IDENTIFICATION OF ECTOPARASITES IN GROUPER FISH (*Epinephelus spp.*) FROM 2 COMMERCIAL FARMS IN KELANTAN

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(D17B0045)

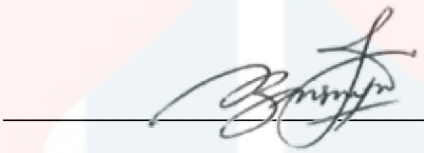
A RESEARCH PAPER SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITI MALAYSIA KELANTAN
IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR
THE DEGREE OF
DOCTOR OF VETERINARY MEDICINE

MAY 2022

UNIVERSITI MALAYSIA KELANTAN

CERTIFICATION

This is to certify that we have read this research paper entitled **'Identification of Ectoparasites in Grouper Fish from Two Commercial Fish Farms in Kelantan'** by Nur Ain Suhaila Binti Che Ujang, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 5436 – Research Project.



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ACKNOWLEDGEMENT

First and foremost I would like to express my greatest gratitude to Allah S,W,T for the strength given to complete this project to fulfill the course DVT 5436 Final Year Research Project.

Special thanks and appreciation to my honourable project supervisor, Dr Basripuzi Nurul Hayyan and co-supervisor, Dr Ruhil Hayati for all the guidance and patience in guiding me in completing this project.

And not to forget to thank Dr Nora Faten Afifah, aquatic laboratory assistant at Universiti Malaysia Kelantan for her willingness to help me throughout this project. Special thanks to all lecturers and staffs of Faculty Veterinary Medicine, Universiti Malaysia Kelantan who involved in this final year research project.

I also would like to take this special opportunity to express my gratitude for having my beloved mother and family throughout this journey for their prayer and constant support.

Lastly, I also would like to thank Aida Hashim, all my groupmates and my classmates for their moral support

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ABSTRACT

Ectoparasitic infestation is the presence of parasites outside the body of host that depend on their host for sustenance, maturation and reproduction. It is one of the most concerning problems in fish farming especially in commercial fish farms because this condition can affect the quality and the production of the fish. This study was conducted to identify the ectoparasites that infest the grouper fish in two commercial fish farms located in Bachok and Pengkalan Chepa, Kelantan.A total of 30 grouper fish were collected in this study. Physical examination was conducted on the fish and ectoparasites were collected for identification. A Chi square test was performed to analyze the data. As for the result, the ectoparasites such as *Zeylanicobdella sp.* (marine leech), *Dactylogyrus sp.* (monogenean) and *Halacarid sp.* (marine mites). were identified from the grouper fish. The prevalence of *Zeylanicobdella sp.* was 80.0% and 0.0% in Farm A and Farm B, respectively. While the both prevalence of *Dactylogyrus sp.* and *Halacarid sp.* was 100.0% in Farm A and 0.0% in Farm B, respectively. The presence of ectoparasites is associated with the lower salinity water. Based on the water quality assessment, the lower salinity can contribute to poor water quality which can induce the infestation of ectoparasites. In this study, *Zeylanicobdella sp* was detected on the skin and gills with prevalence rate of 20.0% and 6.0%, respectively. *Dactylogyrus sp.* and *Halacarid sp.* were detected in the mouth. The prevalence rate of Dactylogyrus sp. was 30.0% on the skin while 33.3% for both gills and mouth. The number of *Halacarid sp.* was lower at the gills and mouth with prevalence rate of 30.0% than the prevalence on the skin (33.3%). As a conclusion, only three ectoparasite genus were identified infesting the fish in Farm A, while no ectoparasites was identified in Farm B. The common sites of ectoparasite infestations were skin, gills and mouth.

