UNIVERSITI PUTRA MALAYSIA

MACROSCOPIC AND MICROSCOPIC LESIONS OF SPECTACLED CAIMAN *(CAIMAN CROCODYLUS)* WITH RESPIRATORY DISEASE

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Macroscopic and Microscopic Lesions in Spectacled Caimans (*Caiman crocodilus)* with Respiratory Disease", by Nurul Fathiah Binti Muhammad Husairy and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD4999 – Final Year Project.

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Table of Contents

ABSTRAK

Abstrak daripada kertas projek yang dikemukankan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek.

LESI MAKROSKOPIK DAN MIKROSKOPIK DALAM BUAYA (*CAIMAN CROCODYLUS*) DENGAN PENYAKIT PERNAFASAN

Oleh

Nurul Fathiah Binti Muhammad Husairy

2023

Penyelia : Profesor Madya Dr. Annas Salleh

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Selama bertahun-tahun, *spectacled caiman* telah dianggap sebagai haiwan yang digeruni. Ini secara beransur-ansur berubah kerana kepentingannya dalam ekopelancongan dan kepentingan ekonomi. Industri penternakan buaya di Malaysia adalah salah satu yang penting dari segi ekonomi kerana pengeksportan barangan kulit

yang boleh dipasarkan. Selain itu, banyak taman tema dan taman hidupan liar mempunyai *spectacled caiman* ini dalam pameran mereka untuk tujuan pendidikan dan pemuliharaan. Penyakit pernafasan adalah salah satu penyakit paling lazim yang menjejaskan *spectacled caiman* dalam kurungan. Ini disebabkan oleh keadaan tekanan yang dihadapi oleh caiman seperti kepadatan yang tinggi, tahap pH yang tidak bersesuaian, dan suhu serta kemasinan yang membawa kepada banyak kematian. Untuk memahami kesan penyakit pernafasan dalam *specacled caiman* (*Caiman crocodylus*), empat ekor *spectacled caiman* yang telah mati dari sebuah taman tema telah dipilih. Nekropsi telah dijalankan untuk memeriksa lesi kasar dan sampel organ kemudiannya diproses secara rutin, diwarnakan menggunakan haematoxylin dan eosin dan dilihat di bawah mikroskop. Antara sampel yang diperoleh ialah mata, kelopak mata, paru-paru, trakea dan epiglotis. Secara makroskopik, paru-paru dan kulit adalah organ yang paling terjejas di kalangan sampel dengan skor lesi tertinggi 2.33 (n=3) untuk paru-paru dan 2.5 (n=4) untuk kulit. Dalam salah satu caiman, secara kasarnya, tanda-tanda klinikal tidak sejajar dengan sampel lain yang mencadangkan kematian akut kerana skor badannya yang baik. Secara mikroskopik, terdapat kemusnahan dinding alveolar dan fibrosis dalam paru-paru, gangguan pada lapisan sel trakea, serta vakuolasi dan fibrosis pada mata dan kelopak mata. Siasatan lanjut diperlukan untuk memberikan gambaran tentang kes ini. Namun begitu, berdasarkan penemuan ini, lesi yang disebutkan di atas mencadangkan Poxvirus, Chlamydia dan Herpesvirus sebagai agen penyebab. Kajian ini penting untuk menambah baik habitat di mana caiman berkaca mata ini disimpan untuk mengurangkan kematian yang tidak diingini yang membawa kepada kerugian ekonomi

Kata kunci: Buaya (Caiman crocodylus), penyakit pernafasan, makroskopi, mikroskopic

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD4999 – Final Year Project.

MACROSCOPIC AND MICROSCOPIC LESIONS OF SPECTACLED CAIMAN *(CAIMAN CROCODYLUS)* WITH RESPIRATORY DISEASE

by

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2023

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Co-supervisor: Dr Mohd Akmal Mohd Noor

For years, spectacled caimans have been perceived as a feared animal, this has gradually changed due to its importance in ecotourism and economic importance. The crocodile farming industry in Malaysia is one of economically important due to the exportation of marketable leather goods. Additionally, many theme parks and wildlife parks have these spectacled caimans in their exhibits for educational and conservation purposes. This research is being carried out due to the lack of data in Malaysia as compared to neighbouring countries such as Thailand. Being said that, respiratory diseases are one of the most common diseases affecting spectacled caimans in captivity. This is due to the stressful conditions the caimans are subjected to such as increased density, poor pH levels, temperature and salinity leading to numerous deaths. To understand the impact of respiratory diseases in spectacled caiman (*Caiman crocodylus*), four spectacled caimans from two different habitats, one a crocodile farm and another a theme park exhibit were selected. Necropsy was carried out to examine gross lesions and organ samples were then routinely processed, stained using haematoxylin and eosin and viewed under magnification. Among samples acquired were the eye, eyelid, lungs, trachea and epiglottis. Macroscopically, the lungs and skin were the organs most affected among the samples with the highest lesion scoring of 2.33 (n=3) for the lungs and 2.5 (n=4) for the skin. In one of the caimans, grossly, the clinical signs did not align with the rest of the samples suggesting an acute death due to its good body score. Microscopically, there was

alveolar wall destruction and fibrosis in the lungs, disruption in the trachea cell layers, as well as vacuolation and fibrosis in the eyes and eyelids. Further investigation is needed provide insight into this case, however based on our findings, the lesions mentioned above suggest Poxvirus, Chlamydia and Herpesvirus as the causative agents. This study is important to improve the habitat in which these spectacled caimans are kept in to reduce unwanted deaths leading to economic losses.

