UNIVERSITI PUTRA MALAYSIA

HISTOPATHOLOGICAL FINDINGS IN SPECTACLED CAIMANS (Caiman crocodilus) WITH RESPIRATORY AND OCULAR SYNDROME

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HISTOPATHOLOGICAL FINDINGS IN SPECTACLED CAIMANS (*Caiman crocodilus*) WITH RESPIRATORY AND OCULAR SYNDROME

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A project paper submitted to the

Faculty of Veterinary Medicine, University Putra Malaysia

In partial fulfilment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan.

October 2023

CERTIFICATION

It is hereby certified that we have read this project paper entitled "Histopathological findings in Spectacled caimans (*Caiman crocodilus)* with respiratory and ocular syndrome", by Sufiah Umaira' binti Noor Kamal Azlin 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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ACKNOWLEDGEMENTS

First and foremost, with His wills, I am very grateful that I am able to finish this project smoothly. A huge thanks to my parents, Noor Kamal Azlin and Suzana Ibrahim for their strong support throughout my journey as a veterinary student. With their prayers, motivation and presence make me where I am right now.

I would like to express my sincere appreciation to my supervisor and co-supervisor, Dr Annas and Dr Akmal for their guidance, support throughout the project and for their valuable input and expertise. Also, my friends that are always there to help each other, going through this rollercoaster journey. Especially to my FYP partner, Nurul, for sharing her thoughts and updates along the way of finishing this project.

Last but not least, I would like to thank myself for not giving up.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 - Projek Ilmiah Tahun Akhir.

PENEMUAN HISTOPATOLOGI DALAM BUAYA *(Caiman crocodilus)* DENGAN SINDROM PERNAFASAN DAN MATA

oleh

Sufiah Umaira' binti Noor Kamal Azlin

2023

Penyelia: Associate Prof. Dr Annas Salleh

Penyelia Bersama: Dr Mohd Akmal Mohd Noor

Penyakit pernafasan telah menjadi satu keprihatinan kesihatan utama dalam kumpulan buaya yang dipelihara. Walaupun negara-negara lain seperti Zimbabwe, Afrika Selatan, dan Thailand sudah membuat laporan tentang wabak sindrom pernafasan dan mata pada buaya, data seumpama itu kurang di Malaysia. Beberapa patogen yang dilaporkan yang diketahui menyebabkan sindrom ini pada buaya muda termasuk poxvirus dan chlamydia. Objektif kajian ini adalah untuk menerangkan dan menilai histopatologi tisu pernafasan dan mata buaya kacamata (Caiman crocodilus) yang terjejas oleh sindrom ini. Sampel paru-paru, trakea, epiglotis, mata, dan kelopak mata dari buaya juvana yang mengalami sindrom pernafasan dan mata antara November 2022 dan Ogos 2023 digunakan dalam kajian ini. Sampel-sampel diproses dan pewarnaan dengan hematoxylin dan eosin (HE), serta pewarnaan khas (pewarnaan Gram, pewarnaan Giemsa, dan pewarnaan Ziehl-Neelsen). Sebarang temuan patologi didokumentasikan dan diberi skor. Dengan menggunakan pewarnaan rutin, paru-paru menunjukkan jenis lesi yang paling banyak, dengan 9 jenis lesi seperti granuloma, emfisema, hiperplasia sel goblet, dan septum falveoli yang tebal. Manakala kelopak mata menunjukkan jenis lesi paling sedikit. Analisis skor keparahan lesi menunjukkan bahawa paru-paru menunjukkan min skor keparahan tertinggi 13.00 ± 1.87, yang tidak signifikan ($p>0.05$) berbanding dengan tisu-tisu lain. Sebaliknya, mata menunjukkan skor keparahan paling rendah 2.00 ± 1.00. Tambahan pula, paru-paru menunjukkan keputusan positif dalam semua ujian pewarnaan khas. Ini bertentangan dengan temuan di epiglotis dan mata, di mana tidak terdapat perubahan di bawah pewarnaan khas. Kesimpulannya, berdasarkan pemeriksaan histopatologi menyeluruh yang dijalankan, kajian ini menekankan bahawa paru-paru menunjukkan temuan yang paling signifikan, yang jelas dalam teknik pewarnaan rutin dan khas. Ejen penyebab yang

mungkin bagi sindrom pernafasan dan mata dalam buaya kacamata ini berdasarkan lesi adalah bakteria Gram-negatif seperti Chlamydia spp., virus seperti herpesvirus atau poxvirus, atau bakteria tahan asid, sebagai contoh, Mycobacteria spp.

