UNIVERSITI PUTRA MALAYSIA

THE CLINICOPATHOLOGY EVALUATION OF *Oreochromis* sp. POST-*Streptococcus iniae* AND *Aeromonas hydrophila* CHALLENGE

AIN MIRZANI AZNI RAES

THE CLINICOPATHOLOGY EVALUATION OF *Oreochromis* sp. POST-*Streptococcus iniae* AND *Aeromonas hydrophila* CHALLENGE

AIN MIRZANI AZNI RAES

A project paper submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfilment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Serdang, Selangor Darul Ehsan

MARCH 2018

CERTIFICATION

It is hereby certified that we have read this project paper entitled "The Clinicopathology Evaluation of *Oreochromis* Sp. Post-*Streptococcus iniae* and *Aeromonas hydrophila* Challenge", by Ain Mirzani binti Azni Raes and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course, VPD 4999 – Final Year Project.

_____

ASSOC. PROF. DR. MD. SABRI MOHD YUSOFF

DVM, MVSc, PhD (UPM)

Senior Lecturer

Department of Veterinary Pathology and Microbiology

Faculty of Veterinary Medicine,

University Putra Malaysia

(Supervisor)

DEDICATION

Every challenging work needs self-efforts as well as guidance of elders especially those who were very close to our heart.

My humble effort I dedicate to my loving

HUSBAND AND PARENTS

Whose affection, love, encouragement and prays of day and night make me able to finish my task,

Along with all hard working and respected

SUPERVISOR

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my main supervisor, Assoc. Prof. Dr. Md. Sabri Mohd Yusoff his excellent guidance, caring, patience, and support in all kinds of ways for without them I would not be able to complete this project. The weeks have been hard on me but he never let me down.

I would like to thank En. Jamil, Madam Latifah and Ms Krish for helping and guiding me in performing laboratory, my FYP-mates Nurhani and Adib Wafri and the staffs at the clinical pathology laboratory and bacteriology laboratory of Faculty of Veterinary Medicine, Universiti Putra Malaysia for helping me out when time constraints.

A very special thanks to my loving husband, Mohamad Hafizi bin Saidon, my family, who helped me, cheered for me, supported me, and stood by me from the very start, until the end.

Finally, I would like to thank my beloved #KJcruise and #KMcruise friends for being my stress reliever, Nabila Farahin and Hanisah for teaching me to do statistical analysis, Siti Nurin Fakhriani for assisting me in great histology pictures, and to my DVM 2018 who were there with me, going through tough times together, making memories and holding me up.

Words could not express my gratitude but I hope Allah will repay all the efforts to everyone who helped me.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATION

A. hydrophila = Aeromonas hydrophila

S. iniae = Streptococcus iniae

CFU = Colony Forming Unit

N = Population size

n = Sample size

i.p. = Intraperitoneal

MS222 = Tricane methanesulfonate

TSA = Trypticase Soy Agar

TSB = Trypticase Soy Broth

°C = Degree Celsius

rpm = Revolutions per minute

PBS = Phosphate Buffer Solution

SPSS IBM20 = Statistical Package for the Social Sciences

% = Percentage

DPX = dystrine, plasticizer and xylene

H&E = Haemosin and Eosin

ABSTRAK

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

PERUBAHAN PATOLOGI PADA *Oreochomis* sp. SELEPAS DIUJI DENGAN JANGKITAN *Streptococcus iniae* DAN *Aeromonas hydrophila*

Oleh

AIN MIRZANI AZNI RAES

2018

Penyelia: Professor Madya Dr Md Sabri Mohd Yusoff

Kajian ini menilai tanda-tanda klinikal, perubahan patologi dalam otak, buah pinggang dan insang pada tilapia hibrid merah (*Oreochromis* sp.) selepas *Streptococcus iniae* dan *Aeromonas hydrophila* challenge. Sebanyak 54 anak ikan tilapia dibahagikan kepada tiga kumpulan dan dijangkiti dengan $\times 10^{10}$ CFU/ ml, Kumpulan A; *Streptococcus iniae* manakala kumpulan B; *Aeromonas hydrophila* dan Kumpulan C; Kawalan. Penyampelan dilakukan setiap selang 12 jam dalam masa 48 jam untuk dinilai untuk lesi bedah siasat secara makroskopik dan mikroskopik. Tanda-tanda klinikal yang ditunjukkan oleh kumpulan A tidak bergerak; lebih banyak lagi di dasar tangki, najis putih panjang dan anoreksia manakala dalam kumpulan B menunjukkan tanda- tanda menelan udara, kematian dan anoreksia. Secara makroskopik, kedua-dua kumpulan mengalami kesebakan

dalam buah pinggang, insang, otak dan pendarahan sirip. Selain itu, terdapat eksopthalmia yang diperhatikan dalam kumpulan A manakala pendarahan operkulum yang diperhatikan dalam kumpulan B. Perubahan histopatologi yang dinilai adalah penyusupan sel-sel radang dan kesebakan di otak, ginjal dan insang dengan tambahan pemvakuolan di otak, penjanarosotan pada saluran-saluran kecil di ginjal dan perubahan lamella sekunder insang, di mana keterukan lesi yang diperhatikan lebih tinggi dalam kumpulan B. Hasil kajian menunjukkan bahawa ikan yang dijangkiti dengan *S. Iniae* dan *A. hydrophila* boleh menunjukkan beberapa tanda klinikal yang sama dan perubahan patologi tetapi secara amnya lebih teruk dalam jangkitan *A. hydrophila*.

Kata kunci: Tilapia, *Oreochromis* sp., *S. iniae*, *A. hydrophila,* histology

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial for the course VPD 4999-Final Year Project

THE CLINICOPATHOLOGY EVALUATION OF *Oreochromis* sp. POST-*Streptococcus iniae* AND *Aeromonas hydrophila* CHALLENGE

by

AIN MIRZANI AZNI RAES

2018

Supervisor: Associate Professor Dr Md Sabri Mohd Yusoff

The study evaluated the clinical signs, pathological changes in brain, kidney and gills of Red hybrid tilapia (*Oreochromis* sp.) post-*Streptococcus iniae* and *Aeromonas hydrophila* challenge. A total of 54 tilapia fingerlings were divided into three groups and infected with $\times 10^{10}$ CFU/ml of Group A; *Streptococcus iniae* while group B; *Aeromonas hydrophila* and Group C; Control respectively. Sampling was done every 12 hours intervals within 48 hours to be evaluated for post-mortem lesion macroscopically and microscopically. The clinical signs shown by group A were motionless; more at the base of the tank, long white faeces and anorexia while in group B showed air gulping, death and anorexia. Macroscopically, both groups developed congestion in kidney, gills, brain and fin haemorrhage. Additionally, there was exophthalmia observed in group A while haemorrhagic operculum observed in group B. Histopathological changes evaluated were infiltration of inflammatory

cells and congestion in brain, kidney and gills with additional of vacuolation in the brain, degeneration of tubules in kidney and secondary lamellar changes in gills, where severity observed was more in group B. The results verified that fish infected with *S. iniae* and *A. hydrophila* may develop some similar clinical signs and pathological changes but generally more severe in *A. hydrophila* infection.

