirus* does not appear to contribute to IBD pathogenesis, even though often coexists with *Reptarenavirus* in snakes with IBD. No association with any pathological findings or clinical signs, but neither, earlier studies were able to associate or rule out their pathological potential. [6]

Hartmanivirus and *Reptarenvirus* natural hosts are reptiles, even though the origin and reservoir hosts of both are still not determined; studies investigating captive and wild-caught native wild snakes with IBD from Costa Rica had showed that IBD occurs in wild boa constrictors, in conjunction with *Reptarenaviruses* and *Hartmaniviruses*. Different cases belonging to collections with only Brazilian indigenous snakes, demonstrate that these viruses circulate in wild snakes. [13]
Hartmanivirus seems to have neuronal and smooth muscle cells as target cells, *Reptarenaviruses* instead are able to replicate in most of the cell types. *Reptarenavirus* is an enveloped virus, presenting glycoproteins on the spherical virion's surface, (Figure 1.) used to mediate efficiently the virus entrance inside the cell. The virion diameter is medium-sized, and ranges from 100 to 200 nm. Like the other members of *Arenaviridae* family (except for *Antennavirus*), *Reptarenavirus* has a negative sense bi-segmented RNA genome; the large segment (L) encodes the RNA-dependent-RNA-polymerase (RdRp) or RNA replicase, an enzyme that catalyzes the replication of RNA from an RNA template (Catalyzes synthesis of the RNA strand complementary to a given RNA template); the small segment (S) encodes the glycoprotein precursor protein (GPC) and the nucleo protein (NP).[7]

Lack of proof-reading ability leads to the accumulation of point mutations during replication, representing the major mechanism driving to divergence and creating new lineages over time.

The available viral replication cycle refers to Arenavirus, primarily related to *Mammarenavirus*, where the cell attachment and entry occurs through receptor-mediated endocytosis; followed by the viral and cell membrane fusion. The ribonucleoprotein complex, then, is released into the cytoplasm, where both replication and transcription are conducted. While the virion assembly is through budding over the plasma membrane. [8]

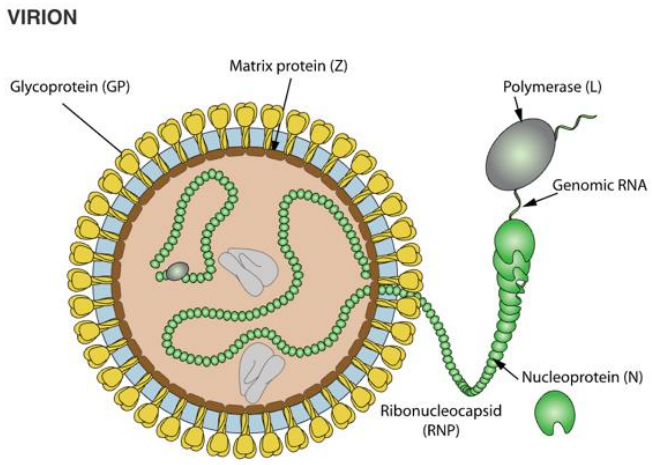


Figure 1. Schematic representation of *Reptarenavirus* virions' cross-section. (https://viralzone.expasy.org/6599)

One of the most important outcomes of the *Reptarenavirus* infection is the structural changes produced within the host cells; the specifically cytopathic effect, in this case, is inclusion body formation. Essential for diagnostic purposes, since it is the only way to be aware of the fact that on the cells there is a viral disease, inclusion bodies are aggregates of and excessive production of viral proteins; for *Reptarenvirus* nucleoprotein (NP) usually cytoplasmic.

It has been documented that, often, snakes with IBD are co-infected with several Reptarenaviruses, enhancing the IBs formation; this co-infection finds a possible explanation with a cross-species transmission, from prey animals carrying different *Reptarenviruses*, which may represent an option for the snakes to obtain the infection. [6]

Previous studies performed in vivo tests, inoculating on both Boas and Pythons, purified *Reptarenaviruses*; impressively during this observation, pythons evolve heavy CNS signs but no IBs, while apparently safe boas incubated large IBs in most tissues.

