UNIVERSITÀ DEGLI STUDI DI PADOVA
Dipartimento di Biomedicina Comparata e Alimentazione
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Developing insights on IBD: understanding its occurrence and impact on Boidae family

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Summary

Inclusion body disease (IBD) is a significant health concern in Boidae, a diverse family of snakes that includes popular pet species such as boas and pythons.

This thesis encompasses a thorough review of existing literature, as well as the analysis of data collected from affected individuals. The etiology of IBD remains elusive, with viral and genetic factors proposed as potential triggers. Initially thought to be caused by a retrovirus, association with members of the Arenaviridae family have been suggested, even though the determination of the definitive causative agent is still lacking.

The pathogenesis involves the formation of intracellular protein aggregates, leading to cellular dysfunction and systemic disease progression. The mechanism leading to the formation of these protein aggregates and its impact on the host immune responses are still areas of research. Clinical manifestations of IBD in boas and pythons are highly variable according to the species, ranging from subtle signs to severe neurological damage and death. To diagnose the disease, different techniques including electron microscopy, immunohistochemistry and PCR-based assays are employed to detect inclusions and viral particles. Management and control in captive snake populations pose significant challenges: strict quarantine protocols, regular health screening and proper husbandry practices are necessary due to the lack of vaccines and antiviral therapies.

This thesis aims to provide and analyze the data gathered from necropsies carried out with the BCA group, emphasizing the clinical signs, findings at macroscopic and microscopic levels, along with the information obtained from bacteriological, parasitological and virological examinations.

Moreover, it encompasses preventive medicine, management of the disease in captive populations and, thanks to the help of an expert veterinarian and many breeders, it delineates the economical and ethical impact of such alignment.

Introduction

The Boidae family encompasses a wide variety of species of boas and pythons, from the Ball python (*Python regius*) (Figure 1), which is also commonly kept as pet, to the peculiar rainbow boa (*Epicrates cenchria*) (Figure 2) found throughout Central and South America.

Figure 1. Adult individual of Ball python (*Python regius*).

Figure 2. Juvenile of rainbow boa (*Epicrates cenchria)*

These two species share some characteristics, such as the fact that they are constrictor snakes, meaning that their method of killing consists of wrapping around the preys and suffocating them to death. Moreover, boas and pythons are considered primitive snakes because they have two functioning lungs and vestigial pelvic spurs (remnants of hind legs and pelvic bones, as they evolved from lizards).

Despite this, there are some aspects that allow them to be distinguished: for example, pythons are found only in the Old World (Africa, Asia and Australia), whereas boas are found in continents of both Old and New World (North, Central and South America and the Caribbean). Furthermore, the reproductive strategy is also different, as pythons are oviparous, while boas give birth to live youngs (ovoviviparity).

A recent study has examined 44 years (1975-2018) of snakes-trade records, whose results calculated that approximately 6.2 million CITES-listed alive snakes were traded worldwide during that period, together with other body parts (Hierink et al., 2020).

Despite this, it is shocking how these numbers are related only to the snake species listed in the CITES Appendix II, which restricts trade for *"species that are not necessarily now threatened with extinction but that may become so unless trade is closely controlled"* (UNEP-WCMC on behalf of the CITES Secretariat, 2023).

The most susceptible species to trade were pythons, with particular emphasis on reticulated python (*Malayopython reticulatus*) and ball pythons (*Python regius*), representing almost half of the trade (Hierink et al., 2020).

Already for many years now, these animals have been facing a serious problem in the form of Inclusion Body Disease (IBD), which is a contagious and deceptive ailment documented both in wild and captive settings.

In the last years, advances in molecular research have hypothesized that the possible causative agent of this illness is a *Reptarenavirus* of the family *Arenaviridae* (Hetzel et al., 2013) (Stenglein et al., 2012), contrary to claims by researchers of the previous years, reporting that the source of the disease was a *Retrovirus*.

To confirm the correlation between *Reptarenavirus* and IBD, researchers focused their work on the origin of IBDP (inclusion body disease protein): a unique 68KDa protein was identified in an electrophoretogram of IBD-infected tissues and, thanks to this, results revealed that inclusion bodies (IB) are mainly composed of the previous mentioned IBDP.

The initial hypothesis about the protein origin, and thus the pathogenesis of the disease, was that IBD could be a viral-induced protein storage disease (LSDs). This condition is the consequence of an intrinsic metabolic error leading to the accumulation of the protein, which should be normally degraded by lysosomes. The absence of these enzymes induces the intracellular build-up of endogenous substances, which are recognized as IB. Inflammation and oxidative stress, often induced by such conditions as suboptimal animal care or other pathological processes, may play a pivotal role in the development of LSDs (Wozniak et al., 2000).

In 2012, further breakthrough in the understanding of IBD was achieved thanks to Next Generation Sequencing (NGS), marking a significant advancement in the IBD frontier. In fact, the strong connection between *Reptarenavirus* and IBD was confirmed through its behavior in cell cultures, where it produced IB that closely resembled the ones observed in the infected specimens. Furthermore, the antibodies developed were shown to bind directly to the inclusions present in the affected animals' cells.

The *Arenavirus* genus, which belongs to the family *Arenaviridae*, comprises 22 species of rodent-borne viruses that are classified into four genera (*Antennavirus, Mammarenavirus, Hartmanivirus* and *Reptarenavirus*), with the last two found in reptilian hosts. Some Reptarenaviruses cause BIBD (Hetzel et al., 2013).

Reptarenavirus produces enveloped virions containing a genome consisting of two to three single-stranded RNA segments that replicate in the cytoplasm of infected cells. Virions, which are the complete viral particles, exhibit a spherical or variable shape, as well as a diameter ranging from 100 to 200 nm. The surface of the virions is covered by trimeric spike structures, each measuring 10 nm in length and spaced at intervals of 11 to 15 nm, and they are formed by the membrane GP subunits, namely GP1 and GP2. Resembling the virions of *Mammarenavirus*, the virions of *Reptarenavirus* possess an additional layer known as zinc-binding protein (Z) layer, located beneath the lipid layer. Unlike the *Mammarenavirus*, *Reptarenavirus* does not possess a second layer under the lipid membrane (Hetzel et al., 2013).

Reptarenavirus has a unique genetic organization, in which the small and large RNAs encode two proteins in separate, non-overlapping open reading frames (ORFs) with opposite polarities. This arrangement is known as ambisense coding. The two ORFs are separated by non-coding intergenic regions (IGRs) (Figure 3).

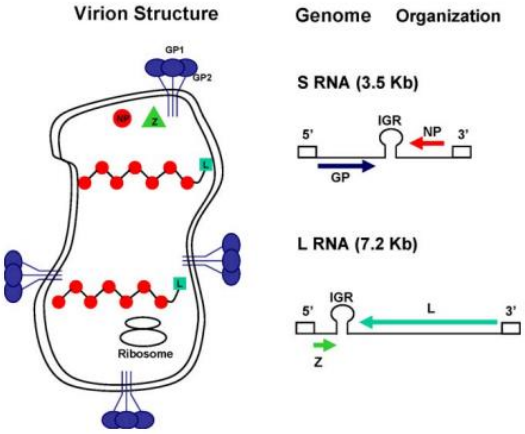


Figure 3. *Arenavirus* virion structure and genome organization (Emonet et al., 2009).

Specifically, the S RNA (small RNA) encodes the NP (Nucleoprotein) in the virus genome's complementary sequence, and the GPC (Glycoprotein Precursor) in the virus genome's sense sequence. On the other hand, the L RNA (large RNA) encodes the L protein in the virus genome-complementary sequence and Z in the virus genome-sense sequence. The intergenic regions (IGRs) form one or more hairpin loop structures, and they play key roles in structure-dependent transcription termination, in the assembly and budding of virions (Stenglein et al., 2012) (Hetzel et al., 2013).

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

The distinctive trait of *Reptarenavirus* infection is the structural changes triggered in host cells. In this case, the specific cytopathic effect is the formation of IB, which are crucial for diagnostic purposes because they are the only visible sign that a viral infection is present in the cell. As previously reported, IB are clusters of an excessive production of viral proteins, with *Reptarenavirus* usually leading to the accumulation of nucleoprotein (NP) in the cell's cytoplasm.

The disease is recognized due to the characteristic clinical manifestations, which differ between the two species affected. In infected pythons, *Reptarenavirus* infection predominantly manifested as central nervous system signs, going from "stargazing", head tremors, disorientation to regurgitation and "corkscrewing". The same signs were also described for positive boas, but milder and many snakes appear to be clinically healthy (Alfaro-Alarcón et al., 2022). Moreover, individuals with IBD display a range of other clinical signs, from anorexia to tumors, which severely impair their quality of life and reproductive success. (Mader D.R., 2006).

The exact transmission routes and the incubation period are not fully understood, but many hypotheses were advanced. Considered the most common infection route, horizontal transmission is the dominant theory and it involves different mechanisms, such as direct contact (biting, scratching or rubbing), venereal contact (sexual intercourse), airborne transmission (infected droplets), or fomite transmission (any object or surface that have been contaminated by the virus, which is able to survive for long time in the environment).

While some studies suggest that horizontal transmission is possible, it's important to underline that the evidence is limited, and further research is needed to confirm this hypothesis (Simard et al., 2020).

Although less common, vertical transmission remains a significant route of infection. It involves the transmission of the infection agent from one generation to the next one and happens across gametes, transplacental transmission, and perinatal infections. Prenatal infection is determining for the preservation of *Arenavirus*, as it allows for the viral genome reassortment (Keller et al., 2017).

Finally, for many years, the possibility of transmission through a vector has also been considered. The common snake mite, *Ophionyssus natricis*, is a blood feeding mesostigmatid mite that parasitizes reptiles, and which has a worldwide distribution. It can be found under scales, especially around eyes, nostrils, pits and cloaca; and heavy infestation of this arthropod can lead to severe anemia, anorexia, lethargy, up to the death of the host individual (Wozniak et al., 2000) (Mader D.R., 2006).
They feed on snakes' blood and body fluids and can become infected with *Reptarenavirus*, being able to then transmit it to non-infected snakes and spread the disease. There is currently little evidence to support this supposition, but the current study reports one of the few proofs available up to date (*Python regius,* AQ536).

Detecting and diagnosing IBD has always been a challenge, with histology being for years the only reliable way. However, advances in the diagnostic techniques, such as reverse transcription-PCR (RT-PCR) and immunohistochemistry, have meaningfully improved our abilities to detect and confirm IBD cases (Hetzel et al., 2013; Chang et al., 2016). Further studies and continued improvement of the methodologies to diagnose the problem are needed, but the achievements to date allow researchers and veterinarians to better understand the prevalence of this disease and implement appropriate management strategies, particularly in captive settings, where the disease can spread rapidly among populations.