***Kata kunci**: Buaya (Caiman crocodylus), sindrom pernafasan dan mata, histopatologi*

ABSTRACT

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

HISTOPATHOLOGICAL FINDINGS IN SPECTACLED CAIMANS (*Caiman crocodilus*) WITH RESPIRATORY AND OCULAR SYNDROME

by

Sufiah Umaira' binti Noor Kamal Azlin

2023

Supervisor: Associate Prof. Dr Annas Salleh

Co-supervisor: Dr Mohd Akmal Mohd Noor

Respiratory disease has emerged as a prominent health concern in captive crocodilians. While other countries such as Zimbabwe, South Africa, and Thailand have reported outbreaks of ocular and respiratory syndrome in crocodilians, such data is lacked in Malaysia. Some of the reported pathogens known to cause this syndrome in juvenile crocodilians are poxvirus and Chlamydiosis. The objective of this study is to describe and assess the histopathology of the respiratory and ocular tissues of spectacled caimans (*Caiman crocodilus*) affected by this syndrome. Samples of lungs, trachea, epiglottis, eyes, and eyelids form spectacled caimans with ocular and respiratory syndrome between November 2022 and August 2023 were used in this study. They were processed and stained with haematoxylin and eosin (HE) stain, as well as special stains (Gram stain, Giemsa stain, and Ziehl-Neelsen stain). Any pathological findings were documented and were scored. Using the routine stain, the lungs displayed the most types of lesions, with 9 different lesions such as granulomas, emphysema, goblet cell hyperplasia and thicken septa. While the eyelid exhibited the least type of lesion. Analysis of the lesion severity score revealed that the lungs showed the highest severity mean 13.00 ± 1.87, which is not significant ($p>0.05$) compared to the other tissues. In contrast, the eyes exhibited the least severe score of 2.00 ± 1.00. Moreover, the lungs exhibited positive results in all special stain tests. This contradicted the findings in both the epiglottis and eyes, where no abnormalities were detected under special staining. In conclusion, based on the comprehensive histopathological examinations conducted, this study underscores that the lungs exhibited the most significant findings, evident both in routine and special staining techniques. The likely causative agents for ocular and respiratory syndrome in these spectacled caimans based on the lesions are Gram-negative bacteria such as

Chlamydia spp., viruses like herpesvirus or poxvirus, or acid-fast bacteria, for example, *Mycobacteria spp*.

Keywords: *Common caiman (Caiman crocodylus), respiratory and ocular syndrome, histopathology*

1.0 INTRODUCTION

Caiman crocodilus is currently acknowledged to consist of four recognized subspecies: *C. c. crocodilus, C. c. fuscus, C. c. chiapasius,* and *C. c. apaporiensis*. Previously, Caiman yacare was categorized as a fifth subspecies, namely *C. c. yacare*. However, it is now widely accepted as a distinct species and is no longer classified as a subspecies (Escobedo-Galvan, et al., 2011).

Respiratory disease is likely one of the prevailing health issues in captive crocodilians (Nevarez, 2006). Nevarez (2006) also mentioned the majority of respiratory infections in crocodilians can be attributed to either bacterial or fungal sources, and certain rhinitis and pharyngitis syndromes have been documented in specific crocodilian species. Some of the previously reported respiratory diseases affecting the crocodilians are poxvirus infection (Nevarez, 2009), chlamydiosis (Inchuai et al., 2021), herpesvirus infection (Shilton, 2016), and other primary or secondary bacterial and fungal infections (Nevarez, 2009). These respiratory diseases often result in almost similar macroscopic lesions which includes conjunctivitis, pharyngitis, systemic lymphoid proliferation with non-suppurative encephalitis, and lymphonodular skin lesions (Shilton, 2016). Macroscopically, crocodiles presented appear to be nutritionally deficient and swollen, red conjunctiva and pharynx, red, edematous lungs and pale, enlarged liver are observed, also pneumonia (Borel et al., 2018). However, microscopically, lesions are different depending on the causative agent. For example, Poxvirus infection is known to cause formation of eosinophilic intracytoplasmic inclusions, ballooning of epidermal cells and Borrel and Bollinger's bodies (Nevarez, 2009).

Techniques in histopathology include the usage of routine hematoxylin and eosin, special stain, and immunohistochemistry. Special stains such as Gram stains are known to be useful for observation and differentiation of bacterial pathogens into Gram-positive and Gram-negative, while Giemsa stains may aid in the observation of certain lesions including chlamydia inclusion bodies. Previously, between November 2022 until August 2023, an outbreak of respiratory and ocular disease has occurred among captivated juveniles spectacled caiman (*Caiman crocodilus*), leading to death of 5 out of 9 of the juvenile caimans. This study aims to score the severity of histopathological changes under routine stains and to document the histopathology lesions in these juvenile spectacled caimans using special stains. Due to the lack of information pertaining to respiratory and ocular disease among crocodilians in Malaysia, this study aims to document the gross and microscopic lesions in juvenile spectacled caimans. The hypotheses for this study are as follows:

H_{01}: Each respiratory organ shows similar lesion severity in caimans with respiratory and ocular syndrome.

