SEROLOGICAL STATUS OF AUJESZKY'S DISEASE AND CLASSICAL SWINE FEVER IN WEST OF PENINSULAR MALAYSIA BETWEEN 2019 AND 2021

By

LI HONGXIA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Veterinary Science

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DEDICATION

MY FAMILY

I am very grateful to my family. Special thanks to my parents, LI HELIANG and CHEN GUILAN, who always support me financially and spiritually and always encourage and comfort me when I encounter difficulties. My sister LI SHUANG is always by my side when I am feeling down. I love you very much.

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For all the support, help, care, and patience throughout the journey and thesis of my study

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Low Suet Ee, who is always there to support me when I need it

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

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Aujeszky's disease (AD) and Classical Swine Fever (CSF) are two common diseases that have spread worldwide. Various porcine viral diseases exist in Malaysia, where AD and CSF are the most common viral endemic diseases in the country. The serological status of AD and CSF in Peninsular Malaysia was reported prior to 2018, with no updated data after that. Hence, in our study, we used commercial samples that were submitted to UPM between 2019 and 2021 to determine the serological status of AD and CSF in swine farms in Peninsular Malaysia. In this study, a convenient sampling method was applied to farms. The ELISA test was conducted using the IDEXX Pseudorabies Virus glycoprotein I (gpl) Antibody Test Kit for AD and the IDEXX Classical Swine Fever Ab ELISA Test Kit for CSF serology diagnosis. Seroprevalence of AD indicates infection status, while seroprevalence of CSF indicates protection status. All tested farms were categorized into four categories according to their location (Perak, Johor, Penang, Selangor & Malacca region). From 2019 to

2021, a total of 2780 samples from 61 farms were submitted for AD ELISA test. Overall infection rate of the AD field strain virus was 18.03% during the 3 years period. The AD infection rate is highly related to the region. Farms in the Johor region had the highest infection rate, while farms in the Perak region had the lowest infection rate. 1-6 weeks old piglets and breeders were more susceptible to infection. Statistical analysis showed that small herd size farms and open-house farms increased the risk of farm infection with AD. For CSF, from 2019 to 2021, a total of 3127 samples from 65 farms were submitted. There are 3 categories of farm status classification (ideal, moderate, and less ideal). 12.31% of farms were classified as ideal farm status with seroprevalence of 62.04% from 2019 to 2021. Farms in Johor region has the highest seroprevalence and the best farm CSF status (ideal), while farms from Penang region has CSF status that is not ideal. Breeder herd were generally having a higher CSF protection lever, especially in sows. The seroprevalence of CSF was also related to herd size, herd type, type of housing system, vaccine brand, and vaccination procedure. Overall, the prevention and control effects of AD have remained good and stable, while the protection of CSF is not ideal in Peninsular Malaysia.

Keywords: Aujeszky's Disease (AD), Classical Swine Fever (CSF), ELISA test, Peninsular Malaysia, Serological status

SDG: GOAL 2: Zreo Hunger, GOAL 3: Good Health and Well-Being

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

STATUS SEROLOGI PENYAKIT AUJESZKY DAN DEMAN BABI KLASIK DI SEMENANJUNG BARAT MALAYSIA ANTARA 2019 DAN 2021

Oleh

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Ogos 2024

Pengerusi : Profesor Madya Ooi Peck Toung, PhD
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Penyakit Aujeszky (AD) dan Demam Babi Klasik (CSF) adalah dua penyakit biasa yang telah merebak ke seluruh dunia. Pelbagai penyakit virus babi wujud di Malaysia, di mana AD dan CSF adalah penyakit endemik virus yang paling biasa di negara ini. Status serologi AD dan CSF di Semenanjung Malaysia dilaporkan sebelum 2018, tanpa data dikemas kini selepas itu. Oleh itu, dalam kajian kami, kami menggunakan sampel komersial yang telah diserahkan kepada UPM antara 2019 dan 2021 untuk menentukan status serologi AD dan CSF di ladang babi di Semenanjung Malaysia. Dalam kajian ini, kaedah persampelan yang mudah digunakan untuk ladang. Ujian ELISA telah dijalankan menggunakan Kit Ujian Antibodi IDEXX Pseudorabies Virus glycoprotein I (gpI) untuk AD dan Kit Ujian IDEXX Classical Swine Fever Ab ELISA untuk diagnosis serologi CSF. Seroprevalensi AD menunjukkan status jangkitan, manakala seroprevalensi CSF menunjukkan status perlindungan. Semua ladang yang diuji dikategorikan kepada empat kategori mengikut lokasinya (Perak, Johor, Pulau Pinang, Selangor & Wilayah Melaka). Dari

2019 hingga 2021, sejumlah 2780 sampel daripada 61 ladang telah dihantar untuk ujian ELISA AD. Kadar jangkitan keseluruhan virus terikan medan AD ialah 18.03% dalam tempoh 3 tahun. Kadar jangkitan AD sangat berkaitan dengan rantau ini. Ladang di wilayah Johor mempunyai kadar jangkitan tertinggi, manakala ladang di wilayah Perak mempunyai kadar jangkitan paling rendah. Anak babi dan penternak berumur 1-6 minggu lebih terdedah kepada jangkitan. Analisis statistik menunjukkan bahawa ladang saiz kumpulan kecil dan ladang rumah terbuka meningkatkan risiko jangkitan ladang dengan AD. Selain itu, sebanyak 3127 sampel daripada 65 ladang telah dihantar untuk ujian ELISA CSF dari 2019 hingga 2021. Status ladang diklasifikasi kepada tiga (3) kategori, iaitu ideal, sederhana dan kurang ideal. 12.31% ladang diklasifikasikan sebagai status ladang ideal dengan seroprevalens 62.04% dari 2019 hingga 2021. Ladang di negeri Johor mempunyai status seroprevalensi CSF yang terbaik (ideal), manakala ladang di negeri Pulau Pinang mempunyai status ladang CSF yang kurang ideal. Kumpulan pembiak biasanya mempunyai tuil perlindungan CSF yang lebih tinggi, terutamanya dalam induk. Seroprevalensi CSF juga berkaitan dengan saiz kumpulan, jenis kumpulan, jenis sistem perumahan, jenama vaksin, dan prosedur vaksinasi. Secara keseluruhannya, kesan pencegahan dan kawalan AD kekal baik dan stabil (ideal), manakala perlindungan CSF tidak ideal di Semenanjung Malaysia.

Kata Kunci: Penyakit Aujeszky (DAN), Demam Babi Klasik (CSF), ujian ELISA, Semenanjung Malaysia, Status serologi

SDG: MATLAMAT 2: Sifar Kelaparan, MATLAMAT 3: Kesihatan dan Kesejahteraan yang Baik

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LIST OF ABBREVIATIONS

%	Percent
>	More than
<	Less than
≤	Less than or equal to
≥	More than or equal to
µl	Microliter
ml	Milliliter
nm	Nanometer
°C	Degrees Celsius
ASF	African Swine Fever
AD	Aujeszky's Disease
ADV	Aujeszky's Disease Virus
CNS	Central Nervous System
COVID-19	Coronavirus Disease 2019
CPE	Cytopathic effect
CSF	Classical Swine Fever
CSFV	Classical Swine Fever Virus
CV%	Coefficient of variation
DIVA	Differentiating Infected from Vaccinated Animals
DVS	Department of Veterinary Services Malaysia
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FVM	Faculty of Veterinary Medicine
gB	Glycoprotein B
gC	Glycoprotein C

gD	Glycoprotein D
gE	Glycoprotein E
gH	Glycoprotein H
gI	Glycoprotein I
gK	Glycoprotein K
gL	Glycoprotein L
gM	Glycoprotein M
gN	Glycoprotein N
gpI	Glycoprotein I
GPE-	Japanese guinea-pig exaltation-negative
HC	Hog Cholera
IDR	Import dependency ratio
LAMP	Loop-mediated isothermal amplification
LPC	Lapinized Philippines Coronel
MDA	Maternal derived antibody
MLV	Modified Live Vaccine
RFLP	Restriction fragment length polymorphism
S/N	Serum to negative ratio
SSR	Self-sufficiency ratio
UPM	Universiti Putra Malaysia
US	United States
VLSU	Veterinary Laboratory Service Unit
VNT	Viral neutralization tests

CHAPTER 1

INTRODUCTION

1.1 Overview

There are a total of 470 farms in operation with a pig population of 1,468,438 head in peninsular Malaysia in 2020. Recently, many small farms are closing due to the African swine fever (ASF) outbreak and other factors such as policies and successors issue, but larger farms have continued to expand production scale and production volume. The shortage of pork supply is mainly solved by importing unprocessed frozen pork from neighboring countries (Federation of Livestock Farmers' Associations of Malaysia, 2020). Import dependency ratio (IDR) was 5.8% in livestock production of Malaysia in 2020 (Department of Statistics Malaysia, 2020). In addition, pork livestock production in 2021 fell by double digits to 10.5% compared to 2020. Malaysia is a country with 93.4% of self-sufficiency ratio (SSR) of pork from its pig industry in 2021 (Department of Veterinary Services Malaysia, 2022). The increase in the pork consumption population in Malaysia has promoted the good development of the pig industry (Aisya Naama et al., 2018). In Malaysia, although almost all pig farms are licensed to operate, pig farms in our country need to undergo regular good laboratory practice audits and undergo the livestock farm accreditation scheme according to the Department of Veterinary Services Malaysia (DVS) (Singh & Fong, 2014).

Globally, Aujeszky's disease (AD) and classical swine fever (CSF) have been identified as viral diseases causing reproductive and respiratory problems in

pigs (Wittmann, 1991). Both diseases are also the most common endemic swine diseases in Malaysia (Low, 2019). AD and CSF cause great economic losses to the pig industry. Routine vaccination against these two diseases has been practiced in Malaysia for about 50 years. The latest serological surveys of AD and CSF were reported in Malaysia in 2018 (Low, 2019). However, data after 2018 have not been updated. The latest status of AD and CSF remain unknown. The status and data on these two diseases are limited in Malaysia. Thus, this is the latest report on the serological status of AD and CSF.

Aujeszky's disease (AD), also known as "mad itch" or "Pseudorabies", is caused by the Aujeszky's disease virus (ADV), a Suid alphaherpesvirus 1 (SuHV-1) (Sehl & Teifke, 2020a). SuHV-1 is an enveloped virus. It has important surface glycoproteins, some of which are essential for the virus to replicate and invade the host (Silva-Junior et al., 2020). The causative agent of AD is an alphaherpesvirus, and it belongs to the herpesviridae family (Andrewes, 1963; Mettenleiter, 2020). An acute, highly contagious infectious disease of pigs, the clinical symptoms are mainly characterized by nervous system disorders and organ damage. Pigs resistant to acute infection with ADV can develop latent infection and become lifelong latent infection. Under the stimulation of stress conditions, the latent ADV can be activated and cause recurrent infection, and the ADV will spread toxins outwards. Pigs are the only species that can survive AD infection. Therefore, pigs become a reservoir host and source of infection for the virus. ADV can infect a wide range of mammals but not humans in previous studies. (Fenner et al., 1987; Marcaccini et al., 2008; Pensaert & Kluge, 1989). However, many reports indicate that ADV can

cross the species barrier from livestock to humans, and that the virus can cause damage to the central nervous system (CNS) in humans (Ai et al., 2018). In a study, researchers isolated an ADV strain from humans with acute encephalitis, so humans can largely be considered as potential hosts of ADV (Liu et al., 2021).

Moreover, so far no effective drugs and treatments have been developed to prevent and control this disease, so AD is a potential threat and challenge for public health (Chen et al., 2022).

In the middle of the 20th century, AD was more prevalent in countries in Eastern Europe and the Balkan Peninsula. Before the 1960s, the symptoms of pigs were relatively mild after infection, so it did not cause major economic losses in the swine industry. However, due to the mutation and evolution of AD strains and the emergence of new virulent strains leading to the formation of virulent strains in the 1960s and 1970s (Beran, 2002). The number of AD outbreaks in pig farms increased significantly, and pigs of various ages could be infected, and the symptoms were significantly aggravated. AD was first discovered in Hungary in 1902 (Aujeszky, 1902), and then spread worldwide. Since the establishment of intensive pig farms after World War II, AD infections have spread rapidly. Through the continuous global circulation of livestock products, the disease is rapidly becoming prevalent and spreading, especially since the 1970s (Mettenleiter, 2020). In 1976, an outbreak of AD was reported in Malaysia (Lee & Porey, 1979). In addition, in 1984 AD was declared as endemic in Malaysia (Too, 1997). AD is a viral disease primarily affecting

mammals, especially pigs. Pigs are the most susceptible animals to the disease, but other domestic and wild mammals are also susceptible. The disease is widespread and present in most countries of the world. The susceptibility of animals to AD is affected by many factors, such as age, virulence, and species (Low, 2019).

Classical swine fever (CSF), also called hog cholera, is caused by the classical swine fever virus (CSFV), or hog cholera virus. CSFV belongs to the family Flaviviridae, genus Pestivirus (Lowings et al., 1996; Paton & Greiser-Wilke, 2003). Since CSF is a highly contagious disease, it causes huge economic losses to the swine industry (Ganges et al., 2020). The World Organisation for Animal Health (WOAH) has classified CSF as a disease on List A (Edwards, 2000). CSFV is a single stranded, positive sense, and enveloped RNA virus (Lindenbach et al., 2013). Two non-translated regions (NTRs) and one large open reading frame (ORF) make up the genome (Collett, 1992; Rümenapf et al., 1991).

The genome has a length of about 12,300 nucleotides (Paton et al., 2000). CSF virus isolates can be classified into a variety of types and subtypes based on genetic similarity (Frías-Lepoureau & Greiser-Wilke, 2002; Paton et al., 2000). The phylogenetic analyses have demonstrated unequivocally that CSF virus isolates with distinct genetic type appear to be typical for specific geographic areas, and their application has grown to be a powerful method for identifying strains within specific outbreaks (Moennig et al., 2003).

In 1895, the state of Perak in Peninsular Malaysia recorded the first case of CSF disease (Aisya Naama et al., 2018). The appearance of this disease at the time was most likely related to the introduction of foreign pig breeds. Since 1975, vaccination with attenuated cell culture (Japanese GPE-strain) vaccines on farms and increased awareness of the disease among pig farmers have resulted in a gradual decrease in CSF infection rates (Too, 1997a). Farmers have also taken a number of measures, including adopting modern farming intensive systems, enhancing biosecurity on their farms, and completely stopping the use of swill to feed pigs. Although pig farms can reduce infection rates by strengthening biosecurity management measures and vaccination with CSF, the disease persists in Malaysia. This is mainly due to the failure of vaccination programs caused by differences in vaccine brands, vaccination systems, and vaccination frequencies on farms (Low, 2019). In 1995, a serious outbreak of CSF occurred in East Malaysia (Too, 1997b, 1997c). The presence of CSF was also detected in Peninsular Malaysia during serological surveys on CSF during 2016 - 2017 (Low, 2019).

In Malaysia, the Department of Veterinary Services requires mandatory vaccination for CSF, but not for AD. Through questionnaire survey and combined with the 2016 research report, it was found that about 95% of farms have implemented vaccination to control AD in Malaysia (Low et al., 2018). In practice, vaccination for AD is recommended at 4 weeks before delivery in pregnant sows.

For the CSF vaccination program, live attenuated vaccines derived from the Japanese GPE-strain are used to regularly vaccinate against CSFV (Allaudin, 2004). In general, the vaccine schedule consists of 1st dose at 4 weeks of age and a booster at 8 weeks of age or only one dose at either 5 - 7 weeks of age or 6 - 8 weeks of age in porker. Mass vaccination at 3 - 4 months in breeder is recommended in Malaysia (Low, 2019).

1.2 Justification

Both diseases have existed in Malaysia for more than 40 years, and the only report on the status of AD and CSF was done in 2018. However, data after 2018 have not been updated. The latest status of the disease remains unknown. The status and data on these two diseases are scarce in Malaysia, since the latest situation of farms is not known or understood. Despite long-standing vaccination practices against ADV and CSFV, the intensity of virus transmission has not been determined on local pig farms after 2018.

1.3 Objectives

The objectives of this research are:

i. To investigate ADV field exposure in Peninsular Malaysia pig farms based on commercial samples submitted to UPM during 2019-2021.

ii. To investigate CSF protection level in Peninsular Malaysia pig farms based on commercial samples submitted to UPM during 2019-2021.

1.4 Hypothesis

It is hypothesized that:

i. ADV field exposure differences across the different regions Peninsular Malaysia.

ii. CSF protection level exhibits differences across the different regions in Peninsular Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Aujeszky's Disease

2.1.1 Origin of AD Virus

In 1813, a new disease called "mad itch" was discovered in the United States. Almost a hundred years later, the writings of the Hungarian scientist Aladar Aujeszky described the disease, for which it was named. In 1902, the disease linked to cattle, dogs, and cats (Aujeszky, 1902). In the first half of the 19th century, "mad itch" was used in the United States to describe a disease of cattle due to the excessive itching caused by AD (Hanson, 1954). The term "mad itch" first appeared in two American farmers' magazines in 1839 and 1844 (Hanson, 1954). In Switzerland, a similar "mad itch" disease was described in cattle in 1889 (Kohler & Kohler, 2003). Since the first discovery of Aujeszky's disease virus (ADV) in Hungary in 1902, this pathogen has been considered as one of the most important pathogens and has caused fatal losses to the swine industry worldwide.

Outbreaks of AD were severe in Europe before 1960, especially in Eastern Europe. In the mid-1970s, an outbreak of the disease began in the United States on a large scale. During this period, the incidence of the disease has increased significantly with the development of intensive farming (Beran, 2002). Since then, AD has been found in several European countries, and the main symptoms of the disease appear in cattle with localized itching. For many years in Europe, AD has been an important cause of mortality in pigs of

different ages, and it is also one of the causes of abortion in sows. Until the late 1960s and early 1970s, the disease was only considered a significant cause of death in piglets in the United States, and occasionally in cattle, sheep, dogs and cats (Thawley et al., 1991).

Like all herpesviruses, the ADV virus consists of a protein coat, capsid, nucleoprotein core, and lipid bilayer membrane which contains the A (glyco) protein (Mettenleiter, 2000).

ADV particles have a typical herpes virion structure. They are quasi-spherical and have an overall diameter between 150nm and 180nm. ADV is an enveloped, double-stranded linear DNA herpesvirus (Zheng et al., 2022), and the total length of ADV genome is about 150kb and a GC content as high as 74% (Böhm et al., 2016). It is known that the viral genome contains more than 70 genomes, which can encode 70 to 100 viral proteins (Lerma et al., 2016). At present, there are 11 kinds of glycoproteins that have been analyzed clearly, including gB, gC, gD, gE, gG, gH, gI, gK, gL, gM and gN (Böhm et al., 2016). gB, gC, gD, gE, gI and AD virus virulence is related. The gE protein is the main virulence factor, which can promote the release of virus particles and spread among cells, promote the fusion between cells and have neurotropism (Mengeling et al., 1997; Nauwynck et al., 1999). The ADV replicates in a variety of mammalian and avian cell cultures and produces eosinophilic intranuclear inclusion bodies (Reissig & Kaplan, 1962). The cytopathic effect (CPE) of ADV in cell culture was found to be bright, shining, and spherical (Kaplan & Vatter, 1959). ADV has a strong affinity for nervous tissue, which

can cause damage to the nervous system. In addition, ADV will cause damage to the immune system, reproductive system, and respiratory system, and clinical symptoms such as meningitis, infertility, and pneumonia.

ADV is a virus with a relatively strong ability to resist harsh environments among herpes viruses. ADV can survive in liquid or solid surface for at least 7 days. The virus on the hay in the pig house can survive for about 46 days in winter, and it can survive for about 1 month in summer. It takes 30-50 minutes at 55-60°C or 3 minutes at 80°C to inactivate. Keeping the virus stable requires a pH between 4 and 9. The optimal storage temperature is -70°C, and the virus cultures preserved in vacuum drying and low temperature storage can be stored for many years (Xiang et al., 2020). Many disinfectants can disinfect this virus, such as 5% NaOH or orthophenylphenol. There is only one serotype of ADV, but different strains have differences in biological characteristics and virulence (Liu et al., 2022).