Despite the current efforts to manage IBD, there is still not a definitive cure against it. In fact, what breeders or zoological parks can do focuses mainly on preventive and supportive care, in order to avoid or at least alleviate clinical symptoms and to enhance the general well-being of the individuals in a collection. Quarantine measures, regular health screenings, proper husbandry practices and disposal of positive animal carcasses have been established as essential measures in captive collections to mitigate the spread of this critical ailment (NSW Dept. of Environment and Conservation, 2004).
The quest for more effective treatments continues, forecasting optimism for improved outcomes in the future.

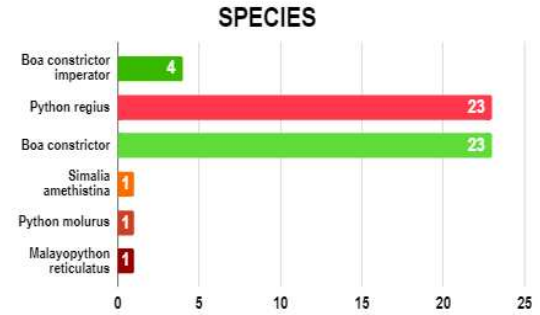
The pathology group of the BCA department decided to contribute to the ongoing research by deepening the diagnostics aspects of the disease and performing a new study on captive snakes in Italy.

Chapter 1: Materials and methods

1.1 Animals

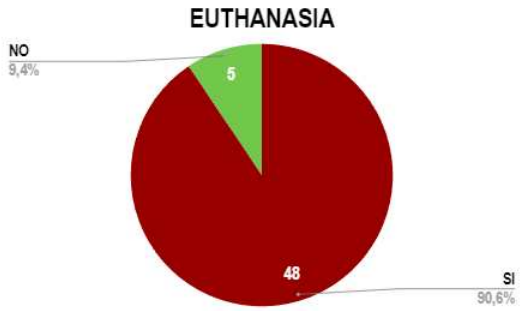
The study was performed on 53 captive snakes, from Italy, submitted for diagnostic purposes by vet and owners to the University of Padua, between 2019 and 2023.

The snakes belong to species of boas and pythons (*Boa constrictor imperator* [4 snakes], *Boa constrictor* [23 snakes], *Python regius* [23 snakes], *Simalia amethistina* [1 snake], *Python molurus* [1 snake], *Malayopython reticulatus* [1 snake]) (Graph 1).



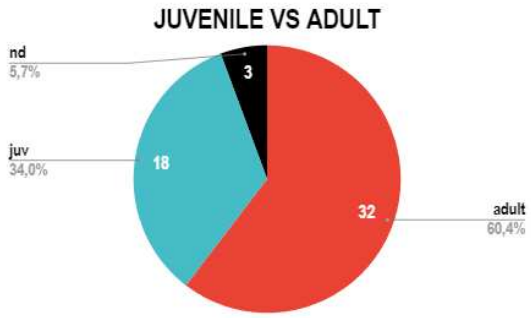
Graph 1. Different species of boas and pythons submitted to the University of Padua.

The snakes died of natural causes (5) or were euthanized (48) by means of intracardiac injection, and a postmortem examination was performed to determine or exclude the correlation with IBD. Animals were euthanized with Tanax (T-61) injection, which is a nonbarbiturate, nonnarcotic mixture of embutramide, mebezonium, iodide, and tetracaine hydrochloride (Leary S. L., 2020) (Graph 2).



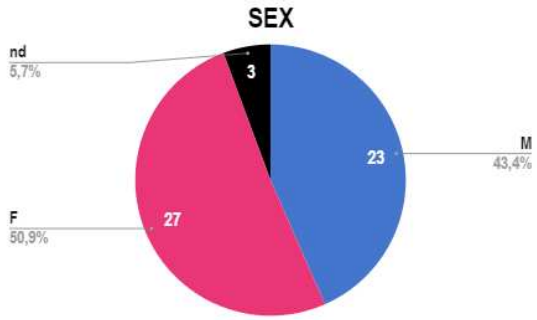
Graph 2. Numbers of snakes that died due to natural causes and because of euthanasia.

The age class was estimated for all the animals according to length and weight, following what is reported in specific snakes' manuals (De Vosjoli P., 1997) (De Vosjoli et al., 2004). In our study, the majority of individuals comprised adult snakes, totaling thirty-two. Due to lack of data, the age class of three individuals could not be determined. The remaining eighteen individuals were classified as juveniles (Graph 3).



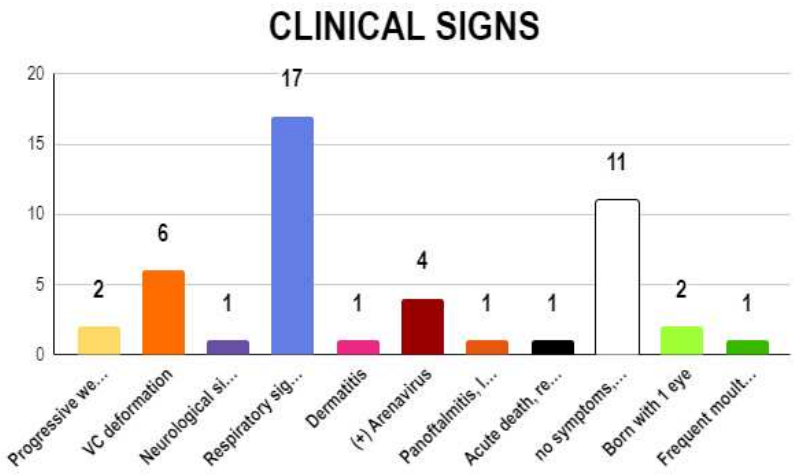
Graph 3. Proportions of juvenile and adult individuals used in the research.

The sex has been determined during the necropsy procedure, with a total of twenty-three males and twenty-seven females. Due to lack of data, the sex of three individuals was not reported (Graph 4).



Graph 4. Proportions of male and female snakes in the study.

As for the clinical signs, when reported by the referring veterinarian, included respiratory signs and vertebral deformation, as the most prevalent (Graph 5).



Graph 5. Clinical signs exhibited by the animals prior to death.

Table 1 provides a comprehensive overview of all the data, including species, age class, sex, year of postmortem, and the cause of death, whether due to euthanasia or other reasons.

Table 1. Animals signalment and clinical signs.

Snake no.	***Species***	***Age****	***Sex***	***Year of PM***	***Clinical signs*** **	***Euthanasia***
1	*B. constrictor imperator*	Juv	M	2022	O	Yes
2	*B. constrictor imperator*	Ad	F	2022	VD	Yes
3	*B. constrictor imperator*	Juv	F	2022	VD	Yes
4	*B. constrictor imperator*	Ad	F	2022	N	Yes
5	*P. regius*	Ad	F	2022	R	Yes
6	*P. regius*	Juv	F	2022	R	Yes
7	*P. regius*	Juv	F	2022	R	Yes
8	*B. constrictor*	Juv	F	2022	VD	Yes
9	*B. constrictor*	Juv	M	2022	VD	Yes
10	*P. regius*	Ad	F	2023	R	Yes
11	*B. constrictor*	Ad	M	2023	O	Yes
12	*P. regius*	Ad	F	2023	R	Yes
13	*B. constrictor*	Ad	M	2023	+	Yes
14	*S. amethistina*	Ad	M	2022	VD	No
15	*P. molurus*	ND	ND	2019	O	Yes
16	*M. reticulatus*	ND	F	2020	O	No
17	*B. constrictor*	Ad	ND	2020	NR	Yes
18	*B. constrictor*	Ad	ND	2021	NR	No
19	*B. constrictor*	ND	M	2022	NR	No
20	*B. constrictor*	Ad	F	2022	O	No
21	*B. constrictor*	Ad	F	2023	NR	Yes

22	*P. regius*	Ad	M	2023	NR	Yes
23	*P. regius*	Ad	F	2023	R	Yes
24	*P. regius*	Ad	M	2023	R	Yes
25	*P. regius*	Ad	F	2023	R	Yes
26	*B. constrictor*	Juv	M	2023	+	Yes
27	*B. constrictor*	Ad	F	2023	+	Yes
28	*P. regius*	Ad	F	2023	R	Yes
29	*P. regius*	Ad	F	2023	R	Yes
30	*B.constrictor*	Juv	M	2023	NR	Yes
31	*P. regius*	Juv	F	2023	R	Yes
32	*P. regius*	Ad	F	2023	R	Yes
33	*P. regius*	Ad	F	2023	R	Yes
34	*P. regius*	Juv	F	2023	R	Yes
35	*P. regius*	Ad	F	2023	R	Yes
36	*B. constrictor*	Juv	M	2023	NR	Yes
37	*B.constrictor*	Ad	M	2023	NR	Yes
38	*B.constrictor*	Ad	M	2023	NR	Yes
39	*B.constrictor*	Ad	M	2023	NR	Yes
40	*B.constrictor*	Ad	M	2023	NR	Yes
41	*B.constrictor*	Juv	M	2023	NR	Yes
42	*P. regius*	Ad	M	2023	NR	Yes
43	*B.constrictor*	Juv	M	2023	NR	Yes
44	*B. constrictor*	Juv	M	2023	NR	Yes
45	*B. constrictor*	Juv	M	2023	NR	Yes
46	*B. constrictor*	Juv	M	2023	NR	Yes
47	*P. regius*	Ad	F	2023	R	Yes
48	*B. constrictor*	Juv	F	2023	O	Yes

49	*P. regius*	Ad	M	2023	+	Yes
50	*P. regius*	Ad	F	2023	R	Yes
51	*P. regius*	Juv	M	2023	O	Yes
52	*P. regius*	Juv	F	2023	VD	Yes
53	*P. regius*	Juv	F	2023	O	Yes

*: Juv=Juvenile; Ad=Adult.
**: VD=Vertebral column deformation; N=Neurological signs; R=Respiratory signs; +=Arenavirus positive; NR=Not Reported; O=Others

1.2 Necroscopy procedure

The animals arrived at the BCA department for a post mortem examination.

As snakes have different features to mammals their necropsy requires a different protocol.

Starting from with weightning, then measurement of the trunk (from snout to vent) as well as the entire body length (from snout to tip of the tail) (Figure 4).

The external inspection refers to skin, eyes, nostrils, ears, mouth, vent, tail, bone protuberances, muscles. Any gross change observed macroscopically (trauma, spectacles opacification, hemorrhages, pathological secretions, …) should be included in the necropsy report with location as accurate as possible. Photography is also mandatory before opening the body cavity (Rizac et. al, 2020).