Keywords: *Common caiman (Caiman crocodylus), respiratory disease, macroscopic, microscopic*

1.0 Introduction

1.1 Background of Study

There is a known worldwide distribution of crocodilian species scattered across the continents. From the Alligatoridae family, the spectacled caiman (*Caiman crocodilus*) with a length of 1.4 - 2.5 m and a weight ranging from 7 - 40 kg. The name comes from the appearance of spectacles due to a bony ridge between its eyes. In captivity, they are often affected by stress and poor living conditions in their habitat. This leads to a plethora of diseases such as chlamydia, herpesvirus, and poxvirus characterized by respiratory conditions.

The respiratory system of spectacled caiman has well-developed lungs consisting of intercostal muscles and diaphragm muscles that act as a divider between the liver and the lungs called septum post hepaticum.

Among viruses that cause respiratory diseases in the spectacled caiman are chlamydia virus, pox virus and herpesvirus. Chlamydial infections in crocodiles are often presented as conjunctivitis, hepatitis, and pharyngitis affecting the liver, heart, kidney, spleen, and entire respiratory tract in spectacled caimans.

The most prominent sign of chlamydia is seen as eyelid swelling and discharge from the eye itself observed as fibrin. Similarly, the same material is seen at the base of the tongue and pharynx. Morbidity and mortality rates are high in juvenile spectacled caimans less than 6 months of age with chlamydiosis (Jerrett et. al., 2008).

Airway accumulation of fibrin material leads to the blockage of the airway causing death. There are nine known species of Chlamydiaceae affecting a wide range of hosts (Everett et all, 1999). Histopathologically, hepatic thrombosis, histiocytic interstitial pneumonia, and necrotizing lymphohistiocytic myocarditis were observed.

Poxvirus is presented as a double-stranded DNA virus causing papillomatosis. In captivity, poxvirus is seen mainly in juvenile crocodiles. However, in adult crocodiles, it is latent until subjected to stressful conditions (Nevarez, 2009). Transmission occurs through direct contact with skin lesions, water, feed, and contaminated fomites.

Macroscopically, pox-like lesions appear on the head, gingiva, palate, maxilla, mandible, and limbs. Occasionally, other lesions such as wart-like nodules that may appear shallow or raised are present. Histopathologically, Bollinger and Borrel's bodies, hyperkeratosis, acanthosis, and necrosis are observed (Nevarez, 2009).

In the spectacled caiman, herpesvirus is presented as Crocodyline herpesvirus (CrHV – 1) with conjunctivitis and pharyngitis syndrome as well as Crocodyline

herpesvirus (CrHV – 2) with systemic lymphoid proliferation, nonsuppurative encephalitis and lymphonodular skin lesions. Other than that, corneal opacity and thickening, oedema and reddening of nictating membrane and conjunctiva with mucopurulent exudate. Microscopically, aggregates of lymphocytes and macrophages are present in the dermis layer. Hyperplasticity and numerous lymphocytes and macrophages are also observed in the tonsils.

These respiratory diseases are not as well-documented in reptiles, especially those in captivity and the pathogenesis and pathophysiology remain unclear. Thus, more studies are carried out to aid in understanding the histopathological lesions of respiratory diseases in specifically spectacled caimans. This study is aimed to assess the histological features of the respiratory system using hematoxylin and eosin stain.

1. 2 Problem Statement

There have been many recent deaths in spectacled caiman crocodiles in captivity. This study uses samples from a crocodile farm in Parit Jawa, Muar, Johor, and a theme park in Ipoh, Perak by the name of The Lost World of Tambun. This study aims to evaluate and identify lesions that are specific to respiratory diseases such as chlamydiosis, poxvirus, and herpesvirus. Additionally, there has not been much documentation regarding respiratory diseases in crocodilians in Malaysia, specifically in spectacled caimans.

1.3 Justification of the study

This study is carried out to provide more information regarding respiratory disease in crocodilians in Malaysia specifically the spectacled caiman. With this further understanding, we aim to improve the crocodile farming industry and wildlife parks in Malaysia.

1.4 Objectives of the study

This research aims to describe and score the severity of the macroscopic lesions of spectacled caimans with respiratory disease and describe the severity of the microscopic lesions of spectacled caimans with respiratory disease.

1.5 Hypothesis

H_0: All of the caimans will show macroscopic and microscopic lesions consistent with respiratory disease in crocodilians and reptiles.

H_A: All of the caimans will show different macroscopic and microscopic lesions compared to the previously reported reptiles and crocodilians with respiratory disease.

2.0 Literature review

2.1 Spectacled Caiman *(Caiman crocodilus)*

The spectacled caiman comes from the alligatoridae family consisting of alligators, caimans and their extinct relatives. It is usually greenish, brownish in colour and has a spectacle-like ridge between their eyes which gives it its name. The spectacled caiman can grow up to 2.5 metres in length and weigh up to 40kg with males being heavier than the females. It is amongst the smallest in the crocodile family as it does not grow as big as it's other counterparts.