***Keywords**: Ectoparasites, grouper fish, identification, water quality*

ABSTRAK

Infestasi ektoparasit adalah kehadiran parasit di luar badan hos yang bergantung pada hos mereka untuk makanan, kematangan dan pembiakan. Ia merupakan antara masalah yang paling membimbangkan dalam penternakan ikan terutama di ladang ikan komersial kerana keadaan ini boleh menjejaskan kualiti dan pengeluaran ikan tersebut. Kajian ini bertujuan untuk mengenalpasti ektoparasit yang menyerang ikan kerapu di ladang ikan komersial di Kelantan. Oleh itu, sebanyak 30 ekor ikan kerapu telah diambil daripada 2 buah ladang ternakan ikan komersial di Kelantan dan pemeriksaan fizikal untuk mengumpul dan mengenal pasti ektoparasit telah dilakukan. Ujian Chi Square telah dijalankan untuk menganalisis data yang diperolehi. Hasilnya, ektoparasit seperti *Zeylanicobdella sp* (lintah laut), *Dactylogyrus sp.* (monogenea) dan *Halacarid sp*. (Tungau laut) dikenapasti daripada ikan kerapu. Kelaziman *Zeylanicobdella sp* adalah 80.0% dan 0.0% di Ladang A dan Ladang B, Masing-masing. Manakala kelaziman *Dactylogyrus sp.* dan *Halacarid sp.*adalah masing-masing 100.0% di Ladang A dan 0.0% di Ladang B. Kehadiran ektoparasit dikaitkan dengan salinity air yang lebih rendah. Berdasarkan penilaian kualiti air, saliniti yang lebih rendah boleh menyumbang kepada kualiti air yang buruk yang boleh mencetuskan infestasi ektoparasit. Dalam kajian ini juga, menunjukkan *Zeylanicobdella sp* berada di kulit dan insang dengan kadar kelaziman masing-masing 20.0% dan 6.0%, manakala kehadiran *Dactylogyrus sp.* dan *Halacarid sp.* juga berlaku di dalam mulut. Kadar kelaziman 30.0% pada kulit manakala 33.3% bagi kedua-dua insang dan mulut yang terjejas oleh *Dactylogyrus sp.* manakala bilangan *Halacarid sp*. lebih rendah pada insang dan mulut dengan kadar kelaziman 30.0% dan kulit lebih tinggi sedikit dengan kelaziman 33.3%. Sebagai kesimpulan, hanya tiga ektoparasit dikenal pasti di Ladang ikan A, manakala tiada ektoparasit dikenal pasti di Ladang B. Dan tempat yang menunjukkan kehadiran ektoparasit ialah kulit, insang dan mulut.

Kata kunci: *Ektoparasit, ikan kerapu, kualiti air, pengenalpastian*

1.0 Introduction

The fisheries industry in Malaysia is growing and provides about 60% of the total animal protein consumed in Malaysia (Abu Talib et al., 2013). Groupers is one of the most famous and important commercial species of fish that has high demand in Malaysia (Rumeida, 2018). According to her, the number of each grouper species in Malaysia was counted, and it was more than in any prior year, reached 11,388 tonnes. The grouper catches were greater in the fishing zone with the most marine parks. Sixty-eight species of groupers from over 500 species worldwide are reportedly found in Malaysia (Ambak et al., 2012). The east coast of Peninsular Malaysia contributed to the highest landing of groupers in Malaysia which due to its geographical location that faces the South China Sea (Haifa et. al., 2008).

Due to high demand in groupers and declining of catches from the ocean, there are increase in cultured fish not only of groupers but of all marine fish as this alternative way to increase the production of the fish (Moksness et al, 2007). However, some studies show cultured groupers were showing higher ectoparasites compared to the wild ones (Seng, 1998). Futhermore, the rise of parasitic infestations has been linked to the development of cultured system. Parasitic infestations lead to a major economic impact on cultured farming system because anti-parasitic treatment for cultured fish is either impossible or costly (Barbara, 2007). In order to control this problem, the fish health management is very important. One of the important factors that contribute to the health and parasites infestation was water quality. There was a positive correlation between prevalence of parasites with dissolved oxygen, pH, and temperature (Wanja, et al.,2020).

2.0 Research problem

Nowadays, aquaculture industry has become the top 15 of global food producers and it is very profitable. However, there are some issues and challenges including ectoparasites infestation. Ectoparasite infestation can result in a reduction of fish quality and production. It can also affect human health. Parasites can have lethal impacts on host populations and their repercussions can result in significant losses for the fishing industry. Several studies have been conducted on the prevalence of ectoparasites in grouper fish in certain state in Malaysia. However, such studies have never been conducted in Kelantan.