Considering *Reptarenavirus* as the cause of the disease, other contributory causes may play a role in providing favorable conditions for the disease to arise.

Health status and living conditions of the animal during the infection can have a huge impact on pathology development; a clear example was given by a study [10] where a group of snakes presented clinical signs similar to IBD but also connectable to parasitic infection, were treated and their housing optimized; in a short period, the symptoms disappeared. Immunocompromised individuals are more susceptible to *Reptarenavirus* infection, that worsens the Immune response and thus snakes are more prone to develop comorbidities. At present, the strongest evidence that *Reptarenavirus* is the etiological agent for IBD is the composition of the IBs, almost entirely made up of *Reptarenavirus* NP. However, this does not preclude the possibility of another unidentified endogenous agent contributing to the development of the disease. Concurrent infections with more than one *Reptarenavirus* are common phenomenon; aid also by Snakes mites (Ophionyssus natricis) that spread infection between animals via contaminated blood.

Pathogenesis

The processes underlying pathogenesis of *Reptarenaviruses* are still unknown. It has been hypothesized that some mechanisms resemble the same mammarenaviral disease. Since *Mammarenavirus* portal of entry is the respiratory system, the lungs are the site where the initial viral replication occurs, similarly in an experimental study a snake was inoculated with the virus through respiratory route (to simulate the potential natural pathway of infection), and later the disclosure of viral RNA appear just in the lung; [9] in the same manner the necropsies performed by the pathology group of the BCA department, resulted in severe pulmonary lesions in almost every specimens, without IBs formation. In *Mammarenavirus* pathogenesis the antigen-presenting cells are the foremost targets during the early stages; since snakes do not possess a proper lymphatic system with lymph nodes, but mostly lymph tissue accumulations and lymphatic sinus (particularly liver and intestine, plus spleen loosely arranged with red and white pulp), it's difficult to describe properly the infection course. Presumably, a following systematic spread occurs, towards other organs and tissues like liver, kidney, CNS, heart, and GIT. What is clear is that the disease has a slow and gradual course.

Clinical signs

IBD has been linked with immunosuppression development and numerous clinical signs, that slightly differ from species; for example, boas tend to present weight loss (significantly greater in female specimens), vertebral column deformation, neuro signs as opisthotonos (stargazing) and disorientation; pythons, instead, are more incline to show respiratory signs, in some cases vertebral column deformation as well, and inability to right itself when placed in dorsal recumbency, or further chronic inflammation, such as dermatitis. Customarily, captive snakes present a highly variable pool of clinical signs, including anorexia, regurgitation linked with stomatitis, pneumonia, and lymphoproliferative disorders. Boid snakes may have subclinical infection and exhibit symptoms afterward. For these reasons is fundamental to achieve an antemortem IBD diagnosis for example by detecting Inclusion Bodies in cytological samples (i.e., blood smear). However, it's easy to overlook inclusions in tissue section, mainly in snakes with few of them, especially if limited to CNS. Boid snakes with IBD may have a subclinical and latent infection that can persist, the amount of afflicted snakes that can develop symptoms is unknown in relation to those that appear unaffected. Severely afflicted specimens can present leukocytosis, relative lymphocytosis, and lower total protein and globulin values. [2]

In 2017 Stenglein and coworkers [7] reported in an experiment they had reproduced IBD in Python regius and Boa constrictor by cardiac injection of purified *Reptarenavirus*. They diagnosed classical IBD, as defined by IB formation. Expressed by the presence of eosinophilic intracytoplasmic inclusion bodies in neurons and glial cells in central nervous system, different organs' epithelial cells, smooth muscle tissue, lymphocytes in esophageal tonsils and outermost blood cells. These conclusions underlying the entanglement of the IBD process and furnish supplementary clues that the disease result may differ not just among viruses but even among species of snakes. [10]

In the post-mortem examinations conducted by the pathology group of BCA department, several of the present snakes showed evident vertebral column deformation (Figure 2a, 2b). Nevertheless, a previous Brazilian study, describes an IBD-positive Boa constrictor's necropsy, that showed restriction of movements, multiple granulomas in dorsal vertebrae and fractures. The swelling points, turn out to be a caseous form of necrosis, attributed then to Salmonella sp. infection. Salmonella sp. resides in microflora of both cold and warm-blooded animals; considered an opportunistic pathogen that can cause gastrointestinal or septic disorders, in reptiles often linked with osteomyelitis. [11]

Figure 2a, Boa constrictor displaying vertebral column deformation.