They are native to Latin America countries such as Brazil and Peru and has been introduced to countries in Asia, America and Australia (Balaguera-Reina & Velasco, 2019) for many purposes such as conservation and economic purposes. Being a carnivorous animal, they usually hunt at night with a diet consisting of small mammals,

crustaceans and small reptiles such as lizards, turtles and snakes. They usually reside in forests, inland bodies of water and the savannah and is intolerant to cold weathers.

According to the International Union for Conservation of Nature (IUCN), spectacled caimans are of lower risk in the Red List of Threatened Species (Balaguera-Reina & Velasco, 2019). Being said that, upon captivity, respiratory signs are reported to be one of the most common presentations in captive crocodilians. Among pathogens responsible are *Chlamydia spp*., poxvirus and herpesvirus.

Figure 1 : Spectacled caiman *(Caiman crocodilus)* (Source: St. Edward's University (2006). *The Spectacled Caiman (Caiman Crocodilus)*

2.2 Diseases of Crocodilians

There are many underlying factors affecting crocodilians in captivity such as immunosuppression and stress. Stress is defined as any event that results in a change in homeostasis (Nevarez, 2020). Among stress factors that affect crocodilians in captivity may vary from excessive noise and handling, diet changes, air and water quality, temperature, and overcrowding (Nevarez, 2020). Diseases in crocodilians may be presented as systemic, dermatological, neurological, respiratory, musculoskeletal, gastrointestinal, and ocular signs.

Studies have shown that the first signs of illness observed in captive crocodiles are non-specific such as lethargy, anorexia, and subsequently death (Nevarez, 2020). Additionally, the captive environment results in runting which by definition is varying growth rates and failure of some individuals to thrive in the environment. This is due to the lack of fitness and hardiness in captivity.

Dermatological lesions in captive crocodilians occur secondary to factors associated with management issues such as poor water quality and poor enclosure design (Nevarez, 2020). The presence of lacerations and breaking of the skin barrier acts as a nidus for infections by bacteria and fungi. Additionally, enclosures that are not routinely cleaned result in a fatty, slimy layer on the water surface.

Interestingly, respiratory signs are one of the most common presentations in captive crocodilians (Nevarez, 2020). This study focuses on the respiratory signs in captive crocodilians. Respiratory signs in these individuals can be characterised as excessive basking, dyspnea, tachypnea, abnormal swimming, and anorexia (Nevarez, 2020). Often times, neurological signs may accompany these respiratory signs.

Musculoskeletal disease in captive crocodilians occur due to either trauma from altercations presented as fractures and partial amputations or changes in environment or incubation temperature. Progression of these lesions often leads to muscle or nerve damage and consequently paresis or paralysis (Nevarez, 2020).

Ocular signs have been reported in cases of chlamydiosis characterized by keratitis and conjunctivitis. Oxytetracycline is effective in these cases however, antimicrobial resistance poses a risk factor and more importantly and safety of meat from these individuals (Nevarez, 2020).

2.2 *Chlamydia spp.*

Chlamydia exists as an obligate intracellular bacterium and a gram-negative coccus. Morphologically, there are two distinct forms of Chlamydia spp. being the infectious form called elementary bodies and the intracytoplasmic, reproductive form

called reticulate bodies (Inchuai, 2022). The elementary bodies will then develop into non-infectious form which are the reticulate bodies that converts back to elementary bodies and subsequently released for infection of other cells.

There are Chlamydia sp. which are host specific such as *C. abortus* in small ruminants. *C. suis* in pigs and *C. felis* in cats. In crocodiles, *C. psittaci, C. caviae,* and *C. pneumoniae* are prevalent with high morbidity and mortality, especially in juveniles. Among the risk factors were seasonal changes, breeding systems, for C. abortus, hygiene, and population density in birds for *C. pstitacci* (Santos et al., 2014).

Chlamydiae attach to cells using hemagglutinin. Inflammation then occurs due to the response of cell-mediated immunity resulting in tissue damage. Since they are unable to metabolize energy, they depend on their host cell for ATP supply and other intermediates. The cell structure of chlamydiae consists of the DNA, RNA, cell wall, and ribosomes.

Chlamydiosis affects a range of hosts ranging from humans, reptiles, mammals, amphibians, and fish. Chlamydia spp. are able to cross the natural host barriers due to its ability to acquire new DNA from the environment and replicate to spread to surrounding cells as a form of self-protection. Transmission occurs through direct contact, fomites, and contaminated feed and water (Edling, 2023).

In crocodiles, clinical signs are manifested as bilateral conjunctivitis, pharyngitis and fibrin-like material in the oral cavity and pharynx. The fibrinous exudate in the pharynx leads to airway obstruction and eventually death. There is a high morbidity level (67%) and low mortality level (29%) in juveniles less than 6 months old recorded in previous cases (Jerrett et. al., 2008). In yearlings and growers, similar conjunctival and oropharyngeal lesions were observed.

Additionally, studies have shown that growers are reservoirs of chlamydia species posing a threat to hatchlings and juveniles resulting in possible disease outbreaks. However, further research into epidemiology factors in the general wild crocodile population will provide more insight into identifying the potential host range of chlamydia. Detection of all members of *Chlamydiaceae* is carried out through polymerase chain reaction (PCR) as it has a higher sensitivity than other immunological detection methods (Edling, 2023).