H_{A1}: Each respiratory organ shows different lesion severity in caimans with respiratory and ocular syndrome.

H_{02}: Specific histopathological changes are not observed using special stains in caimans with respiratory and ocular syndrome.

H_{A2}: Specific histopathological changes can be observed using special stains in caimans with respiratory and ocular syndrome.

2.0 LITERATURE REVIEW

2.1 Crocodillians

Crocodiles are under Archosauria clade, also known as the 'ruling reptiles,' which encompasses a diverse group of ancient reptiles, and they are under the order of Crocodylia (Divers, 2005). Grigg (2015) mentioned, in the Late Cretaceous period, three distinct lineages within Crocodylia managed to endure and persist from their common ancestral roots. These lineages include the gharial lineage *(Gavialoidea)*, the crocodile lineage *(Crocodyloidea)*, and the alligator lineage *(Alligatoroidea).* Within the Crocodylinae subfamily, there are three genera which are *Crocodylus*, or also known as true crocodiles, *Osteolaemus*, and *Tomistoma*. While, the subfamily Alligatorinae consists of four genera, which is alligators (the genuine alligators), caimans, and palaeosuchus, plus, the Gavialinae subfamily encompasses a single genus, Gavialis (Grigg, 2015).

Common question asked regarding these species is, what are the differences? Divers (2005) explained that caiman snouts are narrower than those of alligators but wider than those of crocodile species, and they can be easily distinguished from alligators by the presence of a bony ridge between their eyes. While alligators and crocodiles bear a striking resemblance, one way to differentiate crocodiles is by their elongated, triangular-shaped heads, which feature a distinct fourth mandibular tooth on each side of the mouth (Divers, 2005).

Huchzermeyer (2003) found that the genus Caiman comprises two distinct species: *C. latirostris*, commonly known as the Broad-snouted caiman, and *C. crocodilus*, referred to as the Common caiman, also, both of these species are native

to South America. Male caimans can reach a maximum length of 2.5 meters, whereas females can grow up to 1.4 meters (Griggs, 2015).

Figure 1 Adult *Caiman latirostris* (Source: Verdade, L. M., & Piña, C. I. (2006). Caiman latirostris. *Catalogue of American Amphibians and Reptiles (CAAR)*.)

2.2 Common agents of respiratory and ocular diseases in juvenile crocodilians

In juveniles, there are a few diseases commonly seen that lead to ocular and respiratory signs. Huchzermeyer (2002) mentioned a few of these diseases that were found in juvenile and hatchling crocodilians. The first one would be crocodile pox, which results from an infection with a parapoxvirus, affecting young crocodiles and generating dry, brown lesions within the oral cavity.

While crocodiles under one year of age, two forms of chlamydiosis occurred, acute hepatitis and chronic bilateral conjunctivitis (Huchzermeyer, 2002). Shilton et. al. (2016) found that CP (Conjunctivitis and/or pharyngitis) syndrome was primarily observed in hatchlings. The macroscopic observations during autopsies in CP syndrome were primarily confined to the eyes, pharynx, and larynx, plus, bilateral eye lesions were a common occurrence. In less severe instances, there was thickening, swelling, and redness of the nictitating membrane and palpebral conjunctiva, along with limited mucopurulent discharge. Shilton et.al. studied that conjunctival swab cultures were performed, resulting in no significant growth in 2 cases and light to moderate growth of various bacteria in 6 cases. The predominant organisms included Streptococcus sp. (3 cases), Aeromonas hydrophila (1 case), Morganella morganii (1 case), and Enterobacter sp., Staphylococcus sp., and Corynebacterium sp. (all from 1 case).

2.3 Histopathological stains

2.3.1 Routine stain

Bancroft et. al. (2012) claimed, hematoxylin and eosin stain (H&E) is widely used in histology due to its simplicity and effectiveness in highlighting diverse tissue

structures. He explained that hematoxylin predominantly stains cell nuclei blue-black, providing clear intranuclear detail, while eosin stains cell cytoplasm and most connective tissue fibers in different shades of pink, orange, and red.

2.3.2 Special stains

2.3.2.1 Gram stain

The Gram stain technique enables the differentiation of Gram-positive and Gram-negative bacteria through a process of selective staining using a crystal violet–iodine complex and a safranin counterstain (Coico, 2006). He described that Gram-positive bacteria maintain the violet stain in their cell walls even after exposure to alcohol, causing them to appear purple, while Gram-negative bacteria lose the stain during the same treatment, resulting in a pink colour.