First, blood is taken from the carcass of the animal for preparation of blood smears on a cytology slide using a standard wedge technique, air-drying them for 24 hours and staining them following a precise protocol. This type of analysis allows us to be able to examine and check for viral inclusions in lymphocytes and heterophils.

The skin is observed grossly and with the aid of a magnifier to check the possible presence of ectoparasites (especially in the mouth area) (Rizac et. al, 2020).

The examination continues by placing the animal in dorsal recumbency and by cutting the skin. Two possible techniques can be used, but we mainly performed a single section, starting from between the mandibular branches and ending in the perianal area, from which feces will be collected for the coprological exam (Rizac et. al, 2020).

The organs are eviscerated *as a whole* following the cutting of trachea and esophagus by dissecting the dorsal connecting tissue. As in other species, the pericardium is then

opened to isolate the heart, which is tricameral (presenting two atria and one ventricle) and usually positioned in the first third of the snake's body.

Evisceration proceeds with the removal of the trachea, composed of incomplete rings, of lungs (with the left less developed compared to the right one) and air sac.

It follows with the liver (elongated and bilobed), gallbladder (extrahepatic), pancreas (multilobed and pinkish), and spleen (reddish).

The digestive tract is isolated and composed of the esophagus, stomach, small and large intestine.

The examination concludes with the isolation of kidneys and gonads. The kidneys are multilobed, brick-colored, and positioned asymmetrically, with the right one located cranially. The reproductive organs are positioned similarly, with the testis cranial to the kidneys and the ovaries close by the pancreas. Males also present two hemipenis at the end of the tail (Figure 5-6) (Rizac et. al, 2020).

Figure 4. *B. constrictor*, whole carcass

Figure 5. *S. ametisthina*, whole carcass and eviscerated organs

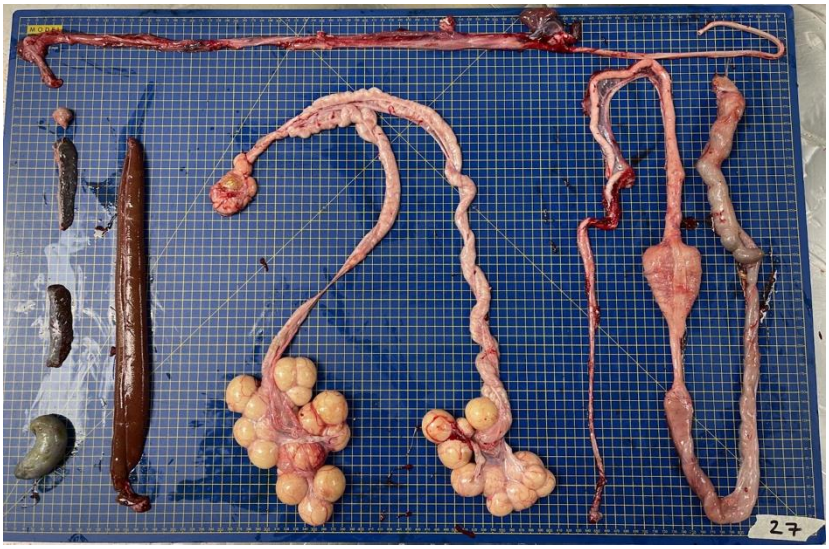


Figure 6. *B. constrictor* isolated organ systems.

1.3 Histological processing

1.3.1 Sample preservation

Following the necropsy, different preservation methods are employed for the collected organ and tissue samples, depending on the type of examination they will undergo.

For all specimens obtained from the snakes, they are fixed in a 10% buffered formalin solution for histological examination. Formalin is a chemical solution based on formaldehyde that exists in a liquid form at room temperature. It is widely used to disinfect and preserve biological materials. However, it's essential to be aware that formalin is a substance with toxic properties, it can be irritating, and there is a potential carcinogenic risk associated with its use. Therefore, when handling formalin, it's crucial to follow safety protocols.

When using formalin for preservation, a volume of solution equal to 10 times the volume of the specimen is typically applied. The container used for this process should be tightly sealed, and it needs to be closed carefully and promptly to prevent the dispersion of potentially irritating vapors that could affect eyes and nasal mucosa.

1.3.2 Slides preparation

Organ specimens preserved in formalin are sent to the veterinary histopathology laboratory within the BCA Department. Here, the preparations are cut under a fume hood to ensure safety and then placed in biocassettes, with the size of the latter chosen to match the dimensions of the cut tissues. Next, the cassettes containing the specimens are

immersed in formalin, and they go through a processing procedure. This processinvolves a cyclic sequence that serves to remove water and formalin and it also entails immersing the sample in a series of alcohol solutions with an increasing gradation scale. The dehydration process is essential because the included substances exhibit hydrophobic characteristics. This means that they won't easily penetrate tissue in presence of water. In some cases, xylene, a substance known for its ability to completely remove water, is used to further aid this process.

Following this step, the tissue is placed in a biocassette and filled with liquid paraffin at a temperature range of approximately 57-59°C. As it cools, the wax transitions into a solid state. It is important that paraffin does not become too hot during this process because excessive heat can lead to the formation of folded-in sheets, which would complicate slide preparation. Paraffin at high temperature tends to assume wax-like characteristics.

Once the paraffin block containing the tissue has fully solidified, it is sliced into thin sections using a microtome. The microtome is a tool that employs a disposable blade to cut the blocks into sheets with a thickness ranging from 3 to 5 micrometers.

These sheets are then affixed to glass slides and, to facilitate their adherence, they are briefly placed in a warm distilled water bath.

Hematoxylin-eosin staining will then be performed using an automated instrument. Hematoxylin, a basic water-based dye, is used to stain the negatively charged nuclei and membranes blue. Conversely, eosin, an alcohol-based dye, is used to stain positively charged cytoplasm red or pink, thus allowing the differentiation of collagen, cellular and mitochondrial proteins.
Before staining, the slide needs to be rehydrated: it will be placed in a heater at 70°C to melt and remove the remaining paraffin. To ensure the staining process proceeds accurately, the following steps are meticulously performed:

1. Dewaxing and rehydration: the slides with the histological preparation are immersed in a xylene solution for 5 minutes to help eliminate the remaining paraffin. They are then placed in ethanol solutions with progressively lower % to concentration (ranging from 100% to 75%) for 2 minutes per solution. Finally, the

slide is immersed first in tap water for 15 seconds and then in distilled water for 20 seconds.

2. Staining: the slide is first immersed in hematoxylin for approximately 5 minutes and then in tap water for 7 minutes. The latter, as it is slightly alkaline, helps change the color of the nuclei and membrane from purple to bluish. The slide is then dipped in distilled water to remove any remaining salts and subsequently placed in the second dye, eosin, for 50 seconds.
3. Dehydration: the slide is sequentially immersed in ethanol solutions with increasing concentrations for about 30 seconds to 1 minute per solution. Then, it is placed in xylene solution for 4 minutes to ensure proper dehydration of the slide. This prepares it for mounting under the fume hood.

To securely mount the coverslip, the slide is dried, and a drop of glue is applied at center position. The slide is moistened with xylene solution, and the coverslip is placed slightly tilted to prevent the formation of air bubbles. After 24 hours, the glue will have completely adhered to the slide, making it ready for observation under a microscope. After 48 hours, it will be ready for shipment and further analysis.

1.4 Parasitological, bacteriological and virological examinations

1.4.1 Parasitology

During the necroscopy process, fecal samples from 41 out of 53 snakes were collected and forwarded to the MAPS – UNIPD Parasitology and Parasitic Diseases Laboratory. The samples underwent qualitative coprological examination using the flotation-sedimentation technique, specifically targeting Coccidia such as *Caryospora* and other relevant parasites.

Additionally, Ziehl-Neelsen staining was applied to fecal smears to identify the presence of *Cryptosporidium.*

1.4.2 Microbiology

The purpose of clinical bacteriology is to provide information regarding the presence/absence of a pathogen. For this research, bacteriological analysis was performed by Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe).

During the necroscopic exam, swabs were taken in different body parts from 21 out of the 53 study subjects and they were then sent to the laboratory. In general, bacterial identification requires 24-96 hours from the time of arrival of the sample.

The test is conducted using live, viable bacterial strains that have been isolated in pure form through culture examination. The primary objective is to assess the in vitro sensitivity of the pathogen to various active ingredients.

Antimicrobial susceptibility is determined by establishing the minimum inhibitory concentration (MIC), which denotes the lowest concentration of antimicrobial substance capable of inhibiting the growth of a bacterium. During interpretation, the laboratory refers to the clinical breakpoint values outlined in globally recognized guidelines. These guidelines help to classify the microorganism as sensitive, resistant, or intermediate to each selected antibiotic.

Breakpoint values and dilution ranges vary depending on the drug and bacterial species; and should be considered alongside clinical information. MIC is expressed as a numerical concentration, corresponding to a classification (sensitive, resistant, intermediate), and by a quotient, obtained by dividing the breakpoint by the detected MIC. The latter offers additional information to clinicians for therapeutic decision-making.

1.4.3 Virology

Through necropsy examination, the collected organs were also carefully stored in sterile containers and maintained at temperatures between 2-8 °C. Subsequently, they were dispatched to the LABOKLIN laboratory for virological analysis using the PCR. Polymerase Chain Reaction is an exceptionally sensitive and precise method employed for the detection of infectious agents. Using PCR, gene sequences related to specific pathogens are replicated and identified, facilitating accurate and efficient diagnosis.

In this study, LABOKLIN test number 8565 aims to detect the presence of various viruses and, to achieve this, specific materials are required based on the parameters outlined for each virus.

For *Adenovirus* detection, samples such as swabs (cloaca or pharynx), tracheal lavage, and tissue (intestine or liver) are needed. *Arenavirus* detection necessitates EDTA blood, blood smear, tissue (liver, brain, kidneys or pancreas) and swab (esophagus).

Paramyxovirus/Ferla Virus detection requires oral and cloacal swabs, tracheal washes, and samples from lung, liver, kidney and pancreas. Finally, *Reovirus* detection involves

oral swabs, lung samples, and feces. The testing is anticipated to take between two and four days to complete.

Chapter 2: Results

2.1 Macroscopic examination

During the necropsy analysis, few gross lesions were detected in 10 individuals (e.g. lungs and liver discolorations, damaged ribs, material inside the ureters).

Nineteen out of 53 snakes (13 *Boa constrictor* and 6 *Python regius*) had a body score condition (BCS) less than 3. Among this group, 6 snakes exhibited vertebral column deformation in various tracts of the vertebral column (Figure 7-8), two had skin-related problems, one had blood mites (*Ophionyssus natricis*), two were born with one eye, and 4 had intestinal constipation.

Figure 7.
Python regius, female, juvenile displaying cervical vertebral column deformation.

Figure 8.
Boa constrictor, sex not determined adult with thoracic vertebral column deformation.