2.1.2 Pathogenesis of AD

The existence of ADV is very extensive, and it is distributed in various countries and regions all over the world. The virus is not only susceptible to pigs, sheep, cattle, dogs and cats (Pomeranz et al., 2005), but also to various carnivores and wild animals. In animal husbandry, some economic animals can also be infected with AD, such as minks. Feeding minks with viscera and bones containing ADV can develop AD and cause large scale morbidity and death.

ADV proliferates in the tonsil and pharyngeal epithelial tissue, and the proliferated virus reaches the spinal cord through the olfactory nerve and glossopharyngeal nerve, where it further proliferates and spreads to the entire brain, eventually leading to brain and spinal cord inflammation (Klingbeil et al., 2015). Under natural conditions, the initial site of ADV infection is the nasopharyngeal epithelium and tonsil, and then the virus spreads from lymphatic vessels to local lymph nodes. After the virus goes into the glossopharyngeal nerve, ambiguous nerve, and olfactory nerve endings, the encapsulated virion or non-enveloped nucleocapsid travels retrograde along the axon to the ambiguous nerve, and finally reaches the CNS. ADV invades the cerebellum of pigs after replicating in the pons and spinal cord. Hence, the virus is latent in the trigeminal ganglion, olfactory bulb, tonsil, and sacral ganglion (Romero et al., 2003; Wheeler & Osorio, 1991).

ADV is carried from the cells to various parts of the body through the blood circulation, through the ingestion of leukocytes in the lesion or the adsorption on the cell surface, especially the placental tissue of pregnant animals, which will lead to abortion or stillbirth in sows.

2.1.3 Epidemiology of AD

The reservoir host and natural host of ADV are pigs (Freuling et al., 2017), but the important source of infection of the disease is infected pigs, diseased pigs and infected mice (Wang et al., 2017). ADV is mainly transmitted by air, direct contact, and vertical transmission. In animal husbandry and breeding, the disease of some animals is mostly related to contact with pigs and rats.

Generally, except for pigs, other infected animals will die from ADV infection. The spread of ADV mainly relies on the shedding of virus from infected pigs to infect healthy pigs. The personnel who come in touch with ADV infected pigs and the equipment utilized in pig farms both contribute significantly to the disease's spread. For example, polluted air, drinking water, feed and production tools in pig farms (Li et al., 2017). The virus can infect brain tissue in pigs through nasal secretions, causing severe neurological symptoms. ADV can also be transmitted to sows during mating through semen (Beran, 1991), and sows can infect piglets vertically via embryos (Bolin & Bolin, 1984). In addition, air is the main route of ADV transmission (Vannier, 1988). Close-range aerosol transmission and direct nose to nose contact are the two basic ways that ADV is transmitted. Animals with ADV infection excrete the virus for 10 to 14 days in their oropharyngeal or nasal fluid (Van Oirschot, 1999). A study showed that ADV can be transmitted by aerosol from one herd to another with a distance of 9 kilometers or more between the two herds (Christensen et al., 1993; Gloster et al., 1984).

2.1.4 Clinical Impact of AD

The viral strain, vaccination dose, vaccination route, and age of the pig are all factors that affect how ADV develops. Prior to the weaning stage, neurological symptoms in pigs are severe, whereas older animals are more resistant to neurological disorders (Papageorgiou et al., 2011). This serious transnational disease has the potential to have a significant economic impact on the swine industry (Low, 2019). According to the age of pigs, the disease is divided into 4 types: newborn piglets, weaned piglets, adult pigs, and old pigs.

Young newborn piglets are the most susceptible, especially suckling piglets below 2 weeks of age with a 100% mortality rate (Nauwynck, 1997). Piglets do not have any symptoms before the disease occurs. Additionally, they periodically tremble and shake violently. Piglets that are infected may show clinical symptoms on the first or second day of life and typically pass away before they reach the age of five days. At 3-4 weeks of age, the mortality rate drops to about 50% (Nauwynck, 1997). Typically, severe neurological disturbances are observed in piglets infected with ADV. With little clinical symptoms, the condition may be characterized by abrupt death. Initially, the newborn piglets behaved normally in all aspects such as feeding and mentation. Then, they suddenly become ill with high fever, with body temperature as high as 41°C or higher. Fever, lethargy, anorexia, vomiting, diarrhea, weakness, incoordination, and convulsions often precede death (Thawley et al., 1991).

After weaning piglets aged 6-10 weeks are infected with ADV, clinical symptoms such as coughing, mouth breathing, sneezing, and runny nose will appear. Piglets severely infected with ADV will show obvious neurological symptoms, vomiting and diarrhea, repeated diarrhea and so on. The morbidity rate of piglets at this age ranges from 30% to 40% in veterinary clinics, and the mortality rate is about 20%.

Severe clinical neurological illness is uncommon in adult pigs aged 5-6 months and beyond. The recovered pigs serve as the primary source of AD infection since they may shed the virus for a very long time.

The clinical symptoms of sows aged 13 months and beyond had body temperatures that rose to 40.5-41°C, loss of appetite, miscarriage, stillbirth, mummified fetus, and weak offspring in sows (Gordon & Luke, 1955; Nauwynck & Pensaert, 1992). Sows mainly produce stillbirths. During gestation, abortion in sows often occurred about 10 days after infection with ADV, and the size of the aborted fetuses was consistent, generally around 264-290mm in length. Abortion and stillbirth in pregnant sows can be caused by newly infected regions, and mainly occur sows before farrowing. The degree of loss in the old epidemic region is related to the immune status of the pig herd and the virulence of ADV. Sows with severe symptoms after infection with ADV can be infertile and even be culled from the farm. Sows of any parity can become ill after being infected with the virus. Although sows gradually recover clinically, latent infection and reduced productivity may occur. Purchasing infected gilts or sows with ADV is how the disease is typically introduced to farms. ADV can be excreted by sows in oral secretions, milk, vaginal secretions, and nasal secretions.

Boars infected with ADV will show bilateral testicular swelling and progressive atrophy, and will gradually develop orchitis (Salogni et al., 2016) and persistent genital tract latent infection, resulting in loss of breeding ability. Boars may emit ADV in their semen and also be sterile (Baskerville, 1981).

ADV can also spread to a wide variety of other animals, including rodents, carnivores, and ruminants. It has a 100% death rate, severe pruritus, and CNS impairment (Sehl & Teifke, 2020b). However, it should be noted that once a

pig is infected with ADV, it will carry the virus for life and become a storage host for the pathogen (Schulz et al., 2015).

The lesions visible to the naked eye are mainly pinpoint bleeding points in the kidney, obvious congestion and hemorrhage in the meninges, and increased cerebrospinal fluid. White scattered necrotic spots appear in tonsils, liver and spleen, pulmonary edema, lobular interstitial pneumonia or hemorrhagic spots. Gastric mucosa had catarrhal inflammation and gastric fundus mucosal bleeding. The brain tissue and skin of the buttocks of the aborted fetus had bleeding spots, kidney and myocardial hemorrhage, and gray-white necrosis in the liver and spleen.

Histopathological changes mainly showed typical non-suppurative meningitis, with obvious vascular sleeve and glial cell necrosis. In brain nerve cells, intranuclear acidic inclusion bodies are seen.

In the middle and late 20th century, ADV mutated and produced a more virulent strain, which caused pigs of different ages to be infected and the symptoms were more severe. After the emergence of virulent strains, the number of pig farms with outbreaks of ADV in countries around the world increased significantly (Hogue et al., 2014), and the virus has been prevalent for decades (Liu et al., 2022). The widespread infection of ADV poses a serious threat to the swine industry worldwide.

ADV outbreaks mostly occurred in the late 1970s. the AD outbreak began in the United States in 1974; the AD outbreak began in the United Kingdom in

1971 (Liu et al., 2017); the large scale AD outbreak occurred in Denmark in 1978 (Zhao et al., 2017); Greece experienced a sharp rise in ADV infection in 1983 (Guo et al., 2016), and Germany experienced a large-scale epidemic of AD in 1977. Prior to this, only sporadic pig farms were affected (Meng et al., 2016); AD outbreaks began in Malaysia in 1976 (Lee & Porey, 1979), and the number of AD cases suddenly increased in 1977 (Low et al., 2018). Despite vaccination, AD outbreaks were reported in different parts of Malaysia in 1976 (Lee & Porey, 1979), 1984 (Too, 1997), and 1990s (Singh & Singh, 1998).

2.1.5 Vaccination and Vaccines of AD

Despite efforts to prevent AD in the swine industry, the epidemic of this disease continued to expand. In the early 1960s, live attenuated vaccines were developed (Mettenleiter, 2020); the first genetically engineered live ADV vaccines lacking virulence determining genes were first used in animal husbandry in the 1980s (Freuling et al., 2017). Since then, AD has been characterized using a labeled vaccine combined with a companion diagnostic ELISA test to DIVA (Differentiating Infected from Vaccinated Animals) (Freuling et al., 2017; Kit et al., 1985; Kit et al., 1987). DIVA refers to the differentiation of infection from vaccinated animals by using labeled vaccines and corresponding serological diagnostic tests (Liu et al., 2022).

With the help of precise differential ELISAs and very potent marker vaccinations, AD eradication programs are now feasible in most parts of the world (Van Oirschot, 1999). The best methods to prevent ADV and lessen the economic damage it causes is by vaccination (Freuling et al., 2017). As far as

AD vaccines are concerned, they are currently divided into attenuated vaccines, inactivated vaccines, gene deletion marker vaccines, recombinant vector vaccines, nucleic acid vaccines, and subunit vaccines.

After the traditional AD vaccine is used to immunize pigs, it is not possible to use serological detection methods to distinguish vaccine virus from wild virus by detecting antibodies. This can only be found through the comparison of antigen sequencing to find the difference in its gene sequence. Compared with traditional AD vaccines, the purpose of marking specific fragments of vaccine strains is to facilitate the use of serological diagnostic methods to distinguish whether they are vaccine antibodies. The advantages of gene deletion marker vaccines are safety, stability, good immunogenicity, and the ability to distinguish wild virus infections.

At present, the most commonly used is the marker vaccine, especially the glycoprotein E (gE) deleted vaccine. Along with gE ELISA development, the gE-deleted ADV vaccine has the benefit of being used for DIVA, and this vaccine is very effective and safe (Wang et al., 2015). It has been demonstrated that when pigs are vaccinated with these DIVA vaccines against ADV, the amount and time of virus shedding from the pigs is reduced. Thus, pigs will be less infectious for ADV DIVA vaccinated herds (Van Oirschot, 1999). In addition, pigs appear to be less susceptible to ADV after vaccination, with only higher doses of ADV causing infection in pig herds (Smet et al., 1992; Van Oirschot, 1988; Wittmann, Ohlinger, & Hohn, 1982).

The focus of prevention and control AD is to inoculate vaccine, control the quality of food such as water and feed, and clean up the environmental sanitation of the pig house to prevent the occurrence of the disease.

The viability of the DIVA idea is supported by the fact that ADV infection in domestic pigs has been effectively managed or even eradicated in many countries using vaccines and diagnostic testing (Liu et al., 2022). Countries such as Europe and North America have used vaccine immunization and corresponding serological detection methods to implement AD decontamination in stages, and have achieved success. Therefore, the prevention and control measures and purification schemes of these countries where ADV has been eliminated are worthy of reference and learning for pig farmers in my country. One of the measures is to vaccinate all the pigs on the farm. The AD epidemic is prevented by the high strength immune gene deletion vaccine of the whole group, and then the positive pigs are eliminated in time with the help of corresponding serological detection methods. In swine medicine, many countries have reduced the prevalence of AD through vaccination strategies and eradication of infected animals. Therefore, available vaccines are well known and highly effective (Bouma, 2005; Nes & A., 2001).

Passive immunity is generally a specific antibody obtained through placenta of the mother, breast milk or yolk body, it is called the maternal derived antibody (MDA), which gives animals immunity to the pathogen of the disease. The amount of colostrum a newborn consumes and the mother's antibody

titers are the two key factors that determine passive immunity (Müller et al., 2010). In addition, related reports state that domestic pigs' maternal antibodies to ADV have an 11.3 day half-life (Tenhagen et al., 1995).

Maternal immunity protects against ADV invasion in the neurological system of newborn pigs, preventing transmission of ADV in young domestic pigs, and maternal immunity levels are correlated with protection against ADV invasion (Bouma et al., 1997; Kritas et al., 1997).

To prevent AD from spreading among domestic pigs, it is very necessary to carry out immunization program for pigs. The formulation of the immunization program should follow the following principles: select excellent vaccines, and immunize domestic pigs regularly and quantitatively. An effective vaccine can help domestic pigs resist the invasion of this virus. Regular and quantitative vaccination can strengthen the immunity of domestic pigs in order to effectively resist the virus.

Each pig farm formulates its own immune program according to the prevalence of AD and the results of antibody testing. It is best to test the level of vaccine antibodies every quarter or half a year. Booster immunization or general immunization should be given to individuals with low vaccine antibody levels or groups with uneven titers. Vaccination is an important means of controlling ADV at present. Pig farms should formulate a reasonable vaccine immunization program, which can be combined with antibody detection to understand the pattern of pig antibodies, in order to determine the appropriate

vaccine immunization time.

In conclusion, the pathogen is still circulating in pig herds in Malaysia despite the availability of effective vaccines widely in pig herds. Although AD vaccination is not mandatory for farms, about 95% of pig farms are vaccinated in Malaysia. In addition, the latest serological survey of AD was completed in 2018, and infection rate of AD field strain virus was 4.25% in Malaysia (Low et al., 2018). The current vaccination system is mass vaccination in 3 to 4 months interval or no AD vaccination (Low, 2019).

2.1.6 AD Diagnostics Tests

Pigs are vaccinated in most countries where AD is endemic. However, vaccination prevented neither infection nor the establishment of wild-type ADV latency. To achieve this, a serological detection and exclusion protocol should be followed (Van Oirschot et al., 1986). According to epidemiology, clinical symptoms, and pathological changes, a preliminary diagnosis of AD can be made, but an accurate diagnosis cannot be made. Therefore, laboratory tests are necessary to ensure a more accurate diagnosis.

The diagnostic methods of the main laboratories are: viral isolation, enzyme-linked immunosorbent assay (ELISA), virus neutralization test (VNT), and other molecular biological approaches targeting ADV genes have been established, such as loop mediated isothermal amplification (LAMP), nano PCR, PCR, and real-time PCR (Liu et al., 2022).

For viral isolation, the virus can be isolated from tissues such as spinal cord, brain tissue, tonsil and spleen of affected pigs.

Virus neutralization test (VNT) is the basic method for detecting virus serum antibodies. This method requires high serum quality, and the virus neutralization test cannot distinguish antibodies produced by vaccines and wild virus infections (Pensaert & Kluge, 1989).

The loop-mediated isothermal amplification method (LAMP) is practical, cheap, convenient, efficient, and fast, so it can be used as a technical means for rapid AD diagnosis and disease purification in farms and grassroots institutions. However, its operating environment is highly demanding, and it is very easy to contaminate and cause false positive results.

Enzyme-linked immunosorbent assay (ELISA) is more suitable for large-scale detection of swine serum. This diagnostic method is fast, simple, sensitive, specific and reliable. ELISA has become one of the routine methods for detecting swine AD in the world (Van Oirschot, 1991). Various commercialized ELISA kits have been widely used in the evaluation of AD immune effect and disease purification, and the gene deletion vaccine can also distinguish vaccine-immunized and wild-type infected animals. At present, detection methods such as gE-ELISA (Van Oirschot et al., 1986), gB-ELISA, gG-ELISA (Freuling et al., 2017), and gI-ELISA have been established by using expressed proteins as antigens. In addition, ELISA could test large number of serum samples in a short period of time (Jasbir, 1998).

Most of the AD vaccines given in Malaysia use vaccines with a deletion of the glycoprotein E (gE) gene, which allows the gE-ELISA test to detect gE antibodies produced by wild AD viruses in pigs. AD has been eradicated in many developed countries by using this gE-ELISA test kit to distinguish between vaccinated and infected pigs.

2.2 Classical Swine Fever

2.2.1 Origin of CSF Virus

Globally, classical swine fever (CSF) is a highly economically significant viral pig disease (Edwards et al., 2000; Moennig et al., 2003). Different international terminology describes the disease, including "European" swine fever and hog cholera (HC).

A cholera-like disease in pigs, subsequently identified as hog cholera, was first described in Tennessee around 1810 (Van Oirschot, 1999). Most researchers admit that the first occurrence of CSF was documented in Ohio in 1833, and the origin of CSF was investigated by Hanson (Hanson, 1957). CSF has broken out in various countries around the world and caused huge economic losses since the 1960s (Cole & Brooks, 1961). Particularly, a severe outbreak of CSF in the Netherlands in 1997-1998, with 429 outbreaks and around 700,000 pigs being slaughtered, and the total economic losses are estimated at more than $2 billion (Elbers et al., 1999; Ganges et al., 2020). Europe, Africa, Americas, Australia, and Asia were among the continents where CSF was distributed globally (Dahle & Liess, 1992). 38 countries in the world have been declared CSF-free, including most of the European Union (EU), Oceania and

all of North America currently (OIE, 2019b). However, CSF is still endemic in some countries in Asia, Central and South America, and Southeast Asia (Frías-Lepoureau, 2002; Ganges et al., 2020; Morilla & Rosales, 2002), particularly in Asia, which currently has the highest diversity of viruses (Paton et al., 2000). There have been reports of sickness in Madagascar, but the situation is unclear throughout most of Africa (Paton & Greiser-Wilke, 2003).

CSFV is an RNA virus, and the encapsulated virion of this virus contains an icosahedral nucleocapsid and glycosylated membrane proteins (Paton et al., 2000). Two non-translated regions (NTRs) surround a single, sizable open reading frame (ORF) in the genome (Collett, 1992; Rümenapf et al., 1991). Four structural and eight nonstructural proteins are produced by the processing of 3898 amino acids of polyprotein encoded by the viral genome by cellular and viral proteases. The structural protein is composed of C, E^{rns}, E1, and E2, and the non-structural protein is composed of N^{pro}, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B (Lindenbach et al., 2007).

For CSFV, genetic variability is significant (Paton et al., 2000; Postel et al., 2012). 11 subgenotypes (1.1, 1.2, 1.3, 1.4, 2.1, 2.2, 2.3, 3.1, 3.2, 3.3, and 3.4) and three genotypes are have been described in CSFV isolates from throughout the world (Beer et al., 2015; Deng et al., 2005; Lowings et al., 1996; Pan et al., 2005; Zhang et al., 2015). Subgenotype 2.1 is further subdivided into sub-subgenotypes 2.1a, 2.1b, 2.1c, and 2.1d (Sun et al., 2013; Xing et al., 2019). There is a clear geographic pattern in the genotype distribution that has not altered much throughout time (Beer et al., 2015; Björklund et al., 1999). In

the last several decades, genotype 2 has become the most common all across the world (Chen et al., 2010; Pereda et al., 2005), and according to earlier research on Asian CSFV isolates, Malaysian isolates from the 1980s were determined to belong to genogroup 2.1 (Lowings et al., 1996; Paton et al., 2000).

2.2.2 Pathogenesis of CSF

CSFV typically enters the host body via the oronasal pathway in natural settings (Dahle & Liess, 1992). No matter how it enters, the virus first infects the tonsillar crypt epithelial cells before spreading to the lymphoid tissues (Ressang, 1973b; Trautwein, 1988).The tonsils are the first most important site and the regional lymph nodes are thought to be the second most important site for viral multiplication (Dahle & Liess, 1992). CSFV infection is followed by initial replication in the tonsils, which is disseminated to neighboring lymphoid tissue (Liess, 1987). The virus is transported to the local lymph nodes after reaching the lymphatic capillaries (Ganges et al., 2020). The virus continues to replicate at this location, and the visceral lymph nodes, bone marrow, and spleen are secondary replication sites where the virus is transmitted by blood (Ressang, 1973a, 1973b). The next step is to penetrate the efferent blood capillaries, which causes viremia, CSFV is spread in body (Cheville & Mengeling, 1969). The parenchymatous organs are attacked late in the viremic phase (Belák et al., 2008; Liu et al., 2011). Additionally immunosuppressive, CSFV can take up to three weeks to cause neutralizing antibodies to develop (Paton & Greiser-Wilke, 2003).