Figure 2b, Simalia amethistina on ventral recumbency, displaying vertebral column deformation.

Additional common symptoms reported in IBD-positive snakes, were significant weight loss, and neurological signs, probably due to intraneuronal inclusions, which may evolve in neurodegenerative disorders based on the biochemical nature of the protein deposits forming the inclusions.

In addition, both in the necropsies performed in BCA dept and in other studies [10], several snakes with histologically detected IBs, tested negative to virological test for *Reptarenvirus*, RT-PCR as well; might have appeared false negative and may have eluded RT-PCR revelation, as result of genetic variations or due to a scarce diagnostic test sensitivity. [10]

Immune response

Adaptive immune system known to be the second line of defense has a slower response because of the mechanism of specific antigen recognition but plays an important role in disease progression and possible development of comorbidities. As well as for the pathogenesis also for the mechanism of the immune response in snakes, little is known, especially for the role of the immune response against *Reptarenavirus*. It has been demonstrated that reptiles like all the other vertebrates own the immune response, which also includes both humoral and cell-mediated factors. Reptiles possess also the correspondence of interleukins (IL), IFNs, and toll-like receptors through which they can arrange their immune response. It has been shown that the immune response is temperature and hormone dependent. Due to the lack of cytokine-mediated development of fever, their poikilotherm status leads them to expose their body to higher environmental temperatures. The cell-mediated component of reptile's immune response, as in mammals, is based on T-cells, whose proliferation depends on seasonal cycle. This immune response seems to be stronger in females than in males.[12] Like in the other vertebrates, reptile immunoglobulins (Ig) compose the humoral component and produce three classes of antibodies: IgY, IgM, and IgD. Whilst M immunoglobulins can be easily compared to the mammal IgM, the molecular features of the IgY, instead, are more similar to the mammalian IgG. In reptiles, exposure to a foreign antigen or contagious agent generates IgM production, which reaches the peak up to 8 weeks later. These divergent timings indicate a different maturation in the immune response of reptiles compared with mammals. Depending on species and antigens, the reptile's IgM reaction may continue until 34 weeks subsequently the exposure, while IgY appears around 31 days after exposure and can last for several years, more similar to the outcome of IgM in mammals. Additionally, the antibody response is influenced by concurrent and individual factors like temperature, gender, season, age, and neuroendocrine status. Evidence that bacterial infections and neoplastic processes are common in snakes with IBD advises that the disease is linked with immunosuppression; this linkage was shown during a study on antibody response to IBD [12] in which they investigated the association of Lymphocytic choriomeningitis virus (LCMV), a prototype of *Arenavirus*, with type I interferon (IFN-I) production inhibition by the NP. Further, the Z protein's ability to re-localize the promyelocytic leukemia bodies (PML), to the cytoplasm; the PML tumor suppression is inhibited and thus promotes the *Reptarenavirus* tumorgenesis. Furthermore, has been proven that *Reptarenavirus* infected snakes with IBs have lower levels of anti-reptarenavirus antibodies, compared with *Reptarenavirus* infected snakes without IBs.

Diagnosis

The celerity and reliability of the pathogen recognition are of paramount importance t for an efficient diagnosis and therefore to contain the disease spreading; notably IBD where outlining a proper diagnosis is crucial. The ideal ante-mortem diagnosis leans on IBs detection; by identification of the eosinophilic intracytoplasmic IBs through histological tissue section, blood smears, and isolated peripheral white blood cells (PWBC) in hematoxylin and eosin (H&E) stain; this pathway can, however, lead to limited result. Searching for the presence of viral infections using oral and cloacal swabs or through esophageal tonsils' samples; and testing them with RT-PCR, searching for viral DNA; may increase the sensitivity for the *Reptarenavirus* detection. The histological tissue or blood evaluation combined with the RT-PCR results can give a great foretelling values for a reliable IBD diagnosis; the only contraindication appears to be the failure to track *Reptarenavirus* RNA in blood, during the early stages of the infection.