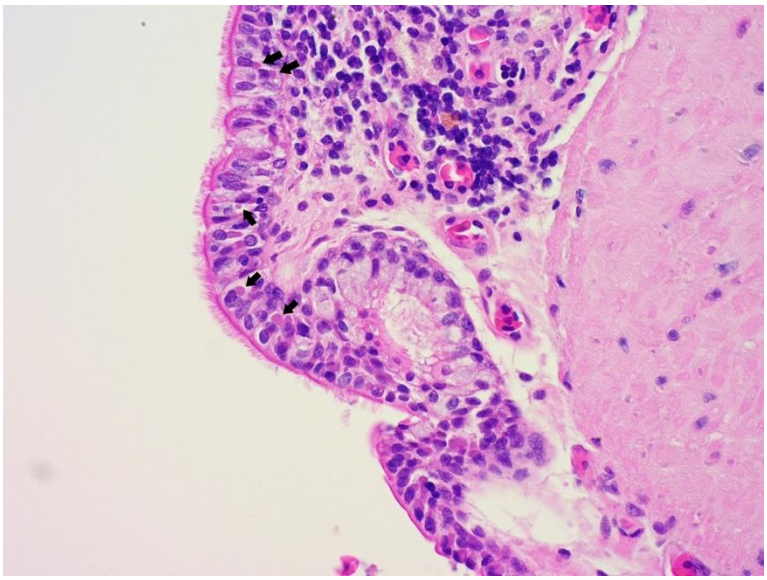
2.2 Microscopic findings

The histopathological examination of tissues and organs coming from 53 snakes confirmed BIBD in 10 individuals (5 *Boa constrictor*, 3 *Python regius,* and 2 *Boa constrictor imperator*). In six individuals (1 *Python molurus*, 1 *Malayopython reticulatus*, and 4 *Boa constrictor*) an inconclusive result was obtained, while the remaining 36 animals were BIBD negative (Table 1).

The IBD diagnosis was based on the presence of the typical eosinophilic hyaline, intracytoplasmic, IB with various size and shape, ranging from 2 to 6 μm in diameter.

2.2.1 Lungs

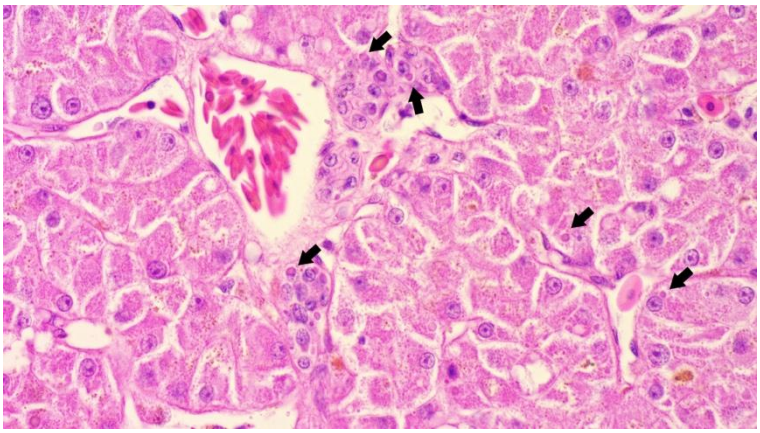
Figure 9.



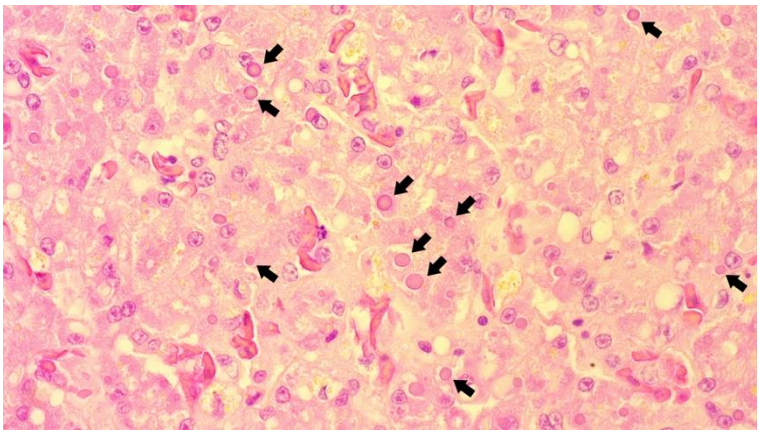
A) *Boa constrictor imperator*, female, adult. *Arenavirus* virologic test positive. Lung section: IB (arrows) are present in within the cytoplasm of the ciliate epithelial cells of bronchioles; H&E, 20X.

2.2.2 Liver

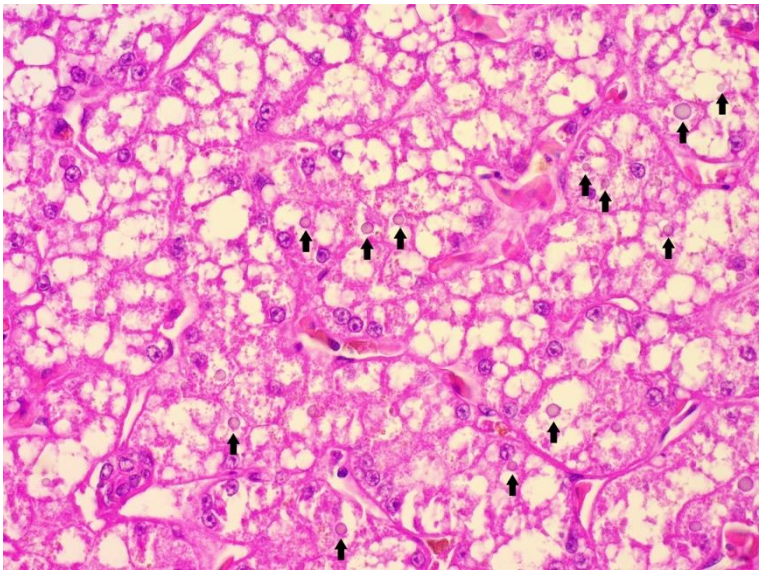
Figure 10.



A) *Boa constrictor*, male, adult. *Arenavirus* virologic test positive. Liver: IB (arrows) are observed in the hepatocytes and in biliary cells ducts; H&E, X40.



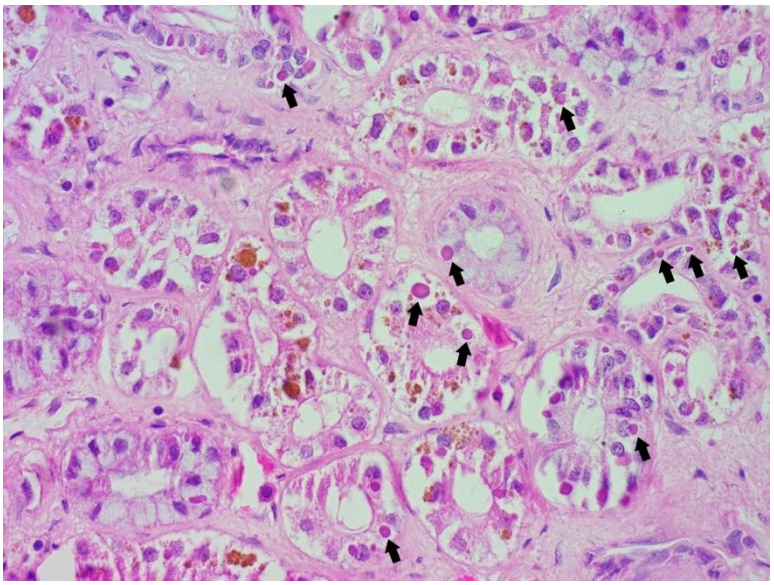
B) *Boa constrictor*, female, adult. *Arenavirus* virologic test negative.
Liver: eosinophilic inclusions (arrows) were detected inside multiple hepatocytes; H&E, X40.



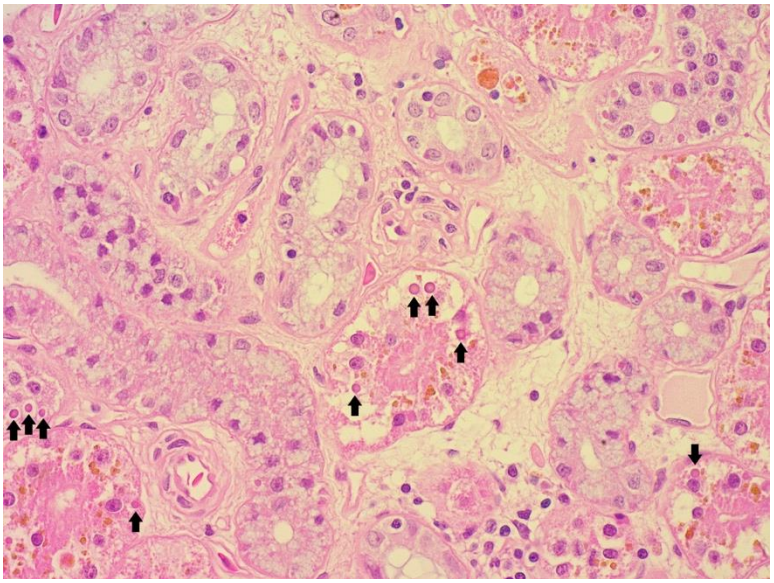
C) *Python regius*, female, juvenile. Histologic section of liver with moderate to severe macrovacuolar lipidosis. IB (arrows) are detected in large number in the hepatocytes; H&E, X40.

2.2.3 Kidneys

Figure 11.



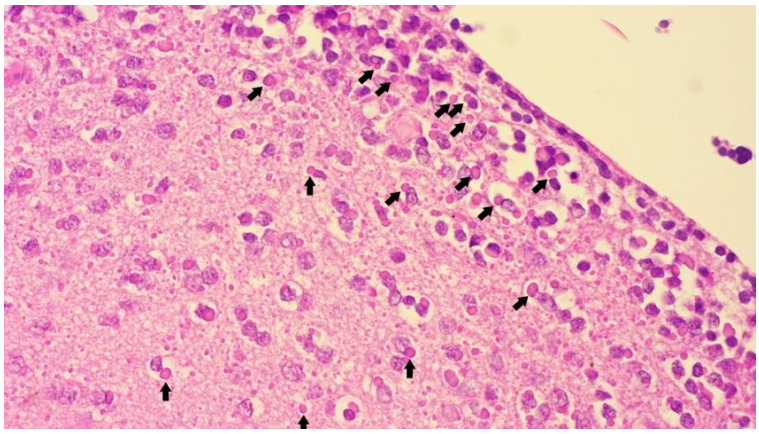
A) *Boa constrictor imperator*, female, adult with IB (arrows).
Kidney: eosinophilic, intracytoplasmic inclusions are present in tubular epithelial cells; H&E, X40.