2.3 Poxvirus

From the Poxviridae family, poxvirus exists as a double-stranded DNA virus that is enveloped. The causative agent of poxvirus lesions in crocodiles belongs to the genus *Crocodylidpoxvirus*. Among crocodilian species that have been reported with poxvirus lesions include *Caiman crocodilus yacare, Crocodylus niloticus, Crocodylus johnstoni,*

Crocodylus porosus and Caiman crocodilus fuscus (Sarker et. al., 2019). Often times, juveniles and hatchlings are affected and show clinical signs whereas in adult crocodiles, it is latent and shows clinical signs when in stressful conditions (Nevarez, 2020).

Transmission occurs through direct contact, fomites, and contaminated feed and water. Replication of poxvirus occurs in cytoplasmic viroplasms of the epithelial cells of the host. There are four developmental stages of lesions which are early active, active, expulsion and the healing stage (OIE, 2020).

During the early active stage, inclusion bodies and grey-white foci are observed. During the active stage, grey-white foci that appear raised are seen accompanied by inclusion bodies. At the expulsion stage, foci, inflammatory cells, and hyperkeratosis are observed (Sarker et. al., 2019). Lastly, foci and hyperkeratosis are observed in the healing stages. The pathogenesis is not known as a whole. During these stages, the poxvirus lesions do not enter the basement membrane layer of the epidermis (Sarker et.al., 2019) hence the quality of the finished leather product is not affected in the process given enough healing time.

2.4 Herpesvirus

Herpesviridae are DNA virus which are large and eveloped and high host fidelity. Transmission occurs through close contact, specifically mucosal via aerosol or vertical. It is usually latent, and persists in neurons and lymphocytes of the host. Often times, clinical signs are exhibited in young and immunocompromised individuals and those in stressful conditions (Shilton, 2016). Upon infection, it is lifelong and often subclinical. In reptiles such as lizards, upon herpesvirus infection results in chronic proliferative stomatitis, acute or necrotizing, pharyngitis, stomatitis and tracheitis.

From the Herpesviridae family, crocodyline herpesvirus exists as two types. Firstly, crocodyline herpesvirus (CrHV-1) whereby conjunctivitis and pharyngitis are observed (Shilton, 2016). Secondly, crocodyline herpesvirus (CrHV - 2) whereby there is systemic lymphoid proliferation with nonsuppurative encephalitis and lymphonodular skin lesions. The pathogenesis is affected by specific and non-specific host defense mechanisms. Transmission occurs through direct contact, fomites, and contaminated feed and water (Shilton, 2016). Initially, the virus replicates in the epithelial cells which eventually spread to the other vital organs.

Lesions are observed in the oral cavity, skin, liver, gastrointestinal system and respiratory system (Shilton, 2016). In hatchlings, lesions in hatchings were first characterised by conjunctivitis and/or pharyngitis. Reddening and swelling of the

conjunctivae of nictating membrane and eyelids are observed in crocodilians infected with herpesvirus. Additionally, fibrinocaseous exudate was also present in the oropharynx of the crocodile (Paungpin et. al., 2021). Polymerase chain reaction is carried out as a diagnostic test to identify the herpesvirus.

2.5 Economic Importance

In Malaysia, crocodile farming, crocodile conservation sites, and recreational zoos are places where the spectacled caiman crocodiles are found. However, being said that, in captivity, they are often placed under stressful conditions that challenge the homeostasis of the animal leading to immunosuppression (Nevarez, 2020). Some infectious agents are latent in the individual and become active due to stress.

A common cause of death in juvenile spectacled caimans in captivity is due to respiratory issues caused by a variety of agents such as Poxvirus, Chlamydia, and Herpesvirus. This directly affects the economy of the country as these crocodiles are bred for educational and tourism purposes.

Additionally, crocodile farming in Malaysia exports meat and skin used to make leather goods (Bernama, 2021). The presence of poxvirus prevents the skin from being sold to the market due to its cosmetic defects.

3.0 Materials and Methods

3.1 Samples and sample background

For this study, the type of samples used are archived FFPE samples from the histopathology lab of the Faculty of Veterinary Medicine, Universiti Putra Malaysia. The samples are from five juvenile spectacled caimans and were previously kept along with other four juvenile spectacled caimans in an exhibit of a theme park located in Lost World of Tambun in Perak, Malaysia.

From November 2022 until February 2023, most of these nine juvenile caimans were reported to be sick. Throughout the period, two of the caimans were dead, showing clinical signs such as inappetence, lethargy, and weakness. Upon observation of clinical signs, affected individuals were quarantined. Following death, they were diagnosed with chlamydiosis based on typical clinical signs of conjunctivitis and fibrinous inflammation of the upper respiratory tract.

3.2 Necropsy and macroscopic examination

Necropsy was carried out in the fresh sample and gross examination and body condition were examined. For the macroscopical lesions, collections of antemortem photographs and postmortem photographs of the five dead caimans from the archives of post mortem lab of Universiti Putra Malaysia, along with their formalin-fixed samples will be used to describe the gross lesions. For the microscopic lesions, available formalin-fixed paraffin-embedded tissue samples of the nictitating membrane, epiglottis, trachea, lungs, and liver will be routinely subjected to histopathology.