2.2.3 Epidemiology of CSF

There are two ways of transmission of CSFV: direct transmission and indirect transmission (Ribbens et al., 2004). Direct contact between pigs is the most effective method of virus transmission, according to a wealth of field data (Moennig, 2000; Terpstra, 1988). CSFV is directly transmitted to other pigs via the oral cavity, nasal cavity, conjunctival fluid, digestive tract, and genitourinary tract (Ressang, 1973a; Ressang et al., 1972). Direct transmission of the CSFV can occur through both within-herd and between-herd transmission. For example, when a susceptible pig herd is introduced into a herd carrying CSFV, direct contact occurs between the herds and causes direct transmission of virus among the herds (Laddomada, 2000). Additionally, intrauterine infection might spread the CSFV from sows to their progeny (Dewulf et al., 2001; Meyer et al., 1981). CSFV can also be transmitted directly from wild boars to domestic pigs (Moennig, 2000). Humans are not susceptible to CSF infection (Moennig, 2015).

Both domestic and wild pigs are susceptible to CSF (Depner et al., 1995; Moennig, 2000). CSFV is transmitted from wild boars to domestic pigs mainly through indirect contact (Kaden, 1998; Kaden et al., 2003). For example, feeding domestic pigs with silage made from green plants contaminated by wild boars or wild boar offal infected with CSFV can cause indirect transmission of the virus from wild boars to domestic pigs (Kaden et al., 1992). Artificial insemination of sows with CSFV-infected semen results in CSFV infection in the sows (Elbers et al., 1999). Indirect transmission of CSFV can also occur through contact with contaminated vehicles and people, feeding

swill without proper disinfection, and contact with neighbors on infected farms (Edwards, 2000; Fritzemeier et al., 2000). In addition, CSFV can also be transmitted through arthropods, birds, ruminants, pets, and rodents (Ribbens et al., 2004; Van Oirschot, 1999).

2.2.4 Clinical Impact of CSF

CSF is a hemorrhagic, highly contagious, and transboundary disease (De Smit et al., 1999; Ganges et al., 2020). Members of the Suidae are the natural hosts of CSFV, which means that wild boars as well as domestic pigs are completely susceptible to the virus (Moennig, 2000).

CSF does not usually present with a febrile illness with typical clinical symptoms and substantial morbidity and death (Dahle & Liess, 1992). The virulence of the viral strain, the host immune status, health condition, the age, concomitant infections, genetic background, breed, as well as exposure history all have a significant role in determining the clinical symptoms of CSF, which are highly variable (Belák et al., 2008; Cao et al., 2018; Kaden et al., 2000; von Rosen et al., 2013). Incubation duration of CSF varies with the virulence of the virus and extends from 3 to 15 days (Douglas, 2002). Young animals typically suffer greater damage than done adults (Moennig, 2000). CSF can be divided into 2 forms of disease: acute form and chronic form.

2.2.4.1 Acute form CSF

For acute CSF, which is most common in piglets under 12 weeks of age, it is usually an infectious disease caused by highly and moderately virulent strains

(Ganges et al., 2020; Muñoz-González et al., 2017; Postel et al., 2018). It is an almost fatal and extremely serious disease. The initial clinical feature is persistent high fever. Within 2-6 days after being exposed to the virus, the body temperature often increases and is higher than 40°C, with the typical peak being between the fourth and eighth day of sickness (Belák et al., 2008; Dahle & Liess, 1992; Mittelholzer et al., 2000). Death will occur 10-20 days after pigs are infected with CSFV, and the mortality rate can reach 100% (Floegel‐Niesmann et al., 2003; Moennig et al., 2003). The pigs also showed signs of trembling, lethargy, depression, vomiting, loss of appetite, and huddling with pigs (Douglas, 2002; Moennig, 2000; Tarradas et al., 2014).

The most typical characteristic of acute CSF is a hemorrhagic syndrome, including cutaneous hemorrhages in the tail, abdomen, ears, and inner limbs during the 2-3 weeks after infection with CSFV (Moennig et al., 2003; Postel et al., 2018). At the same time, there are also neurological symptoms, such as convulsions, staggering, standing with hunched back, and uncoordinated movements (Blome et al., 2017; Postel et al., 2018).

Pathological changes visible through autopsy: edema, hemorrhage, and enlargement of lymph nodes (Gómez-Villamandos et al., 2006). The gastrointestinal tract may also be affected, for example: button ulcers in the colon, catarrhal enteritis in the small and large intestine, and bleeding in the fundus area of the stomach (Douglas, 2002). A minor inflammation in the tonsils that subsequently progresses to necrotic tonsillitis or, as a result of a secondary bacterial infection, to a suppurative tonsillitis are two examples of

pathological tonsil symptoms (Dahle & Liess, 1992). Splenic infarction and interlobular blood vessel thrombosis were found (Dahle & Liess, 1992). Punctate bleeding can also occur in the heart, epiglottis, bladder, viscera, kidneys, throat, and serous membranes (Blome et al., 2017; Postel et al., 2018).

2.2.4.2 Chronic form CSF

For chronic CSF, the clinical symptoms are similar to the acute CSF, but the chronic CSF is less severe than the acute CSF. The incubation period of the virus is longer, about a week (Douglas, 2002). In general, only non-specific clinical signs are seen in afflicted animals (Blome et al., 2017). Clinical manifestations include occasional fever, mild drowsiness, diarrhea, developmental delays, and anorexia (Ganges et al., 2020). Only a tiny percentage of infected pigs, especially young ones, develop chronic CSF (Postel et al., 2018). CSFV can survive in animals for 2-3 months. Infected pigs continuously shed large amounts of virus from their bodies throughout the course of chronic CSF (Blome et al., 2017; Postel et al., 2018).

The reason for the formation of chronic CSF is that when pigs are infected with CSFV, the body cannot establish an effective immune response to the virus to eliminate the disease (Moennig et al., 2003; Petrov et al., 2014; Tarradas et al., 2014). Antibodies are produced by the immune system, but they are unable to stop the virus from spreading (Postel et al., 2018). Neutralizing antibodies can be detected in serum samples at the end of the first month after pig infection, but only temporarily. Neutralizing antibodies last for a few days and

then disappear (Depner, 1996; Mengeling & Packer, 1969).

Pathological changes are atypical, particularly the absence of bleeding in serosa and organ. In animals with chronic CSF, necrotic and ulcerative lesions can be observed in the colon, ileum, and ileocecal valve when the animal has symptoms of diarrhea. A wide variety of other diseases must be taken into consideration in the differential diagnosis because the clinical symptoms and pathological changes of chronic CSF are very nonspecific (Blome et al., 2017; Moennig et al., 2003; Postel et al., 2018).

2.2.5 Vaccination and Vaccines of CSF

Currently, no effective drugs have been developed to treat CSF, so vaccination is the first choice to control and prevent the spread and outbreak of the disease. There are two types of CSF vaccines. One is a modified live vaccine (MLV), and the other is the marker subunit DIVA vaccines.

2.2.5.1 Modified Live Vaccines (MLV)

Inactivated vaccines were often used for the prevention and control of CSF, but it has been reported that they cannot prevent CSF (Low, 2019). In the 1960s, many live attenuated vaccines against CSF have been developed (Paton & Greiser-Wilke, 2003). In places where CSF is an endemic disease, vaccination is essential for its prevention. The modified live vaccine (MLV) is the most extensively used vaccine in commercial herds because it is inexpensive, safe, and highly effective (Chang et al., 2023).

Currently the most common modified live vaccines (MLV), including the Chinese C-strain, the French Thiverval strain, the lapinized Philippines Coronel (LPC) strain, or the Japanese guinea-pig exaltation negative (GPE−) strain (Postel et al., 2018).

For Chinese C-strain vaccine, it is a safe and effective live attenuated vaccine, which was developed by China in 1954 and has been widely used ever since (Zhou, 2019). C-strain vaccine can produce early immune protection mechanism very quickly after immunization. The fact that vaccinated pigs are shielded from strong strain CSFV infections as soon as 5 days following immunization serves as evidence of their effectiveness, and these animals vaccinated with the C-strain had lifelong immunity. The C-strain vaccine can also be administered orally to wild pigs, which can develop immunity against CSFV (Moennig, 2000; Rossi et al., 2015). In addition, C-strain vaccine can provide comprehensive preventive immune protection, and it can also play a good immune effect on pregnant sows, and is safe (Tong et al., 2022; Wei et al., 2021). The C-strain vaccine provides protection for at least 6 to 18 months, and even lifelong immunity (Van Oirschot, 2003).

Although MLV vaccine can provide effective protection for pigs, these vaccines have a common disadvantage in that it does not have the functionality of DIVA in terms of serological testing(Postel et al., 2018).

2.2.5.2 Marker Subunit DIVA Vaccines

To overcome these shortcomings of traditional vaccines, marker subunit DIVA vaccines and accompanying diagnostic tests have been developed. DIVA vaccine is not only able to induce a rapid, safe, and effective immune response in domestic pigs, but it can also be used for oral immunization against CSFV in wild boar, so DIVA vaccine is an extremely important option for controlling CSF (Postel et al., 2018).

One or more antigenic proteins are present in DIVA vaccines (Van Oirschot, 1999). The first generation of marker DIVA vaccines were first developed using insect cell-expressed recombinant CSFV glycoprotein E2 (Depner et al., 2001), and animals vaccinated with this type of vaccine cannot induce immune responses to CSFV NS3 and E^{rns}, which makes it easy to realize the function of DIVA. The marked E2 subunit diva vaccine that is expressed in the baculovirus system could decrease the spread of wild-type CSFV between pigs (Van Oirschot, 1999).

Subsequently, the second generation modified CSFV DIVA vaccine was developed in recent years, which is a chimeric marker vaccine (CP7_E2alf) (Postel et al., 2018). CP7_E2alf is a new live attenuated marker vaccine and was licensed by the European Medicines Agency in 2014 (Zhou, 2019). Experimental studies have shown that the vaccine can play an immune protective role against CSFV within a few days, and it has a high effectiveness (Eble et al., 2013).

Although currently available vaccines are sufficient to provide effective immune protection against CSF, they do not provide 100% protection. Therefore, in order to be able to eradicate CSF, continuous vaccination of pigs and enhanced biosecurity measures on farms are necessary.

In addition, herd immunity is also related to the control and prevention of CSF. Improving the immunity of animal groups can greatly reduce the transmission between animals and the spread of pathogens, especially in areas where high and virulent CSFV strains are prevalent, where herd immunity vaccination is very much needed. Compared with the E2 subunit vaccine, the C strain vaccine may be a better choice because the C strain vaccine induces herd immunity 1 to 2 weeks earlier than the E2 subunit vaccine (Chang et al., 2023). In order to maintain a high level of herd immunity, supplemental vaccination should be given once a month to newly introduced piglets and non-immunized young pigs (Van Oirschot, 2003).

In Malaysia, CSFV is controlled via routine vaccination applying MLV. The overall seroprevalence of CSF in the recent serological survey was 69.81% in 2016 and 2017 (Low, 2019).

2.2.6 CSF Diagnostics Tests

CSF can be diagnosed by recognition of clinical signs and postmortem results by the veterinarians, but the pathological finding and clinical signs of CSF infected pigs vary with the health status and age of the pig, as well as the virus strain (Greiser-Wilke et al., 2007). The clinical signs of CSF in pigs are quite

diverse and might be mistaken for those of other diseases caused by viruses (Wang et al., 2020). In addition, because CSF usually has an incubation period of several weeks, it is difficult to diagnose by clinical signs in the early stages of virus infection in a herd (Paton & Greiser-Wilke, 2003). Although the clinical signs and pathological changes of CSF are very helpful in diagnosing the disease, there are many pig diseases and complex signs, and latent infection and atypical signs can easily lead to misdiagnosis. Therefore, laboratory diagnosis of CSF is essential.

There are many laboratory testing methods, but the convenient, fast, and low-cost methods are the most practical when ensuring correct results. Therefore, the most commonly used methods to detect CSFV are Enzyme-linked immunosorbent assay (ELISA), viral neutralization tests (VNT), and PCR techniques.

Enzyme-linked immunosorbent assay (ELISA) is an internationally recognized detection method, which can detect results in a large number, quickly and reliably. Large-scale serological testing of CSFV is possible by using different commercially available ELISA kits (Colijn et al., 1997), so it is also used as the main way for CSFV antibody detection and the primary means for monitoring immune antibody levels in pigs. The essence of ELISA is a reaction in which enzyme labeling technology specifically combines with antibodies or antigens (Dewulf et al., 2004). It is an antigen or antibody combination between an enzyme-labeled anti-antibody and a solid-phase carrier. The color reaction further reflects the content of the antibody or antigen. This method has high

sensitivity and specificity (Ji et al., 2018). ELISA detection technology is of great significance to the purification of CSF.

The "gold standard" in testing for viruses is known as VNT, and it is the most specific and sensitive diagnostic test for antibodies to the CSFV. The test virus was neutralized by antibodies to CSFV in the serum (Moennig, 2000). Although it takes a long time and the operation process is complicated because it is based on cell culture technology, but the accuracy is high. VNT is the best approach for differentiating CSFV-specific antibodies from those generated after pigs were infected with other pestiviruses (Postel et al., 2018). However, the VNT is unable to differentiate between modified live CSFV vaccines and CSFV field infection, making it unsuitable for large tests on samples (Greiser-Wilke et al., 2007).

PCR technology has become a research and diagnostic technology that every laboratory must master, and this technology has gradually become a routine diagnostic technology for CSFV in most laboratories. For the detection of CSFV and differentiation of C-strain and wild-type CSFV, different PCR diagnostic methods have been developed (Luo et al., 2014). RT-PCR is a detection method for antigens, and it is currently one of the most sensitive detection methods for detecting CSFV (Dewulf et al., 2004). However, the shortcomings of this method are also obvious. The operation is cumbersome, cannot be automated, expensive, and professional laboratory personnel must be used for detection (Paton & Greiser-Wilke, 2003).

CHAPTER 3

SEROLOGICAL STATUS OF AUJESZKY'S DISEASE

3.1 Introduction

3.1.1 Overview

Acute and frequently fatal, Aujeszky's disease (AD) primarily affects pigs but can also affect other domestic and wild animals. The disease was initially identified in 1813 in cattle who were extremely itchy. Consequently, it was initially referred to as "mad itch". The disease is clinical similarity to rabies led to the usage of the term "Pseudorabies" (Papageorgiou et al., 2011). It is a double stranded and enveloped DNA virus (Mettenleiter, 1996). ADV is a lethal disease that can infect many different species of animals, including opossums, mice, goats, cattle, and sheep. ADV causes severe economic disease in domestic pigs and may lurk in the trigeminal ganglia of naturally infected domestic and wild pigs (Yang et al., 2016). The most efficient way to spread AD is by direct contact between pigs, which typically happens when they come into touch. Other oral routes for transmission include contaminated water and colostrum from infected sows to nursing piglets. AD can also be spread through the air. When infected pigs breed, it can also be passed on through the vaginal mucosa or semen (Beran, 1991). Pigs can contract ADV infection at various ages. Humans have recently been found to have isolated transitory infections, however, that were still huge debate about this findings (Heynen, 1941; Sehl & Teifke, 2020a). Only pigs survive ADV infection of all species (Pensaert & Kluge, 1989).

As far as AD vaccines are concerned, they are currently divided into attenuated vaccines, inactivated vaccines, gene deletion marker vaccines, recombinant vector vaccines, nucleic acid vaccines, and subunit vaccines. These vaccines have a certain control and prevention, but the effect is not very satisfactory and cannot completely eliminate the disease. This is because the prevalence of the disease has not been fundamentally controlled. However, since the advent of gene deletion marker vaccines in the late 1980s, the control of the disease has achieved good results. The application of the gE gene deletion vaccine and the corresponding gE-ELISA monitoring technology have achieved good results in the elimination plan of the disease (Nes & A., 2001; Pensaert et al., 2004).

In Malaysia, AD has existed for nearly 50 years. After the outbreak of the disease was first reported in Malaysia, farmers did not consider taking control measures because most farmers knew little about AD prevention and control measures. Therefore, the disease repeatedly spread in the country. With the development of vaccines and farmers' emphasis on AD, AD vaccination has now been regarded as an important means of basic prevention and control of the disease. Only a serological status of AD in Peninsular Malaysia was reported at 2018 (Low, 2019), but data and information remain limited. To this end, the focus of this study is to provide an up-to-date serological status on AD in Peninsular Malaysia between 2019 and 2021 because this period is the beginning and end of my research.

3.1.2 Objective

The objectives of this study are:

i. To determine the serological status of AD in pig farms in Peninsular Malaysia based on commercial samples submitted to UPM between 2019 and 2021.

ii. To investigate the field challenges of ADV in pig farms in Peninsular Malaysia based on commercial samples submitted to UPM between 2019 and 2021.

3.2 Materials and Methods

As shown in Figure 3.1, our research methodology has five main steps

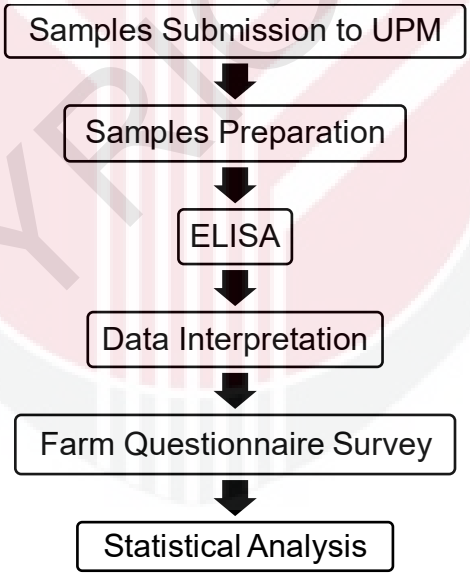

Figure 3.1 : Methodology framework for the study of Aujeszky's Disease

3.2.1 Sample Collection

Swine serum samples that were submitted to Veterinary Laboratory Service Unit (VLSU) of the Faculty of Veterinary Medicine, Universiti Putra Malaysia between 2019 and 2021 were used for this study which used a convenient sampling technique. This approach was chosen because most pig farms in Peninsular Malaysia are inaccessible and frequently have tight visitors' policies and biosecurity restriction. The farm requires that serum samples be tested for ADV by ELISA. The samples used in this study must meet the criteria mentioned below, and the samples that do not meet the criteria will be excluded. There are samples of the following age groups: 1-6 weeks, 7-12 weeks, 13-20 weeks, gilts (8-10 months old), sows (13-months-old and beyond). Minimum 4 samples each listed age group.

3.2.2 Region Categorization

Four regions were used to group the pig farms (Perak, Johor, Penang, and Selangor & Malacca) according to their geographical location and number of farms (Figure 3.2). Due to the small number of farms and samples in Selangor and Malacca, Selangor and Malacca are classified as one region.