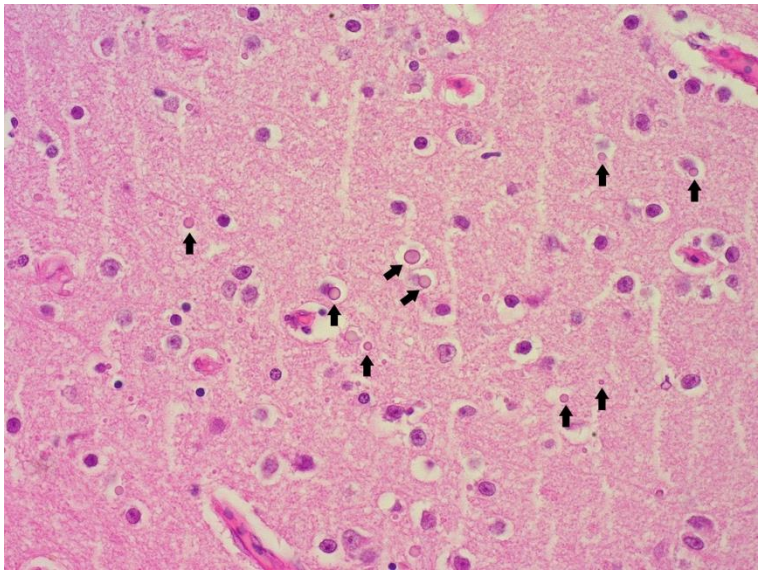
B) *Python regius*, female, adult. *Arenavirus* virologic test negative. Kidney: IB (arrows) are detected inside the cytoplasm of tubular epithelial cells; H&E, X40.

2.2.4 Brain

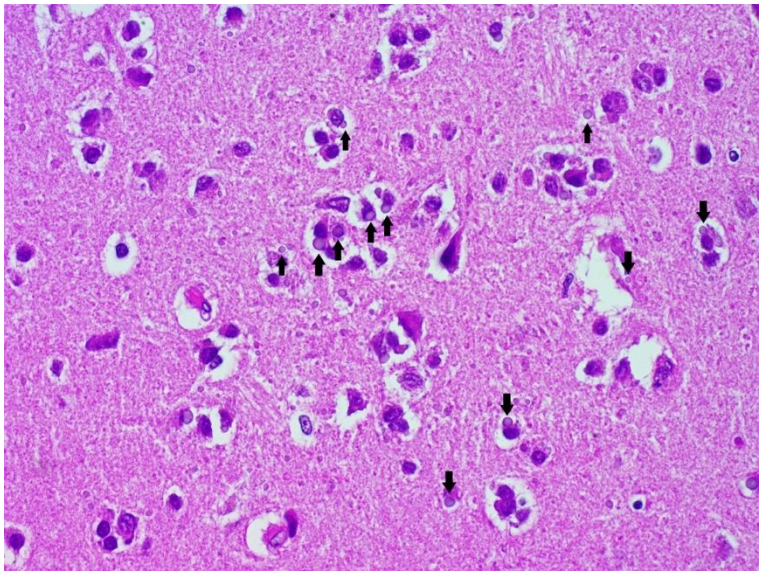
Figure 12.



A) *Boa constrictor imperator*, female, adult, positive to IBD. CNS: eosinophilic, intracytoplasmic, medium-sized inclusions found in large number inside neurons and glial cells; H&E, X40.



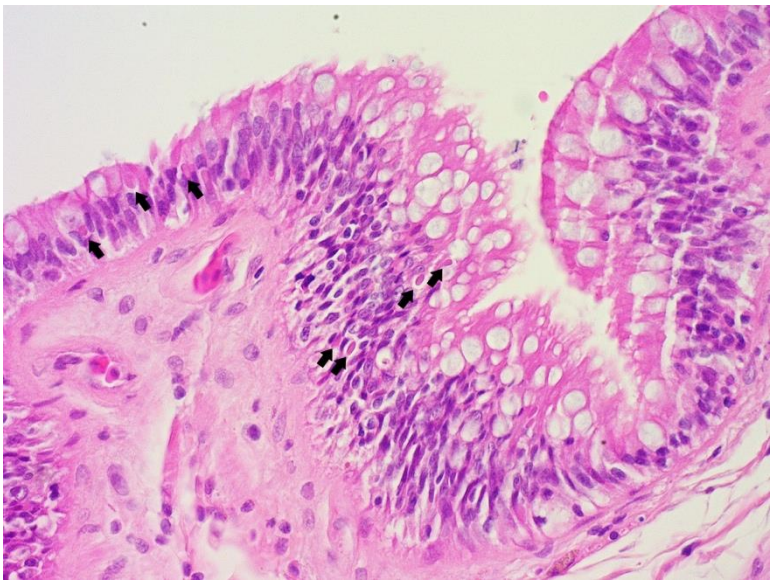
B) *Python regius*, female, adult. *Arenavirus* virology test negative.
CNS: IB (arrows) inside glial cells; H&E, X40.



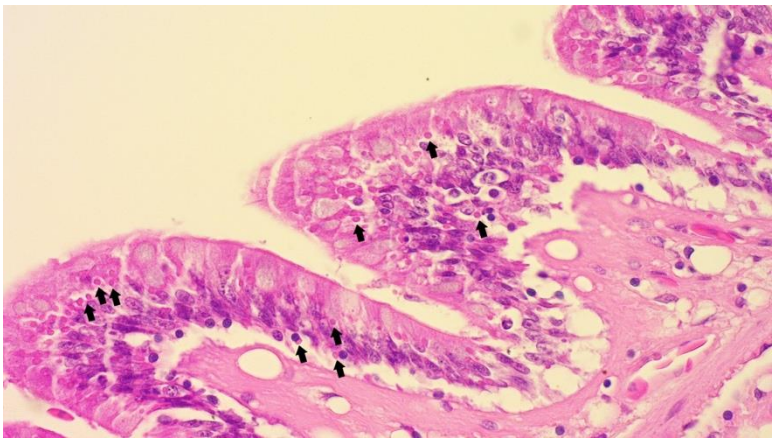
C) *Boa constrictor*, female, adult. Serological test positive for *Arenavirus*. CNS: IB (arrows) are identified inside neurons; H&E, X40.

2.2.5 Intestine

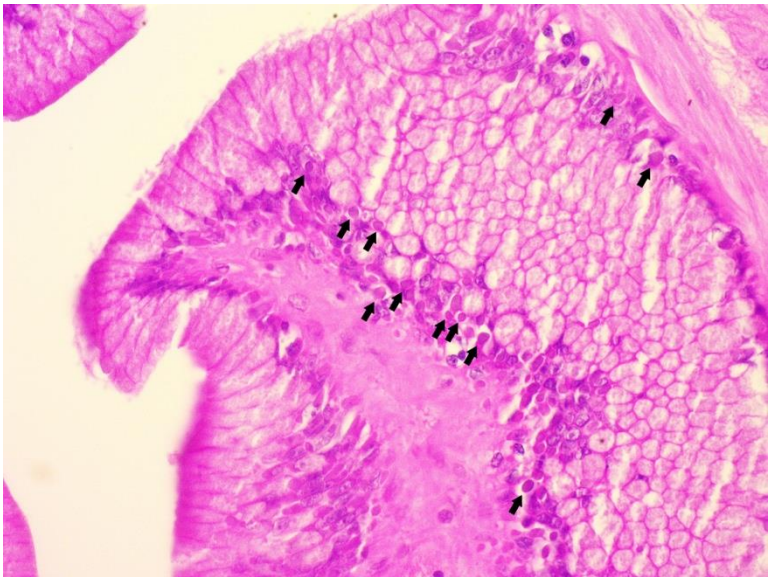
Figure 13



A) *Python regius*, female, adult**.** *Arenavirus* virologic test negative. Histology of the intestine with intraepithelial inclusions (arrows) H&E, 40X.



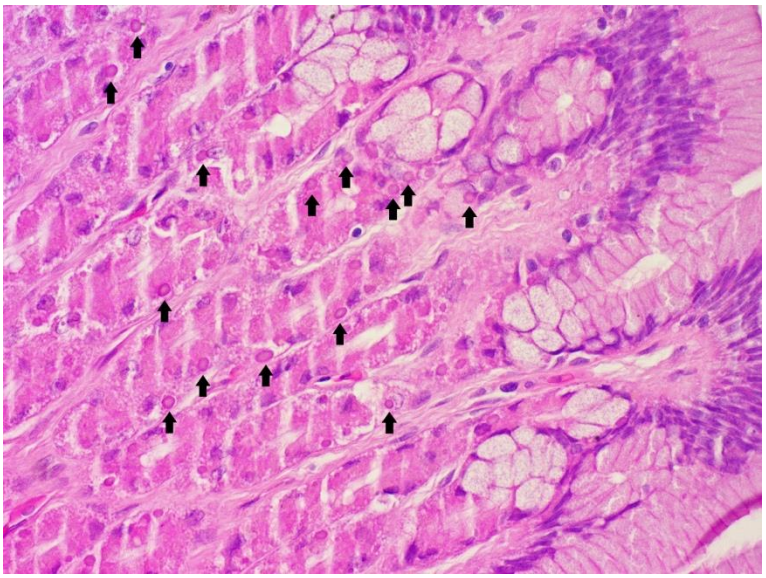
B) *Boa constrictor*, male, adult, positive to IBD. Histologic section of the intestine, where IB (arrows) are present inside the epithelial cells; H&E, 40X.



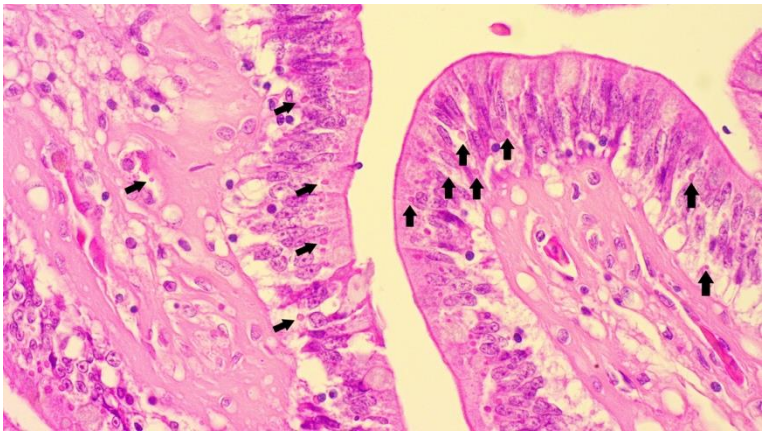
C) *Python regius*, female, juvenile. Histology of intestine shows eosinophilic inclusions (arrows); H&E, 40X.