Additionally, two samples were obtained from the Lost World of Tambun. The lesions will be described. In each tissue, the types of lesions will be identified, and they will be semi-quantitatively scored. In general, the score will be as follows: 0 for normal, 1 for mild, 2 for moderate, 3 for severe, and 4 for very severe. These carcasses were previously acquired from a crocodile farm located at Parit Jawa, Muar, Johor as well as the Lost World of Tambun, Ipoh.

3.3 Histopathology

The samples taken were the eyelid, lung, kidney, spleen, small and large intestine, trachea, epiglottis, stomach, heart, and liver. These tissues were then routinely sectioned, stained using haemotoxylin and eosin stain, and examined with the aid of image analyser.

4.0 Results

4.1 Macroscopic Lesions

The necropsy was conducted on four crocodiles. Three out of four of the animals (n=¾) were in poor nutritional status with a low body condition score of about 1.5 - 2 out of a 5-point system. Upon presentation, the coccygeal muscle on the proximal tail appeared to have undergone atrophy and was visibly small.

The conjunctivae of all caimans appeared to be bilaterally red and swollen as seen in (A) and (B) of Figure 1. On the ventral abdominal area, coalescing to macular lesions that appear grey in colour were observed with a diameter ranging from 1 to 2 mm in diameter. Similar lesions were also found on the ventral neck, limbs, and root of the tail

as observed in (C) and (D) of Figure 1. No similar lesions were found on the dorsal portion of the caimans.

The oral mucosa of two of the caimans appeared markedly reddened and swollen whereas the other two appeared pale and yellow. The kidneys appeared to be dark red to brown in colour. In one of the caimans, there was a single white cyst adhering to the kidney. The livers were pale and enlarged.

All lungs were red, edematous and congested. There was also the presence of caseous material upon cross-section. Additionally, one of the lungs appeared to have coalescing granuloma which replaced 80% of the normal lung parenchyma which could be due to a bacterial infection as shown in (E) and (F) of Figure 1.

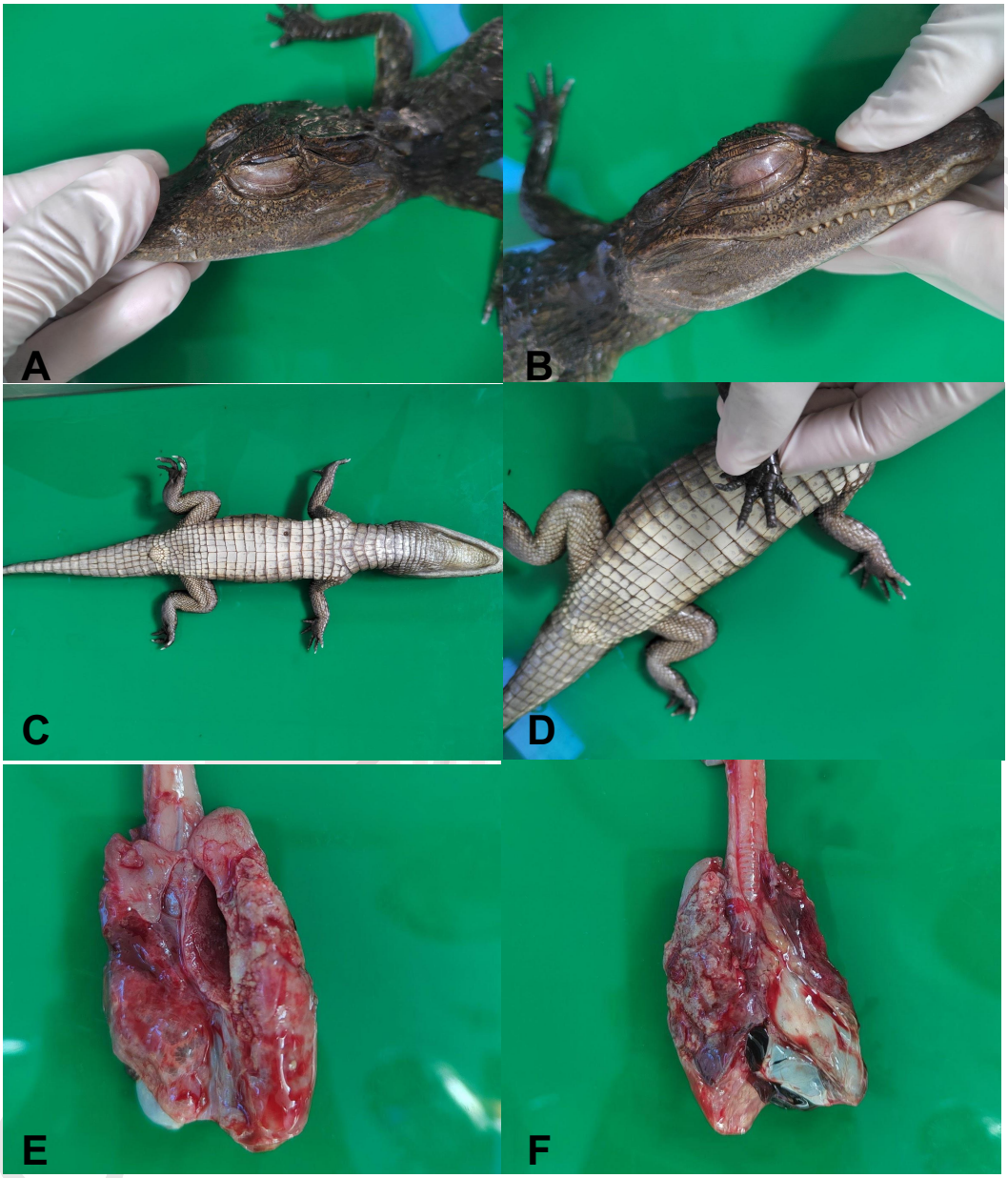

Figure 2 – Gross findings upon necropsy. **(A)** and **(B)** Bilateral reddening and swelling of conjunctiva and nictating membrane. **(C)** and **(D)** Presence of grey, coalescing to macular lesions on the ventral abdomen of a juvenile spectacled caiman. **(E)** and **(F)** Presence of coalescing granuloma replacing 80% of normal lung parenchyma