Keywords: Tilapia, *Oreochromis* sp., *Streptococcus iniae*, *Aeromonas hydrophila,* histology

1.0 INTRODUCTION

1.1 Study Background

1.1.1 Background:

Tilapia, *Oreochromis* sp. nowadays become an important fish cultured in Southeast Asia because of their hardy environmental tolerant characteristics and has high demand in Malaysia (Hassan et al., 2013). Red tilapia has contributed approximately 90% of the total tilapia production in Malaysia with generally cultured in ponds, cages and tanks as well as in pen culture system (Hamzah et al., 2007). In Morrison et al. (2006) stated that since the tilapia fish can breed well in captivity and also in wide variety of water condition, it becomes so famous and important to the aquaculture industry whereby it can grow in pH water ranging from pH 5 to pH 9, survived in dissolved oxygen as low as <2 mg/L and ammonia levels at 50mg/L longer than other fish. Although *Oreochromis* sp. is well known for its hardy criteria (Hassan et al., 2013), there are still some species of bacteria that can infect causing septicaemia in this fish especially in the presence of stressors which are *Streptococcus iniae* (Suanyuk et al., 2010) and *Aeromonas hydrophila* (Pachanawan et al., 2008). Therefore, this study is conducted to observe, evaluate and compare the clinical signs and lesions developed in *Oreochromis* sp. resulted from the infection of these two bacteria species after 48 hours of exposure.

1.1.2 Overall Objective

1) To observe the clinical signs shown by Red hybrid tilapia post *Streptococcus iniae* and *Aeromonas hydrophila* challenge in 12 hours sampling intervals within 48 hours

2) To compare the severity of lesions in the brain, gills and kidney post-inoculation of *Streptococcus iniae* and *Aeromonas hydrophila* challenge in 12 hours sampling intervals within 48 hours.

1.1.3 Justification:

From the literature, there were limited studies focusing on the comparison in pathogenicity of *Streptococcus iniae* and *Aeromonas hydrophila* in fishes with 12 hours sample intervals of post inoculation. Thus, this study is done to compare the clinicopathology of *Streptococcus iniae* and *Aeromonas hydrophila* in Red hybrid tilapia (*Oreochromis* sp.). Both bacteria are opportunistic bacteria which can cause diseases in fish, thus contribute to the lost in the aquaculture industry.

1.1.4 Hypothesis:

Ho = There is no difference in clinical signs observed in Red hybrid tilapia (*Oreochromis* sp.) *s*hown post-*Streptococcus iniae* and *Aeromonas hydrophila* challenge in 12 hours sampling intervals within 48 hours.

Ha = There are difference clinical signs observed in Red hybrid tilapia (*Oreochromis* sp.) *s*hown post-*Streptococcus iniae* and *Aeromonas hydrophila* challenge in 12 hours sampling intervals within 48 hours.

Ho = There are no difference degrees of severity of the lesions seen in Red hybrid tilapia (*Oreochromis* sp.) of the brain, gills and kidney post *Streptococcus iniae* and *Aeromonas hydrophila* challenge in 12 hours sampling intervals within 48 hours

Ha = There are difference degrees of severity of the lesions seen in Red hybrid tilapia (*Oreochromis* sp.) of the brain, gills and kidney post *Streptococcus iniae* and *Aeromonas hydrophila* challenge in 12 hours sampling intervals within 48 hours

2.0 LITERATURE REVIEW

2.1 Tilapia (*Oreochromis* sp.)

Tilapia is a freshwater fish, belongs to the family of Cichlidae that inhabits in the fresh and brackish water of Africa, Middle East, Coastal India, Central and South America (Soto et al., 2010). In the second half of the 20th century, Tilapia fish had been introduced into many tropical, subtropical and temperate regions and it was first introduced to Malaysia in mid- 1980's. Tilapia farming has a potential economic value with high international demand became one of the most important sources of aquaculture industries in Malaysia. Aquaculture sector in Malaysia put a major focus on tilapia farming as it accounts for more than one third of the total freshwater aquaculture production in the country (Low et al., 2015). According to (Soto et al., 2010), tilapia are hardy, prolific and fast growing tropical fish which suits for various types of aquaculture farming, perceived as a palatable, marketable and nutritious product. Red hybrid tilapia (*Oreochromis* sp.) is the cross between Nile tilapia (*Oreochromis niloticus*) and Mozambique tilapia (*Oreochromis mossambicus*).

2.2 *Streptococcus iniae* Infection

Streptococcosis caused by *S. iniae* is an important pathogen in aquaculture for marine and freshwater fish, which may cause considerable economic losses and infect different species of fish, and also a zoonotic disease to human (Pretto-Giordano, 2015). *S. iniae* is characterised by a sphere shaped, Gram-positive cocci, and a catalase negative bacterium (Rattanachaikunsopon & Phumkhachorn, 2010). Fish can be infected with *S. iniae* through a variety of routes either oral, bath exposure with or without the presence of abrasions, olfactory route, through direct contact in crowded conditions, or through cannibalism of moribund or dead infected fish (Agnew & Barnes, 2007). It was stated in Pretto-Giordano (2015) that the most common clinical signs of streptococcosis include exophthalmia, ascites, erratic swimming, lethargy, melanosis, meningoencephalitis, septicaemia and high mortality. Macroscopically, the lesion could be congested liver, kidneys and spleen of the infected fish (Rahmatullah et al., 2017).

2.3 Aeromonas hydrophila Infection

Aeromonas hydrophila causes disease in fish known as "Motile Aeromonas Septicaemia" (MAS) which also known as "Hemorrhagic Septicemia," "Ulcer Disease," or "Red-Sore Disease. All the names given are related to the lesions developed upon infection including septicaemia where the bacteria or bacterial toxins are among the most common bacteria in freshwater habitats throughout the world (Ciprino, 1984) and also present within numerous organs of the fish, and ulcers of the fish's skin (Swann & White, 1991). Additionally, *A. hydrophila* is a Gram-negative aerobic and facultative anaerobic, oxidase-positive motile bacterium

that dwells in aquatic environments and even found in gastrointestinal tracts of healthy fish, which can also act as an opportunistic biological agent that contributes to the occurrence of a fish disease and its deterioration (Laith & Najiah, 2013) especially with other contributing factors such as stress (Ciprino, 1984). The clinical signs associated with *A. hydrophila* infection range from sudden death in otherwise healthy fish to lack of appetite, abnormalities in swimming, pale gills, bloated appearance, and skin ulcerations; may occur at any site on the fish and often are surrounded by a bright rim of red tissue (Swann & White, 1991). Yardimci & Aydin (2011) stated that clinical signs were observed associated with *A. hydrophila* infection include weakness, anorexia, swimming closer to the surface, darkness in colour with hyperaemia and lysis of the fins. Macroscopically, a lesion that could be observed includes congestion in liver, kidney and spleen (Ibrahem et al., 2008).

3.0 MATERIALS AND METHOD

3.1 Fish

A total of 54 Red hybrid tilapia (*Oreochromis* sp.) fingerlings obtained from a fish farm in Beranang, Selangor with body length range from 7-10 cm were divided into three groups. The tanks were cleaned, disinfected and filled with dechlorinated water before the fish was randomly transferred equally into the tanks. All the tanks were aerated continuously throughout the study was carried out. The fish was acclimatised in the aerated glass tanks for a week before any procedures were done to the fish. The fish was fed twice a day with commercial fish pellet in the morning and evening.