Other diagnostic processes, as nucleic acid-based approaches, have been considered too challenging and time-consuming, because of the high genetic diversity; and too low sensitivity. The recent identification of *Reptarenavirus* nucleoprotein (NP), as the major component of IBs, favorably enhances the possibility of using the immunohistochemistry (IHC) staining as a diagnostic tool; reinforcing the specificity of the antemortem diagnosis, validated as well in early infection stages.

Additionally, monoclonal antibody has been developed against inclusion body disease protein, currently used for immunodiagnostic assays.

Inclusion Bodies

The first effects of the replication in permissive cells by cytocidal viruses are the morphologic effects called cytophathic effect (CPE). Namely the alteration in cell morphology following the viral infection that brings structural and biochemical effects. Common effects are rounding of the altered cell, junction with neighboring cells (syncytial cells formation), apoptotic process induction but also Inclusion bodies (IBs) formation; which became then, the hallmark of IBD development. This morphologic effect can be a useful tool for diagnostic purposes, the virologist, in fact, is thus able to identify and isolate the virus. The IBs can be nuclear but usually cytoplasmic and may exhibit either altered host cell structures or accumulations of viral components. In some cases, the cell itself might become the inclusion due to structure alteration caused by the viral replication, by means of electron microscopy the composition can be then determined.[14]

Thus, the cytopathic effect, that characterizes IBD, is the presence of histologically eosinophilic hyaline, intracytoplasmic, and ultra-structurally inclusion bodies in almost all cell types. These aggregates are formed by granular electron-dense material, not defined by a membrane; they may contain previral material or storage material of dysfunctional cells, further the antigenetically distinct “68-kDa protein” that was characterized from nonverbal inclusion in IBD-infected Boa Constrictors.[4] IBs might vary in size and shape, usually have a 2 µm diameter, but average size and density can increase with infection duration; larger ones may reach 3-6 µm in diameter expressed as membrane bound aggregates of amorphous to granular material mixed with membrane like fragments.[5] other nonverbal inclusions containing granular to fibrillar electron-dense material have been identified in cells linked with Retroviruses infection, but no IBs of IBD were ever been associated with any retroviral infection. It appears, also, that *Reptarenavirus* infection, does not readily induce detectable IBs formation; thus, suggesting additional factors involved in pathogenesis process. It has been noted that *Reptarenavirus* affects several snake species, but IBs formation is linked only to Boid (and Pythonidae), and similar species, vertical transmission, coinfection with other viruses (e.g., *Hartmanivirus* or *Chuvirus*), or even the host’s genotype (impossible to demonstrate) could be considered a prerequisite for IBs formation. Down below few histologic pictures in H&E stain from the tissues collected during the necropsies held in the BCA Department (Fig. 3-4-5-6).