3.0 Research questions

3.1 What are the genus of ectoparasites infesting grouper fish that are reared in the identified commercial fish farms in Kelantan?

3.2 What is the prevalence of ectoparasites infesting the grouper fish in the commercial farms?

4.0 Research hypothesis

The grouper fish reared in the identified farms to be infested with *Zeylanicobdella* spp. (marine leech), *Monogenea* spp. and *Digenea* spp. that are found on the surface of the body and gills while *Caligus* sp. (sea lice) will be found at the fish operculum.

5.0 Objectives

5.1 To identify the genus of ectoparasites that infest grouper fish that are reared in the identified commercial fish farm in Kelantan.

5.2 To determine the prevalence of ectoparasitic infesting the grouper fish in the commercial farms.

6.0 Literature review

6.1 Ectoparasite infestation in grouper fish

Humpback grouper (*Cromileptes altivelis*), tiger grouper (*Ephinephelus fuscoguttatus*), grouper fish *(E. fuscoguttatus x E. polyphekadion*), and cantang grouper (*Epinephalus sp*) are examples of cultured groupers that can be grown in hatcheries (Sudirman et.al.,2020). Mucus, fins, gills, and mouths had the highest infestations of ectoparasites. *Zeylanicobdella sp., Brooklynella hostilis, Caligus,* and *Dactylogyrus sp*. are among the ectoparasites identified in grouper (Yusni, 2019). The higher infestation of parasites may caused by the higher stocking density of the fish. As the stocking density above than the limit, it will cause the parasites easily to propagate in the fish body (Abe, 2020). However, the parasites infestation most affecting the cultured grouper compared to wild grouper that may due to the nature of the water condition (Ihwan *et. al*, 2013).

6.2 Clinical signs of ectoparasite infestation

According to a study of Sudirman *et. al.,* 2020, the prevalence of affected fish that were showing the lesions was 43.3%. The lesions were including the wound on the skin and gills. This condition may cause death to the grouper as the fish difficult to eat. Besides, hemorrhage and swelling on the attachment and feeding sites of the leech which cause consequent loss of large amount of

blood, the probable secondary effects of multiple feeding wounds, and the loss of appetite of the affected fish. The ectoparasite affected grouper will be moving abnormally (sideways), feeble, having blistered skin, fins, and gills, as well as breathing on the saltwater surface, or leaning (possessing a big head and slim body) (Erlida *et al*., 2000).

6.3 Poor water quality induce parasitic infestation

The most important parameters in water quality assessment are temperature, pH, dissolved oxygen (DO) and salinity. Based on a study conducted by Saha *et al*., (2013), acidic pH, lower DO and high temperature can induce the infestation and multiplication of ectoparasites. The low of dissolve oxygen may due to the oxidation of excessive feed. The acidic water pH can cause stress to the fish which lead to immune suppress to the fish (Machado et al, 2020). Fish can be susceptible to parasites infestation when pH rises above 11 as this can cause lesion on the gills, eyes and corneal of the fish. This condition is caused by the toxicity when the pH increase to become more alkaline. (Ojwala *et. al.*, (2018).

7.0 Materials and methods

7.1 Water quality analysis

Water quality was analysed by testing the water sample collected from the concrete tank that grouper fish being reared. The parameters used to analyse the water quality include temperature, dissolve oxygen, pH, and salinity of the water by using thermometer, DO meter, pH meter and refractometer respectively.

7.2 Fish collection and examination

Thirty grouper fish (*Epinephalus* sp.) were collected from two commercial fish farms in Kelantan which located at Bachok (Farm A) and Pengkalan Chepa (Farm B). The fish were transported in plastic bags with oxygen supplied once the water and fish have been introduced. A twisted rubber band was used to close the bag after filled with oxygen. Whenever feasible, the first plastic bag should be placed inside a second bag to prevent leaks. These containers may carry fish for up to 48 hours without needing to replace the water if properly wrapped.

Upon arrival at the aquatic laboratory, the size and weight of the fish were measured. The weight was measured by using weighing scale while the total length and standard length were measured by using a ruler.

The fish were examined for abnormal colour changes, eye lesions, swelling of the coelomic cavity and musculoskeletal abnormalities. The gills were examined by lifting the operculum. Fins were spread to be examined, and evaluation of the oral cavity was also conducted.