3.2 Bacteria

Two species of bacteria from stock; *Streptococcus iniae* and *Aeromonas hydrophila* were cultured in two different TSA plates, incubated at 37°C for 24 hours. As to increase the virulence of each bacteria species, both of the bacteria were inoculated separately into 5 tilapia fish. Before that, 24 hours at 30°C TSB bacteria broth was prepared and 0.2 mL from broth was inoculated into the fish through intraperitoneal (i.p.) route. After 24 hours, a swab from brain, kidney and eye were taken and streaked onto the TSA, incubated at 37°C for 24 hours. New bacteria broths were prepared in the TSB from the cultured swab for each bacteria species and kept in shaking incubator for 22 hours at 30°C for the further experiment to be carried out.1 mL of each TSB broths was transferred into two micro centrifuges, followed by centrifugation at 3000 rpm for 15 minutes. The supernatant was discarded and the centrifugated bacteria were diluted with 1mL PBS. The diluted solution then was

compared with the McFarland to determine the concentration of the bacteria in the cultured broth. The final concentration of the bacteria was recorded at 10^{10} CFU/ml.

3.3 Experimental Design

This study was designed by acclimatising N=54 tilapia fish in three different tanks size with 18 fish for each tank for a week before bacterial inoculation. The tanks were labelled as Group A; infected with *Streptococcus iniae* while group B; infected with *Aeromonas hydrophila* and Group C; Control respectively. 0.2 mL bacteria from the prepared fresh broth were administered into the fish through (intraperitoneal) i.p. route. The study was done in 48 hours period where for every 12 hours interval starting from the point of bacteria was administered, three samples (n=3) were selected randomly to be examined. The three sample (n=3) taken was first immersed in the overdose MS222 to anaesthetise and euthanized by pitting method before any further procedure was done. The general appearance of each fish was observed before proceeding to the post-mortem. Eye and brain swab were taken for bacterial cultured for further conformation test. Brain, gills and kidney were taken for histology examination. Any clinical signs, macroscopic and microscopic lesions observed throughout the experiment were recorded.

3.4 Bacterial isolation and Identification

The swab from brain and eye were taken and immediately streaked onto the TSA and incubated and incubated at 37°C for 24 hours. The colony suspected to be *S. iniae* and *A. hydrophila* were then subculture again at 37°C for 24 hours to get pure culture which is needed for further conformation test. Then Gram staining was performed and observed under a microscope to confirm the cell morphology of both bacteria. Finally, the obtained pure colonies then further subjected to biochemical test related to the identification of both *S. iniae* and *A. hydrophila.*

3.5 Histology

The organs collected for histopathology evaluation in this experiment were brain, kidney and gills. The organ samples were first fixed in the 10% formalin and processed in the automatic tissue processor. The organ samples were further processed into paraffin blocks and sectioned serially at 3µm thickness and stained with haematoxylin and eosin. Stained slide was then mounted with DPX glue overnight. Finally, the slides were examined under a microscope for lesion scoring. The lesion was categorised into 0= none, 1= mild, 2= moderate and 3= severe.

3.6 Statistical Analysis

Statistical analysis was performed using SPSS, version IBM20 and tested at 5% level of significance. The test used to determine the significance of the lesion scoring was a nonparametric test; Kruskal Wallis test.

4.0 RESULTS

4.1 Clinical Signs and Macroscopic Lesion

The most prominent clinical signs shown by Group A were anorexia and motionless; where the fish were observed to be more at the base of the tank with limited movement. In addition, this group also produced long white faeces attached to the anus. Meanwhile, for Group B, the first clinical sign observed was the sudden death of 3 fish in the first few hours after infected with the bacterium and followed by anorexia to the remaining fish. The fish in this group was observed showing moderate air gulping and swimming closer to the water surface.

Macroscopically, both groups developed congestion in kidney, gills, brain and fin haemorrhage especially at the base of the fin, however, it is noticed that Group B looked more severe compared to Group A. Additionally, there was exophthalmia with cloudy eyes observed in group A while haemorrhagic operculum observed in group B.

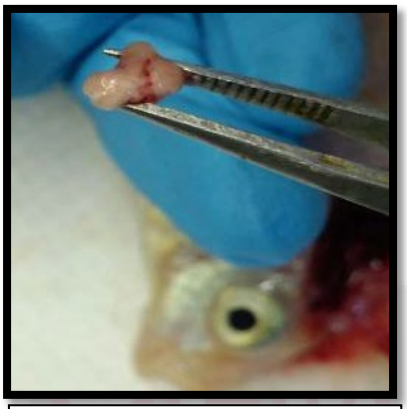


Figure 1: Congested brain

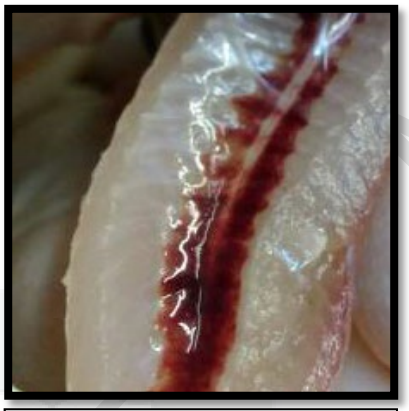


Figure 2: Congested kidney

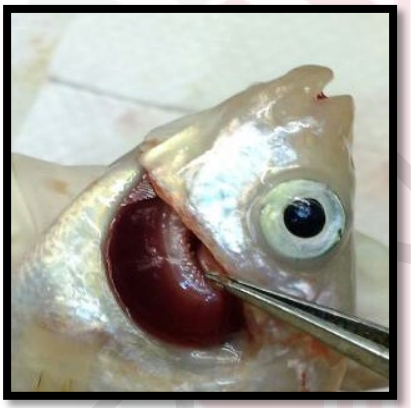


Figure 3: Congested gills

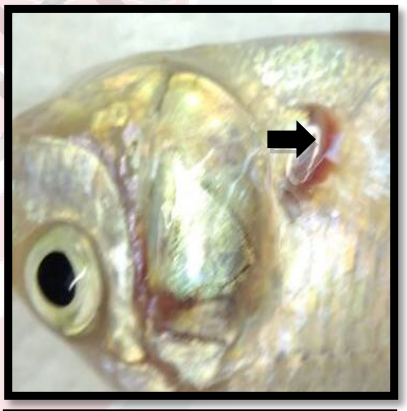


Figure 4: Fin haemorrhage

Figure 5: Exophthalmia with cloudy eye

Figure 6: Haemorrhagic operculum

4.2 Bacterial Isolation and Identification Result

The suspected bacteria from swab culture to be *S. iniae* and *A. hydrophila* were isolated to obtained pure culture. Then, Gram staining from thepure culture from Group A showed Gram positive cocci while from Group B showed Gram negative rod bacteria. The pure cultures were further subjected to biochemical test and the results were obtained as in Table 1 and Table 2 respectively. Both bacteria fulfilled the criteria for *S. iniae* and *A. hydrophila* accordingly.