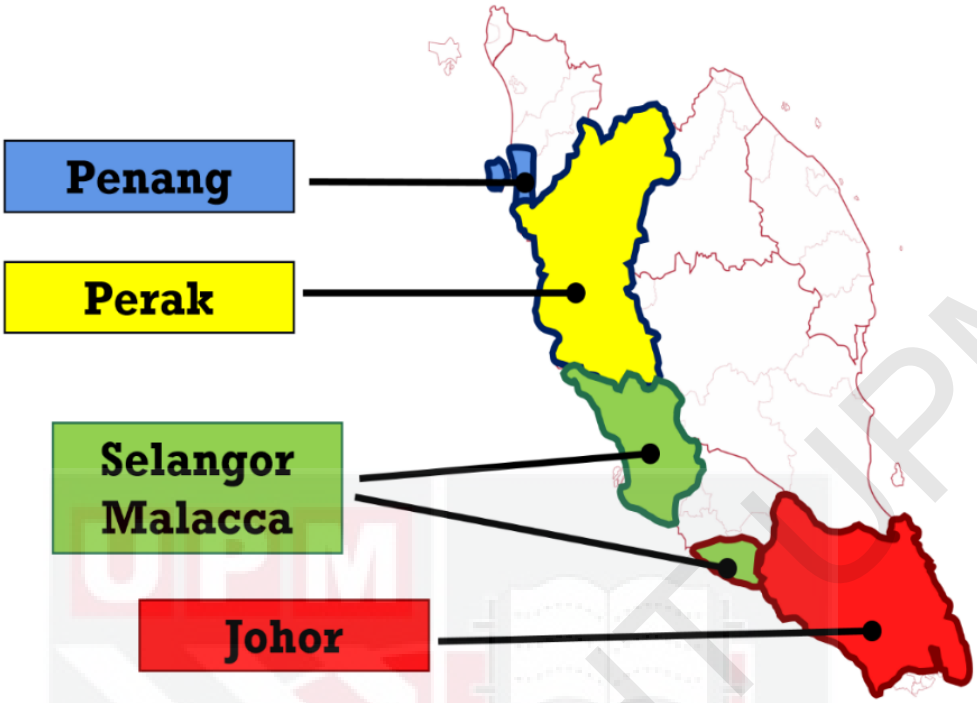

Figure 3.2 : Distribution of the region of the samples submitted to FVM, UPM in 2019-2021

3.2.3 Using Enzyme-Linked Immunosorbent Assay (ELISA) to Detect AD Antibody

According to the instructions provided by the manufacturer, the serum samples were analyzed using a commercial Pseudorabies Virus gpI Antibody Test Kit (IDEXX Laboratories, Inc., US). Glycoprotein I (gpI) is also known glycoprotein E (gE) (Stegeman, 1995). This test kit detects antibodies to the gpI/gE antigen of the Pseudorabies virus/Aujeszky's disease virus in swine serum. Antibodies to gpI/gE were signs of exposure to field strains of PRV or vaccinations that contain the gpI/gE antigen. The test relies on the identification of antibodies that were specific for the gpI/gE of the ADV. The kit has a sensitivity of 96.25% and a specificity of 98.75% (Jasbir, 1998).

Firstly, using a two-fold (1:2) serum dilution, the IDEXX PRV/ADV gpI/gE test was conducted on an ADV antigen-coated microwell. Prior to usage, all reagents must reach at room temperature. Next, 100µl of the negative and positive controls were dispense into the designated well. The plate was incubated for one hour at 18-26°C (room temperature). The solution in the wells was removed and each well was washed 3 times with 300µl of washing solution with a Filter Microplate Washer (Bio Tek ELx50^{TM}, US). After the third wash, the plate was pat on absorbent paper to remove residual wash solution. Then, 100µl of conjugate was added to each well. Incubate at 18-26°C (room temperature) for 20 minutes. After incubation, the plate was washed again 3 times. 100µl of TMB substrate was dispensed into each well and a third incubation was performed for 15 minutes at 18-26°C. Finally, add 50µl of stop solution to each well. Using an Absorbance Microplate Reader (Biotek ELx808^{TM}, USA), data from samples and controls were measured and recorded. The color intensity absorbance used was 650 nm. IDEXX XChek Software (Version 4.1.1.14, Inc., US) was used to evaluate the data, and the result was indicated by a sample to negative ratio (S/N) value. "S" is the absorbance value or optical density (OD) that was measured from the test sample well. "N" is the absorbance or OD value that was measured from a negative control well or a blank well. By comparing the computed S/N ratio to the standard advised by the test kit, as stated in Table 3.1 below, the serological status of samples is ascertained.

Table 3.1 : AD ELISA test kit interpretation

AD Serological status		
S/N ≤ 0.60	**0.60 < S/N < 0.70**	**S/N ≥ 0.70**
Positive	Suspect	Negative

3.2.4 Data Analysis

Analysis was performed on each farm based on all serum samples submitted within the farm during 2019-2021. The S/N value detected by the ELISA kit can determine the serological status of the serum sample, which is divided into three categories: positive, suspected, and negative. Since gE/gpl deletion vaccines are the most commonly used AD vaccines in pig farms in Malaysia, the presence of gE/gpl antibody indicates that the pigs in the farm were exposed to AD field strains. This means that the farm was infected with AD. The entire herd is considered seropositive for any positive serum sample detected on farm (Evans et al., 2008). Therefore, farms with seropositive results were classified as "AD field strain exposed farms". In contrast, any negative serum sample detected within the farm, the entire herd is considered seronegative. Seronegative farm was classified as "no AD field strain exposure farm" (Low, 2019).

3.2.5 Farm Questionnaire Survey

A questionnaire was sent online to the farms that submitted serum samples, and the farmer or farm manager filled in the basic information of the farm from 2019 to 2021. The content of the questionnaire included the location of the farm, herd size, housing type, site, neighboring farm, biosecurity measures and vaccination protocol, among others. Farm questionnaire survey form is in

Appendix I.

3.2.6 Statistical Analysis

For assistance with statistical analysis, IBM® SPSS Statistics 28 was utilized.

Use the Chi-square test to determine whether AD status is associated with different regions.

In addition, Multinomial Logistic Regression was used to carry out co-relationship analysis to determine whether the housing type, site, neighboring farm, biosecurity measures and vaccination protocol are related to the infected farms.

Finally, $p < 0.05$ and 95% confidence interval were used to determine statistical significance for each test that was performed.

3.3 Results

3.3.1 Overall Farms and Sample Received

In the AD study conducted in 2019–2021, there were 2780 samples collected from 61 farms (Table 3.2). 16 farms overlapped in these 3 years. From all the farms received, a total of 879 samples from 20 farms were received in 2019, 937 samples from 22 farms in 2020, and 964 samples from 19 farms in 2021.

Table 3.2 : Number of farms and serum samples submitted to FVM, UPM for AD ELISA testing in each year in 2019-2021

Region	Number of Farms	Number of Samples
	2019	
Perak	10	527
Johor	2	71
Penang	3	107
Selangor & Malacca	5	174
Total	20	879
	2020	
Perak	13	568
Johor	1	40
Penang	5	188
Selangor & Malacca	3	141
Total	22	937
	2021	
Perak	10	466
Johor	5	311
Penang	2	78
Selangor & Malacca	2	109
Total	19	964
Grand Total	61	2780

According to the regional research records of AD samples in 2019-2021 (Table 3.3). A total of 1561 samples were received from 33 farms in Perak, 422 samples were received from 8 farms in Johor. The number of farms from Johor region is limited, the data collected was not sufficient to represent the entire region, it only provided a representation number. 373 samples were received from 10 farms in Penang, and 424 samples from 10 farms were received in Selangor & Malacca.

Table 3.3 : Number of farms and serum samples submitted to FVM, UPM for AD ELISA testing in each region in 2019-2021

Region	Number of Farms	Number of Samples
Perak	**2019-2021**	
	10	527
	13	568
	10	466
Total	33	1561
Johor	**2019-2021**	
	2	71
	1	40
	5	311
Total	8	422
Penang	**2019-2021**	
	3	107
	5	188
	2	78
Total	10	373
Selangor & Malacca	**2019-2021**	
	5	174
	3	141
	2	109
Total	10	424
Grand Total	61	2780

3.3.2 Overall AD Seroprevalence

In this study, the AD ELISA test kit was used and it is a DIVA test kit that can identify field virus exposure from pig serum results. When seropositive result is detected, the farm is considered as a field ADV exposed farm. Otherwise, it is regarded as a non-field ADV exposed farm. There were 11 farms in all that had seropositive results, representing 18.03 % (11/61) of the farms in this study. 42 samples were positive, accounting for 1.51% of the samples (42/2780) in this study. Overall, field ADV exposed farms and positive samples were the highest in 2019. The serological status of AD in 2019-2021 is shown in Table 3.4.

Table 3.4 : Number of farms and samples status in 2019-2021

Year	Number of Farms	Infected Farms	Suspected Farms	Non-infected Farms
2019	20	6	2	12
2020	22	2	0	20
2021	19	3	1	15
2019-2021	61	11	3	47
Year	**Number of Samples**	**Positive Samples**	**Suspected Samples**	**Negative Samples**
2019	879	23	3	853
2020	937	5	1	931
2021	964	14	1	949
2019-2021	2780	42	5	2733

The percentage of farms and sample status of AD in 2019-2021 can be seen in Figure 3.3. In 2019, a total of 20 farms provided 879 serum samples. 30% (6/20) of the farm was positive with seroprevalence of 2.62% (23/879). In 2020, a total of 22 farms provided 937 serum samples. 9.09% (2/22) of the farm was positive with seroprevalence of 0.53% (5/937). In 2021, a total of 19 farms provided 964 serum samples. 15.79% (3/19) of the farm was positive with seroprevalence of 1.45% (14/964). Overall, the percentage of ADV infected farms and positive samples gradually decreased from 2019 to 2021.

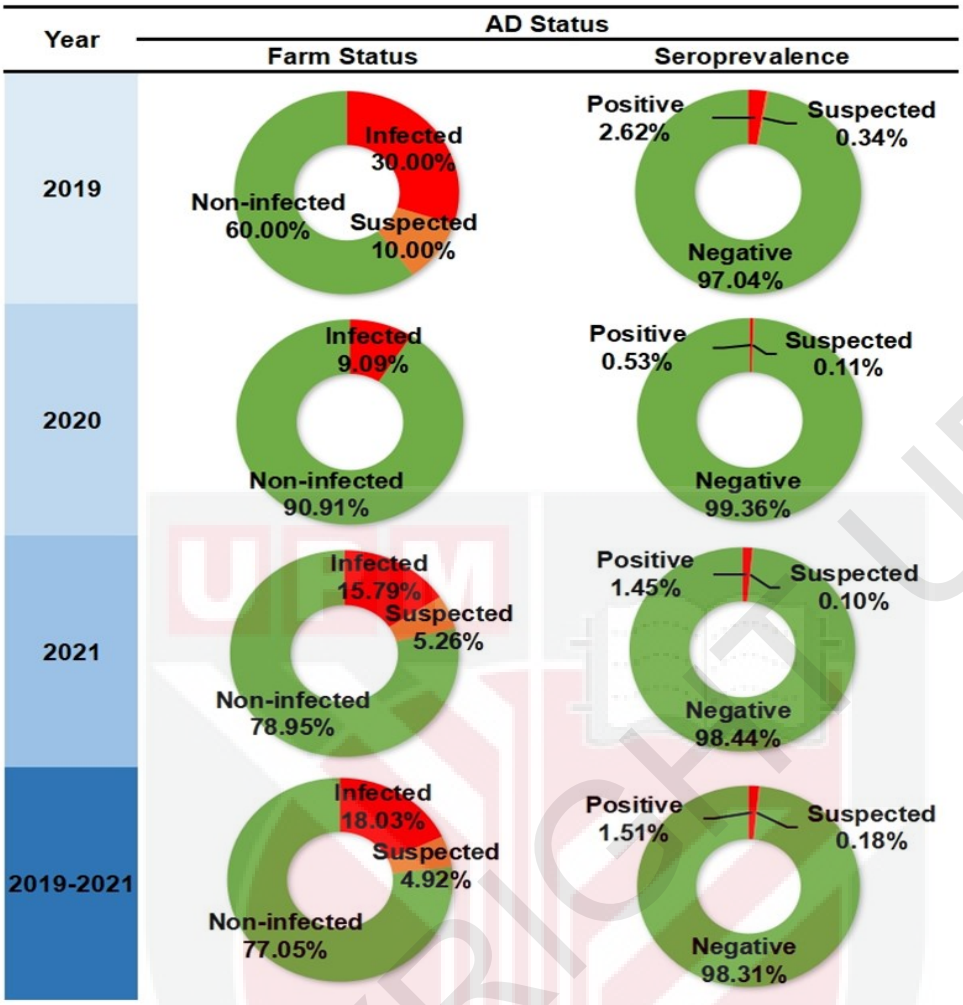

Figure 3.3 : Summary of farm AD status and seroprevalence of AD in each year in 2019-2021

3.3.3 AD Seroprevalence Across Region

In Table 3.5, the overall seroprevalence rate of AD wild strains in pig farms from 2019 to 2021 in Peninsular Malaysia is shown. The overall seropositive rate in Perak region is zero. In the Johor region, 50% (4/8) of the farms were exposed to AD field strains, and the overall seroprevalence rate was 5.92. In the Penang region, only 10% (1/10) of the farms were detected to AD field strain, and the seroprevalence rate was 0.27. In Selangor & Malacca region, 60% of the farms were exposed to AD field strains, the seroprevalence rate

was 3.77. From 2019 to 2021, 18.03% of the farms were detected to AD field strain, with a seroprevalence of 1.51. In general, there is no exposure to AD field strains in the Perak region, while the exposure to AD field strains in the Johor region is higher in 2019-2021, followed by Selangor & Malacca, Penang.

Table 3.5 : Summary of farm AD status and seroprevalence of AD in each region in 2019-2021

Region	Infected Farms	Seropositive samples	Overall seropositivity (%)
Perak	0 (33)	0 (1561)	0.00
Johor	4 (8)	25 (422)	5.92
Penang	1 (10)	1 (373)	0.27
Selangor & Malacca	6 (10)	16 (424)	3.77
Total	11（61）	42（2780）	1.51

Note: The values inside the brackets represents the number of farms or sample, whereas the values outside the brackets represents the number of infected farms or seropositive sample

Statistics and evaluation from all samples submitted to UPM in each region, there was no exposure to positive AD field strains in the Perak region during the three-year period from 2019 to 2021, and even the seronegative rate of samples in 2020 reached 100 %. In contrast to the Johor region, the seropositive rates of samples were 16.90%, 10%, and 2.89% from 2019 to 2021. In the Penang region, there was no exposure to positive AD field strains during 2019 and 2020, and the seropositive rate of samples until 2021 was 1.28%. For the Selangor & Malacca region, the seropositive rates of samples from 2019 to 2021 are 6.32%, 0.71%, and 3.67%, respectively. Based on the overall seropositivity, the exposure of AD field strain was higher in the Johor region and lower in Perak regions in Peninsular Malaysia from 2019 to 2021 in Figure 3.4 & 3.5.

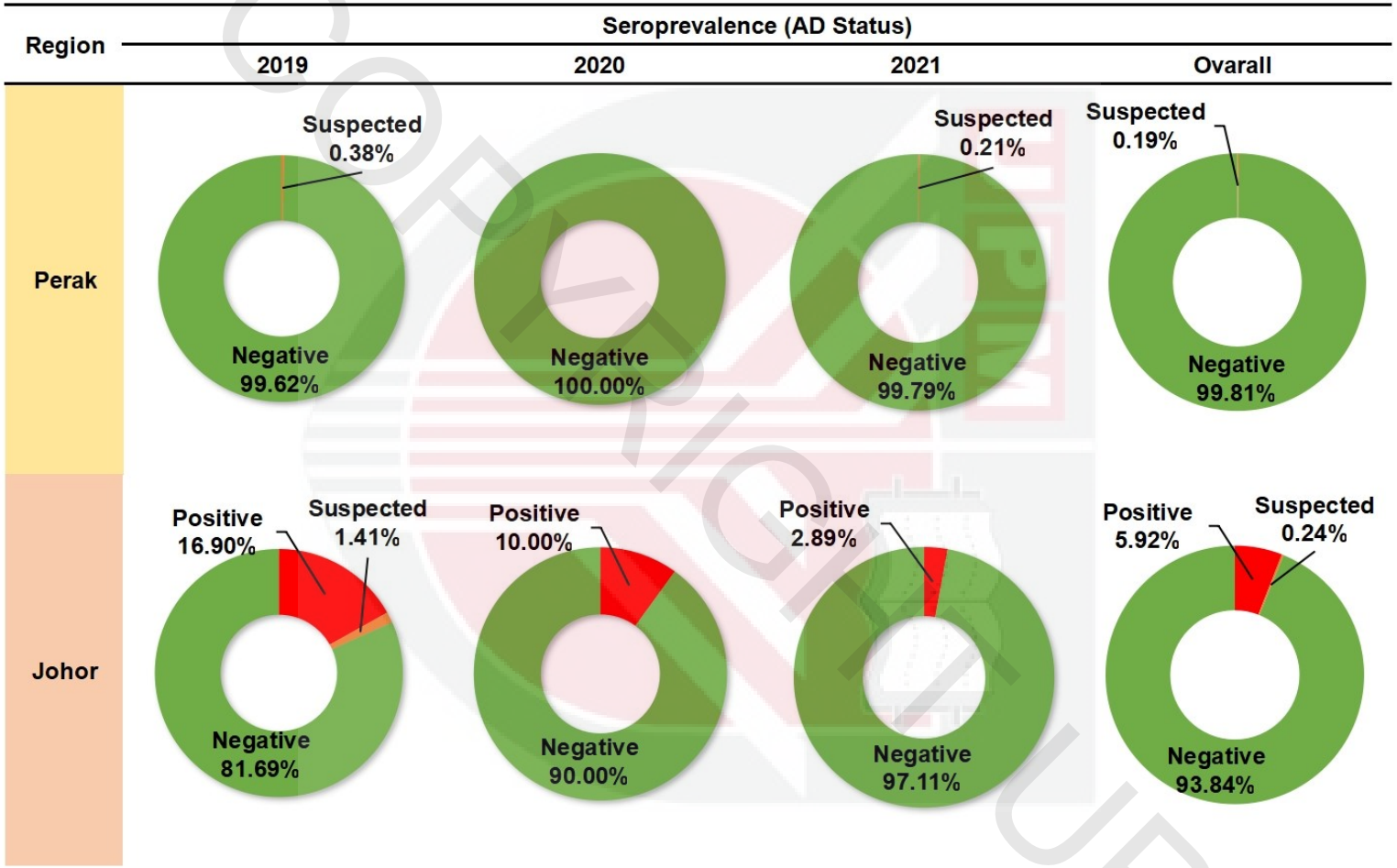

Figure 3.4 : Summary of the serological status of AD in different regions based on serum samples submitted to FVM, UPM in 2019-2021

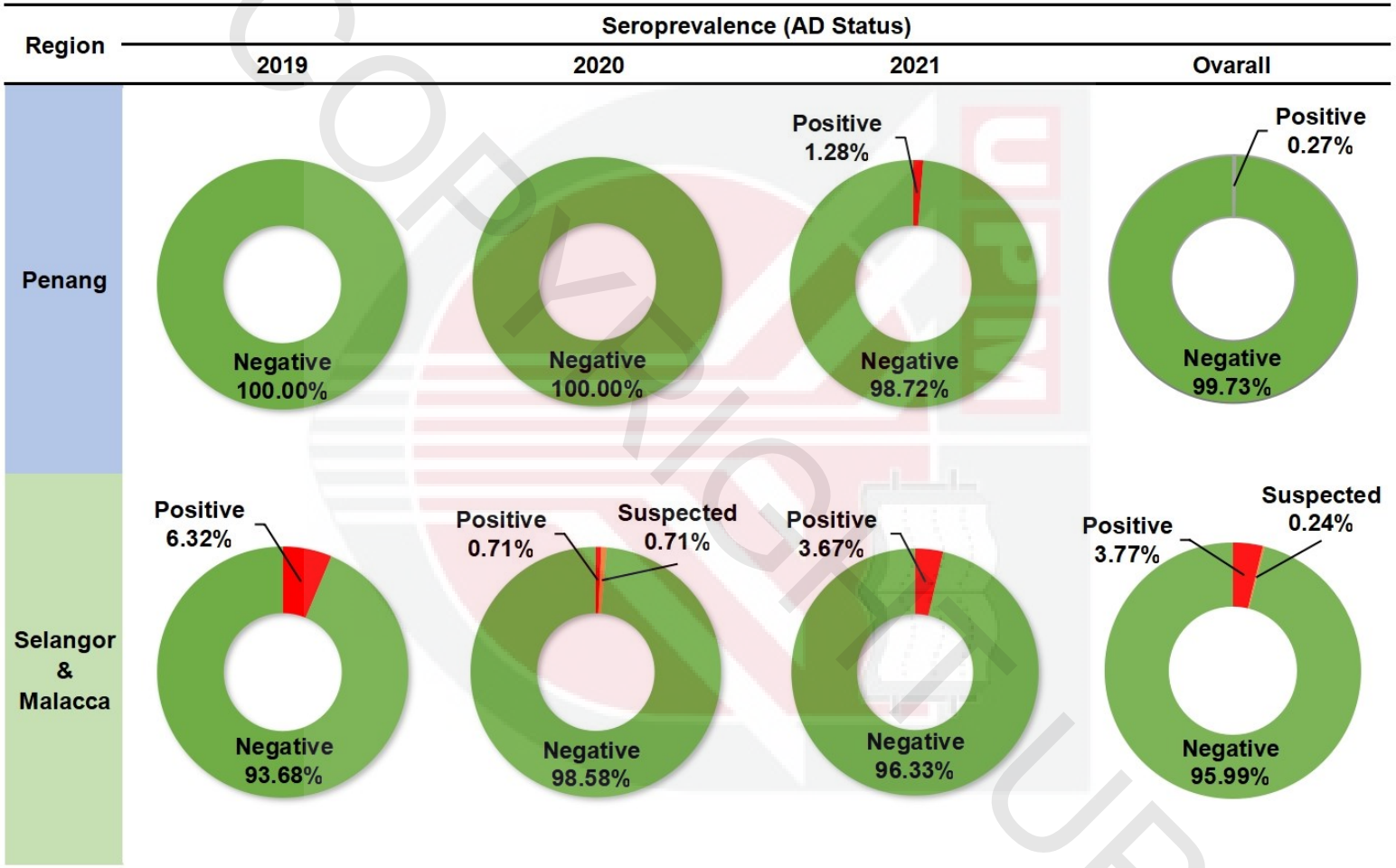

Figure 3.5 : Summary of the serological status of AD in different regions based on serum samples submitted to FVM, UPM in 2019-2021

The association between AD status and various regions was observed using statistical analytic techniques; the chi-square test was performed to confirm this relationship, and the results are shown in Table 3.6 below.