7.3 Ectoparasites examination and identification

The ectoparasites that can be observed by naked eyes were collected manually from the fish body. After removal of the ectoparasites, the body surface, fins, gills, and oral cavity of the fish were gently scraped with scalpel blade. In addition, some part of gills and fins will be gently cut to be observed under a dissecting microscope.

The collected ectoparasites were observed grossly and microscopically for their morphology. For gross observation, the ectoparasites were taken directly from from the body of the fish and placed on a board. The colour, size and the structure of the parasites were recorded. For microscopic examination, the specimens were placed under the microscope for further identification.

7.4 Statistical Analysis

A Chi-square test was used to determine the prevalence of ectoparasites collected from the fish (Marta et al., 2011). The test was performed using the SPSS Version 27 software. The prevalence was calculated based on the following formula:

$$\text{Prevalence (\%)} = \frac{No.of\ cases}{No.of\ individuals\ in\ the\ study} \text{x100}$$

8.0 Results

Table 8.1: Genus of ectoparasites among the collected grouper fish.

Magnification factor	The appearance of ectoparasites under the microscope	Identified genus	Site	Number of collected ectoparasite	Number of infested fish
4x		*Zeylanicobdella sp.*	• Skin	40	8
40x		*Dactylogyrus* sp.	• Skin • Gills • Mouth	229	10
100x		*Halacarid sp.*	• Skin • Gills • Mouth	121	10

Table 8.1 shows the identified genus of ectoparasites which are *Zeylanicobdella sp.* (marine leech), *Dactylogyrus sp*. (monogenean), and *Halacarid sp* (marine mite) which were found at the skin, gills and mouth. This ectoparasites were identified based on morphological characteristics.

Table 8.2: The prevalence of ectoparasites based on genus in Farm A and Farm B.

Number of infested and non-infested fish	Ectoparasite genus		
	Zeylanicobdella sp.	*Dactylogyrus sp.*	*Halacarid sp.*
Farm A			
Infested fish	8	10	10
Non-infested fish	2	0	0
Prevalence (%)	80	100	100
Farm B			
Infested fish	0	0	0
Non-infested fish	20	20	20
Prevalence (%)	0	0	0

Table 8.2 shows the prevalence of ectoparasites based on the genus which are *Dactylogyrus sp., Zeylanicobdella sp., and Halacarid sp.*

Table 8.3: The prevalence of ectoparasites based on the affected sites

Sites	Prevalence (%)		
	Zeylanicobdella sp.	*Dactylogyrus sp.*	*Halacarid sp.*
Skin	20.0	30.0	33.3
Gills	6.0	33.3	30.0
Mouth	-	33.3	30.0

*based on the presence of parasites at the skin, gills and mouth

Table 8.3 shows the prevalence of ectoparasites at the common sites of infestation. The prevalence was calculated by using the stated formula and the result showed that *Zeylanicobdella sp.* has higher prevalence rate on the skin in comparison to gills which were 20% and 6%, respectively. While the prevalence of *Dactylogyrus sp.* infesting the skin was lower than gills and mouth which were 30% and 33.3%, respectively. *Halacarid sp.* was recorded to infest the skin (33.3%), as well as the gills and mouth of the fish.

Table 8.4: Water quality parameters in Farm A and Farm B

Water Quality Test	Range	Farm A	Farm B
pH	6.80-7.80	6.09	7.01
Temperature (˚C)	23-27	25	32
Dissolved Oxygen (my/L)	6.00-8.00	5.85	3.27
Salinity (ppt)	15-25	7.48	16

Table 8.4 shows that the water quality parameters of Farm A was within normal range for temperature but lower in pH, dissolved oxygen and salinity. However, Farm B has higher temperature and lower dissolved oxygen than the recommended range which was not a good environment for the cultured fish.

9.0 Discussion

The findings show that there were three identified genus of ectoparasites based on the morphologic characteristic that were detected in this study namely *Zeylanicobdella sp.* (marine leech), *Dactylogyrus sp.* (monogenean) and *Halacarid sp.* (marine mites). The ectoparasites were identified based on the morphology observed under a light microscope. The identification can only be conducted at genus level. Molecular identification need to be conducted to confirm the species. Among the three genus of ectoparasites that have been detected, *Zeylanicobdella sp.* (marine leech) was the biggest ectoparasites that can be seen by naked eyes. However, the observation under the microscope provides clearer view on the morphology of the leech. In this study, *Dactylogyrus* sp was identified as the trematode that infesting the grouper fish.