Test	**Result**
Catalase	Negative
Soluble haemolysin	Positive
NaCl	Negative
Bile	Negative
Lactose	Positive
Sorbitol	Negative
Trehalose	Positive

Table 1: Group A

Test	**Result**
Oxidase	Positive
TSI	-Acid -No HS produced -Gas present
Urea	Negative
SIM	Positive
Citrate	Negative

Table 2: Group B

4.3 Microscopic -Histology

The microscopic evaluation, the organs were focused on brain kidney and gills for both groups. Under the light microscope, all three organs were observed for congestion and infiltration of inflammatory cells. However, there was another one lesion that observed specifically in each organ involved vacuolation, degeneration of tubules and secondary lamellar changes in brain, kidney and gills respectively.

4.3.1 Brain

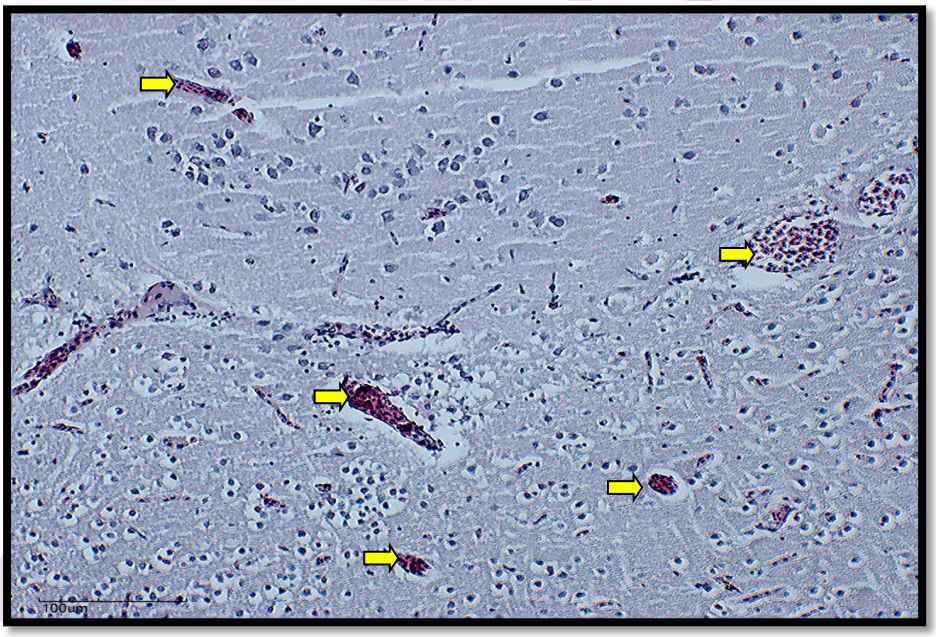


Figure 7: Congestion in brain (H&E x200)

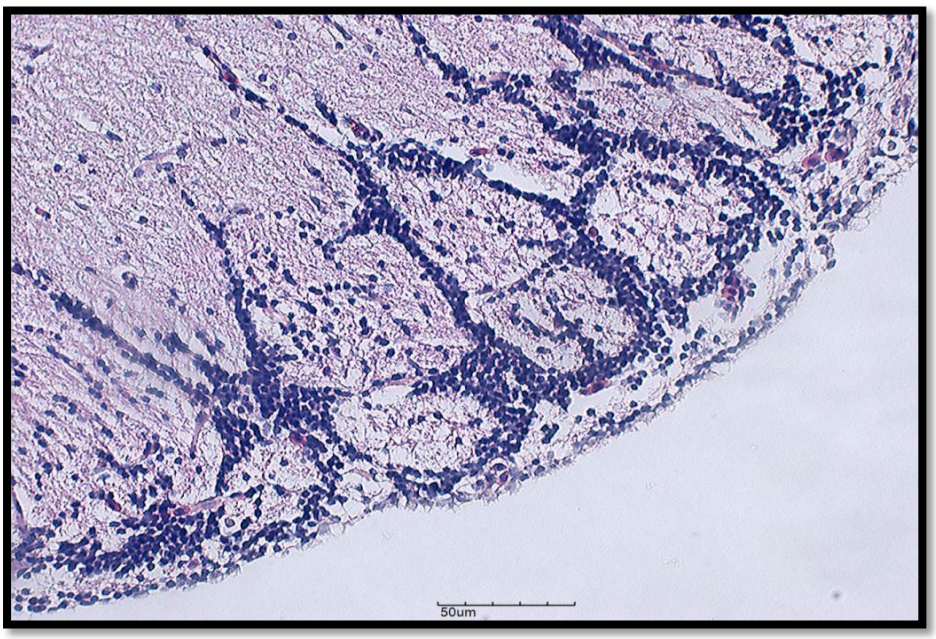


Figure 8: Meningitis (H&E x400)

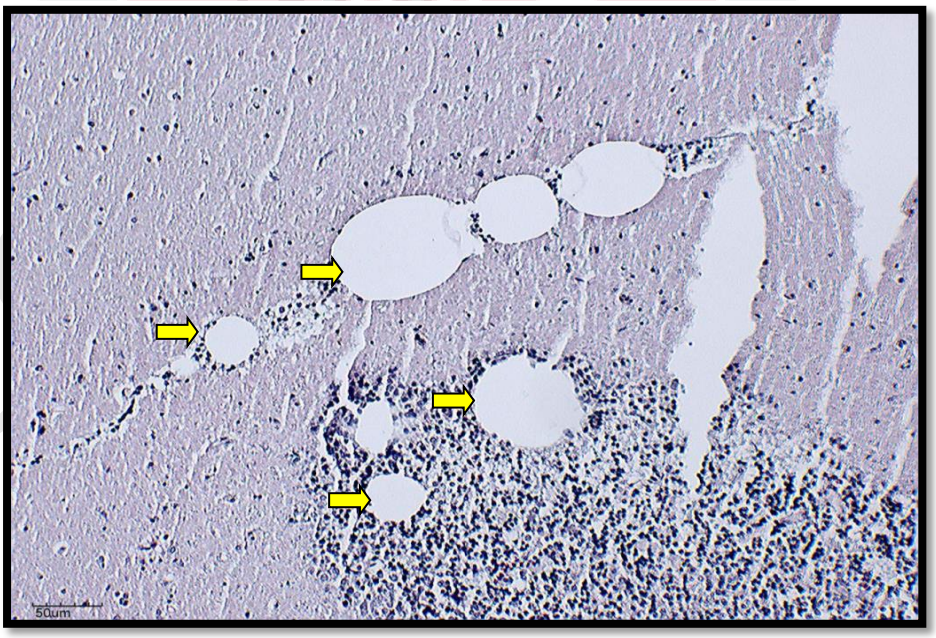


Figure 9: Vacuolation (H&E x200)