2.3.2.2 Giemsa stain

The utilization of Giemsa staining reduces ambiguity in distinguishing chlamydial inclusions from bacterial debris (Mittal et. al., 1993). It is apparent that Giemsa staining gives a somewhat more conservative estimate of the prevalence of Chlamydia compared to FA-stained smears (Yoneda et.al., 1975).

2.3.2.3 Ziehl-Neelsen stain

Wulff et. al. (2004) mentioned a bacterial family of particular diagnostic significance is the Mycobacteria family, such as Mycobacterium tuberculosis and Mycobacterium leprae, are nonmotile rod-shaped bacteria that typically aggregate into cordlike groups. These bacteria produce distinctive waxy substances called mycolic acids, which covalently attach to peptidoglycan in the bacterial cell wall, they possess

a unique resistance to alcohol decolorization after staining with hot carbol-fuchsin. This characteristic, known as acid-fast, serves as an excellent diagnostic marker.

3.0 METHODS AND MATERIALS

3.1 Study period and location

This study was conducted in September 2023 at Veterinary Histopathology Lab, Faculty of Veterinary Science, University Putra Malaysia, Serdang, Selangor.

3.2 Caimans and sample collection

A total of 4 juvenile spectacled caimans from a petting zoo in Lost World of Tambun in Ipoh were collected for this study. Respiratory and ocular signs were shown by all caimans prior to death. Three of the samples are archived samples from November 2022 to July 2023 cases. For the remaining sample, the animal was reported as sudden death at the end of August and postmortem examination was performed. The findings from the necropsy were pulmonary edema, congested lungs, heart, and kidney, and urates seen generally across the kidney. Tissue samples, which were composed of the lungs, trachea, epiglottis, eyelid, and eyes were collected from the representative affected animals. All types of collected tissues were kept at −10°C. The samples collected were fixed in neutral buffered formalin and subsequently processed. These tissues will be routinely processed using paraffin-embedded techniques. They will be sectioned at 4 μm, and will be transferred onto glass slides.

3.3 Identification of histological findings in Caimans with ocular and respiratory syndrome

Once the samples were processed using standard techniques, the samples were stained with hematoxylin and eosin to identify morphological changes, gram stain to enhance identification of gram-negative and positive bacteria, giemsa stain for

Chlamydia inclusion bodies detection and Ziehl-Neelsen to highlight Acid-Alcoholic Resistant Bacilli (AARB).

Following initial screening of all slides, aspects of tissues that subjectively seemed to differ among caimans were quantified. Pathological findings identified in each tissue section on slides stained with routine stain were scored as 0 (none), 1 for 1-25% lesion (mild), 2 for 26-50% lesion (moderate), 3 for 51-75% lesion (severe) or 4 for 76-100% lesion (very severe). Digital images were taken for the following histological features. While lesions found in special stains samples were identified and described.

3.4 Statistical Analysis

The data of lesion severity scores were recorded, accumulative scores of each organ were compared between each other organ. Statistical data were then analyzed using Kruskal-Wallis test with significance difference at $p<0.05$.

4.0 RESULTS

In general, the lungs showed the most types of lesions compared to the other tissues on both the routine and special stains. Raw data and statistical outputs of the study are presented in Appendix A.

4.1 HEMATOXYLIN AND EOSIN STAIN (H&E STAIN)

4.1.1 Histological description

Using the HE stain, significant tissue alterations were observed in all of the collected tissue samples of caimans with respiratory and ocular syndrome. The lung samples, in particular, displayed notable abnormalities, characterized by the enlargement of air spaces accompanied by the destruction of alveolar walls, indicative of evident fibrosis. Additionally, coalescing granulomas obscured the normal pulmonary architecture, while the airway epithelium revealed signs of goblet hyperplasia, with goblet cells clustered together (Figure 1 B) exhibiting an expansion of mucin-secreting granules. These changes collectively resulted in a noticeable thickening of the alveoli. Further investigation into other organs revealed a degeneration of the epithelial cells in the trachea, epiglottis, eyes, and eyelids, marked by vacuolation. Some lung samples also exhibited the loss of bronchiole linings, suggesting necrosis, likely attributed to pyknotic cell nuclei. Severe congestion and infiltration of heterophils and multinucleated giant cells were observed in most of the examined organs. In the trachea samples, the tunica adventitia, tunica submucosa, and tunica mucosa were notably absent, although the hyaline cartilage remained intact. The congestion in the trachea was minimal, primarily located in the tunica submucosa. The epiglottis displayed swollen cells with clear cytoplasm and eccentric, irregularly

shaped, hyperchromatic nuclei, characteristics consistent with koilocytes. In both the eyes and eyelids, epithelial cell vacuolation was observed in conjunction with fibrosis. Intranuclear inclusion bodies were found in the epithelial layer of the eyelid. Overall, the histological analysis of these samples reveals a complex array of pathological changes in the lung and associated organs, shedding light on the underlying conditions and potential causes of these observed alterations. Figure 2 shows the histopathological changes.