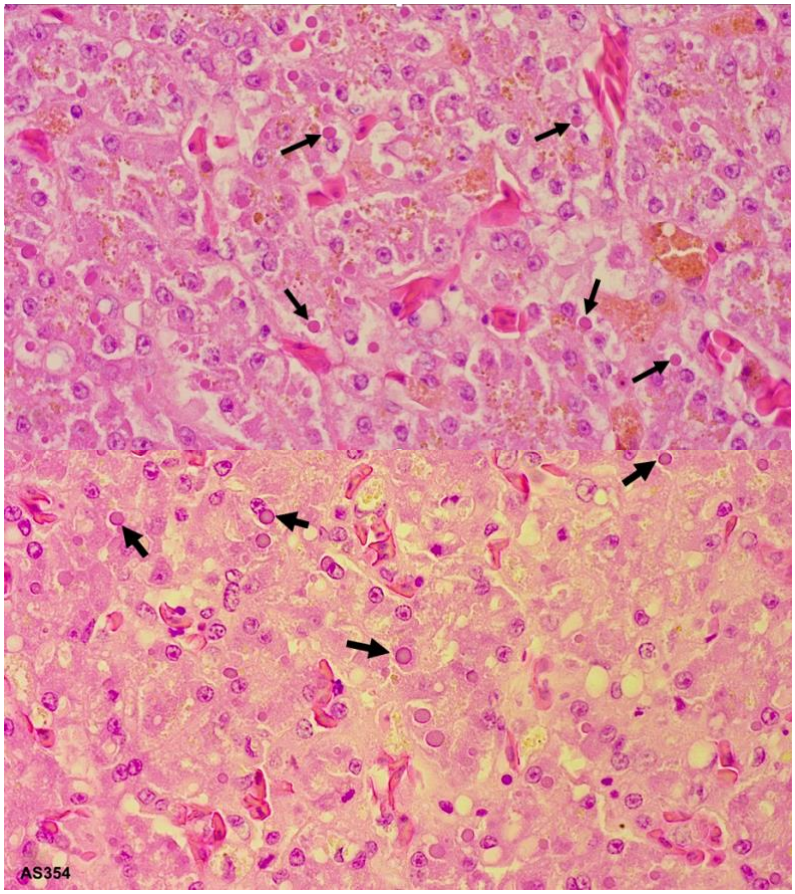


Figure 3a
Boa constrictor female, Arenavirus virologic test positive. Inclusion bodies in liver (arrows). H&E stain, magnification 40x.

Figure 3b
Boa constrictor female, Arenavirus virologic test negative. Inclusion bodies in liver. H&E stain, magnification 40x.

Figure 4a
Python regius female, Arenavirus virologic test negative. Inclusion bodies in CNS. H&E stain, magnification 40x.

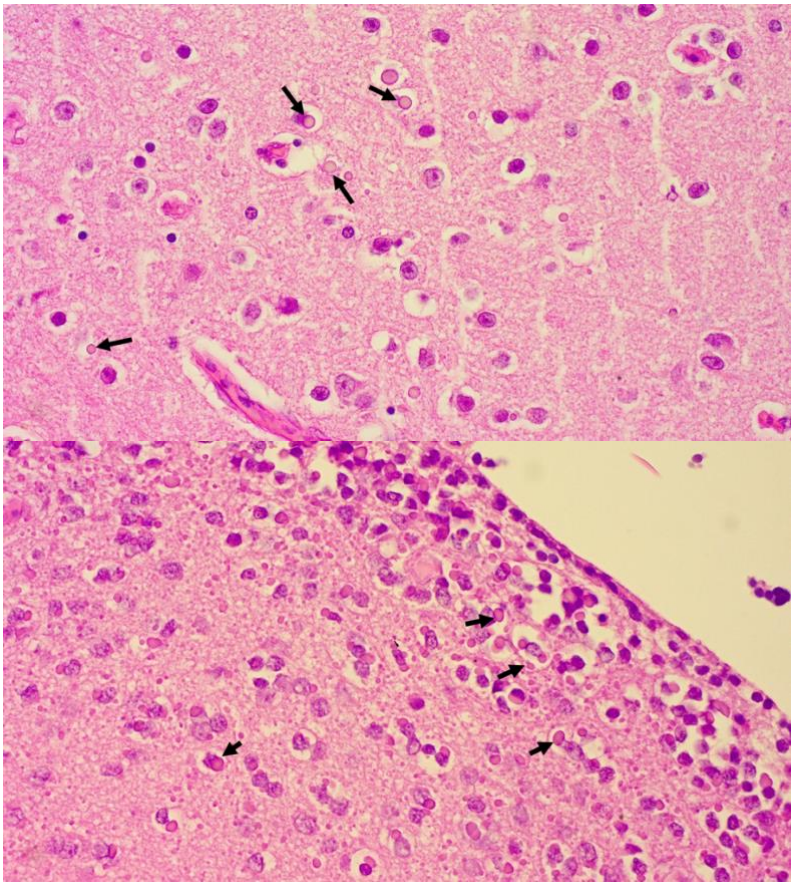


Figure 4b
Boa constrictor imperator female, Arenavirus virologic test positive. Inclusion bodies in CNS. H&E stain, magnification 40x