In this study, the prevalence of groupers affected by *Zeylanicobdella sp.* was 80% in Farm A and 0.0% in Farm B. While both *Dactylogyrus sp.* and *Halcarid sp.* have the prevalence rate of 100% in Farm A and 0.0% in Farm B. This result showed high prevalence rate of ectoparasites infestation in Farm A while there was no ectoparasite found in Farm B. Poor water quality may contribute to the infestation and propagation of ectoparasites among the grouper fish. Declined water quality may cause stress to the fish which can lead to immunosuppression. This condition may induce the introduction of other infectious diseases including endoparasite infestation (Dunier, 1996). However, the findings in this study showed that Farm A has higher prevalence compared to farm B even though most of the parameters of water quality tested in the Farm A and Farm B were poor as Farm A has slightly low pH and dissolved oxygen and very low in salinity, meanwhile for Farm B were high in pH and temperature but low in dissolved oxygen. Both condition may induce the infestation of the ectoparasites, however poor health of the grouper fish also might be caused by other factors that were not a concern in this study.

On the other hand, in this study, the skin has the highest number of *Zeylanicobdella sp.* with a prevalence rate of 20% compared to gills at 6.0% and mouth with prevalence rate of 0%. This may be due to the easier accessibility of the skin for attachment and feeding. While the number of *Dactylogyrus sp.* was higher at gills and mouth as both prevalence rate of 33.3%, followed by skin with prevalence rate of 30.0%. But the gills and mouth has lower number of *Halacarid sp.* with prevalence rate of 30.0% and skin slightly higher with the prevalence of 33.3%.

10.0 Conclusion

In conclusion, there were three genus of ectoparasites that have been identified in this study namely *Zeylanicobdella sp.* (marine leech), *Dactylogyrus sp.* (monogenea) and *Halacarid sp.* (marine mites). The study demonstrates a prevalence rate of 80% for *Zeylanicobdella sp.*,100.0% for both *Dactylogyrus sp.* and *Halacarid sp.* among collected groupers in Farm A. The common sites for *Zeylanicobdella sp.* was the skin followed by gills. *Dactylogyrus sp.* and *Halacarid sp.* presence on skins, gills, and mouth. In this study the presence of ectoparasites associated with the lower levels of salinity.

11.0 Recommendations and future work

There are several suggestions and recommendations to improve if the same study design is conducted in the future. Firstly, I would like to increase the sample size to get more valid and accurate results. To calculate the sample size, researcher can use the following formula:

$$\text{Sample size} = \frac{(Z\text{-}score)^2 x\ StdDev\ x\ (1-StdDev)}{(confident\ interval)\text{^}2}$$

In addition, to get valid results for identification, the processed samples should be observed immediately under a microscope as some of the parasites may already died and undergo morphological changes when detached from their host and left dry on a microscope slide. Thus it is advisable to process and observe the samples on the same day of collection.

Appendix A

Appendix A.1: Environment of Farm A in Bachok, Kelantan.

Appendix A.2: Fish tanks in Farm B, Pengkalan Chepa, Kelantan.

Appendix B

IACUC UMK/ CONFIDENTIAL

Universiti Malaysia KELANTAN

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
UNIVERSITI MALAYSIA KELANTAN

Application for Approval
of a Project Involving the Use and Care of Animals

NOTE:

1. Please complete the application form in accordance to the Animal Ethics Committee Guidelines. Incomplete application will result in the return of the application and delay in the granting of the approval.
2. Attach a copy of the proposal (research / elective / teaching / other).
3. Application must be word-processed or typewritten and forwarded to the Chairperson, Animal Ethics Committee of the Faculties/Centres (ACUC PTj), Universiti Malaysia Kelantan (UMK).