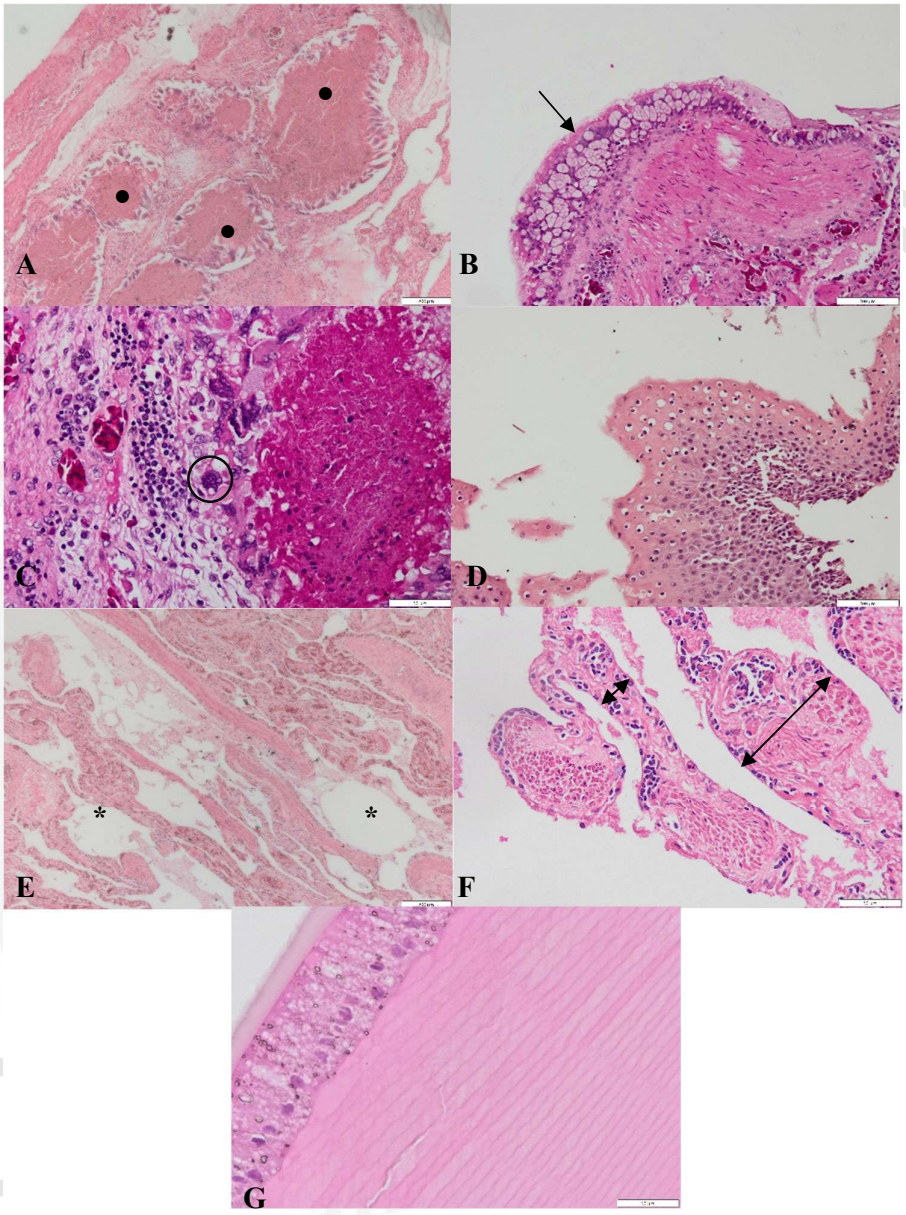

Figure 2 Histopathological findings of organs under H&E stain. **(A)** Granulomas seen in lungs (black dots). **(B)** Goblet cells hyperplasia (black arrow) in the lungs. **(C)** Giant cell (black circle) found in lungs. **(D)** Koilocytes seen in epiglottis section. **(E)** Congested and emphysema (asterisk) of lungs. **(F)** Thicken falveoli septa (double arrow lines). **(G)** Cytoplasmic vacuolation of corneal epithelium.