4.1.2 Macroscopic Lesions Assessment

In this evaluation of organ samples, the following mean scores were obtained for the organs that were taken for sampling, varying from the lungs, trachea, eyelid, eyes, oral mucosa, skin, heart and liver. The organ that exhibited the most severe lesions was the skin with a mean score of 2.50 ± 0.57 with lesions that resembled poxvirus lesions and were scored based on the percentage of lesion distribution on the ventral abdomen.

The second organ with the most severe lesions was the lungs with a mean score of 2.33 ± 0.57 underlying the presence of suppurative pneumonia, congestion, and emphysema. Conversely, the organs with the least severe lesions were the eyes, eyelids, and trachea with a mean score of 1.00 ± 0.00. This indicates the least pathological changes within these tissues.

The heart exhibited a mean score of 2.00 ± 1.00 underlying the pathological changes in the heart such as hydropericardium and congestion. The liver exhibited hepatomegaly, congestion, and discoloration with a mean score of 2.00 ± 1.00. The oral mucosa of the samples exhibited varying results; one sample showed pale with fibrinous material, another was reddish and the other was pale.

Organs	Samples & scoring				Mean	SD
	November	February	July	August		
Lungs (N=3)	2	-	3	2	2.33	0.577
Trachea (N=3)	1	-	1	1	1.00	0.000
Eyelid (N=4)	1	0	2	1	1.00	0.000
Eyes (N=2)	1	-	-	1	1.00	0.000
Oral Mucosa (N=3)	2	1	-	2	1.67	0.577
Skin (N=4)	3	2	2	3	2.50	0.577
Heart (N=3)	1	-	3	2	2.00	1.000
Liver (N=3)	2	-	1	3	2.00	1.000

Table 2 Summary of lesion score of organs under H&E stain

Organs	Lungs	Trachea	Eyelid	Eyes	Oral Mucosa	Skin	Heart	Liver	P value
Lesion score (Mean ± SE)	^{b}2.33 ± 0.33	^{a}1.00 ± 0.00	^{ab}1.00 ± 0.00	^{a}1.00 ± 0.00	^{a}1.67 ± 0.33	^{b}2.50 ± 0.33	^{ab}2.00 ± 0.58	^{ab}2.00 ± 0.58	P < 0.065

Table 3 Summary of lesion score of organs under H&E stain with P-value

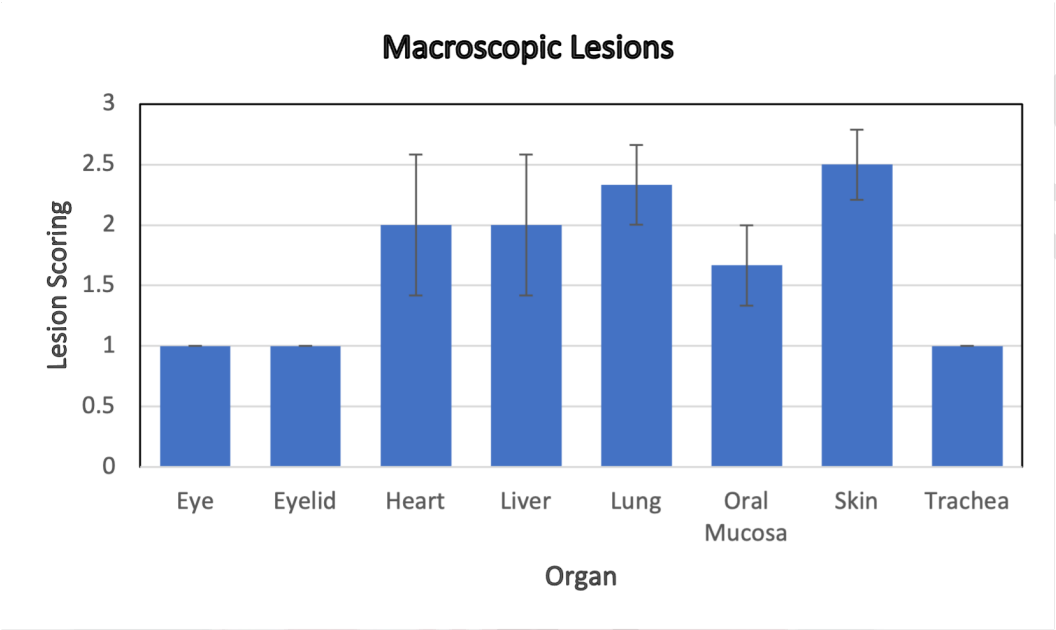

Figure 3 Summary of lesion scoring with standard error mean

4.2 Microscopic Lesions

Histopathological examination revealed infiltration of multinucleated giant cells, lymphocytes, and macrophages resulting in multifocal to coalescing thickening and erosion of the conjunctival epithelium. There was also the presence of intracytoplasmic inclusion bodies found in the eyelid as seen in Figure 4A. Additionally, cytoplasmic vacuolation in the corneal epithelium (Figure 4D).