4.3.2 Kidney

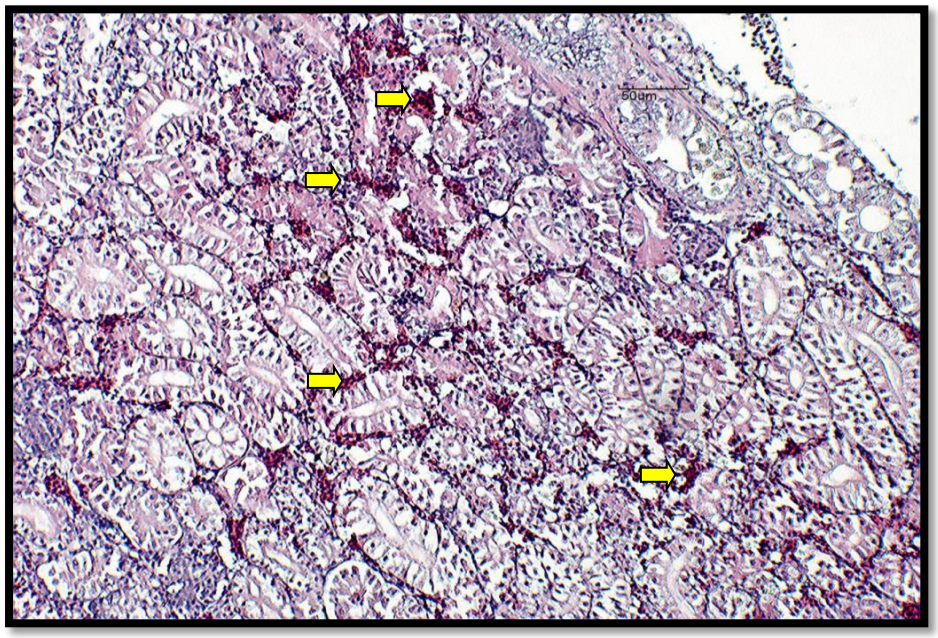


Figure 10: Congestion (H&E x200)

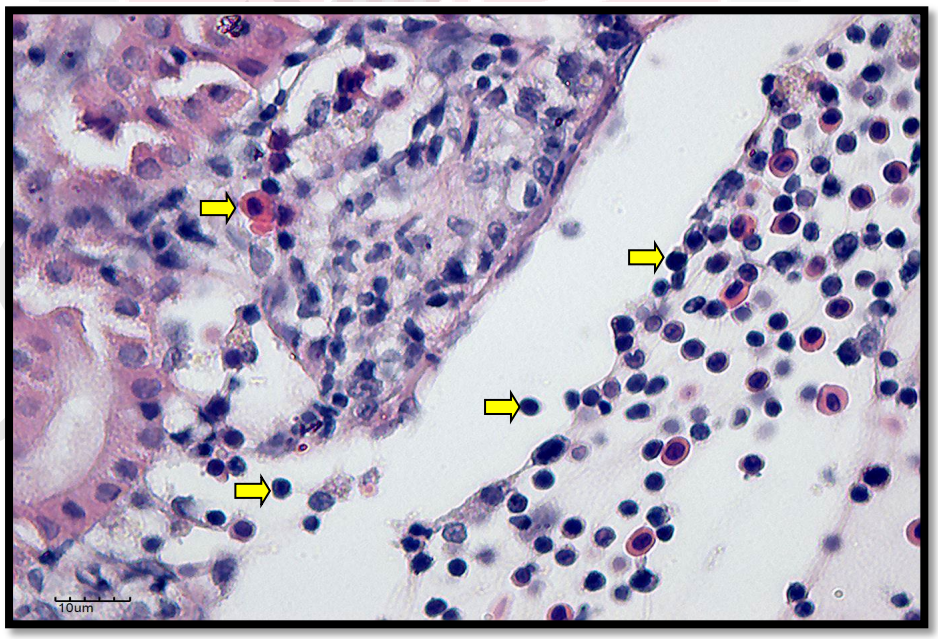


Figure 11: Inflammatory cells (H&E x1000)

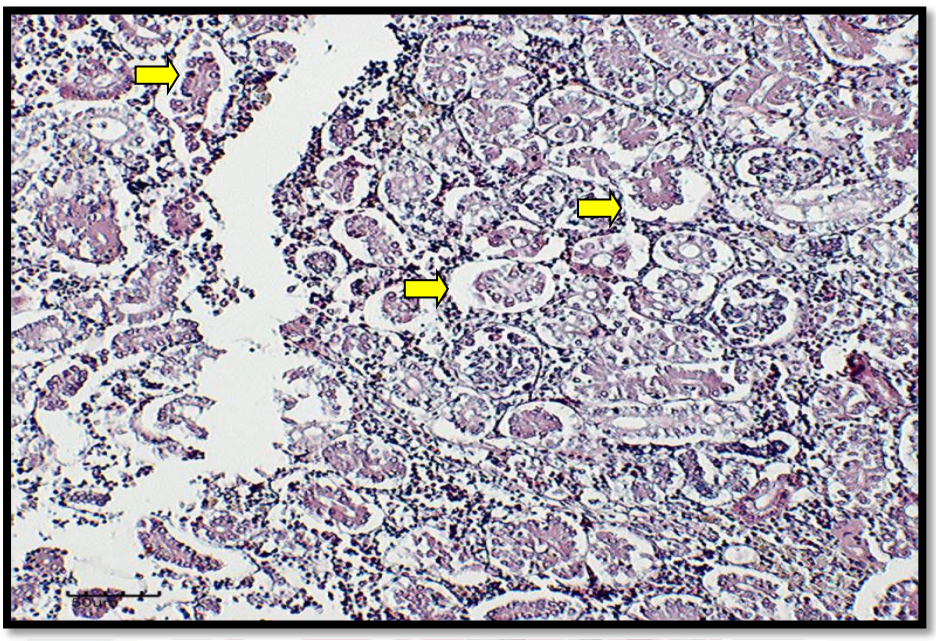


Figure 12: Degeneration of tubules (H&E x200)

4.3.3 Gills

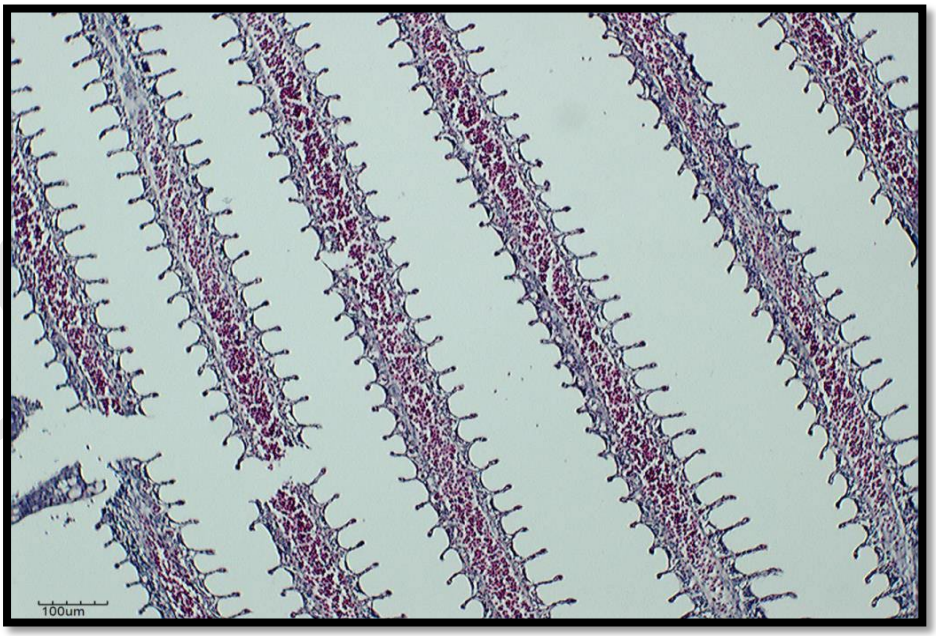


Figure 13: Congestion (H&E x400)