Table 3.6 : Summary of farm AD status associations across regions based on IBM® SPSS Pearson Chi-Square Test in 2019-2021

Infected * Region Crosstabulation

			Region				
			Perak	Johor	Penang	Selangor Malacca	Total
Field Strain Exposed	Infected	Count	0	4	1	6	11
		Expected Count	6.0	1.4	1.8	1.8	11.0
		P-value	0.0001*	0.0117	0.4715	0.0002*	
	Non-infected	Count	33	4	9	4	50
		Expected Count	27.0	6.6	8.2	8.2	50.0
		P-value	0.0001*	0.0117	0.4715	0.0002*	
Total		Count	33	8	10	10	61
		Expected Count	33.0	8.0	10.0	10.0	61.0

*Significant Difference as Compared with Adjusted Bonferroni P-Value **(0.00625)**

3.3.4 AD Seroprevalence across Age Group

The age groups exhibit varying degrees of seropositivity toward the AD field strain, as shown in Figure 3.6. During 2019-2021, the seropositivity rates of serum samples aged 1-6 weeks were 4.55%, 1.08% and 1.91%, respectively. Among serum samples aged 7-12 weeks, the seropositivity rate was low in 2019 and 2021, and no seropositivity was detected in 2020. Among the 13-20 weeks of age, no seropositivity was detected in 2019, and the seropositivity rates of serum samples in 2020 and 2021 were lower. Gilts display the highest rate by more than 8% seropositivity in 2019, and no seropositivity in 2020 and

2021. In sows, the seroprevalence rates of serum samples were 1.88%, 0.88%, and 3.26% from 2019 to 2021, respectively.

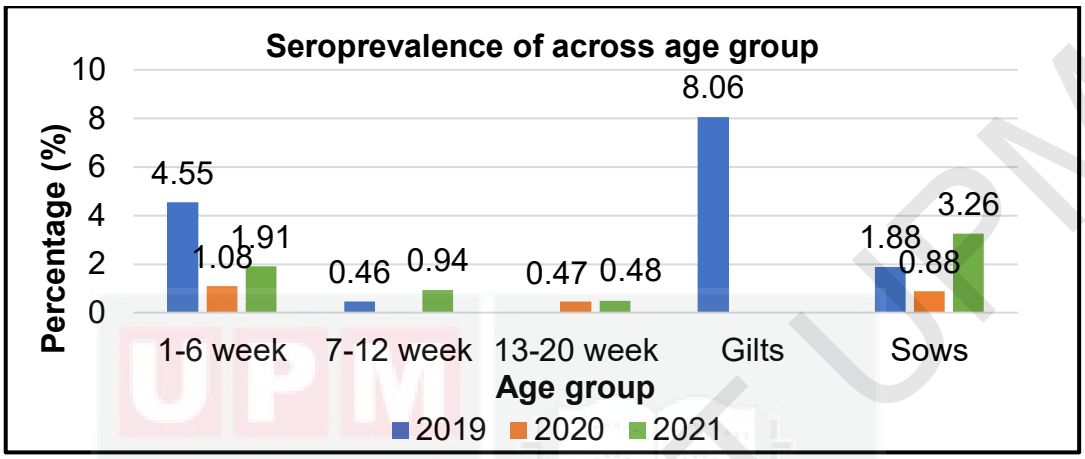

Figure 3.6 : Overall seroprevalence of AD in different age groups in 2019-2021

In Table 3.7, in order to understand the distribution of positive serum samples in more detail, the seropositivity of the samples are divided into different age groups.

Table 3.7 : Summary of seropositive samples of AD in different age groups in 2019-2021

Region	Seropositivity (%)				
	1-6w	7-12w	13-20w	Gilts	Sows
			2019		
Perak	0 0/82	0 0/132	0 0/108	0 0/67	0 0/138
Johor	30.00 3/10	50.00 1/20	0 0/11	0 0/10	40.00 8/20
Penang	0 0/21	0 0/24	0 0/24	0 0/13	0 0/25
Selangor & Malacca	9.76 4/41	0 0/41	0 0/28	12.50 3/24	10.00 4/40
Total	4.55 7/154	0.46 1/217	0 0/171	2.63 3/114	5.38 12/223
			2020		
Perak	0 0/104	0 0/128	0 0/129	0 0/63	0 0/144
Johor	16.67 1/6	0 0/5	10.00 1/10	0 0/5	14.29 2/14
Penang	0 0/44	0 0/42	0 0/43	0 0/23	0 0/36
Selangor & Malacca	4.76 1/31	0 0/31	0 0/32	0 0/15	0 0/32
Total	1.08 2/185	0 0/206	0.47 1/214	0 0/106	0.88 2/226
			2021		
Perak	0 0/103	0 0/103	0 0/102	0 0/57	0 0/101
Johor	2.99 2/67	2.94 2/68	0 0/66	0 0/41	7.25 5/69
Penang	0 0/17	0 0/16	6.25 1/16	0 0/8	0 0/21
Selangor & Malacca	9.09 2/22	0 0/25	0 0/25	0 0/13	8.33 2/24
Total	1.91 4/209	0.94 2/212	0.48 1/209	0 0/119	3.26 7/215

Note: Number of seropositive samples/Total number of samples

3.3.5 AD Seroprevalence in Each Age Group across the Region

A summary of AD field strain farms and affected age groups in 2019 can be observed in Table 3.8. There is a total of 20 farms, of which 6 farms were exposed to AD field strains. 2 farms are from Johor region, 4 farms are from

Selangor & Malacca region. Perak region and Penang region are none.

Table 3.8 : Summary of AD field strain exposure farms and infected age groups in 2019

2019			
Region	**Farm Code**	**Age Group**	**Seropositivity (%)**
Perak			
Johor	J01	Sows (2)	5.71
	J02	1-6w (3) 7-12w (1) Sows (6)	27.78
Penang			
Selangor & Malacca	SM02	Sows (1)	2.27
	SM03	1-6w (2) Sows (2)	12.5
	SM04	Gilts (3)	10.34
	SM05	1-6w (2) Sows (1)	8.82

Note: Values inside brackets indicate number of positive serum samples. Samples were taken according to ages groups: 1-6 weeks, 7-12 weeks, 13-20 weeks, gilts, and sows. Age group not reported in this table represents negative for the disease.

Among the 22 farms in 2020, 2 farms were exposed to AD field strains. There is 1 farm in the Johor region, and 1 farm in the Selangor & Malacca region. Perak region and Penang region are none. It is summarized in Table 3.9 below.

Table 3.9 : Summary of AD field strain exposure farms and infected age groups in 2020

2020			
Region	**Farm Code**	**Age Group**	**Seropositivity (%)**
Perak			
Johor	J03	1-6w (1) 13-20w (1) Sows (2)	11.11
Penang			
Selangor & Malacca	SM07	1-6w (1)	1.49

Note: Values inside brackets indicate number of positive serum samples. Samples were taken according to ages groups: 1-6 weeks, 7-12 weeks, 13-20 weeks, gilts, and sows. Age group not reported in this table represents negative for the disease.

In 2021, 3 out of 19 farms were exposed to AD field strains. There is 1 farm each in Johor region, Penang region, and Selangor & Malacca region. Perak region is none. It is listed in Table 3.10 below.

Table 3.10 : Summary of AD field strain exposure farms and infected age groups in 2021

2021			
Region	**Farm Code**	**Age Group**	**Seropositivity (%)**
Perak			
Johor	J05	1-6w (2) 7-12w (2) Sows (5)	11.11
Penang	PG09	13-20w (1)	2.50
Selangor & Malacca	SM10	1-6w (2) Sows (2)	7.27

Note: Values inside brackets indicate number of positive serum samples. Samples were taken according to ages groups: 1-6 weeks, 7-12 weeks, 13-20 weeks, gilts, and sows. Age group not reported in this table represents negative for the disease.

3.3.6 AD Seroprevalence across Herd Size

From 2019 to 2021, a total of 61 farms submitted serum samples for AD diagnosis, but only 37 farms provided information on herd size in this study.

Farm herd sizes are divided into 3 sizes based on the number of sows in each farm, namely: small, medium and large. Farms with less than 500 head of sows are classified as small herd sizes, farms with between 500 and 1,000 head of sows are classified as medium herd sizes, and farms with more than 1,000 head of sows are classified as large herd sizes. Table 3.11 is detailed data compiled based on information provided by farms. The number of small herd size farms is only 6 and it is the smallest. The number of medium herd size farms is the largest, and it accounts for 51.35% of all farms. 12 farms are

classified as large herd size.

Table 3.11 : Summary of the number of farms based on herd size in 2019-2021

Herd Size	Number of Sows (head)	Number of Farms	Percentage (%)
Small	<500	6	16.22
Medium	500-1000	19	51.35
Large	>1000	12	32.43
Total		37	

As shown in Figure 3.7, 50% of small herd size farms are exposed to AD field strains, which is the highest proportion. The proportions of medium herd size farms and large herd size farms exposed to AD wild strains were relatively low, 10.53% and 16.67% respectively.

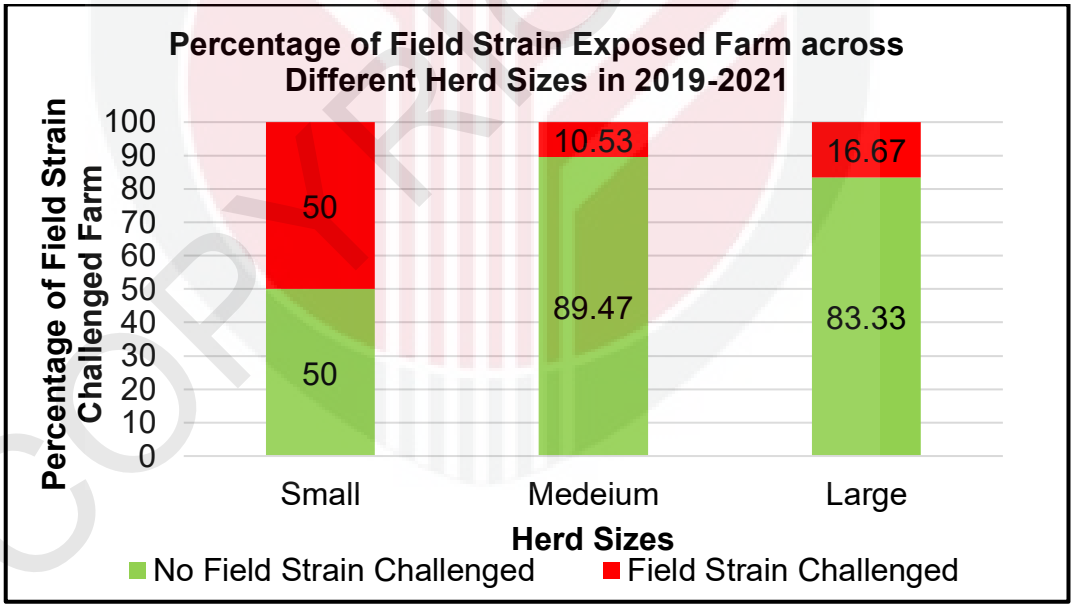

Figure 3.7 : Summary of the percentage of farms exposed to AD field strains in different herd sizes in 2019-2021

Most farms with exposure to AD field strains are from small herd size farms, as shown in Figure 3.8. In 2019, the proportion of farms exposed to AD field

strains was the same among small, medium, and large herd size farms, all at 33.33%. In 2020, 50% of AD-infected farms were from small herd size farms, and 50% of infected farms were from large herd size farms. In 2021, half of the farms challenged with AD field strains were from small farms, and half were from medium herd size farms.

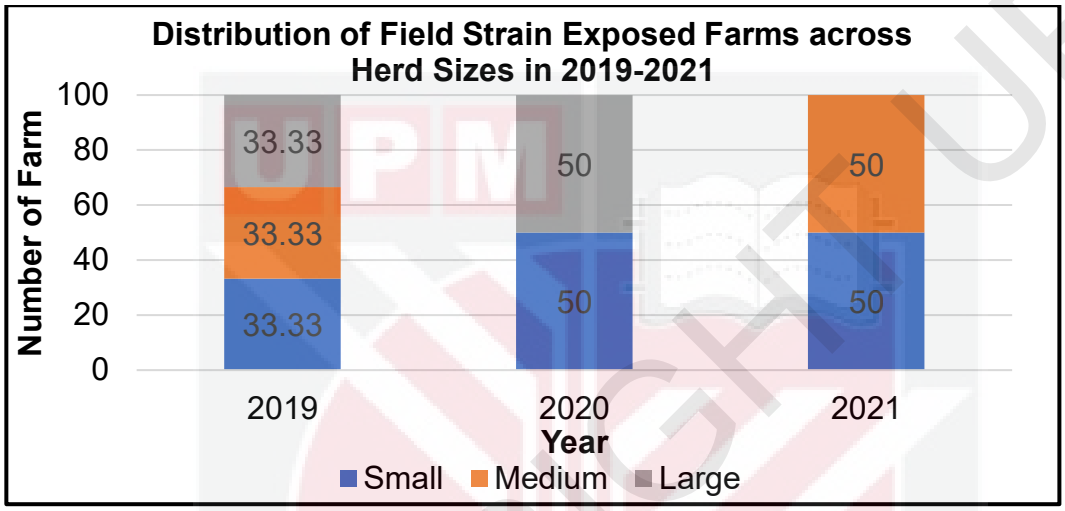

Figure 3.8 : Summary of the distribution of farms exposed to AD field strains in different herd sizes in 2019-2021

3.3.7 Risk Factor Analysis

A total of 61 farms submitted serum samples for AD ELISA test in 2019-2021, but only 34 farms provided information through the questionnaire. Because some farmers are unwilling to disclose farm information, only these 34 farms were included in this study. A summary of the data obtained for various risk factors is shown in Table 3.12.

From the perspective of farm structure, most of the farms are open house type and closed house type, and only 20.59% of the farms have a combination of

open and closed house types. In addition, 73.53% (25/34) of the farms are single sited.

Biosecurity measures include vehicle dip, gate and fence, isolation and holding room, foot dip, and neighboring farm perimeter. In the investigation of biosecurity measures, it was found that all the farms submitted to the questionnaire had gate and fence. Vehicle dip (88.24%), isolation and holding room (64.71%), and foot dip (79.41%) were also more common in farms. In addition, 76.47% of farms had neighboring farms.

In terms of vaccination protocol, mass vaccination (75%) was commonly carried out in breeder herds to control AD on the farms surveyed, and only 25% of farms carried out vaccination in porker herds.

Table 3.12 : Summary of AD infected farm based on various risk factors

Risk Factors		Number of farms	Infected farms	Seropositivity (%)
Farm structure				
Housing Type	Open-house	14	5	35.71
	Open and Closed house	7	2	28.57
	Closed House	13	0	0
Site	Single Sited	25	7	28.00
	Multi Sited	9	0	0
Farm Management				
Biosecurity Measures	Vehicle dip	30	5	16.67
	No Vehicle dip	4	2	50.00
	Gate & Fence	34	5	14.71
	No Gate & Fence	0	0	0.00
	Isolation and Holding room	22	4	18.18
	No Isolation and Holding room	12	3	25.00
	Foot dip	27	4	14.81
	No Foot dip	7	3	42.86
Neighboring Farm	Neighboring Farm Perimeter	26	7	26.92
	No Neighboring Farm Perimeter	8	0	0.00
Vaccination Protocol	Porker Vaccination	6	1	16.67
	No Porker Vaccination	18	2	11.11
	Mass Vaccination for breeders	21	3	14.29
	Other type of Vaccination for breeders	7	2	28.57

Various risk factors were examined using multinomial logistic regression tests, and farm housing type was associated with farm infection rates. From the results in Table 3.13 it was found that open houses increase infections on farms.

Table 3.13 : Summary of AD infected farm for various risk factors from investigated farms based on IBM® SPSS Multinomial Logistic Regression in 2019-2021

AD Infection[a]		B	Std. Error	Sig.	Exp(B)
Infected	Intercept	-20.793	.837	.000	
	Open-house	19.918	.992	.000	446846340.520
	Combination of open and close house	19.877	.000		428972486.899

Note:
a. The reference category is: Non-infected.
Yellow highlight indicates significant association.

3.4 Discussion

3.4.1 Overall AD Status in 2019-2021

In Malaysia, the gene E deletion vaccine is generally used in commercial pig farms. Therefore, we can detect AD field strains by using the matching commercial Pseudorabies Virus gpI Antibody Test Kit (IDEXX Laboratories, Inc., US). When the test result of the sample is positive, it indicates that the farm has been exposed to the AD field strain in the past, and also indicates that the farm might been threatened by the AD field strain.

A total of 2780 samples from 61 pig farms were sampled for AD detection in such a study. From the overall results of 2019-2021, the infection rate of AD field strains is lower than 19%. In 2019, AD infection in pig farms was the highest, and 30% of pig farms were detected to be exposed to AD field strains. However, in both 2020 and 2021, the infection rate will not exceed 16% in both years. This may be related to the Coronavirus Disease 2019 (COVID-19) that led to movement restriction and outbreak of ASF in Malaysia in 2021. In order to control and prevent the further spread and epidemic of ASF in Malaysia,

farms strengthen biosecurity and farm management, such as controlling the movement and entry of personnel, carrying out disinfection and cleaning (Khoo et al., 2021). Based on this study, the infection situation of AD in the past three years is relatively stable in pig farms in Peninsular Malaysia. The seroprevalence of samples decreased from 55.4% in 1998 and 25% in 2019 to lower than 18.03% at present based on previous studies (Low, 2019).

In addition, from the first reported AD outbreak in 1976 to sporadic outbreak reports in different regions, the outbreak cases of the disease have gradually decreased in Malaysia in recent years. Therefore, the risk of natural infection with AD is low, probably due to the preventive measures of the disease by farmers and the widespread vaccination of pigs. Vaccinated pigs have reduced mortality and do not show severe clinical symptoms when pigs infected with ADV (Van Oirschot et al., 1990). In addition, vaccination can not only greatly limit the infectivity of the virus (Van Oirschot & Gielkens, 1984), they also seem to increase the quantity of virus needed to cause infection (Wittmann, Ohlinger, & Höhn, 1982).

3.4.2 AD Status across Region

The results of the four regional farms in 2019-2021 do not represent the overall AD status in the entire region, and they only represent the sample farms.