The lung showed various-sized coalescing granulomas which caused expansion of the parabronchial submucosa and air capillary interstitium extending into the air spaces

and alveoli. The granulomas have central necrotic debris and are surrounded by epitheloid macrophages. Between the granuloma, there are macrophages, lymphocytes, and heterophils. There was also the presence of heterophils and lymphocytes in low numbers. Destruction of the alveolar wall and alveolar air space enlargement and fibrosis were also observed in the lungs.

Similar coalescing granulomas were found in the liver and spleen.

The heart showed marked vascular congestion and thickening of the alveolar septa as shown in Figure 4(B). There were no significant changes in the brain. In the intestine, there was infiltration of lymphocytes and plasma cells as well as necrosis in the villi.

There was also the presence of koilocytes in the epiglottis characterised by irregular shape and hyperchromatic nuclei as shown in Figure 4(C). The trachea exhibited congestion but the hyaline cartilage still remained intact.

In one lung sample, there was presence of hemosidirin deposit as shown in Figure 4(E) which indicates haemorrhage and could be due to recent injury to the lung tissue.

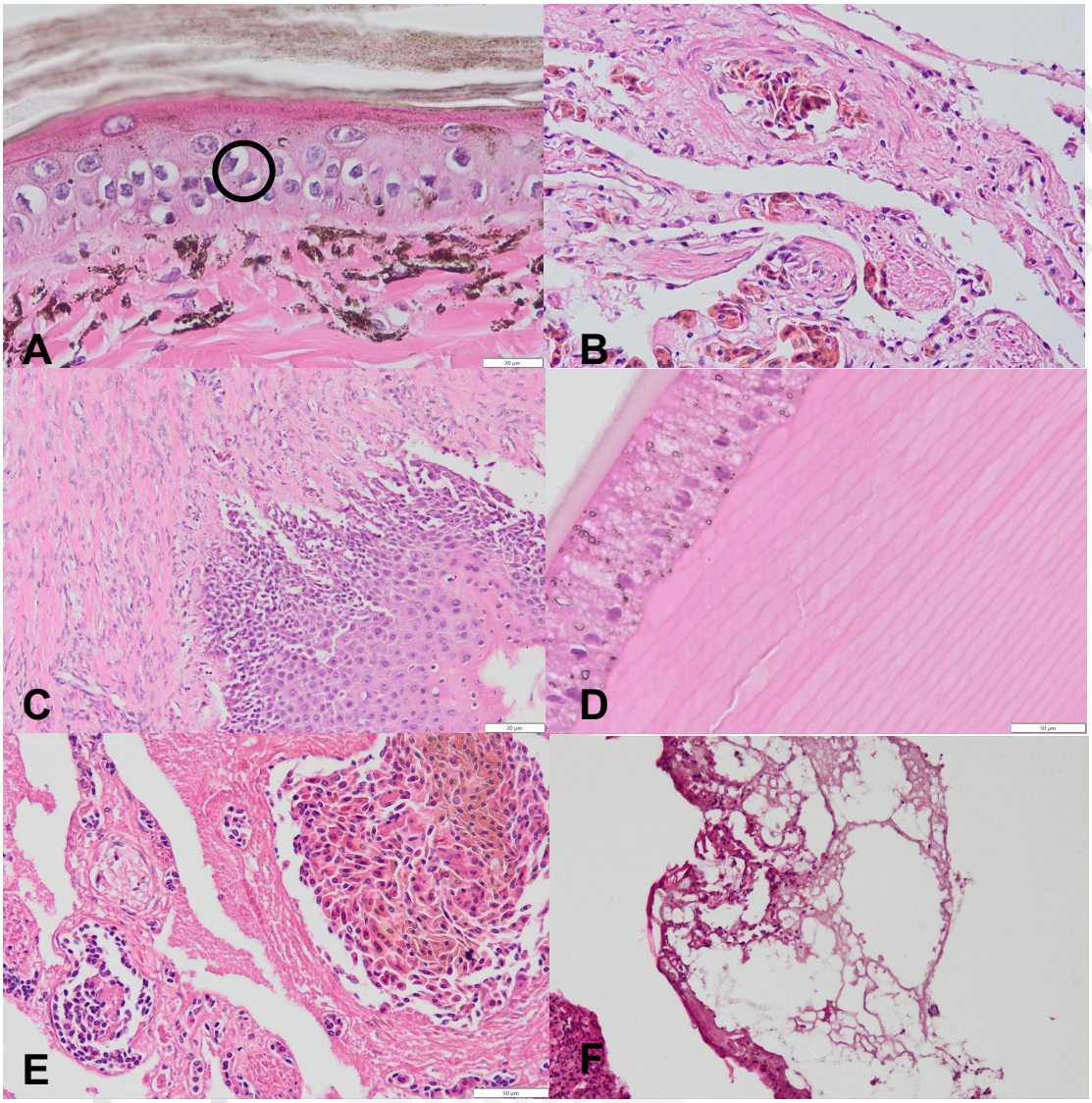

Figure 4 - Histopathological findings of organs under H&E stain. **(A)** Presence of intracytoplasmic inclusion bodies found in the eyelid. **(B)** Thickening of alveolar septa in the lungs. **(C)** Presence of koilocytes in the epiglottis. **(D)** Cytoplasmic vacuolation in the corneal epithelium. **(E)** Haemosiderin deposits and congestion in the lungs. **(F)** Presence of emphysema in the lungs.