TYPE OF APPLICATION: [Please tick (/)]

RESEARCH (/) / ELECTIVE ( ) / TEACHING ( ) / OTHER ( ) Please specify: _____

If teaching / elective project, state course name and code:

FINAL YEAR PROJECT (DVT 5463)

NAME OF PRINCIPAL INVESTIGATOR / CO-ORDINATOR / SUPERVISOR / CHAIRPERSON:

DR. BASRIPUZI NURUL HAYYAN BINTI HASSAN BASRI

DR RUHIL HAYATI BINTI HAMDAN

FACULTY / CENTRE:

FACULTY OF VETERINARY MEDICINE

PROJECT TITLE:

IDENTIFICATION OF ECTOPARASITES IN GROUPER FISH (IPINEPHELUS SP.) AT A COMMERCIAL FISH FARM IN BACHOK, KELANTAN.

Received by Secretary, Animal Ethics Committee

ACUC PTj File No: **Date:**

Appendix B.1: Ethic form for permission to perform the project

UMK/FPV/LAB/FYP F02

VETERINARY DIAGNOSTIC & SERVICES UNIT
FACULTY OF VETERINARY MEDICINE
UNIVERSITI MALAYSIA KELANTAN

PLEDGE FORM

Please (✓) the relevant box :			
Undergraduates	/	Research (*GRA / RA / Enumerator*)	
Postgraduates (*MSc / PhD)*		Others (please specify) :	

A. Application Details

Name : Nur Ain Suhaila Binti Che Ujang Matric No. : D17B0045

Email : Suhaila.d17b0045@siswa.umk.edu.my Phone No. : 0142234856

Supervisor's name : Dr. Basripuzi Nurul Hayyan Binti Hassan Basri

B. Laboratory Rules

i. **SAFETY IS PRIORITY**. Please ensure that you have read, understand and adhere to all rules and regulations in the labs.
ii. Labs and preparation room are considered as **PROHIBITED AREA** for students. Students are **NOT ALLOWED** to enter the labs and preparation room without seeking any permission from the lecturers or laboratory staffs.
iii. Eating, drinking, gum chewing, applying cosmetics, manipulating contact lenses, and other unsafe activities are not permitted in the laboratory.
iv. **ALWAYS** wear a lab coat during laboratory experiments. Lab coats should not be worn outside the laboratory.
v. Long hair, dangling jewelleries, and loose or baggy clothing are a hazard in the laboratory. Long hair **MUST** be tied back, and dangling jewelleries and baggy clothing must be secured. Shoes must **COMPLETELY** cover the foot. Sandals or open-toed shoes are **NOT ALLOWED** in the laboratory.
vi. Make sure to wear safety glasses or face shields when working with hazardous materials and/or equipments.
vii. Be prepared for your work in the laboratory. Read all procedures thoroughly before entering the laboratory. Never fool around in the laboratory. Horseplay, practical jokes, and pranks are dangerous and prohibited.
viii. Unauthorized experiments, work and preparations are **NOT ALLOWED**.
ix. Labels and equipment instructions must be read carefully before use. If you do not understand how to use the equipment, please ask for help.
x. **NEVER** leave on-going experiment unattended.
xi. Solid chemicals, metals, matches, filter papers, broken glasses, and other materials designated by the instructor are to be deposited in the proper waste containers, not in the sink. Follow the directions for waste disposal.
xii. Do not return unused chemicals to the reagent container. Follow the directions for the storage or disposal of these materials.
xiii. Keep hands away from face, eyes, mouth, and body while using chemicals or lab equipment. Wash your hands with soap and water after performing all experiments.
xiv. Work areas should be kept clean and neat at all times. Work surfaces are to be cleaned at the end of each laboratory or activity.
xv. All equipment or apparatus in the laboratories and preparation room belongs to the university. Students are **NOT ALLOWED** to bring any of the equipment or apparatus out of the rooms without any permission.
xvi. Be **ALERT** and proceed with caution at all times in the laboratory. Notify the laboratory staffs immediately of any unsafe conditions or unsafe acts you observe.
xvii. Know the location of all safety equipments in the room. These include the eye wash station and fire extinguisher.
xviii. In case of emergency, follow the instructions of the laboratory staffs.
xix. Emergency call after office hours 09 – 7717080 / 09-7717083 (City Campus Security Office)

Appendix B.2: Pledge form

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