33 farms submitted serum samples in the Perak region of Peninsular Malaysia, and this region had the largest number of farms and samples in this study, which is highly consistent with the serological surveys done by Low on AD in

2016 and 2017 (Low, 2019). However, no seropositive samples were detected in this region from 2019 to 2021. No exposure to AD field strains were detected at Perak region during these 3 years, which may be attributed to the prevention and control measures, feeding management, and regular vaccination with high efficiency vaccines on the farm (Low et al., 2018). If the farm needs to introduce pigs, it must be tested for ADV and ensure that the imported pigs are negative (Moynagh, 1997). In addition, regular AD vaccination can enhance the antivirus ability of pigs and reduce AD infection in this region. Although no seropositive samples were detected in the Perak region, it still does not mean that AD does not exist in the region. Therefore, farms still need to maintain a high degree of attention to AD and routine performance for prevention.

The number of farms and samples were lesser in the Johor region, while this region has the highest seropositivity rate. In this study, it is (5.71%) in J01 farm and (27.78%) in J02 farm in 2019 (Table 3.8), (11.11%) in 2020 (Table 3.9), and (11.11%) in 2021 (Table 3.10) for AD seropositive pigs in infected farms. Furthermore, the seropositive rates of samples were 16.90%, 10%, and 2.89% from 2019 to 2021 in Johor region (Figure 3.4). However, the number of AD seropositive pigs in Johor region was still higher than other regions. It can be observed from this research report that the exposure risk of AD field strain virus in Johor region is higher, and the risk has always existed.

The high infection rate may be related to the small number of samples obtained from the Johor region. The reason for the small sample size in the

Johor region is mainly because it does not match the age group criteria in the samples collection study, so these samples are not eligible for inclusion in the study and cannot be counted.

Although the detection data of these samples cannot be completely equal to the actual situation of the whole pig farm, it would be able reflect the situation of the AD antibody level of the pig farm to a certain degree. In 2019, the emergence of high seropositivity largely reflected that the main cause of AD infection was internal factors from farms in the region. Based on the research and statistics of Low in 2016-2017, farm breeding density in Johor region is higher than other regions (Low, 2019). Therefore, internal reasons may include improper the farm management, such as high breeding density, poor breeding environment, and failure of rodent control, and introduction of pigs. These reasons can increase the risk of AD exposure. Lack of preventive measures may be the main reason for increasing AD infection, such as unreasonable immunization procedures, poor vaccine quality, and failure to detect and eliminate positive pigs in time. Although the AD infection rate was high in 2019, the situation has improved greatly in 2020 and 2021. The farmers might begin to strengthen the feeding management of the farm and took control measures, such as compulsorily killing seropositive pigs, updating pig herds, and vaccinating after detected field AD challenge.

For the Penang region, the region submitted the least number of samples and only one farm was infected with AD in 2019-2021. The seropositive rate of samples in this region is low, with only 1.28% of positive samples in 2021, and

no positive samples were detected in 2019 and 2020 (Figure 3.5). The small sample size might contribute to the low sample seropositivity rate.

One reason for the small sample size in the Penang region is that it did not match the age group criteria in the study, so these samples were not eligible for study inclusion. Another reason may be related to the outbreak of ASF in the Penang region of Peninsular Malaysia in 2021, which led to culling measures and closures in many farms. The number of pigs and farms in the Penang region has decreased significantly, so the sample size obtained is the smallest.

Although the sample size was small, the samples submitted by the P01 farm were detected to be seropositive in 2021. The presence of a field strain-positive farm in Penang suggests that there is a risk of AD field strain exposure in this region, which could also be a potential threat to the pig herd and could lead to other infections. Overall, the seropositivity rate in the Penang region is low, which can be attributed to good vaccination efforts, husbandry, and good farm biosecurity on the farm. Another reason may be that the number of samples collected that met the criteria for this study was small, resulting in a low seropositivity rate result.

For the Selangor & Malacca region, the seropositive rates of samples from 2019 to 2021 are 6.32%, 0.71%, and 3.67%, respectively. Six out of 10 farms were infected with AD in 2019-2021, especially 4 farms were infected in 2019. this region has the higher seropositivity rate. Overall, it is gradually decreasing

from (2.27%-10.34%) in 2019 and (1.49%) in 2020 to (7.27%) in 2021 for AD seropositive pigs in infected farms. The density of farms is high, so airborne transmission is the main cause of ADV infection on farms. Additionally, pig farms are concentrated in coastal towns in the region. A moist environment can increase the stability of enveloped viruses and promote the formation of aerosols (French et al., 2023), and ADV remains active for a longer time in a humid environment (Verreault, 2010). Therefore, wet conditions are likely to cause ADV to spread from farm to farm by aerial transmission (Solymosi et al., 2004). Both high-density and high-humidity pig farms increase the risk of AD infection.

3.4.3 AD Status across Age Group

The age groups show some degree of seropositivity towards AD field strain, it mainly focuses on 1-6 weeks old piglets and sows in 2019-2021.

It has more seropositivity rate on gilts for 2019, followed by 1-6 weeks old, sows, and 7-12 weeks old. For gilts, which are themselves susceptible to ADV under normal circumstances, so the seropositive rate of pigs in this age group is very high. Another reason could be that if gilt is purchased from outside sources that contain ADV, ADV may unintentionally enter the farm (Leontides et al., 1994; A. Stegeman et al., 1994; J. A. Stegeman et al., 1994). However, no positive serum samples were detected in the gilts group samples in 2020 and 2021, which may be affected by the COVID-19 epidemic. Due to the outbreak of the epidemic around the world, many countries have banned the export and import of breeder animals, so no purchase and introduction of new

gilts within this 2020 till 2021 duration.

In the 1-6 weeks old group, the seroprevalence is high for piglets in this age group from 2019 to 2021. On the one hand, piglets aged 1-6 weeks mainly rely on MDA obtained from placenta or breast milk, and they have not been vaccinated against AD, so pigs in this age group are very vulnerable to the threat of AD. The high seropositivity in 1-6 weeks old piglets may be that the sows were infected with ADV during gestation, and the newborn piglets have been infected with the virus from the placenta (Laval & Enquist, 2020; Sehl & Teifke, 2020b; Van Oirschot, 1999). Serum samples tested positive for ADV after piglets were born.

For finisher pigs of 7-12 weeks and 13-20 weeks old, these two groups of pigs had the lowest degree of infection with seroprevalence less than 3%. In a study of 2 and 15 weeks old domestic pigs infected with ADV, researchers found that 2 weeks old piglets infected with the virus developed severe neurologic and respiratory disease, but did not present these clinical symptoms in 15-week-old pigs. This is because of the immature development of the nervous and immune systems of 2 weeks old piglets (Verpoest et al., 2018), which also indirectly proves that the 15 weeks old pigs have stronger immunity compared to other age groups. The immunity of the finisher pigs is better. The pigs in the two age groups have been vaccinated, so a certain number of antibodies have been produced in the pigs. Therefore, pigs aged 7-12 weeks and 13-20 weeks were less susceptible to AD.

Sows are most susceptible to AD. The seroprevalence of AD in sows was higher compared with pigs of other age groups, which is the same as the findings of Low et al in Malaysia in 2016 (Low et al., 2018). Younger pigs were lower seropositive rate than older pigs, which may be related to the age of pigs (Low et al., 2018). In addition, farm purchases of sows carrying ADV can also increase seropositivity in sow herds (Baskerville, 1981). Once sows are infected with AD, they can continuously spread the ADV on the farm through milk, feces and other excretions, which makes more pigs sick.

3.4.4 AD Seroprevalence across Herd Size

Exposure to AD field strains was detected in half of the small herd size farms. The main factor responsible for the high proportion of infected farms in small herd size farms might be cost constraints. Moreover, because no AD outbreaks have been reported, some farmers may not take the disease seriously or took the necessary biosecurity measures to control it.

Farms infected with AD field strains are rare among medium and large herd size farms compared to small herd size farms. This may be because biosecurity measures are stricter on medium and large herd size farms. For example, it is mandatory to vaccinate pigs to improve vaccine concentration density and immunity quality (Chen et al., 2022). To avoid significant losses due to disease, modern and mature medium and large herd size farms usually purchase highly effective vaccines and regularly vaccinate their herds. Investing in vaccination measures to control AD infection was beneficial for farms (Colomer et al., 2020).

3.4.5 Risk Factor Analysis

Data obtained through questionnaires suggest that exposure to AD field strains on farms may also be associated with other risk factors.

In terms of farm housing type, open house farms are more susceptible to AD field virus challenges than combined open and closed house farms and closed house farms. This may be related to the way AD is transmitted by air. In a study it was shown that ADV could be wind-borne over a distance of more than 40km in areas with dense farm and pig populations (Christensen et al., 1990), so this may increase the risk of AD infection in open house farms.

Moreover, AD can be transmitted through different methods, good biosecurity measures are crucial (Pritchard et al., 2005). Good biosecurity measures not only reduce the risk of ADV introduction on the farm, but also reduce the spread of pathogens on the farm (Alarcón et al., 2021). In this research investigation, it was once again proven that taking biosecurity measures can reduce infection with the disease.

However, not all infected farms have poor biosecurity or are surrounded by neighboring farms. The introduction of infected breeding pigs to the farm is also a possible factor in farm infection. The virus can be controlled through vaccination and suitable programs for gilts and sows, good husbandry by vaccination can control and prevent the diseases circulation within the farm (Colomer et al., 2020). Rapid elimination of the source of infection is an important factor in reducing the prevalence of AD (Aznar et al., 2022). Many

countries had successfully reduced the prevalence of AD through vaccination (Stegeman et al., 1997; J. Stegeman et al., 1994), and it can be clearly observed from the results of the study that AD has been well controlled in Peninsular Malaysia.

3.5 Conclusions

In summary, from the overall results, the infection rate of AD field strains is less than 19% in our study from 2019 to 2021. The seroprevalence was 2.62% in 2019, 0.53% in 2020, and 1.45% in 2021. In addition, the infection rate of AD is related to the region. ADV field exposure exhibits differences across the different regions in Peninsular Malaysia. In Johor region, the infection rate of farms was the highest, followed by that of farms in Selangor & Malacca region. The farm infection rate in Penang region is lower than Johor region and Selangor & Malacca region. Farms were not infected with the AD field strain in Perak region. In this study, the number of farms submitting AD serological diagnosis is the least in the Johor region, so the infection rate of farms in this region cannot represent the entire region, but can only represent the infection rate of a certain farm. Meanwhile, we found that the infection rate of AD was correlated with the age of pigs. AD primarily targets piglets (1-6 weeks of age) and breeder (gilts and sows), so pigs at these ages are usually associated with higher exposure to AD field strains. Furthermore, ADV infection was found to be related to farm size, housing type, and biosecurity measures. Small herd size farms have the highest proportion of infected farms, whereas infected farms are rare among medium and large herd size farms. Open farms are more susceptible to challenges from AD field strains than farms with a

combination of open and closed houses and farms with closed houses. Farms that adopt biosecurity measures such as vehicle dip, isolation and holding room, foot dip, and vaccination can reduce the spread of AD. Vaccination can reduce the prevalence of AD on farms to a great extent.

Although field strains of AD were detected in collected samples by serological diagnosis, especially in Perak region where there was severe infection, there have been no reports of AD outbreaks in any region or farm in Peninsular Malaysia in recent years. This may indicate that there is still AD field exposure, but it did not cause threat and challenge to the swine industry. Therefore, it also reflects that farmers have taken a good control and prevention measure against the disease, including effective vaccination, good feeding management, and strict implementation of various biosecurity measures.

CHAPTER 4

SEROLOGICAL STATUS OF CLASSICAL SWINE FEVER

4.1 Introduction

4.1.1 Overview

Despite being a long-standing illness that first appeared more than 200 years ago, CSF continues to pose a threat to the pig industry in most of the world, especially in the South American and Asian countries where it is sporadic or occasional (Ganges et al., 2020; Postel et al., 2018).

Different countries have different strategies for controlling CSF. For most CSF free countries, such as the United States, Brazil, Canada, and European Union countries. The strategy to control CSF is quarantine and cull infected herds. However, in endemic infected areas in Asia, the Americas, Eastern Europe, and some African countries, the disease is usually controlled through preventive vaccination (Blome et al., 2017; Paton & Greiser-Wilke, 2003). In Malaysia, it has implemented a mandatory vaccine immunization strategy for CSF for many years, but due to constraints from many factors, CSF still cannot be eradicated.

Since 1975, scheduled vaccination with live attenuated vaccines has been used to manage the CSFV in Malaysia, which has resulted in a decline in the prevalence of CSF (Too, 1997a). Although there have been no reports of large-scale outbreaks of CSF in our country in recent years, the potential etiology of CSFV still exists.

Furthermore, there are very few studies and reports on the serological status of CSF in the pig industry in Peninsular Malaysia, and the most recent survey on CSF status was in 2018. Therefore, in order to promote the elimination of CSF in our country, it is necessary not only to regularly vaccinate, but also to carry out serological monitoring of CSFV and improve the biosecurity of pig farms. To this end, the focus of this study is to provide an up-to-date serological status of CSF and level of CSF protection in pig farms in Peninsular Malaysia between 2019 and 2021 because this period is the beginning and end of my research.

4.1.2 Objectives

The objectives of this study are:

i. To determine the serological status of CSF in pig farms in Peninsular Malaysia using commercial samples submitted to UPM between 2019 and 2021.

ii. To investigate the level of CSF protection in pig farms in Peninsular Malaysia using commercial samples submitted to UPM between 2019 and 2021.

4.2 Materials and Methods

Similar to AD, the methodological framework of CSF is shown in Figure 3.1.

4.2.1 Sample Collection

The sample collection for CSF is similar to that for AD (Section 3.2.1). The farm requires that serum samples be tested for CSFV by ELISA.

4.2.2 Region Categorization

The regional classification of CSF and AD is similar, which is also based on the number and geographical location of farms, Section 3.2.2 can be referred to.

4.2.3 Using Enzyme-Linked Immunosorbent Assay (ELISA) to Detect CSF Antibody

Classical swine fever virus (CSFV) was detected by Classical Swine Fever Ab ELISA Test Kit (IDEXX Laboratories, Inc., US). ELISA is performed according to standard procedures provided directly by the kit manufacturer. Microplates coated with CSFV antigen are used in a blocking ELISA test. The blocking percentage value is a key indicator for evaluating the level of antibodies against CSFV in serum samples.

Before use, all reagents reached a temperature 18-26 °C (room temperature). By lightly turning or shaking, the reagents were combined. 50µl of the sample diluent was given to each test well and each control well. 50µl of the positive control and negative control were dispense into the designated well. Then, 50µl of the swine serum sample was added to the rest of the wells. Lightly shook the microplate to mixed the solution in the wells.

Microplate was sealed and incubated for 2 hours at 18-26°C (room temperature). The solution in the microplate was removed and each well was washed 3 times with 300µl of washing solution with a Filter Microplate Washer (Bio Tek ELx50TM, US). Patted the microplate on absorbent paper to remove

residual wash solution. Then, 100µl of the conjugate was added to each well. Microplate was sealed and incubated at 18-26°C (room temperature) for 30 minutes. After incubation, the microplate was washed again 3 times. 100µl of TMB substrate was dispensed into each well and third incubation was performed for 10 minutes at 18-26°C (room temperature) in a dark place. Finally, 100µl of stop solution was added to each well. Using an absorbance microplate reader (Biotek ELx808TM, US), the color change was measured according to the of optical density at 450 nm.

The blocking percentage value was obtained using IDEXX XChek software (Version 4.1.1.14, Inc., US). The blocking percentage of the serum sample was calculated from the optical density (OD450) of the negative control and the OD450 of the test sample, and in the absence of specific antibodies, the maximum color rendering effect was a negative result. Negative, suspected, and positive results for pig serum samples were distinguished according to the instructions provided by the manufacturer. By comparing the computed blocking percentage value to the standard advised by the test kit, as stated in Table 4.1 below, the serological status of samples is ascertained.

Table 4.1 : CSF ELISA test kit interpretation

CSF Serological Status		
Blocking% ≤ 30	**30 < Blocking% < 40**	**Blocking% ≥ 40**
Negative	Suspect	Positive

4.2.4 Data Analysis

There is a prevalence of CSF in Malaysia and there is no official farm classification when using the IDEXX ELISA for diagnostic testing of CSF. Through communication with IDEXX field experts, the data analysis was classified into two levels in this study: one is age group level and the other is farm level.

4.2.4.1 CSF Status Classification of Age Group Level

Through communication and guidance with IDEXX field experts, according to the percentage of positive, suspected and negative serology detected in samples from each age group, the status of CSF is divided into 3 categories, namely "Good", "Moderate" or "Poor", it is shown in Table 4.2.

Table 4.2 : Classification criteria for age group CSF status

Percentage of Positive Samples	Percentage of Suspected Samples	Percentage of Negative Samples	Classification
100%	0%	0%	Good
Less than 80%	Less than 20%	0%	Good
	0%	Less than 20%	Good
Less than 60%	Less than 40%	0%	Good
	Less than 20%	Less than 20%	Moderate
	0%	Less than 40%	Moderate
Less than 40%	Less than 60%	0%	Moderate
	Less than 40%	Less than 20%	Moderate
	Less than20%	Less than 40%	Poor
	0%	Less than 60%	Poor
Less than 20%	Less than80%	0%	Moderate
	Less than 60%	Less than 20%	Moderate
	Less than 40%	Less than 40%	Poor
	Less than 20%	Less than 60%	Poor
	0%	Less than 80%	Poor
0%	100%	0%	Moderate
	Less than80%	Less than 20%	Moderate
	Less than 60%	Less than 40%	Moderate
	Less than 40%	Less than 60%	Poor
	Less than 20%	Less than 80%	Poor
	0%	100%	Poor

4.2.4.2 CSF Status Classification of Farm Level

Referring to Table 4.3, the farm status is classified according to the criteria suggested by IDEXX. The CSF status of the farms was classified into three categories, 'Ideal', 'Moderate' or 'Less Ideal', according to the overall level of farm in each age group. Table 4.4 provides an illustration of the classification system, which can be used as an example.

Table 4.3 : Classification criteria for farm CSF status

Farm Status	Criteria
Ideal	A farm has more than or equal to 75% of serum samples that are seropositive. For weaner to finisher stage, 1-6 weeks old and 13-20 weeks old could be either good, moderate or poor, but 7-12 weeks old must be in category of good. Age group categorization of gilts could be in the category of good, moderate, or poor, but age group categorization of sows must be good.
Moderate	A farm has less than 75% of serum samples that are seropositive. For weaner to finisher stage, 1-6 weeks old and 13-20 weeks old could be either good, moderate or poor, but 7-12 weeks old must be in category of good. Age group categorization of gilts is in the category of moderate, or poor, but age group categorization of sows is good.
Less Ideal	A farm has less than 75% of serum samples that are seropositive. Age group that does not meet the criteria for any of the other categories will be classified into this category by default.

Table 4.4 : Example of farm CSF status classification

ID	Serological status					Sero-positivity (%)	Grade
	1-6w	7-12w	13-20w	Gilts	Sows		
P14	Good	Good	Poor	Good	Good	85.00	Ideal
P07	Moderate	Good	Good	Good	Good	74.29	Moderate
P03	Moderate	Poor	Poor	Good	Good	57.14	Less Ideal

4.2.5 Farm Questionnaire Survey

The farm questionnaire survey of CSF and AD is similar. Section 3.2.5 can be referred to.

4.2.6 Statistical Analysis

For assistance with statistical analysis, IBM® SPSS Statistics 28 was utilized.

Descriptive Analysis is used to determine the reliability and accuracy of the farm status classification system, which is based on the farm classification criteria established with the suggestions of technical experts in the field of IDEXX.

Multinomial Logistic Regression was used to carry out co-relationship analysis to determine whether the herd type, age group, or vaccination frequency is related to the seroprevalence. Additionally, in order to compare the differences between the seroprevalence results from age group and vaccination brand, Kruskal Wallis statistical analysis was utilized because seroprevalence is qualitative data that is non-parametric.

Moreover, the connection between age groups in terms of their CSF status was analyzed using a Bivariate Correlations test. Furthermore, analyze the impact of various risk factors on seroprevalence by using Linear Regression.

Finally, $p < 0.05$ and 95% confidence interval were used to determine statistical significance for each test that was performed.

4.3 Results

4.3.1 Statistical Analysis of Farm Status Classification System

Descriptive analysis is used to determine whether a farm status classification system based on farm classification criteria is valuable and correct. Farm status was classified as "Ideal", "Moderate", "Less Ideal" according to the CSF status of each age group, coefficient of variation (CV%), and seropositivity of each farm.