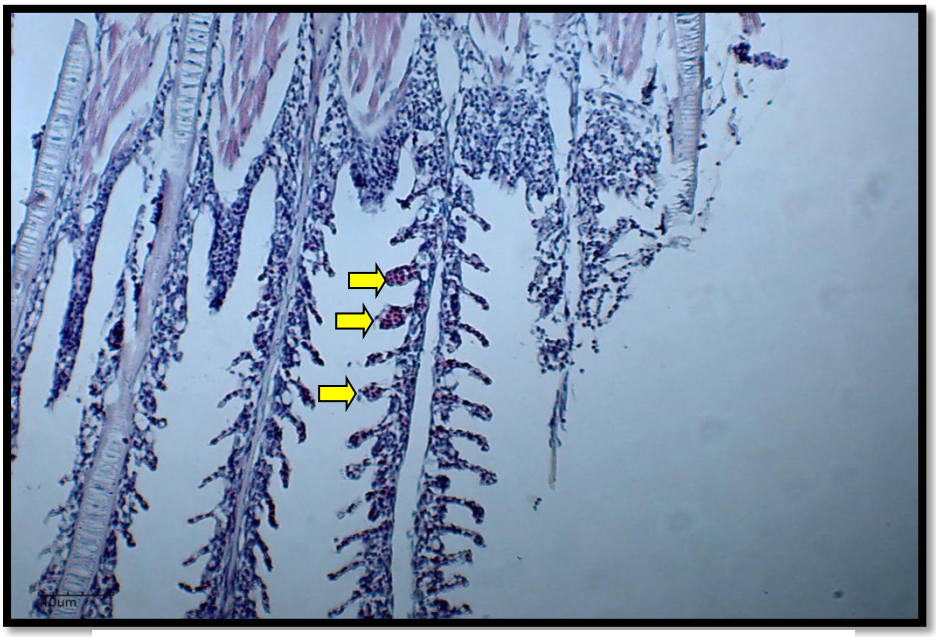


Figure 14: Aneurism of secondary lamella (H&E x200)

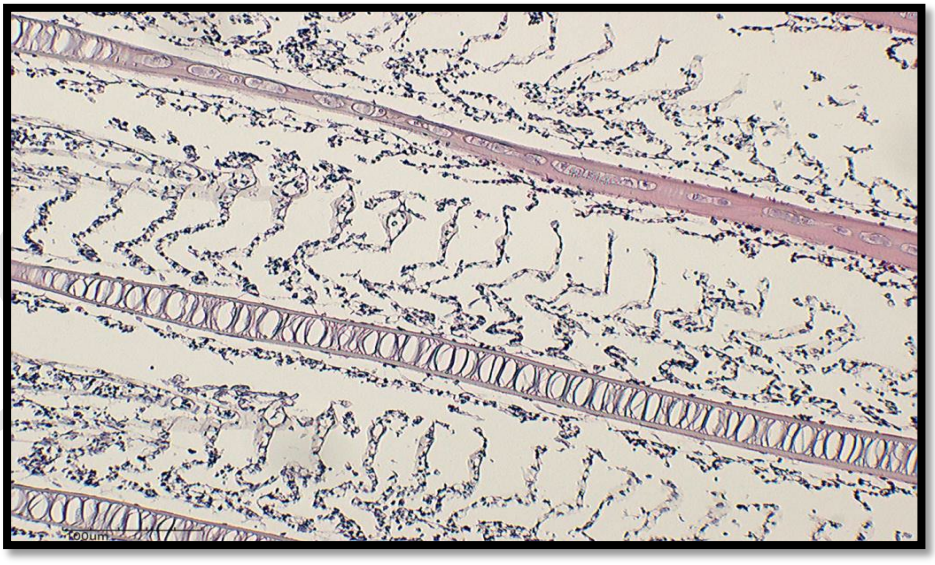


Figure 15: Curling of secondary lamellar (H&E x200)

4.4 Statistical Analysis

4.4.1 Comparison between Organ and Group

The microscopic lesions in specific organs obtained were tested for significance in comparing the severity of the lesion in each organ for both groups. The results as in Table 3 showed that the only organ has a significant difference when tested at 5% level of significance was in the gills at P value < 0.007. However, based on the bar graph, Group B showed high graph number in each organ compared to Group A.

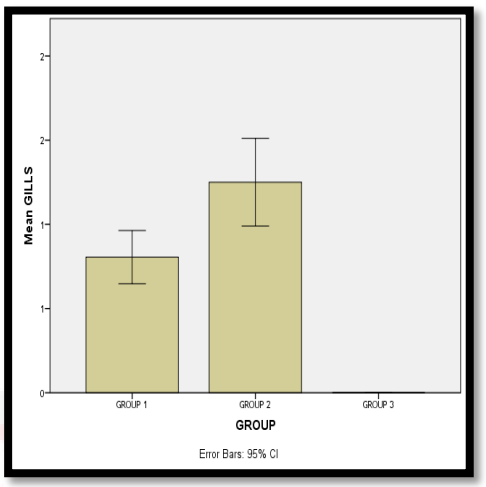
Test Statistics[a,b]

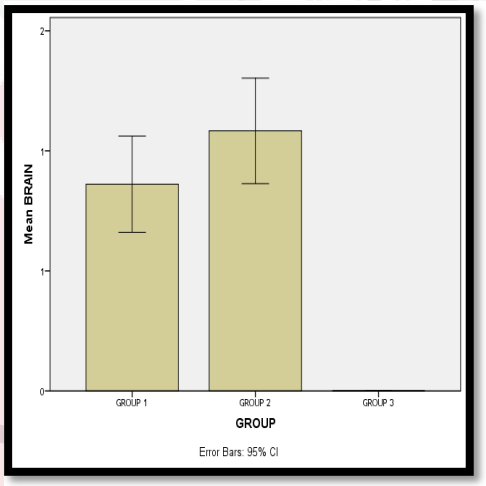
	BRAIN	KIDNEY	GILLS
Chi-Square	2.021	3.261	7.288
df	1	1	1
Asymp. Sig.	.155	.071	.007

a. Kruskal Wallis Test

b. Grouping Variable: GROUP

Table 3: Organ and Group





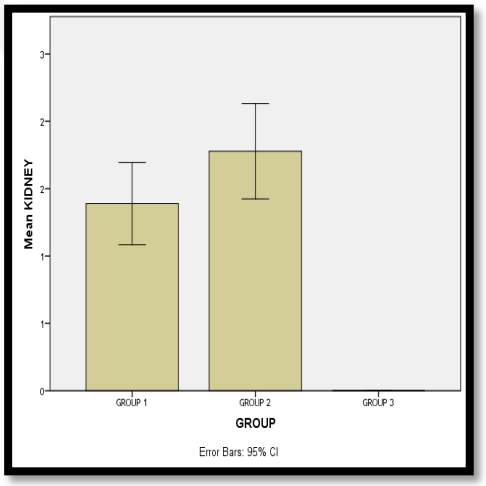


Figure 16, 17, 18: (from top) No significant difference in all graphs

4.4.2 Comparison between organ-hour-group

The lesions were further tested by comparing between organ-hour-group for significance at 5% level of difference. It was observed that only at 12 hours sampling showed significant difference except in the brain. For the next samplings, 24 hours, 36 hours and 48 hours, there were no significant different at all.