4.1.2 Histological Assessment

In the evaluation of organ samples, mean scores were assessed for various organs, focusing on the lungs, tracheas, epiglottis, eyelids, and eyes. The statistical analysis revealed notable variations in the extent of lesions among these organs. The most pronounced lesions were observed in the lungs, with a mean score of 13.00 ± 1.87. This finding underscores the significance of pathological changes within the pulmonary tissue when compared to the other organs examined. Conversely, the eyes displayed the fewest lesions, with a mean score of 2.00 ± 1.00. This indicates a relatively lower level of pathological alterations in this particular organ. While for trachea, epiglottis and eyelids exhibited a mean score of 7.50 ± 3.50, 8.00 ± 0.00 and 4.50 ± 2.50, respectively (Table 1). In summary, our analysis highlights the variation in lesion severity across different organs, with the lungs demonstrating the most significant pathological alterations, followed by the epiglottis, trachea and eyelid, while the eyes exhibited the least extent of lesions.

Organs	**Lungs** (n=4)	**Trachea (n=2)**	**Epiglottis (n=1)**	**Eyelid (n=2)**	**Eyes (n=2)**
Lesion score (Mean ± SE)	[b]13.00 ± 1.87	[ab]7.50 ± 3.50	8.00 ± 0.0	[a]4.50 ± 2.50	[a]2.00 ± 1.00

Table 1 Summary of lesion score of organs under H&E stain with P-value.

4.2 SPECIAL STAINS

In general, lung samples showed positive results for all three stains, while both epiglottis and eyes showed negative results for all stains. The summary of special stains findings is tabulated in Table 2. Raw data and statistical outputs of the study are presented in Appendix B.

4.2.1 Ziehl-Neelsen Stain

Among the lung samples, half exhibited a positive reaction to this stain. Acid-fast bacilli appeared in striking pink was observed in the necrotic tissues of the lung parenchyma, while other tissue components remained a serene blue, as shown in Figure 3A.

4.2.2 Gram Stain

From the observations, it was revealed that only the trachea displayed a positive result for Gram-negative bacteria, characterized by a vivid pink upon staining. In contrast, both eyelid and lung samples exhibited positive results for Gram-positive bacteria, showing purple coloration (see Figure 3B).

4.2.3 Giemsa Stain

Remarkably, Chlamydia inclusion bodies were identified in all samples of the trachea, eyelid (see Figure 3C), and in two out of three lung samples. The melanin granules were bluish to purplish in these Giemsa-stained smears.