The result is shown in Table 4.5. It can be seen that when we use the above classification methods, the farm classification system developed is effective, and the accuracy of each farm being classified into the correct category is up to 98.5%.

Table 4.5 : Summary of the CSF farm status classification system by category based on IBM® SPSS Discriminant Analysis

Classification Results [a,c]						
Grade		Status	Predicted Group Membership			Total
			Ideal	Moderate	Less Ideal	
Original	Count	Ideal	8	0	0	8
		Moderate	0	1	0	1
		Less Ideal	1	0	55	56
	%	Ideal	100.0	.0	.0	100.0
		Moderate	.0	100.0	.0	100.0
		Less Ideal	1.8	.0	98.2	100.0
Cross-validated[b]	Count	Ideal	8	0	0	8
		Moderate	1	0	0	1
		Less Ideal	0	1	55	56
	%	Ideal	100.0	.0	.0	100.0
		Moderate	100.0	.0	.0	100.0
		Less Ideal	.0	1.8	98.2	100.0

Note:
a. 98.5% of original grouped cases correctly classified.
b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
c. 96.9% of cross-validated grouped cases correctly classified.

4.3.2 Overall Farm and Samples Received

In the ELISA test on CSF in 2019–2021, a total number of 3127 serum samples were collected from 65 farms. 13 farms overlapped in 2019-2021. For these samples, a total of 979 samples were collected from 20 farms in 2019, a total of 1131 samples were collected from 24 farms in 2020, and 1017 samples were collected from 21 farms in 2021. Details on the number of samples and farms submitted to FVM, UPM from different regions can be seen in Table 4.6.

Table 4.6 : Number of farms and serum samples submitted to FVM, UPM for CSF ELISA testing in each year in 2019-2021

Region	Number of Farms	Number of Samples
	2019	
Perak	11	646
Johor	2	73
Penang	2	85
Selangor & Malacca	5	175
Total	20	979
	2020	
Perak	14	604
Johor	1	39
Penang	6	216
Selangor & Malacca	3	272
Total	24	1131
	2021	
Perak	11	494
Johor	6	354
Penang	2	78
Selangor & Malacca	2	91
Total	21	1017
Grand Total	65	3127

4.3.3 Overall Ideal Farm Status and CSF Seroprevalence

In 2019, 10% of farms were in ideal status and 63.23% of samples were seropositive. There were 16.67% of farms with ideal status and 61.45% of samples were seropositive in 2020, and farms in ideal status is the most in that year. The proportion of farms with ideal status was 9.52%, and the seropositive samples were 61.55% in 2021. From the overall data, 62.04% of swine serum samples were detected with seropositive of CSF, but only 12.31% of farms in ideal status between 2019 to 2021, as shown in Table 4.7.

Table 4.7 : Summary of ideal farm status and seroprevalence of CSF in each year in 2019-2021

Year	Ideal Farm Status		Seropositive Samples	
	Quantity	Percentage	Quantity	Percentage
2019	2 (20)	10.00%	619 (979)	63.23%
2020	4 (24)	16.67%	695 (1131)	61.45%
2021	2 (21)	9.52%	626 (1017)	61.55%
Overall	8 (65)	12.31%	1940 (3127)	62.04%

Note: The value inside the brackets represents the number of farms or samples, whereas the value outside the brackets represents the number of ideal farms or seropositive samples.

4.3.4 CSF Seroprevalence and Farm Status across Region

The overall data on ideal farms and seropositive samples in each region in 2019-2021, and it is shown in Table 4.8. Overall, a total of 65 farms submitted serum samples for the CSF ELISA test, of which 8 farms (12.31%) were classified as ideal farm status, and a total of 3127 serum samples were submitted, of which 1940 samples (62.04%) were seropositive.

The Perak region has the largest number of farms and samples. A total of 36 farms submitted serum samples, of which 5 farms (13.89%) were classified as ideal farm status, and a total of 1744 serum samples were submitted, of which 1037 samples (61.53%) were seropositive in the Perak region during the three years period. A total of 466 serum samples came from 9 farms in Johor region, of which 2 farms (22.22%) were ideal farm status and 319 samples (68.45%) were seropositive, and the seroprevalence was higher than other regions. For the Penang region, no farms were classified as ideal farm status, Although the number of serum samples was the smallest with only 378 serum samples, 233 samples were seropositive and the seroprevalence was 61.48% in the region. The seroprevalence was almost similar in Perak (61.53%) region and Penang (61.48%) region. Only one farm (10.00%) was in ideal farm status, and a total of 538 serum samples, of which 315 samples (58.55%) were detected seropositive in the Selangor & Malacca region.

General data on seroprevalence of CSF in different regions (Figure 4.1).

Table 4.8 : Summary of ideal farm status and seroprevalence of CSF in each region in 2019-2021

Year	Ideal farm Status		Seropositive samples	
	Quantity	Percentage	Quantity	Percentage
	Perak			
2019	1(11)	9.09%	393(646)	60.84%
2020	3(14)	21.43%	381(604)	63.08%
2021	1(11)	9.09%	299(494)	60.53%
Overall	5(36)	13.89%	1073(1744)	61.53%
	Johor			
2019	0(2)	0.00%	46(73)	63.01%
2020	1(1)	100.00%	39(39)	100.00%
2021	1(6)	16.67%	234(354)	66.10%
Overall	2(9)	22.22%	319(466)	68.45%
	Penang			
2019	0(2)	0.00%	57(85)	67.06%
2020	0(6)	0.00%	137(216)	63.43%
2021	0(2)	0.00%	39(78)	50.00%
Overall	0(10)	0.00%	233(379)	61.48%
	Selangor & Malacca			
2019	1(5)	20.00%	123(175)	70.29%
2020	0(3)	0.00%	138(272)	50.74%
2021	0(2)	0.00%	54(91)	59.34%
Overall	1(10)	10.00%	315(538)	58.55%
Grand Total	8(65)	12.31%	1940(3127)	62.04%

Note: The value inside the brackets represents the number of farms or samples, whereas the value outside the brackets represents the number of ideal farms or seropositive samples.

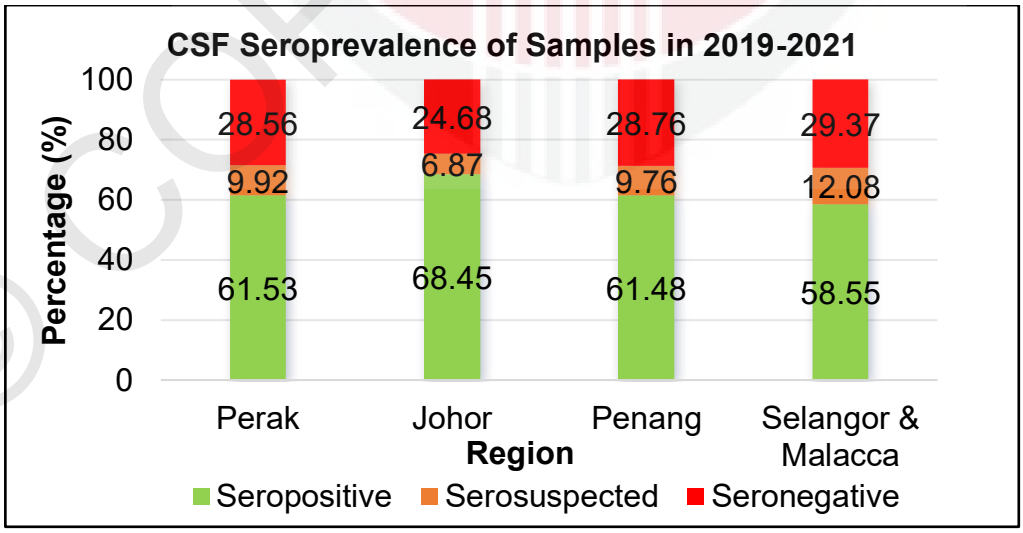

Figure 4.1 : Overall seroprevalence of CSF in different regions in 2019-2021

From the overall results, the CSF status was not ideal in Penang region, and the farm CSF status was slightly better in Johor region. This result may be due to the small number of farms and samples, especially since only one farm submitted serum samples in the Johor region in 2020, the result did not represent the whole region due the samples submitted were limited. For the Penang region, the CSF status of farms has been poor and has not improved from 2019 to 2021. For the Perak region, 9%, 21%, and 9% of the farms were classified as ideal farm status in 2019, 2020, and 2021 respectively. Farm CSF status improved in 2020, but it dropped again in 2021 to the same level as in 2019. In Selangor & Malacca, the CSF status of the farm was good in 2019, but the CSF status of the farm was less ideal in the following two years. As shown in Figure 4.2 & 4.3.

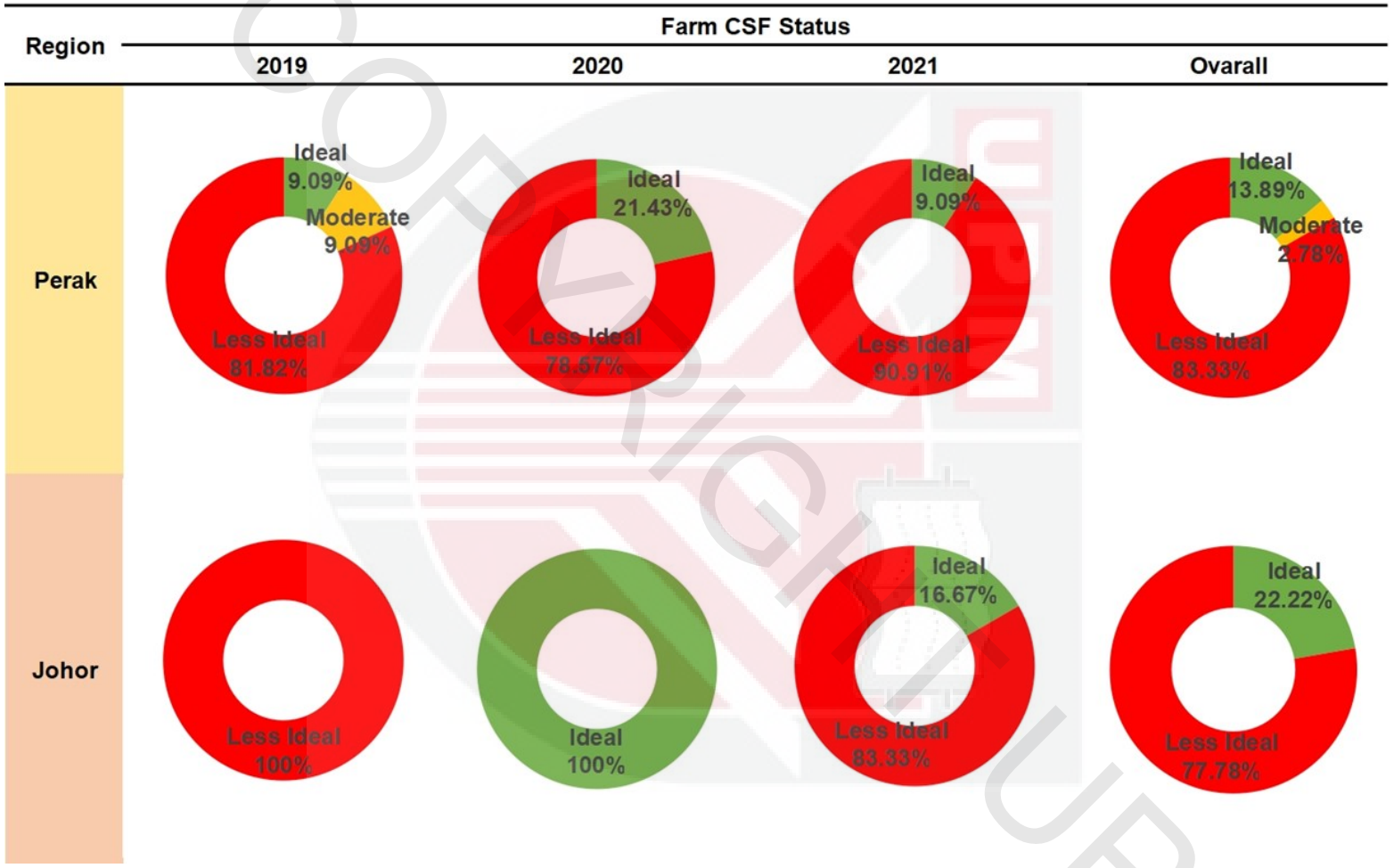

Figure 4.2 : Summary of farm CSF status in different regions based on serum samples submitted to FVM, UPM in 2019-2021

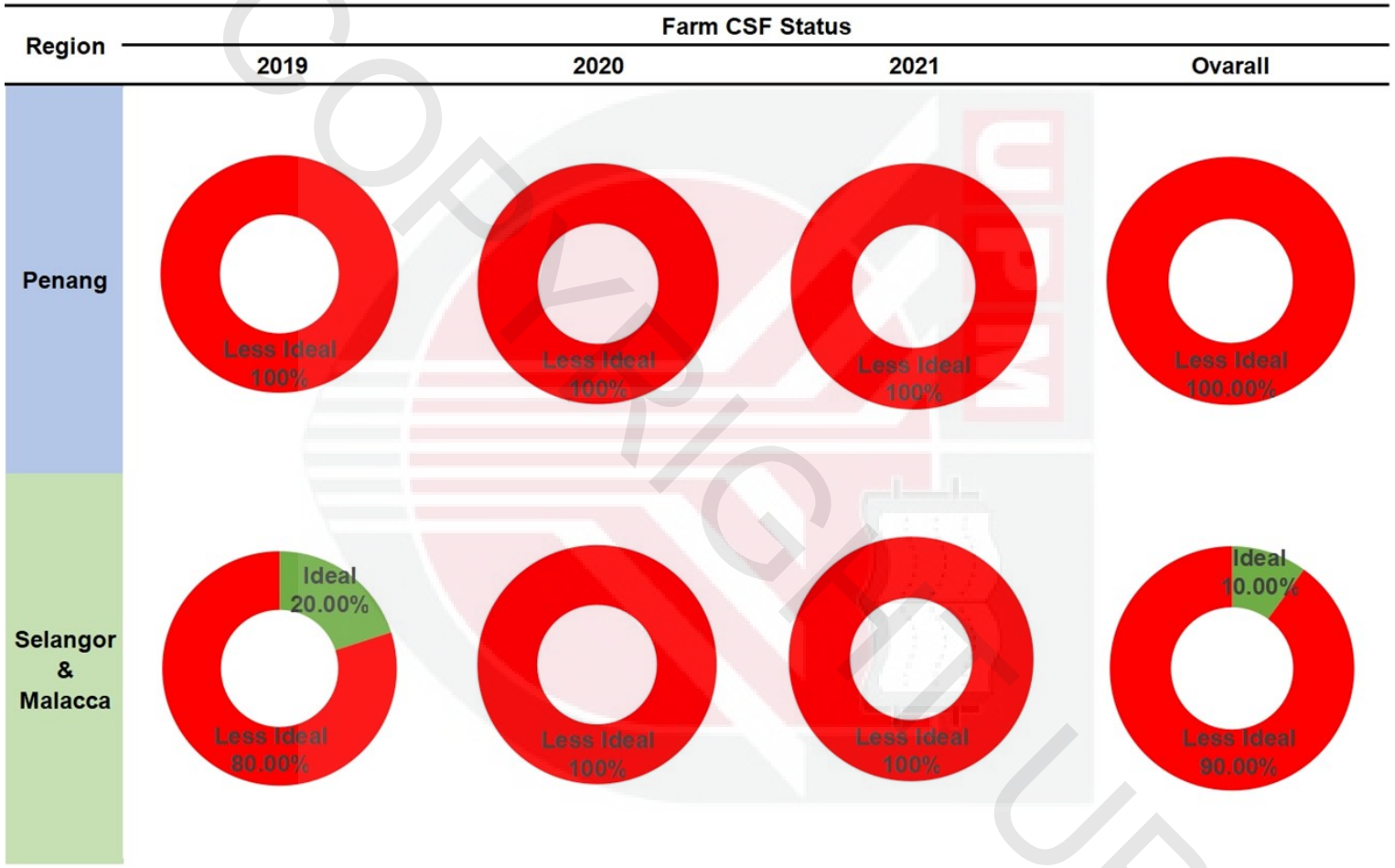

Figure 4.3 : Summary of farm CSF status in different regions based on serum samples submitted to FVM, UPM in 2019-2021

4.3.5 CSF Seroprevalence across Herd Size

A total of 65 farms submitted serum samples for CSF ELISA test in 2019-2021, but only 40 farms provided information on herd size. Therefore, only these samples farms were included in this study. Farm herd sizes are divided into 3 sizes based on the number of sows in each farm, namely: small, medium and large. Farms with less than 500 head of sows are classified as small herd sizes, farms with between 500 and 1,000 head of sows are classified as medium herd sizes, and farms with more than 1,000 head of sows are classified as medium herd sizes are classified as large herd sizes. Table 4.9 is detailed data compiled based on information provided by farms. The number of small herd size farms is only 7 and it is the smallest. The number of medium herd size farms is the largest, and it accounts for 50% of all farms. 13 farms are classified as large herd size.

Table 4.9 : Summary of the number of farms based on herd size in 2019-2021

Herd Size	Number of Sow (head)	Number of Farms	Percentage (%)
Small	<500	7	17.50
Medium	500-1000	20	50.00
Large	>1000	13	32.50
Total		40	

The overall seroprevalence of CSF in each region across different herd sizes is shown in Figure 4.4. The majority of farms in Johor and Penang region are small herd size farms. Farms in the Perak region is generally medium or large herd size farms.

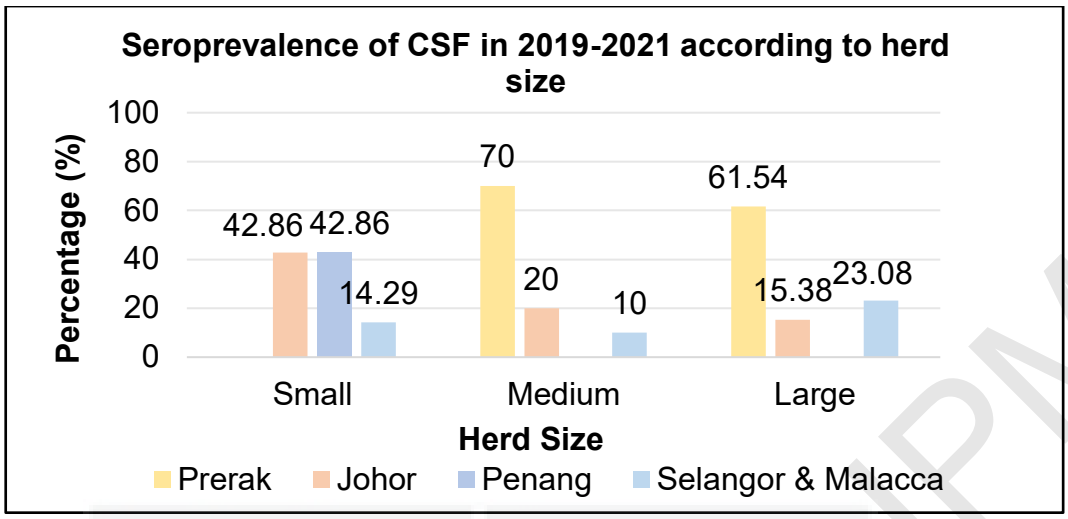

Figure 4.4 : Overall seroprevalence of CSF in different herd sizes in 2019-2021

Farm CSF status and seroprevalence of samples are shown in different herd sizes (Figure 4.5). It was observed that seroprevalence of samples was highest on farms with small herd sizes, but only 14% on farms with ideal status. Among farms with medium herd sizes, farms with ideal status were the largest with 58% seroprevalence in the sample. 14% of farms were in ideal status and 65% of samples were seropositive in large herd size farms.