Test Statistics[a,b]

	BRAIN	KIDNEY	GILLS
Chi-Square	2.507	8.242	8.553
df	1	1	1
Asymp. Sig.	.113	.004	.003

a. Kruskal Wallis Test

b. Grouping Variable: HOUR

Table 4: Organ-Hour-Group

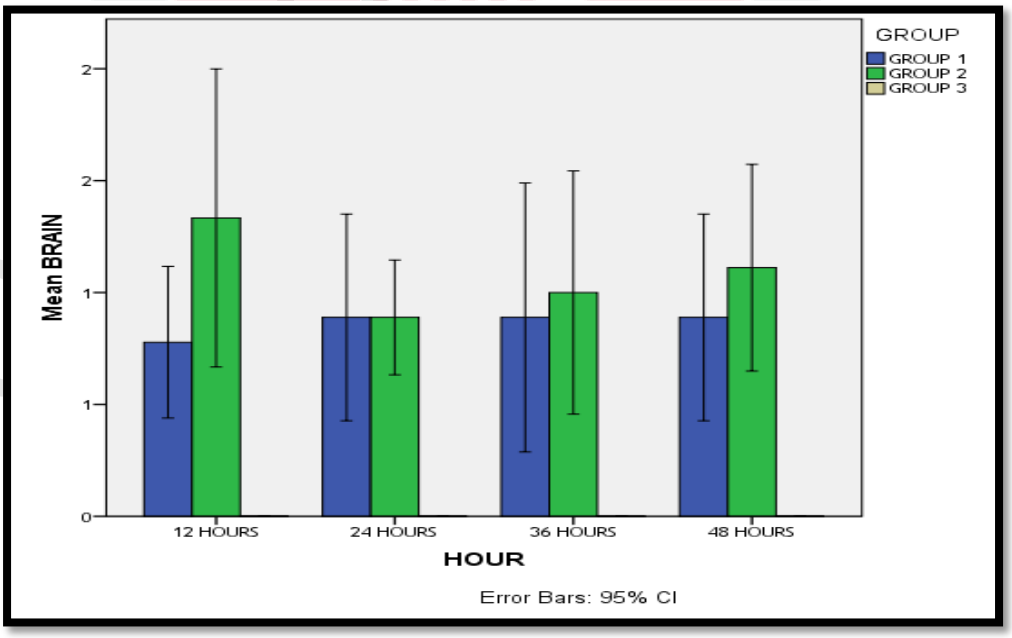


Figure 19: Organ- hour-group comparison in brain

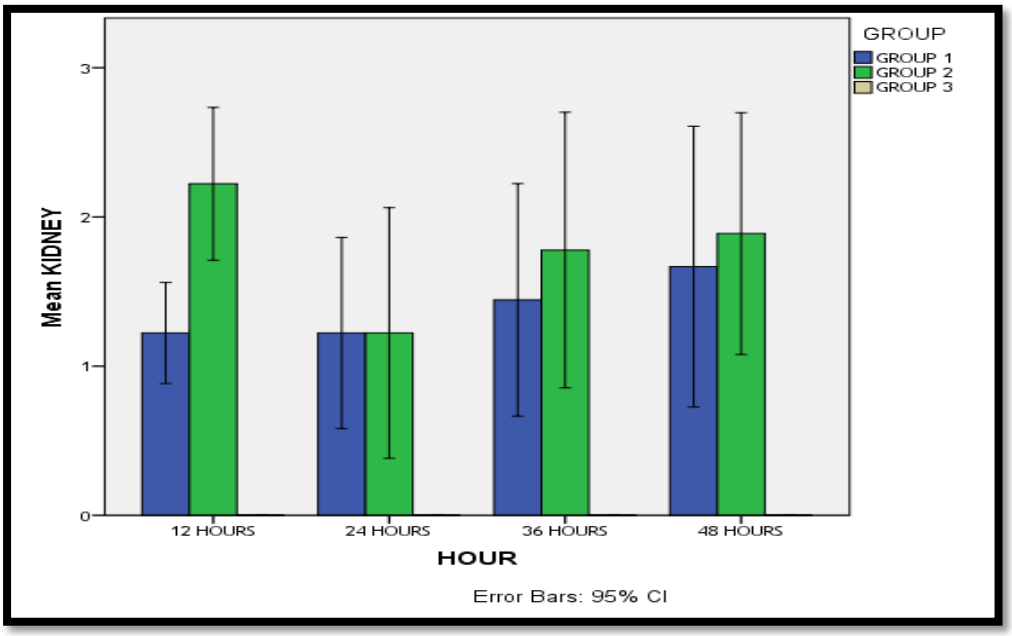


Figure 19: Organ- hour-group comparison in kidney

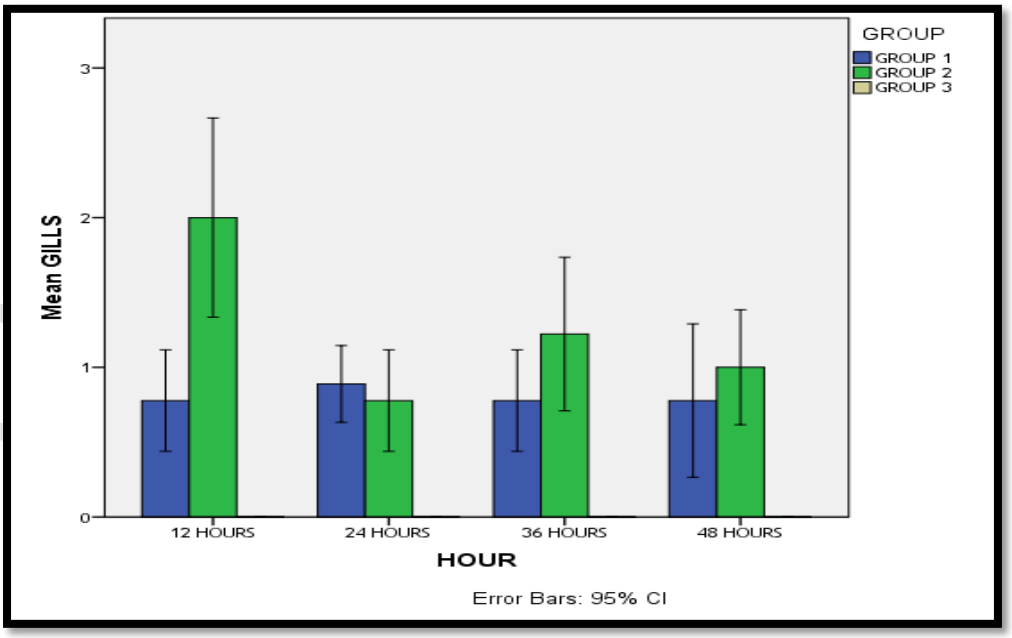


Figure 20: Organ- hour-group comparison in brain

5.0 DISCUSSION

In this study, the *Oreochromis* sp. challenged with *S. iniae* and *A. hydrophila* had shown various clinical signs that resembling the natural infection. The clinical sign and macroscopic lesions showed by Group A were anorexia, motionless, congestion in kidney, gills, brain and fin haemorrhage especially at the base of the fin along with exophthalmia with cloudy eyes. Some similar findings were also found in Chen et al. (2007) stated that clinical sign observed such as erratic swimming, anorexia, lethargy, and congestion of the internal organs. While in Group B, fish showed sudden death followed by anorexia to the remaining fish. The fish in this group was observed showing moderate air gulping and swimming closer to the water surface. These were agreed in Yardimci & Aydin (2011) who found that tilapia infected with *A. hydrophila* showed hyperaemia of the fin bases and fin rot, hyperaemia in gills, kidney and heart, focal hyperemia of the skin over the pectoral fins and swimming closer to the surface.