5.0 Discussion

The purpose of our study was to examine macroscopic and microscopic lesions in spectacled caiman with respiratory disease and examine the results relating to 3 emerging disease syndromes in the spectacled caiman crocodile.

There are a few pathogens that can be suggested from the lesions observed. Poxvirus lesions are primarily characterised by skin lesions. The virus has been reported in crocodiles in captivity in countries such as southern Asia, Australia and southern Africa (Gieger and Furmuga, 2020). Crocodile poxvirus have zoonotic potential and are transmitted through direct contact. Upon the death of the host, the virus remains infectious by existing in inclusion bodies and exits the host cell when another host is found.

In the crocodilian family, the epidermis consists of squamous cells arranged in a straight layer. This is covered by tough rigid scale keratin over the scales and by a more flexible interscale keratin in the fold between the scales (Huchzermeyer et. al., 2009). Poxvirus lesions in this case occur as circumscribed lesions with moderate proliferation of the epidermis, resulting in a depression in the underlying loose dermis (Sarker et. al., 2019). The samples exhibited generalised early active poxvirus lesions charaterised by well demarcated, grey white foci with normal to pin-point keratin damage. On one of the samples, there was a large, depressed foci with abnormal keratin formation with necrotic crust indicating the expulsion stage of poxvirus. Poxvirus lesions were observed on all

samples in various stages and percentage of distribution. Microscopically, poxvirus was also ruled in due to presence of intracytoplasmic inclusion bodies in the keratinocytes. Keratinocytes are present when there is a central plug during the active stage of poxvirus lesions.

Other possible pathogens include chlamydia and herpesvirus. Significant morbidity and mortality is associated with chlamydial and herpesvirus infections in crocodiles in captivity (Paungpin et. al., 2021). Worldwide, the prevalence rate of chlamydia among crocodilians is approximately 57.3% whereby infection was reported in both normal and sick animals. On the other hand, prevalence of herpesvirus is 54% from the alphaherpesvirus family. Infection for both pathogens have been previously reported in countries such as Thailand, South Africa, and Australia (Paungpin et. al., 2021).

Coinfection of chlamydia and herpesvirus is usually presented as ocular and respiratory symptoms in crocodilians (Paungpin et. al., 2021). Additionally, hydropericardium was reported whereby there is an accumulation of fluid in the pericardial cavity which can eventually lead to opacity and thickening of the pericardium.

The oral cavity was presented with reddening and swelling with fibrinocaseous material in the pharynx associated with both herpesvirus and *Chlamydia spp*. infections. This leads to airway obstruction in the caiman being a possible cause of death. Ocular

symptoms such as corneal opacity indicate mild infection. In more severe cases, rupture of the cornea and filling of the ocular chamber with fibrinocaseous exudate was observed (Shilton et. al., 2016).

6.0 Limitation of Study

Due to the lack of samples, the results may not be representative. The carcasses may have not been fresh before tissue sectioning as the carcasses have been preserved in the refrigerator. As three of the samples were processed beforehand, macroscopic lesions were assessed and scored. Due to some images being not available and inconsistent, they might not have been assessed properly. Most of the samples were also limited to the respiratory tract only. Due to a shortage of time and materials, confirmatory diagnostic methods such as PCR were not able to be performed.

7.0 Conclusion

To conclude, macroscopically, the results have demonstrated that the organs most affected in spectacled caiman with respiratory diseases are the lungs and skin. Microscopically, the most prominent findings were the presence of inclusion bodies both

intranuclear and intracytoplasmically. Respiratory disease in the spectacled caiman are not well documented and the pathogenesis is not well understood, and thus further investigation is needed to further understand this.

8.0 Recommendations

In the future, we suggest that the study utilizes larger samples and increasing the study period. A larger sample size would increase data accuracy and provide better representation of the data. Furthermore, increasing the study period will allow for more samples to be collected as well as allowing confirmatory diagnosis such as polymerase chain reaction (PCR) to be conducted for further diagnosis. Additionally, carcass quality should be maintained and controlled across all samples to allow for quality control during necropsy. Furthermore, when doing qualitative data analysis for the macroscopic lesions, it should be conducted by the same individual to avoid discrepancy amongst samples.

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Appendix

Organ	Sample Score				Mean Scoring
	Nov 22	Feb 23	June 23	Aug 23	
Eye (n = 2)	1	/	/	1	1
Eyelid (n = 4)	1	0	2	1	1
Heart (n = 3)	1	/	3	2	2
Liver (n = 3)	2	/	1	3	2
Lung (n = 4)	2	/	3	2	2.33
Oral Mucosa (n = 3)	2	1	/	2	1.67
Skin (n = 4)	3	2	2	3	2.5
Trachea (n = 3)	1	/	1	1	1

Table 1 Summary of lesion score of organs under gross examination