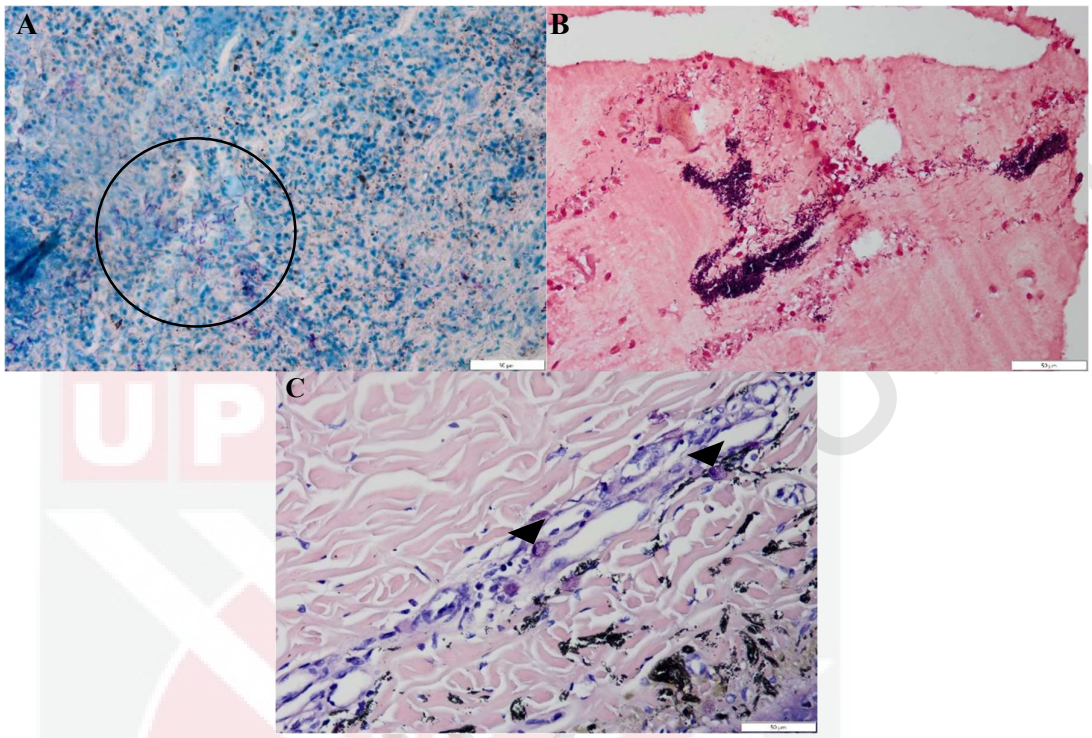

Figure 3 Histopathological findings of organs under special stains. **(A)** Acid fast bacilli detected in lungs under Ziehl-Neelsen stain (black circle). **(B)** Gram-positive bacteria colonies observed in lungs under Gram stain. **(C)** Chlamydia inclusion bodies (black arrowhead).

Organs	Gram stain	Giemsa stain	Ziehl-Neelsen	Total	Mean
Lungs (N=10)	2	2	2	6	1.2
Trachea (N=6)	1	2	0	3	1.0
Epiglottis (N=3)	0	0	0	0	0
Eyelid (N=6)	1	2	0	3	1.0
Eyes (N=3)	0	0	0	0	0

Table 2 Summary of lesion score of organs under special stains.

5.0 DISCUSSION

Compared to adult crocodilians, juvenile and hatchling crocodiles are much more sensitive to open environments. Stressors such as the inability to hide, handling, capture, and sudden environmental noise or movement are common issues for these reptiles. Despite being poikilotherms, crocodiles strive to maintain their body temperature within a specific range of 28°C to 33°C. They achieve this by utilizing the thermogradient present in their natural habitat, which includes exposure to both sunlight and shade, warm surfaces, cold deep water, and burrows. Additionally, these caimans might have experienced compromised immunity due to unusually low environmental temperatures before the appearance of clinical symptoms. There is a chance that the animal was releasing bacteria into its surroundings, and individuals working in that area might have encountered the organisms through water exposure, either by aerosolization and inhalation or, to a lesser degree, ingestion while in the water.

Based on a few literatures, there are a number of common agents of juvenile crocodilians with respiratory and ocular syndrome. For instance, chlamydia, parapoxvirus (Huchzermeyer, 2002), herpesvirus (Paungpin, 2021) and Mycobacteria (Ariel, 1997). After performing histopathology staining procedure, a few differential diagnoses were listed based on the findings obtained.

The first suspected agent for this case is *Chlamydia* spp. This agent is a known pathogen in both freshwater and saltwater crocodiles. This is due to the presence of intracytoplasmic inclusion bodies under Giemsa stain. Paungpin (2021) explained that these inclusion bodies can be observed in random tissue samples, while in this case,

they were observed in the eyelid tissue sample. These inclusion bodies can be identified by iodine staining of glycogen present in the cytoplasm in infected cells. The second reason is Paungpin (2021) claimed that chlamydiosis in crocodilians are typically recognized by ocular and respiratory signs. Upon necropsy, pulmonary haemorrhage, swollen conjunctiva and fibrin accumulation under the nictitating membrane that leads to cloudy eyes were observed in these juvenile caimans. Other than those findings, interstitial pneumonia and conjunctivitis are also indications of chlamydiosis in crocodilians (Carossino, 2022). Thicken septa of the falveoli and heterophil cells infiltrations are the findings observed in this case that shows the characteristics of interstitial pneumonia.

The other agent that was highly suspected is *Mycobacterium* spp. The reason is because positive results of red rod-shaped bacilli that are slightly curved and straight are observed in the lungs tissues under Ziehl-Neelsen stain. This is highly suspected of Mycobacterium. In previous studies, mycobacterium species that were found in crocodilians are *Mycobacterium intracellulare* (Kik, 2013) that was found in broad-snouted caiman (*Caiman latirostris)*, *M. lentiflavum* and *M. szulgai* in Brown Caiman (*Caiman crocodylus)* (Maluta et.al., 2022). Mycoplasma alligatoris and Mycoplasma crocodyli are new species of mycoplamsa that have been identified in American alligators (*Alligator mississippiensis)* (Clippinger et.al., 2000).. In addition, a high number of granulomatous inflammations featuring multinucleated giant cells throughout the lungs, showing mycobacteriosis in reptiles (Soldati, 2004). The granulomas were composed of typical central necrosis surrounded by multinucleated giant cells and interstitial lymphocytic infiltrations.