The microscopic lesion was analysed with Kruskal Wallis Test for significance, based on the comparison result between organ and group, all three organs showed no significant, but Group B demonstrated higher bar graph value which indicated more severe lesion compared with Group A thus, we can say that *A. hydrophila* is more pathogenic than *S. iniae*. This finding is supported in Griffin et al. (2013) highly virulent strain of *A. hydrophila* has emerged to be the primary pathogen in cultured freshwater fish.

From the result that compared the lesion between organ-hour-group, it can be observed that there was a significant difference in kidney and gills which P value

less than 0.05 with the exception in the brain in the first 12-hours sampling. Based on the graph, it also is shown that in the first 12-hours sampling period, Group B showed immediate and had the highest infection compared to Group A and changed constantly over the period. This explained that the most infective period for *A. hydrophila* was at the earlier stage. It was found that in the acute form of Aeromonad infection, a fatal septicaemia may occur so rapidly and fish died before developing clinical signs (Yardimci & Aydin, 2011).However, in Group B, the infection by *S. iniae* showed by the graph was more or less similar in all organ and sampling period or slowly progressive over the 48 hours. This can be supported by Rahmatullah et al. (2017) *S. iniae* infection can be carried asymptomatically but can be associated with sporadic disease outbreaks.

6.0 CONCLUSION AND RECOMENDATIONS

Since there were some different clinical signs observed in *Oreochromis* sp., therefore we can reject the first Ho. In addition, since there was a different degree of severity of the lesions seen in gills, $P<0.05$, observed between groups, we reject the second Ho for gills but fail to reject the second Ho in brain and kidney. As overall, fish infected with *S. iniae* and *A. hydrophila* may develop some similar clinical signs and pathological changes but generally more severe in *A. hydrophila* infection.

As for further study, some recommendations can be taken into consideration. First, we can try to have uniform size of the samples and increase the sample size for more accurate data. Furthermore, it is recommended if the experiment is conducted in an enclosed area to fix the environmental factor. Last but not least, we can also take other organ samples such as eyes and liver for further information of the bacterial infection.

REFERENCES

Agnew, W., & Barnes, A.C. (2007). *Streptococcus iniae*: an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. Veterinary Microbiology 122(1-2): 1-15.

AMN, A., Nur-Nazifah, M., Siti-Zahrah, A., Sabri, M. Y., & Zamri-Saad, M. (2008). Determination of LD50 for Streptococcus *agalactiae infections* in red tilapia and GIFT.

Chen, C., Chao, C., & Bowser, P., R., (2007). Comparative histopathology of *Streptococcus iniae* and *Streptococcus agalactiae*-infected tilapia, *Bulletin-European Associataion of Fish Pathologist, 27(1), 2.*

Cipriano, R.C., Bullock, G.L., & Pyle, S.W. (1984).*Aeromonas hydrophila* and motile aeromonad septicemias of fish.US Fish & Wildlife Publications 134.

Griffin, M. J., Goodwin, A. E., Merry, G. E., Liles, M. R., Williams, M. A., Ware, C., & Waldbieser, G. C. (2013). Rapid quantitative detection of Aeromonas hydrophila strains associated with disease outbreaks in catfish aquaculture. *Journal of Veterinary Diagnostic Investigation*, *25*(4), 473-481.

Hamzah, A., Nguyen, N.H., Ponzoni, R.W., Kamaruzzaman, N., & Subha, B. (2008). Performance and survival of three red tilapia strains (*Oreochromis*spp.) in pond environment in Kedah state, Malaysia. In International Symposium on Tilapia in Aquaculture 8: 199-211.

Hassan, M., Zakariah, M.I., Wahab, W., Muhammad, S.D., Idris, N., & Jasmani, S. (2013). Histopathological and behavioural changes in *Oreochromis* sp. after exposure to different salinities.J. Fisheries Livest. Prod. 1(103): 2.

Laith, A.R., &Najiah, M. (2013).*Aeromonas hydrophila*: antimicrobial susceptibility and histopathology of isolates from diseased catfish, *Clarias gariepinus* (Burchell). Journal of Aquaculture Research and Development 5: 215.

Morrison, Carol M., Kevin Fitzsimmons, and James R. Wright Jr. (2006). Atlas of Tilapia Histology. The World Aquaculture Society, Baton Rouge, Louisiana 70803. United State.

M. Rahmatullah, M. Ariff, M. Kahieshesfandiari, H.M. Daud, M, Zamri-Saad, M.Y. Sabri, M.N.A. Amal & M.Y. Ina-Salwany (2017). Isolation and pathogenicity of *Streptococcus iniae* in cultured red hybrid tilapia (*Oreochromis* sp.) in Malaysia, Journal of Aquatic Health29(4): 208-213.

Pachanawan, A., Phumkhachorn, P., &Rattanachaikunsopon, P. (2008). Potential of *Psidiumguajava* supplemented fish diets in controlling *Aeromonas hydrophila* infection in tilapia (*Oreochromis niloticus*). Journal of Bioscience and

Pretto-Giordano, L.G., Scarpassa, J.A., Barbosa, A.R., Altrão, C.S., Ribeiro, C.G G., & Vilas-Boas, L.A. (2015). *Streptococcus iniae*: an unusual important pathogen fish in Brazil. Journal of Aquaculture Research & Development 6(9): 1.

Rattanachaikunsopon, P., & Phumkhachorn, P., (2010). Potential of cinnamon (Cinnamon verum) oil to control *Streptococcus iniae* infection in tilapia (*Oreochromis niloticus*). Fisheries Science Science, 76(2), 287-293.

Suanyuk, N., Sukkasame, N., Tanmark, N., Yoshida, T., Itami, T., Thune, R.L., & Supamattaya, K. (2010). *Streptococcus iniae* infection in cultured Asian sea bass (*Lates calcarifer*) and red tilapia (*Oreochromis* sp.) in southern Thailand.Songklanakarin Journal of Science & Technology 32 (4): 341-348.

Soto, E., Fernandez, D., Thune, R., & Hawke, J. P. (2010). Interaction of Francisella asiatica with tilapia (*Oreochromis niloticus*) innate immunity. *Infection and immunity*, *78*(5), 2070-2078.

Swann, L., & White, M.R. (1991). Diagnosis and treatment of" *Aeromonas hydrophila"* infection of fish. Aquaculture Extension, Illinois-Indiana Sea Grant Program.

Yardimci, B., &Aydin, Y. (2011).Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*). Ankara Univ Vet FakDerg 58: 47-54.