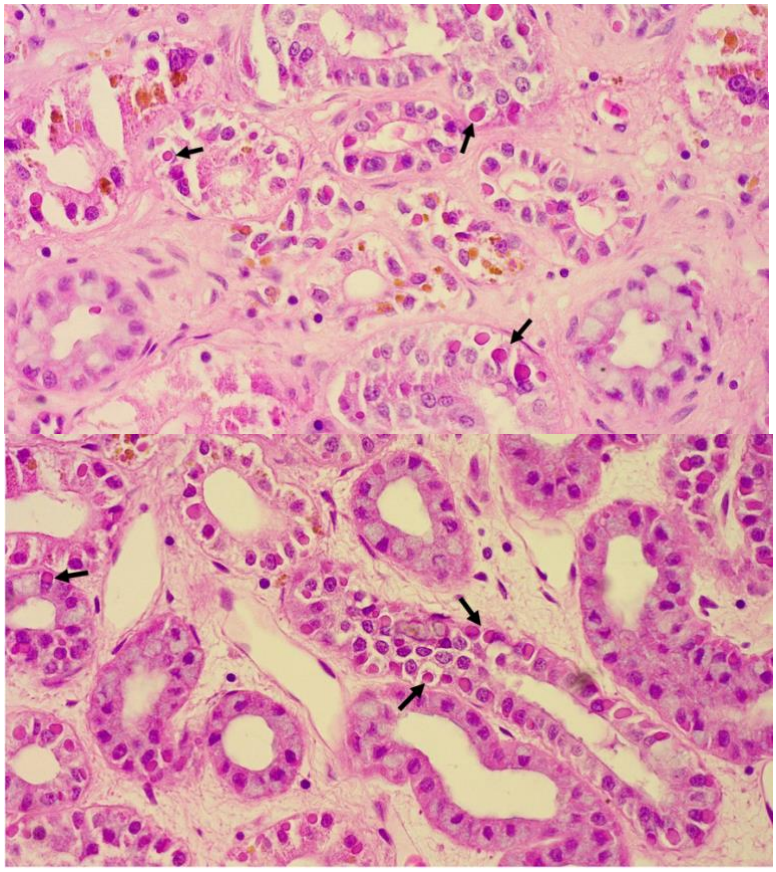
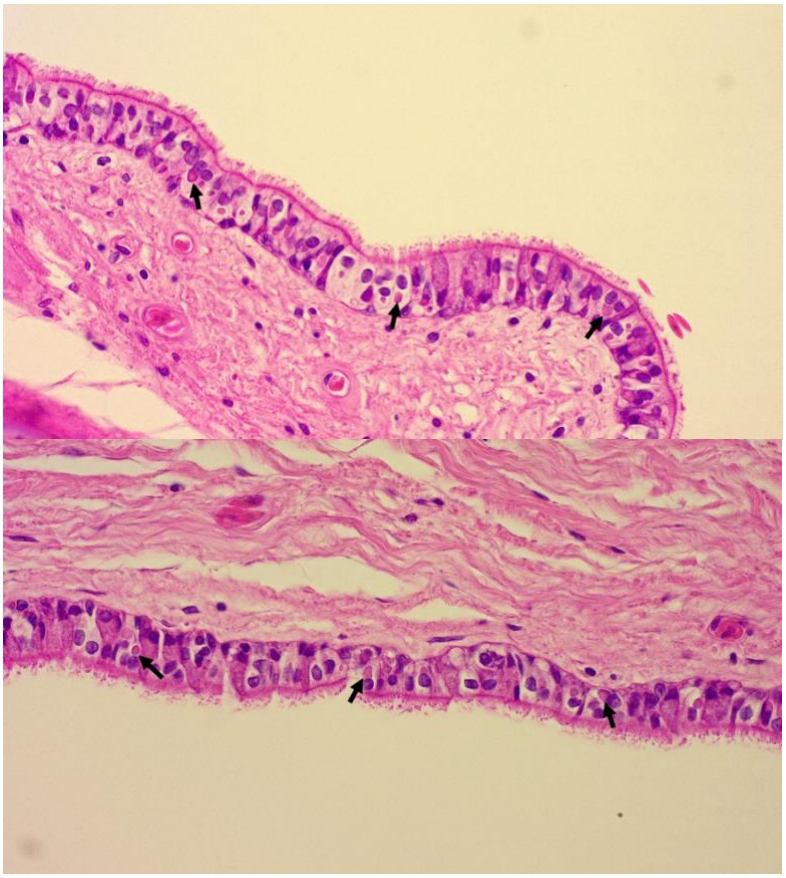