Those were the bacterial agents suspected. For viruses, *Poxvirus* spp was one of the lists. This is because eosinophilic intracytoplasmic inclusions were observed within hypertrophied epithelial cells, with margination of chromatin in Caimans with poxvirus (Ariel, 2011) and in this case, they were found in the epithelium layer on eyelids of affected caiman. This virus infects cells and typically produce inclusions within the cytoplasm of the infected cells. These inclusion bodies became a histologic clue to the diagnosis, and these are commonly called as Guarnieri bodies. The characteristics of these cells appear multiple, granular and eosinophilic.

All data were analyzed using a Statistical Analysis Software, SPSS under a non-parametric Kruskal Wallis H test. The results showed that the p-value is higher than 0.05, which indicates that there is no significant difference between lesion severity of each organ. Hence, null hypotheses are accepted.

6.0 CONCLUSION

In conclusion, there is no significant difference in lesion severity in each respiratory and ocular organs in caimans with respiratory and ocular syndrome. Plus, lungs exhibited the most significant findings, evident both in routine and special staining techniques. Next, the agents for ocular and respiratory syndrome in these spectacled caimans are gram-negative bacteria such as *Chlamydia* spp, viruses like poxvirus, or acid-fast bacteria, for example, *Mycobacteria* spp. For further studies, it is recommended to perform further diagnostic tests to narrow down the differential diagnosis, for example, bacteria or virus isolation and PCR.

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Appendix A

Organs	Sample								total
	February		July		August		November		
	Findings	Scoring	Findings	Scoring	Findings	Scoring	Findings	Scoring	
Lungs	Heterophil infiltration cells Emphysema Congestion Bronchiole lost lining	3 3 1 1 8	Granuloma Bacteria colonies Goblet hyperplasia Congestion Heterophil cell infiltration Emphysema	2 2 2 4 4 3 17	Thicken septa Hemosiderin Granuloma Emphysema Congestion Heterophil cell infiltration	4 1 2 3 2 2 14	Granuloma Bacteria colonies Goblet hyperplasia Congestion Emphysema Heterophil infiltration cells	3 1 1 2 2 4 13	

Appendix A

Trachea	Loss tunica adventitia	4 4	[redacted]	Necrotized epithelium Congestion Vacuolation of epithelial cells	4 3 4 11
Epiglottis	[redacted]			Heterophil cell infiltration Epithelial cell vacuolation Congestion Swelling cells	2 4 1 1 8

Appendix A

Eyelid	Heterophil cell infiltration Cytoplasmic vacuolation of cornea epithelial	1 1 2	[redacted]		Cell swelling Loss epithelial structure Vacuolation of epithelial cells	2 3 2 7	[redacted]	
Eyes	[redacted]		Epithelial cells vacuolation Heterophil cell infiltration	1 2 3	Cytoplasmic vacuolation of cornea epithelial	1 1	[redacted]	

Table 1 Summary of microscopic findings/lesions and scoring under H&E stain.

Scoring:0 = none; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-100

Appendix B

Raw data of lesions score under special stains

Organs	Special staining	Expected findings	Sample				Total of (+)
			Feb	July	Aug	Nov	
Lungs	Gram	Gram-positive bacteria	+		-	+	2/3
		Gram-negative bacteria	-		-	-	0/3
	Giemsa	Chlamydia intracytoplasmic inclusions	+		-	+	2/3
	Ziehl-Neelsen	Acid-fast bacteria	-	+	-	+	2/4
Trachea	Gram	Gram-positive bacteria	-		-		0/2
		Gram-negative bacteria	+		-		1/2
	Giemsa	Chlamydia intracytoplasmic inclusions	+		+		2/2
	Ziehl-Neelsen	Acid-fast bacteria	-		-		0/2
Epiglottis	Gram	Gram-positive bacteria			-		0/1
		Gram-negative bacteria			-		0/1
	Giemsa	Chlamydia intracytoplasmic inclusions			-		0/1
	Ziehl-Neelsen	Acid-fast bacteria			-		0/1
Eyelid	Gram	Gram-positive bacteria	-		+		1/2
		Gram-negative bacteria	-		-		0/2

Appendix B

	Giemsa	Chlamydia intracytoplasmic inclusions	+	[redacted]	+	[redacted]	2/2
	Ziehl-Neelsen	Acid-fast bacteria	-	[redacted]	-	[redacted]	0/2
Eyes	Gram	Gram-positive bacteria Gram-negative bacteria	[redacted]	[redacted]	- -	[redacted]	0/1
	Giemsa	Chlamydia intracytoplasmic inclusions	[redacted]	[redacted]	-	[redacted]	0/1
	Ziehl-Neelsen	Acid-fast bacteria	[redacted]	[redacted]	-	[redacted]	0/1

Table 2 Summary of microscopic findings/lesions and scoring under special stains.

Scoring:

(+) = present

(-) = absent