2.2.6 Other organs

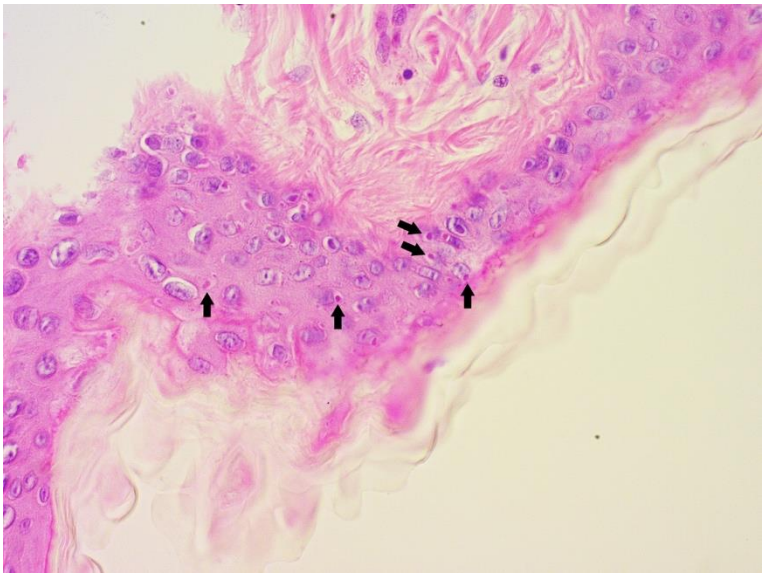
Figure 14.



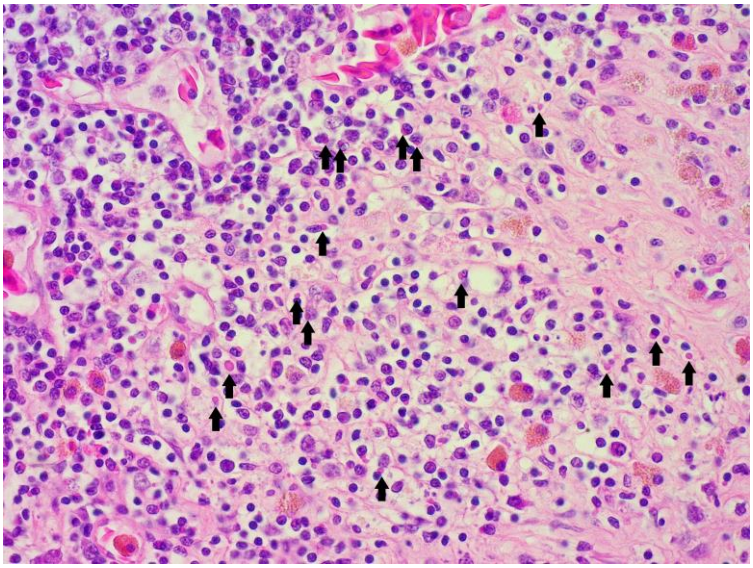
A) *Python regius*, female, adult. *Arenavirus* virologic test negative.
Stomach: IB (arrows) in epithelial and inflammatory cells; H&E, 40X.



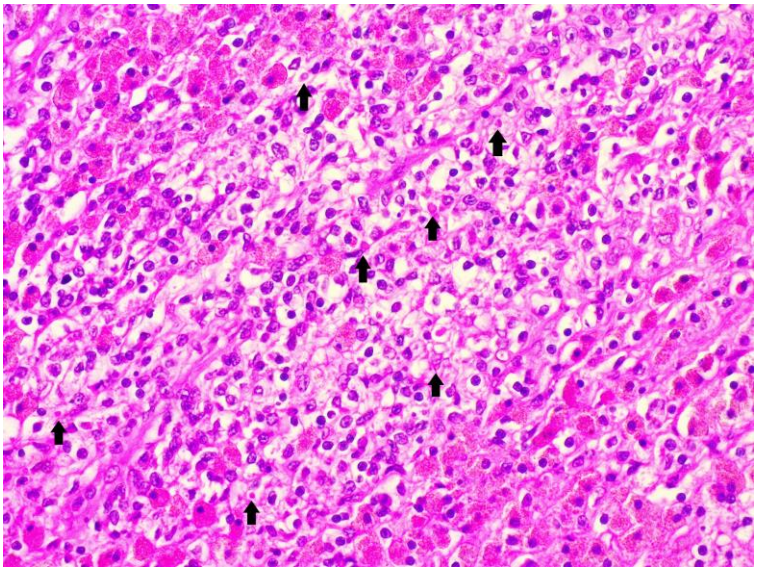
B) *Boa constrictor*, male, adult, positive to IBD.
Stomach: IB (arrows) present inside epithelial cells; H&E, 40X.



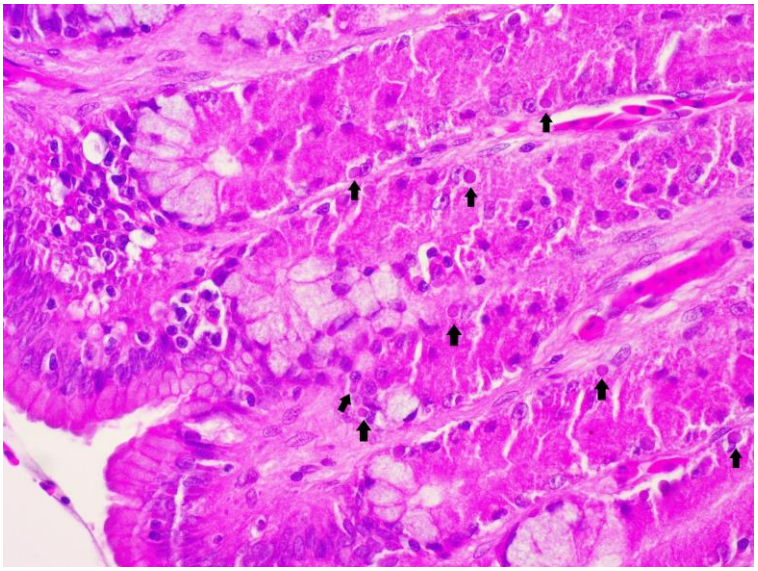
C) *Boa constrictor*, male, adult, positive to IBD and reported with dermatitis. Histology of skin shows the presence of IB (arrows) in keratinocytes; H&E, 40X.



D) *Boa constrictor*, male, adult, positive to IBD. Histology of spleen: IB (arrows) inside the lymphocytes; H&E, 40X.



E) *Boa constrictor*, female, adult. Serological test positive for *Arenavirus*. Spleen: IB (arrows); H&E, 40X.



F) *Python regius*, female, juvenile. Stomach: eosinophilic inclusions (arrows) in epithelial cells; H&E, 40X.

2.3 Parasitological, bacteriological and virological results

2.3.1 Parasitological results

A coprological exam was perfomed on feces coming from 53 snakes, and 6 tested positive to different protozoa (Table 2).

Moreover, 2/6 were also IBD-positive subjects.

Of remarkable importance is a case of a snake with progressive weight loss, the viral infection and a poor husbandry condition could have led to a secondary infection (*Cryptosporidium serpentis*), for which the animal presented the typical signs (gastritis and dilated, ectatic intestinal crypts with attenuated epithelium).

Table 2. Results of the coprological exams from the Laboratory of parasitology and parasitic diseases (UNIPD – MAPS).

Snake no.	*Species*	*Age*	*Sex*	*Coprological exam*	*ZN stain*
1	*B. constrictor imperator*	Ad	M	Neg	Cryptosporidium pos
8	*B. constrictor*	Juv	F	Coccidia pos	Neg
9	*B. constrictor*	Juv	M	Coccidia pos	Neg
10	*P. regius*	Ad	F	Coccidia pos Caryospora pos	Neg
11	*B. constrictor*	Ad	M	Caryospora pos	Neg
12	*P. regius*	Ad	F	Coccidia pos Caryospora pos	Neg

2.3.2 Microbiology results

Microbiological results come from 25 snakes. Swabs were taken from cloaca, trachea or, depending on reported clinical signs, other sites such as ureters or nostrils were tested.

The results showed positivity of 12 snakes and are referrable to various species of bacteria (Table 3).

Interestingly, 5/12 were also IBD-positive subjects.

Some of the bacteria identified (namely *Proteus vulgaris, Salmonella spp., Morganella morganii*) are thought to be part of the Gram-negative microflora of reptiles, which turn out to be pathogenic for humans.

Table 3. Results of bacteriological swabs.

Snake no.	*Species*	*Age*	*Sex*	*Swab site*	*Result(s)*
10	*P. regius*	Ad	F	Cloaca	*Pseudomonas moneilii, Providencia rettgeri, Stenotrophomonas maltophilia*
11	*B. constrictor*	Ad	M	Cloaca	*Proteus vulgaris, Pseudomonas aeruginosa*
12	*P. regius*	Ad	F	Cloaca	*Pseudomonas aeruginosa, Providencia rettgeri*
13	*B. constrictor*	Ad	M	Cloaca	*Proteus vulgaris, Pseudomonas aeruginosa*
14	*S. ametisthina*	Ad	M	Ureter-Kidney-VC	*Citrobacter freundii, Pseudomonas monteilii, Stenotrophomonas maltophila, Alcaligenes faecalis, Enterococcus faecium*
15	*P. molurus*	nd	nd	Trachea	*Klebsiella spp.*
				Lung	*Bordetella spp., Salmonella spp.*
				Nostrils	*Enterobatteriacea, Salmonella spp. S. enterica subsp. Houtenae, Morganella morganii, Bordetella spp.*
16	*M. reticulatus*	nd	F	Liver	*Klebsiella oxytoca, Citrobacter braakii*
21	*B. constrictor*	Ad	F	Trachea-Pharynx-Cloaca	*Morganella morganii, Pseudomonas aeruginosa*
22	*P. regius*	Ad	M	NA	*Acinetobacter pittii, Salmonella spp.*
23	*P. regius*	Ad	F	Trachea	*Pseudomonas aeruginosa Stenotrophomonas maltophila*
				Cloaca	*Citrobacter spp., Salmonella spp.*
24	*P. regius*	Ad	M	Trachea	*Salmonella spp.*
				Cloaca	*Morganella morganii, Salmonella spp., Citrobacter freundii Providencia rettgeri*

					Proteus spp.
25	*P. regius*	Ad	F	Trachea	*Stenotrophomonas maltophila*
				Cloaca	*Escherichia coli, Morganella morganii*

2.3.3 Virological results

Tissue samples from brain, liver, lungs, kidney, intestine and occasionally other organs (e.g. spleen and stomach) of the 53 constrictor snakes were tested by PCR. The results of 28 snakes sampled, unfortunately, are still pending (14 *Boa constrictor* and 14 *Python regius*)

Between all the animals tested (25), 11 were PCR-positive for *Arenavirus*: 4 were *Boa constrictor imperator,* 2 *Boa constrictor* and 4 *Python* regius. In 7/11 no IB were found at histology (2 *Boa constrictor imperator* and 5 *Python regius*).