Figure 5a

Boa constrictor imperator female, Arenavirus virologic test positive. Inclusion bodies in kidneys. H&E stain, magnification 40x

Figure 5b

Figure 6a



Boa constrictor male, Arenavirus virologic test positive. Inclusion bodies in lungs. H&E stain, magnification 40x

Figure 6b

Transmission routes

The exact transmission routes nor the incubation period of *Reptarenavirus* are not fully understood, but there are different hypotheses; the dominant one involves the horizontal transmission throughout the three main portals of entry, via ingestion, inhalation, or direct contact with infected feces, urine, or saliva. Other hypothesis concerns vertical transmission, which can occur from an infected mother to her offspring during pregnancy or childbirth; and vector transmission resulting from the direct contact induced by a vector, in this case, a blood snake mite (*Ophionyssus natricis*).

- Horizontal transmission

  Considered the most common route of infection; can occur through direct contact, such as biting, scratching, or rubbing, between infected and uninfected snakes. Conversely, via direct contact with infected droplets or aerosols, the virus can be shed by *Reptarenavirus* positive snakes. Beyond the body fluids, the virus may be also spread across fomites, any objects or surfaces that have been contaminated by the virus, able to survive for long periods in the environment; thus, healthy snakes result infected by coming into contact with fomites. Study demonstrates that horizontal transmission is possible, *Reptarenavirus* infection may occur through direct or indirect contact with infected snakes, but tests have been performed on few specimens, and evidence are limited; further research is needed to prove this hypothesis. [10]

- Vertical transmission

  Vertical transmission is considered to be the transport of any infectious agent from one generation to the subsequent, counting transmission across gametes, transplacental, and perinatal infections. Less widespread but still a noteworthy route of infection; prenatal infection plays an important role in *Arenavirus* preservation, findings suggest that vertical transmission could have significantly influenced *Reptarenavirus* evolution since co-infection allows viral genomes' reassortment. Also, in previous studies [2] vertical transmission hypothesis has been contemplated, in both egg-lying (oviparous) and live-bearing snakes (viviparous). Reptiles, namely, Pythons and Boas are respectively egg-lying and live-bearing, both displaying embryonic membranes. Viviparous snakes, as boas, possess a simple placenta, responsible for gas exchange, water, and nutrient supply; the thin eggshell that divides the fetal and maternal placenta, deteriorates later during gestation, allowing direct

contact. Ordinarily, the two epithelia remain intact, and maternal and fetal blood do not mix but studies on reptile vertical transmission are scarce.

A 2017 study on *Reptarenavirus* vertical transmission conducted on Boa constrictor, found that not all offspring obtained the full parental "reptarenavirome", and were able to confirm IBD development in 2 months old offspring; but in vitro approach, cell cultures rapidly developed IBs and promoted viruses' replication. [15]

- Vector transmission

  In the vector transmission hypothesis, *Reptarenavirus* is carried by mites or other infected insects and transferred to snakes through infective bites. Transmission is specifically attributed to arthropod *Ophionyssus natricis* (figure 7), commonly found in snakes. They feed on the snake's blood and fluids and can become infected with *Reptarenavirus* and then transmit the virus to other snakes. There is currently too little evidence to support the claim, but in 2015 a study sustained the "transmission through a vector" hypothesis, reporting a growth of *Reptarenavirus* in arthropod cell lines. [16]

Figure 7 Snake blood mites, Ophionyssus natricis (research gate source)

Evidence suggests that both horizontal and vertical transmission of *Reptarenavirus* is possible in snakes; subclinical infections together with each route of transmission is likely to be the reasons behind *Reptarenavirus* co-infections, considered a rule rather than an exception in snakes with IBD.