Four *Python regius*, 1 *Simalia amethistina* and 3 *Boa constrictor* resulted BIBD-negative, but between these 3/8 presented IB at histology (2 *Python regius* and 1 *Boa constrictor*). In six individuals (1 *Python molurus*, 1 *Malayopython reticulatus*, and 4 *Boa constrictor*) an inconclusive result was obtained (Table 4).

Table 4. Results of virological exams from LABOKLIN.

Snake no.	*Species*	*Age*	*Sex*	*Result(s)*						
				Lungs	*Liver*	*Kidney*	*Brain*	*Intestine*	*Spleen*	*Skin*
1	*B. constrictor imperator*	Ad	M	Neg	Neg	Neg	Neg	**Pos**	Neg	Neg
2	*B. constrictor imperator*	Ad	F	**Pos (IB)**	**Pos (IB)**	**Pos (IB)**	**Pos (IB)**	**Pos (IB)**	Neg	Neg
3	*B. constrictor imperator*	Ad	F	**Pos (IB)**	**Pos (IB)**	**Pos (IB)**	**Pos (IB)**	**Pos (IB)**	Neg	Neg
4	*B. constrictor imperator*	Ad	F	Neg	**Pos**	Neg	Neg	Neg	Neg	Neg
5	*P. regius*	Ad	F	Neg	Neg	**Pos**	Neg	Neg	Neg	Neg
6	*P. regius*	Juv	F	Neg	Neg	Neg	Pos	Neg	Neg	Neg
7	*P. regius*	Juv	F	Neg	**Neg (IB)**	Neg	Neg	Neg	Neg	Neg
8	*B. constrictor*	Juv	F	Neg	Neg	Neg	Neg	Neg	Neg	Neg
9	*B. constrictor*	Juv	M	Neg	Neg	Neg	Neg	Neg	Neg	Neg
10	*P. regius*	Ad	F	Neg	**Neg (IB)**	**Neg (IB)**	**Neg (IB)**	**Neg (IB)**	**(IB)**	Neg
11	*B. constrictor*	Ad	M	**Pos (IB)**	**Pos (IB)**	**Pos (IB)**	**Pos (IB)**	**Pos (IB)**	**(IB)**	**(IB)**
12	*P. regius*	Ad	F	Neg	Neg	Neg	Neg	Neg	Neg	Neg
13	*B. constrictor*	Ad	M	**Pos (IB)**	**Pos**	**Pos**	**Pos**	**Pos**	Neg	Neg

14	*S. amethistina*	Ad	M	Neg	Neg	Neg	Neg	Neg	Neg	Neg
15	*P. molurus*	nd	nd	ND	ND	ND	ND	ND	ND	ND
16	*M. reticulatus*	nd	F	ND	ND	ND	ND	ND	ND	ND
17	*B. constrictor*	Ad	nd	ND	ND	ND	ND	ND	ND	ND
18	*B. constrictor*	Ad	nd	ND	ND	ND	ND	ND	ND	ND
19	*B. constrictor*	nd	M	ND	ND	ND	ND	ND	ND	ND
20	*B. constrictor*	Ad	F	**ND (IB)**	**ND (IB)**	**ND (IB)**	**ND (IB)**	**ND (IB)**	ND	ND
21	*B. constrictor*	Ad	F	Neg	Neg	Neg	Neg	Neg	Neg	Neg
22	*P. regius*	Ad	M	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	Neg	Neg
23	*P. regius*	Ad	F	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	Neg	Neg
24	*P. regius*	Ad	M	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	Neg	Neg
25	*P. regius*	Ad	F	Neg	Neg	Neg	Neg	Neg	Neg	Neg
26	*B. constrictor*	Juv	M	Pending						
27	*B. constrictor*	Ad	F	Pending						
28	*P. regius*	Ad	F	Pending						
29	*P. regius*	Ad	F	Pending						
30	*B. constrictor*	Juv	M	Pending						
31	*P. regius*	Juv	F	Pending						
32	*P. regius*	Ad	F	Pending						
33	*P. regius*	Ad	F	Pending						
34	*P. regius*	Juv	F	Pending						
35	*P. regius*	Ad	F	Pending						
36	*B. constrictor*	Juv	M	Pending						
37	*B. constrictor*	Ad	M	Pending						
38	*B. constrictor*	Ad	M	Pending						
39	*B. constrictor*	Ad	M	Pending						
40	*B. constrictor*	Ad	M	Pending						
41	*B. constrictor*	Juv	M	Pending						
42	*P. regius*	Ad	M	Pending						
43	*B. constrictor*	Juv	M	Pending						
44	*B. constrictor*	Juv	M	Pending						
45	*B. constrictor*	Juv	M	Pending						
46	*B. constrictor*	Juv	M	Pending						
47	*P. regius*	Ad	F	Pending						
48	*B. constrictor*	Juv	F	Pending						
49	*P. regius*	Ad	M	Pending						
50	*P. regius*	Ad	F	Pending						
51	*P. regius*	Juv	M	Pending						
52	*P. regius*	Juv	F	Pending						
53	*P. regius*	Juv	F	Pending						

2.4 Correlation between clinical signs and IBD positivity

The combination of histological, virological, coprological and becteriological tests contributes to a more thorough understanding of the health status and infections present within the study population.

The results can be summarized as follows (Table 5):

- Snakes positive by histology: 10 out of 53;
- Snakes positive by virology (PCR): 11 out of 25;
- Snakes positive by parasitology: 6 out of 53;
- Snakes positive by microbiology: 12 out of 25.

Table 5. Snakes diagnosed with IBD, which also yielded positive results in one or more of the additional tests conducted.

*Snake no.**	*Clinical signs***	*Histology*	*PCR*	*Parasitology*	*Microbiology*
1	O	Neg	Intestine (+)	Cryptosporidium	Neg
2	VD	Pos	All organs (+)	Neg	Neg
3	VD	Pos	All organs (+)	Neg	Neg
5	R	Neg	Kidney (+)	Neg	Neg
7	R	Pos	Neg but blood mite (+)	Neg	Neg
10	R	Pos	Neg	Coccidia and Caryospora	Cloaca: *Pseudomonas moneilii, Providencia rettgeri, Stenotrophomonas maltophilia*
11	O	Pos	All organs (+)	Caryospora	Cloaca: *Proteus vulgaris, Pseudomonas aeruginosa*
13	(+)	Pos	All organs (+)	Neg	Cloaca: *Proteus vulgaris, Pseudomonas aeruginosa*
21	NR	Pos	Neg	Not sampled	Trachea-Pharynx-Cloaca: *Morganella morganii, Pseudomonas aeruginosa*
22	NR	Neg	All organs (+)	Not sampled	*Acinetobacter pittii, Salmonella spp.*
23	R	Neg	All organs (+)	Not sampled	Trachea: *Pseudomonas aeruginosa Stenotrophomonas maltophila* Cloaca: *Citrobacter spp.,*

					Salmonella spp.
24	R	Neg	All organs (+)	Not sampled	Trachea: *Salmonella spp.* Cloaca: *Morganella morganii,* *Salmonella spp.,* *Citrobacter freundii* *Providencia rettgeri* *Proteus spp.*
25	R	Neg	All organs (+)	Not sampled	Trachea: *Stenotrophomonas maltophila* Cloaca: *Escherichia coli,* *Morganella morganii*

*: Orange: B.c. imperator; Red: B. constrictor; Purple: P. regius

**: VD=Vertebral column deformation; N=Neurological signs; R=Respiratory signs; +=Arenavirus positive; NR=Not Reported; O=Others

Chapter 3: Prevention, Treatment and Management

Up to date, no effective treatment has been developed against this disease.

Before acquiring or introducing a new individual in a collection, gathering information about the snake's history (including its source) and the possible contact with other snake species is fundamental. In instances where no such information is available, a thorough risk assessment should be conducted and the main way to manage an outbreak is through a strict quarantine plan (Mader D.R., 2006). Of utmost importance are the tests that must be conducted during the latter: serological test and virology (by PCR) are the main methods available to detect the virus. In this way, positive individuals can be isolated and excluded from breeding, as cases of vertical transmission have been reported in the last years (Keller et al., 2017).

3.1 Husbandry consideration: cleaning and disinfection

Immunosuppression in snakes is commonly caused by inappropriate husbandry practices by the keeper, with improper temperature and humidity, poor hygiene and excessive handling resulting in stressed individuals (Van Waeyenberge et al., 2018).

The best way to prevent the introduction of any pathogen into a collection is to have a cleaning and disinfection plan in place that is organized to cause the least amount of stress to the individuals present, but effective in terms of timing and products used.

In addition, from an organizational standpoint, it is important to keep in mind that the research associated with IBD is still evolving and we still do not know for sure how many and which species may be affected. Therefore, it is good practice to keep boas and pythons separated from each other, but especially from snakes of other families (such as vipers) because they may serve as carriers of the disease (Mader D.R., 2006).

In any collection or facility, it is of paramount importance to have a written record of all the animals kept, as it is the best way to become aware of the changes that are taking place. Snakes can take from six to twelve months to develop clinical signs, which is why monitoring the health of the individuals in the collection can help identify an underlying problem (NSW Dept. of Environment and Conservation, 2004).

For this reason, it is appropriate to report everything you know about the animal (species, breed, age, sex, reproductive status, CITES or microchip), details and dates regarding

medical history (e.g. vaccination status or whether the animal is under current medication and any problem related to it), and finally diet-related information.

The first thing to consider is your personal hygiene, which will help in reducing the risk of spreading the disease. Wear gloves or at least wash your hands thoroughly with warm water and soap after handling animals or cleaning their cages or equipment used for their housing. Cover any cuts or open wounds before coming in contact with the animals. If they become contaminated, you are bitten by a non-venomous snake, or they scratch your skin, wash your hands again with warm water and antibacterial soap, rinse, and apply antibacterial skin treatment.

Keep reptiles and their enclosures away from human food preparation and consumption areas (NSW Dept. of Environment and Conservation, 2004).

Regarding the animals themselves, it is crucial that you have a well-organized cleaning plan in the facility. As a good practice, cages should be cleaned of feces and other solid waste on a daily basis.