Husbandry and Welfare

Reptiles comprise about 20% of the global live animal trade, [17] ten million exotic reptiles are estimated to be kept as pets in homes, which means that even a 1% mortality rate equates to millions of animals. Considering the data's lack, the numbers could be much higher due to illegal activity across the pet industry. Even though is now globally established they are sentient, hence able to feel negative and positive emotional states. Humans tend to relate with them less than other pets (like cats and dogs), so less consideration of their needs is accepted in common perception, for example is believed they do not demand so much space, a stimulating environment, or a high husbandry level. In accordance with the CITES trade database, more than three million Royal python specimens have been exported from West Africa since the first recorded trade in 1975. [18]

Also known as Royal pythons (*Python regius*), Ball pythons are often advertised by sellers, as "easy-to-keep" and "low maintenance" species. Clear evidence is provided by the massive component in the BCA collection of post-mortem (13 out of 25 specimens) of Royal pythons, among the specimens on which the necropsies were performed.

A survey on housing and husbandry on pet snake welfare declared that about 75% of pet reptiles perish by the end of the first year of adoption, labeling snakes as the most often succored pet reptile in England. [19] Furthermore, in a survey involving 200 veterinarians, just 19.5% of them stated that the welfare of pet snakes and their needs are "well met" or "very well met". [20] Compliance with the standards necessary for snake welfare is directly linked to the effective maintenance of biological parameters fundamental for: metabolism, thermoregulation, metabolism, and especially immune system response. Poor housing conditions, combined with *Reptarenavirus* infection, create the perfect environment for IBD to thrive. Snake owners should take precautions to prevent the spread of *Reptarenavirus,* to do so, is needed to know the factors behind viral transmission. For instance, a six-month quarantine is suggested as prophylaxis before a new animal release into a stable collection, plus biopsies (i.e., tonsils) and blood smear evaluation as well. [15] Being aware of the possibility of subclinical infections is of paramount importance, because lone cases of IBD are uncommon, usually where there is one case, there are others. Keeping snake's enclosure clean and free from debris and also, preventing mites from entering a collection, and eliminating established infestations are essential tools of a preventive medicine program. Unfortunately, there are no effective vaccines or therapies against such infection and disease; reason why preventive medicine is fundamental. Snakes showing signs of IBD should be immediately displaced from the primary cluster and settled in an isolated area or submitted for a complete necropsy evaluation. The

enclosure of sick or dead animals should be sanitized with bleach and drained in the sunlight for a while. [2] Furthermore, public awareness, should come in contact with the exotic pet trade issue and avoid purchasing animals in poor welfare conditions just because their market price is economical, and they come from unknown individuals.

Conclusions

This work's aim is to analyze the characteristics of IBD and its impact on snake welfare. IBD is a transmissible and progressively viral disease among Boas and Pythons, caused by *Reptarenavirus.* The infection can remain latent or give clinical signs such as weight loss, stargazing, vertebral column deformation, and also its hallmark the inclusion bodies. The lack of vaccination and effective treatments, for this disease, places a greater burden on pre-mortem diagnosis. Diagnosis should be a process that combines several tests to increase the sensitivity and accuracy of the result. The best way to avoid this disease's spreading is, therefore, to combine an effective pre-mortem diagnosis with a focus on snake welfare, improving husbandry, and applying preventive medicine. A universal tool to decrease the danger of this disease is to increase public awareness of the illegal exotic pet trade (especially Ball pythons) and also to make sure that guidelines and recommendations for pet owners are rooted in science. In conclusion, information on IBD is scarce, and much research still needs to be done, in order to improve the knowledge of this disease and the Boas and Pythons welfare.

Acknowledgments

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