Moreover, enclosures should be thoroughly cleaned at least weekly, and an example of a routine may be as follows (NSW Dept. of Environment and Conservation, 2004):

1. Remove the animal and place it in a temporary cage that has been previously cleaned and disinfected, such as a plastic box with rounded corners.
2. If you have a bioactive terrarium, cleaning should be done at least every 6 months. Otherwise, pet owners normally choose between natural soil or artificial substrate (e.g. paper towels or newspaper). In the first case, replace the soil and throw it in a closed container, and never use it for composting, since it could encourage the spread of pathogens. Whereas in the case of paper, remove it carefully without scattering your animal's waste and replace it.
3. Clean the cage meticulously with warm water, soap (e.g. dish soap) and a brush. Throw away anything that cannot be sanitized and reused or that is too old.
4. Rinse and then disinfect (more commonly used and effective products will be listed below). The cage and the materials should remain in contact for at least 15 minutes.
5. Finally, rinse with tap water, allow to dry and, if possible, leave in the sun for a few hours before putting new substrate and animals back inside.

When selecting a disinfectant, determination of what organisms or pathogens are targeted is necessary. More than one may be needed to prevent the spread of a disease. Halogen or halogen-containing compounds, iodophor solutions, chlorine, and phenol-based compounds are all effective disinfectants against viruses.

Disinfectants should be mixed following the manufacturer's instructions: too dilute concentrations are inefficient, while ones too strong may be difficult to rinse, toxic and expensive.

Ammonia and bleach are normally the choice as they are readily available disinfectants and at an affordable price. Ammonia can be used undiluted (full strength) and bleach should be diluted to a working concentration of 5% (50mL to 1 L of water). Either of them is adequate to kill most common pathogens.

Ammonia can be rinsed with water. Chlorine bleach can be neutralized by adding dechlorinator to water or by placing items in direct sunlight for a few hours (sunlight deactivates chlorine) (Mader D.R., 2006).

As mentioned above, the snake mite (*Ophionyssus natricis*) (Figure 15) might be involved in the transmission of IBD, therefore if it is present in your collection rapid action should be taken. The mites may also be transmitted to humans, where it is known to cause skin hypersensitivity (Mader D.R., 2006).

Treatment protocols for the control of this parasite must address a variety of factors before initiating any therapy: the condition and the number of the animals treated, the caretaker's budget, and the area of the infested area. The complex life cycle of the mite (some stages on the animal, others free-living in the environment) and its intense migratory ability make it difficult to eradicate and control it.

Housing for mite-infested animals should be kept as simply furnished as possible.

Figure 15. Snake blood mite, *Ophionyssus natricis.* (Vancraeynest et al., 2006)

Enclosures should be emptied, scrubbed in depth, and refurbished at least twice per week. Hide boxes, substrate and all the furniture should be made of disposable, non-porous material and minimize the microhabitat sought by the parasites.

Waste products from infected individuals should be bagged, sealed, treated with insecticides, incinerated, autoclaved and totally removed from the facility.

A pyrethrin or organophosphate-containing insecticide could be used, but never be applied to live animals. Surfaces must be allowed to dry and air out for a minimum of 24 hours before the return of live animals (Mader D.R., 2006).

3.2 Quarantine protocols

The main goal of quarantine in your collection is to prevent the virus from entering the collection, therefore the key is to start with a strict hygiene protocol.

From an organizational point of view, it is best to have a building separated from the main collection, with a footbath at the entrance. If this is not possible, there should be no air exchange between the quarantine area and where the collection is. In addition, the instruments should not be shared between facilities and, most importantly, everything must be washed and sanitized after handling each animal.

When economically feasible, each animal in quarantine should have its personal equipment. Personal hygiene is another fundamental issue to consider (NSW Dept. of Environment and Conservation, 2004).

The most indicated substrate for quarantined animals is paper, since it is easier to detect signs of disease, parasites or any abnormality.

Although a period of three months is recommended for all reptiles, Boidae and Pythonidae should be observed for a minimum of six months, especially in the case of large and valuable collections (NSW Dept. of Environment and Conservation, 2004).

Snakes with poor body condition, lethargy and persistent infections should never be housed together with other individuals, even if diagnosis is inconclusive.

At both the beginning and at the end of quarantine, the veterinarian should perform a complete physical examination, analyze fecal samples and collect venous blood samples for hematologic and plasma biochemistry determinations (Mader D.R., 2006).

All the individuals that die during this period or confirmed cases of IBD that have been euthanized should be necropsied, and samples from major organs should be collected for histopathology and future studies.

All the equipment that has been in contact with positive individuals should be carefully disinfected or discarded at the end of quarantine (NSW Dept. of Environment and Conservation, 2004).

3.3 Supportive care

Regrettably, there is no effective treatment against inclusion body disease, reason why the options for positive individuals within a collection are limited: attempts to alleviate the suffering may involve administering fluids, antibiotics to address secondary infections, and force-feeding (Mader D.R., 2006).

It's insightful to bring in perspectives from veterinary professionals involved in the study of the disease. One of these veterinarians agreed to answer some questions regarding the complexities associated with IBD in snakes. The observation that bacterial and protozoal infections are frequently found in conjunction with IBD positivity aligns with the understanding that immunocompromised individuals are more prone to secondary infections (Simard et. al, 2020). The approach of performing bacteriological swabs with antibiogram as well as collecting fecal samples and identifying possible parasites is a comprehensive strategy. This allows for individualized treatment plans that address both bacterial and protozoal infections. Combining antibiotic coverage, like fluoroquinolones, with protozoal drugs such as metronidazole reflect a multimodal approach. Furthermore, if the presence of *Cryptosporidium* is confirmed, the veterinarian suggests the use of paromomycin as an excellent choice in treating the affected animal.

Hydration and nutrition should be ensured with the use of gastric esophageal probing, which in this species is particularly smooth and easy to perform. Intracoelomatic fluid therapy is another option, but it should be reserved for severe cases or in situations in which the other route is not practical.

It is also common experience to supplement animals' diet with vitamin C (ascorbic acid) and B-complex to strengthen the immune response.

Chapter 4: Impact on captive snake populations

4.1 Economic implications

Global demand for exotic pets has created a thriving industry and, among the array of unconventional companions, snakes have secured a high-profile place in the hearts and homes of enthusiasts.

The main factor that determines the variation in prices in snake species is the morph, or in other words, the color or pattern that characterizes the skin. However, the distinctiveness of these morphs is influenced by several interconnected drivers. Market demand plays a key role in determining the commercial value: rare and highly sought-after morphs tend to have higher prices, which can then decrease when they become more readily available. In addition, genetic selection and breeder efforts contribute to uniqueness and desirability of a morph. Combination with recessive genes are often difficult to obtain and do not always yield positive results. Some morphs may present management difficulties, such as refusing meals during developmental stages.

Data obtained from a survey submitted to fifty breeders and hobbyists shed light on the general expenses enthusiasts face in owning snakes and the potential losses in the event of infection like *Reptarenavirus.* On average, a breeder with a collection of 150 snakes spends between 10,000 and 15,000 euros annually. This includes veterinary control expenses, thawed food, maintenance of display cases/racks (substrate, temperature and humidity control devices, electricity), and minor expenses like cleaning products and enrichments.

For private individuals with around 4 snakes, maintenance costs range from 600 to 1,000 euros per year.

The major difference lies in the acquisition of individual specimens for privates, with pythons ranging from 50 to 20,000 euros, and boa species ranging from 30 to 5,000 euros.

The survey reveals that 75% of respondents consider annual veterinary visits necessary, and 60% conduct follow-up tests for new acquisitions.

A significant challenge highlighted is the perception among reptile breeders that veterinarians may lack specialized knowledge acquired by them through direct experience. Reluctance to consult them may stem from concerns about the generalist nature of veterinary training and potential gaps in understanding reptile needs. As a

result, many breeders prefer a “do-it-yourself" approach or rely on word-of-mouth advice.
The veterinarian emphasizes the need for veterinary professionals to be up-to-date, knowledgeable, and professional. Providing breeders with a well-structured proposal that includes preventive expenses, timing of testing, and protocols in case of positivity/negativity can help break down the barriers between these two worlds and establish a common path for the proper handling of these animals.

4.2 Welfare and ethical consideration

Dealing with positive individuals for conditions like *Reptarenavirus* is a complex matter without a universally agreed-upon approach. There is consensus that positive individuals, deemed unsuitable for breeding and cohousing, present a challenge due to the highly variable susceptibility of snakes to etiological agents. Some positive snakes may be in good health, asymptomatic and capable of giving birth to healthy offspring, leading to the consideration that, over time, they might become negative or, at least, find a balance with the virus.

In the discussion led by the veterinarian, it is proposed that in cases where the snake is healthy and asymptomatic, a viable solution could be to transfer the animal to private individuals. This involves transparent communication about its condition. However, when faced with a visibly ill boa or python, where poor quality of life and the risk of spreading disease to other snakes are significant concerns, the difficult decision of euthanasia may be deemed more “acceptable”, albeit emotionally challenging.
The study of BIBD is an ongoing process, and active engagement with universities and research facilities, dialogue with specialists and involvement of breeders in the process are seen as vital components.
“Collective contributions to understanding the disease is the only one possible key for getting to the bottom of how these etiological agents move in different species of reptiles. Growing together, veterinarians and owners is the high road to modern, profitable captive breeding that respects the overall well-being of these beautiful animals”.

Conclusion

This thesis aims to present and analyze data collected on postmortem performed on snakes reported to the pathology group of the BCA Department from 2019 to the present with the specific goal of identifying signs of Inclusion Body Disease (IBD). The subjects involved exhibited nonspecific symptoms for this disease, with only a few displaying the typical neurological signs.

As for the presence and the histological appearance of the inclusion bodies (IB) one of the possible explanations could be that the size and shape of the IB could be correlated with the duration of the disease. The inclusions are characterized as membrane-bound aggregates of amorphous to granular material mixed with fragments resembling membranes. In this light, the formation of inclusions might be a gradual process occurring as the infection progresses rather than an immediate or early symptom.

As mentioned earlier, individuals testing positive for IBD, and consequently considered immunocompromised, are more vulnerable to secondary infections and/or parasitism. This susceptibility may account for the presence, in our case series, of protozoa such as Cryptosporidium, Caryospora, and bacteria such as *Pseudomonas aeruginosa* notably known to induce diseases in immunocompromised individuals (Ciobotaru et al., 2009). Histologically, evidence of pneumonia, pulmonary edema and hepatic lipidosis are observed in the snakes affected by this bacterium.

In our cases, it seems that the correlation between histological and virological positivity is unremarkable underlying the fundamental importance of implementing the diagnostic tests to achieve a more accurate diagnosis in every stage of the disease.

Solving this issue is not solely the responsibility of researchers and veterinarians. Establishing an open and transparent channel of communication with breeders, actively involving them in this effort, is the key to making a meaningful impact and guaranteeing a dignified life for these animals.

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