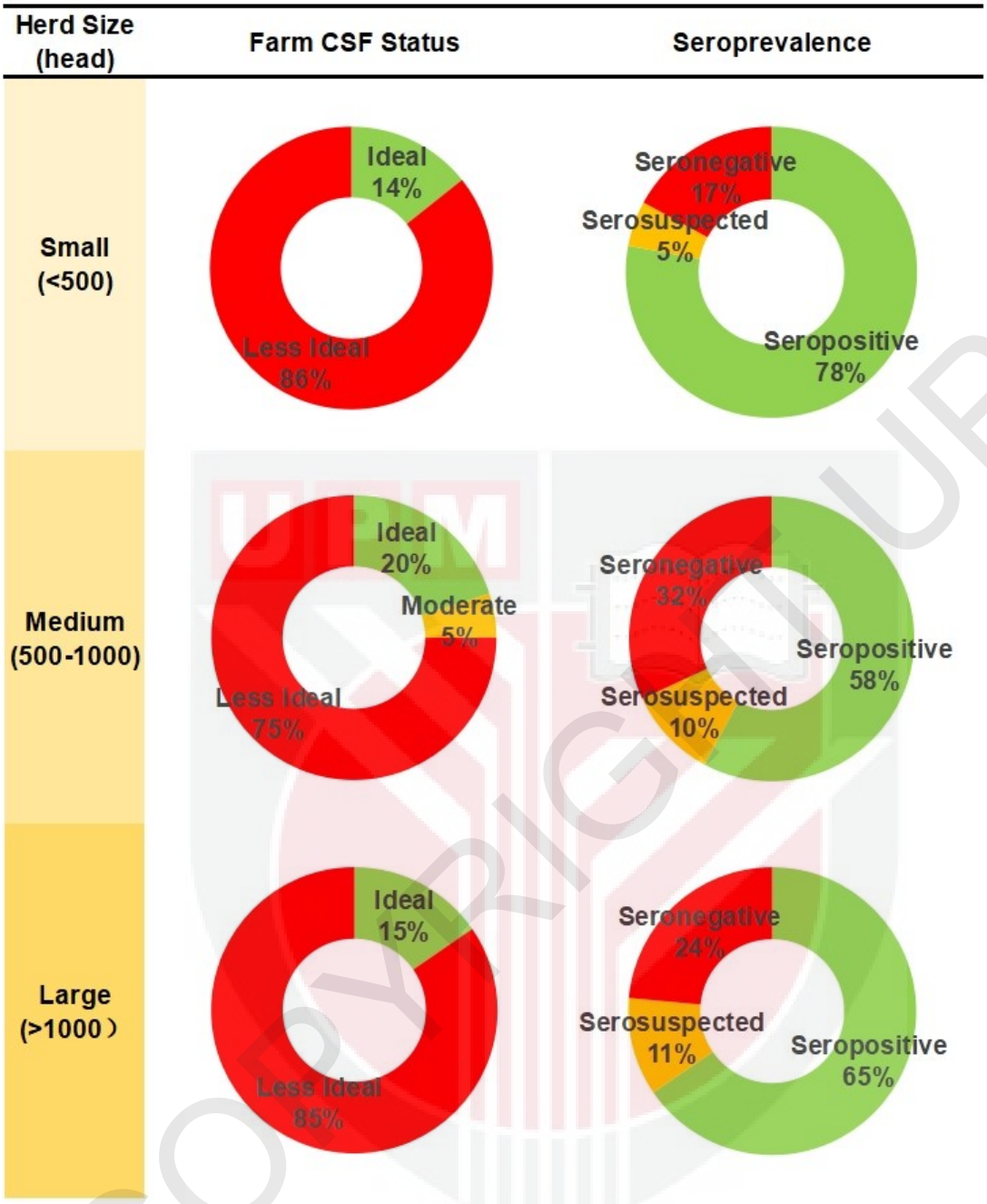

Figure 4.5 : Summary of farm CSF status and seroprevalence of serum samples in different herd sizes in 2019-2021

4.3.6 CSF Seroprevalence across Each Age Group

Overall seroprevalence of CSF in different age groups in 2019-2021 (Figure 4.6). From the overall study, it can be seen that the seroprevalence of CSF is lowest in 7-12 weeks old and the seroprevalence of CSF is highest in sows.

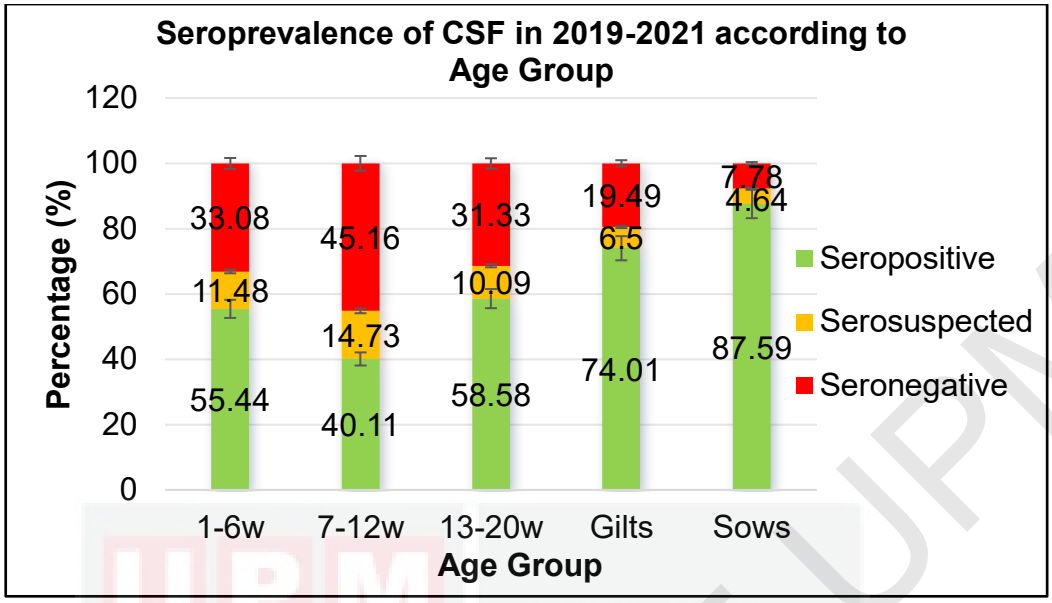

Figure 4.6 : Overall seroprevalence of CSF in different age groups in 2019-2021

The association between different age groups and seropositivity of CSF in 2019-2021 was shown in Table 4.10. A significant association was observed between sows and seronegative.

Table 4.10 : Summary of seroprevalence among different age groups based on IBM® SPSS Multinomial Logistic Regression in 2019-2021

Seroprevalence of CSF [a]		B	Std. Error	Sig.	Exp(B)
Sero-suspected	Intercept	165.117	.000	.	
	1_6wks	-3.935	2342.432	.999	.020
	7_12wks	-1.944	2936.366	.999	.143
	13_20wks	.644	7995.092	1.000	1.903
	Gilts	.500	8304.575	1.000	1.650
	Sows	-1.861	1894.788	.999	.156
Seronegative	Intercept	5.186	3.951	.189	
	1_6wks	-.029	.037	.433	.972
	7_12wks	.071	.040	.075	1.074
	13_20wks	-.066	.044	.132	.936
	Gilts	-.004	.044	.925	.996
	Sows	-.104	.047	.027	.901

Note:
a. The reference category is: Seropositive
Yellow highlight indicates significant association.

Table 4.11 is a summary of the differences among different age groups. It was observed from the result that there are different between each age group. Significant differences were found between the 7–12 weeks old pigs and the other age groups of pigs. This finding can determine that there is a statistically significant difference in seroprevalence between 7–12 weeks old pigs and other groups. For gilts, significant differences also exist between them and other age groups except pigs aged 13-20 weeks and sows. However, there was no significant difference between pigs aged 1-6 weeks and pigs aged 13–20 weeks. In pairwise comparison of seroprevalence among different age groups, as shown in Figure 4.7, the average ranking of the seroprevalence in sows is the highest, and pigs aged 7-13 week is the lowest among all age groups. This indicates that the seroprevalence in sows contains larger values compared to other groups.

Table 4.11 : Summary of seroprevalence among different age groups based on IBM® SPSS Independent Samples-Kruskal Wallis Statistical Analysis in 2019-2021

Dependent Variable	Age Group		Significance	Adjusted Significance
Seroprevalence	1-6 weeks	13-20 weeks	.725	1.000
		Gilts	.002	.023
		Sows	.000	.000
	7-12 week	1-6 weeks	.001	.010
		13-20 weeks	.000	.003
		Gilts	.000	.000
		Sows	.000	.000
	13-20 weeks	Gilts	.007	.068
		Sows	.000	.000
	Gilts	Sows	.031	.310

Note: Yellow highlight indicates significant difference.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

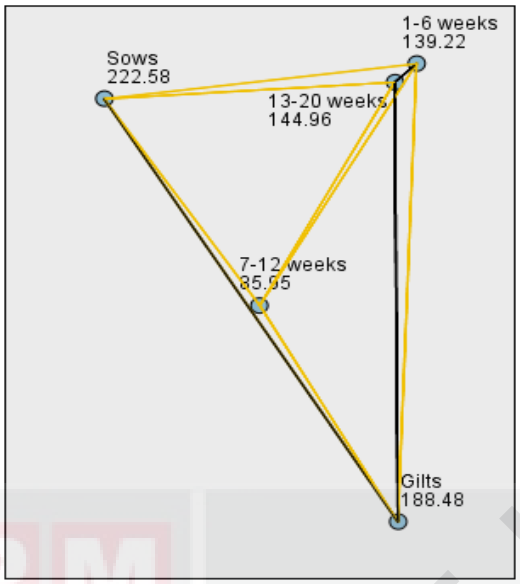

Figure 4.7 : Summary of seroprevalence in different age group based on IBM® SPSS Pairwise Comparison of Independent Samples- Kruskal Wallis Statistical Analysis in 2019-2021
Note: Each node shows the sample average rank of Age.
Yellow line indicates significant difference; black line indicates non-significant.

The interaction between seroprevalence rates in different age groups were studied through correlation analysis between different age groups (Table 4.12). From the data analysis, it can be seen that the significance (p-value) is < 0.05 between the age groups, which shows that the correlation is statistically significant between the age groups, and CSF status was affected in different age groups each other.

Table 4.12 : Summary of the correlation between different age groups based on IBM® SPSS Bivariate Correlations of Spearman's rho Statistical Analysis in 2019-2021

Correlations							
			1-6 weeks	7-12 weeks	13-20 weeks	Gilts	Sows
Spearman's rho	1 - 6 weeks	Correlation Coefficient	1.000	.326**	.307**	.295**	.422**
		Sig. (2-tailed)	.	.000	.001	.004	.000
		N	143	129	117	93	102
	7 -12 weeks	Correlation Coefficient	.326**	1.000	.550**	.283**	.207*
		Sig. (2-tailed)	.000	.	.000	.003	.026
		N	129	171	132	106	115
	13 - 20 weeks	Correlation Coefficient	.307**	.550**	1.000	.414**	.479**
		Sig. (2-tailed)	.001	.000	.	.000	.000
		N	117	132	143	94	102
	Gilts	Correlation Coefficient	.295**	.283**	.414**	1.000	.651**
		Sig. (2-tailed)	.004	.003	.000	.	.000
		N	93	106	94	115	99
	Sows	Correlation Coefficient	.422**	.207*	.479**	.651**	1.000
		Sig. (2-tailed)	.000	.026	.000	.000	.
		N	102	115	102	99	125

Note: **. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
Yellow highlight indicates significant difference.

Overall seroprevalence of CSF in different age groups in 2019-2021 (Figure 4.8 & 4.9). In pigs aged 1-6 weeks, the seroprevalence was 61.15% in 2019, but the protective status of CSF decreased in 2020, and the seroprevalence reached around 60.29% in 2021. In pigs aged 7-12 weeks, the seroprevalence of CSF has been very low in 2019-2021, only about 40%. In pigs aged 13-20 weeks, seroprevalence remained around 60% in 2019 and 2020, but protection status slipped in 2021. For gilts, the protective status is good, with seroprevalence around 70% in 2019 and over 75% in 2020 and 2021. In sows, CSF protection level was the best, and seroprevalence was also the highest

at approximately 90% during these three years.

The seropositivity and the number of samples in each age group in different regions in 2019-2021, it can be seen in Table 4.13.

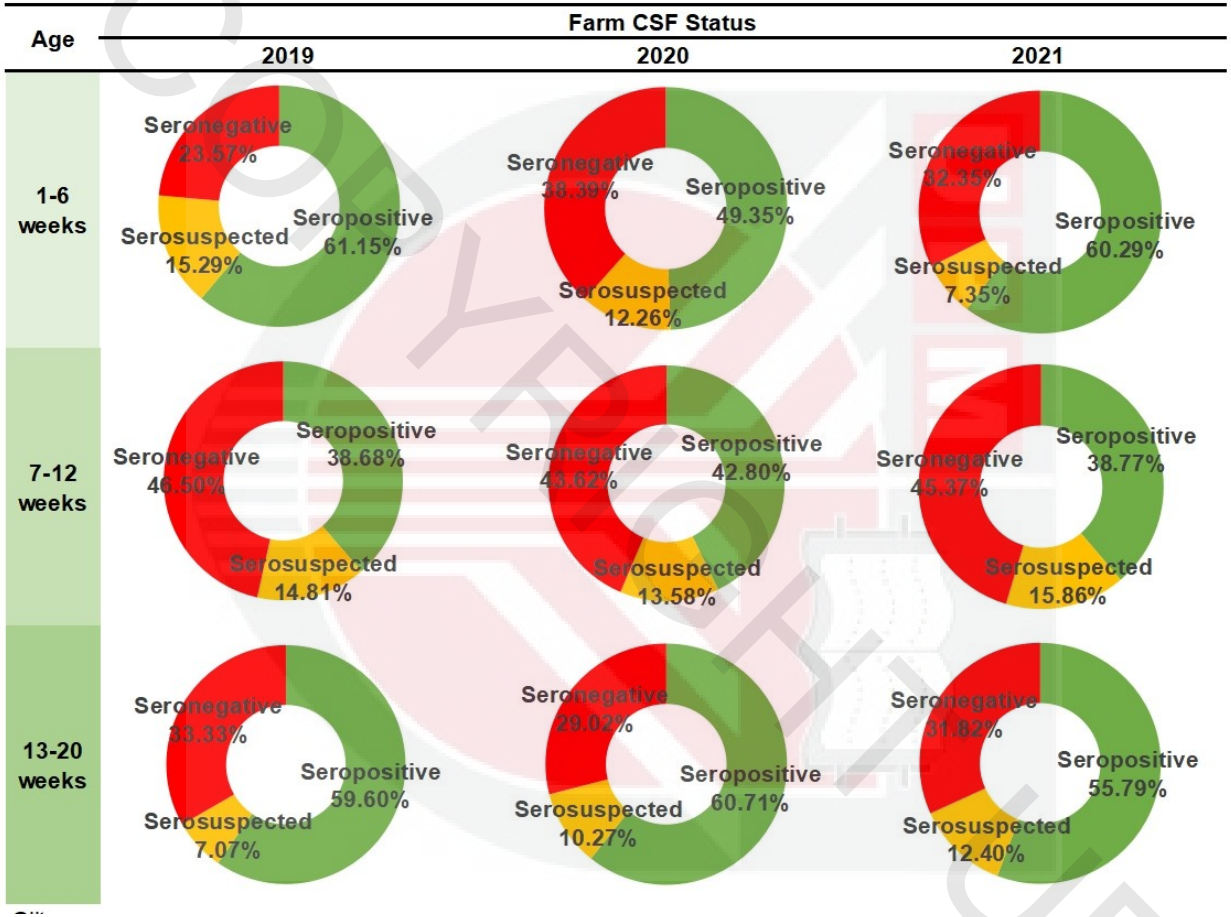

Figure 4.8 : Summary of farm CSF status in different age groups based on swine serum samples submitted to FVM, UPM for CSF LEISA test in 2019-2021

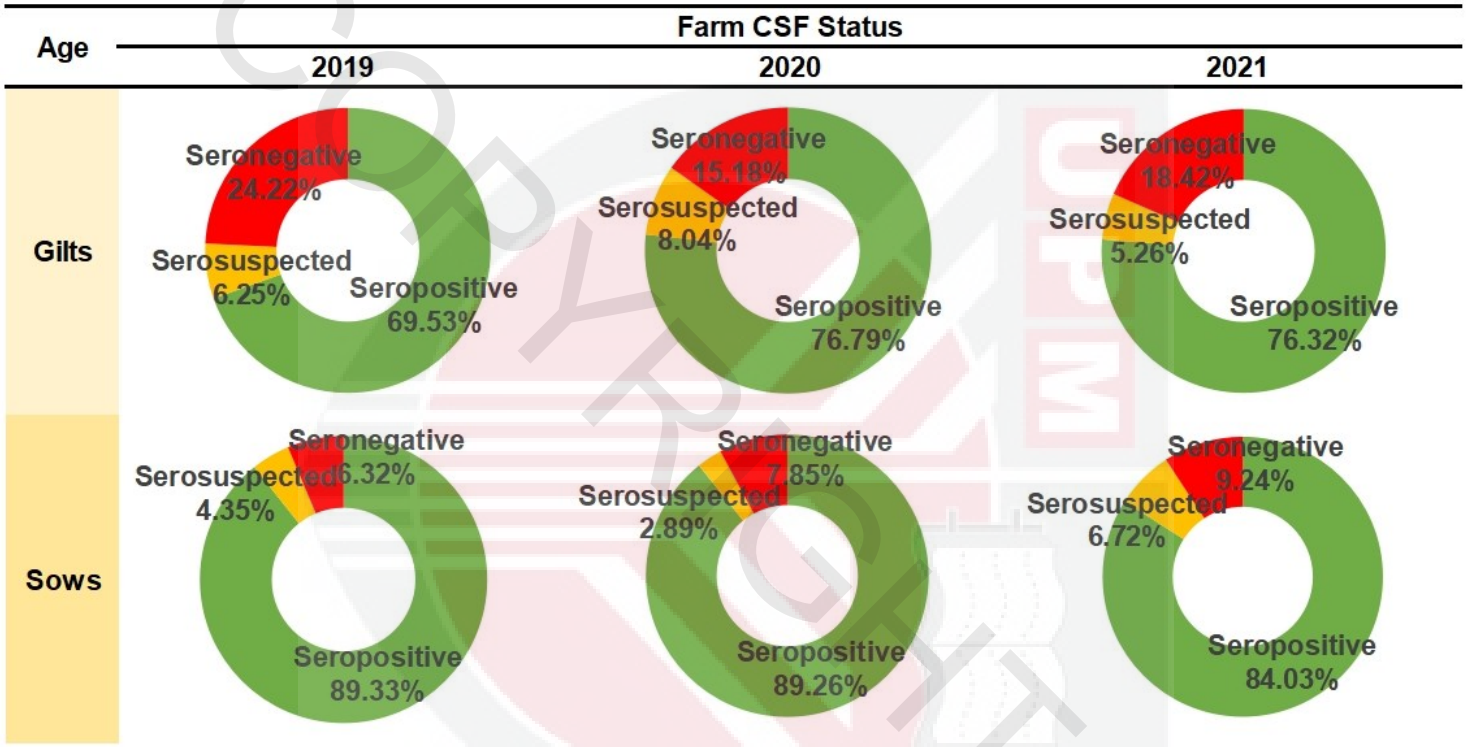

Figure 4.9 : Summary of farm CSF status in different age groups based on swine serum samples submitted to FVM, UPM for CSF LEISA test in 2019-2021

Table 4.13 : Summary of seropositive samples of CSF in different age groups in 2019-2021

Region	Seropositivity (%)				
	1-6w	7-12w	13-20w	Gilts	Sows
			2019		
Perak	51.16	39.02	55.32	73.49	87.79
	44/86	64/164	78/141	61/83	151/172
Johor	45.45	35.00	63.64	63.64	100.00
	5/11	7/20	7/11	7/11	20/20
Penang	52.63	38.89	66.67	90.00	95.00
	10/19	7/18	12/18	9/10	19/20
Selangor & Malacca	90.24	39.02	75.00	50.00	87.80
	37/41	16/41	21/28	12/24	36/41
Total	61.15	38.68	59.60	69.53	89.33
	96/157	94/243	118/198	89/128	226/253
			2020		
Perak	57.76	46.97	47.76	79.10	87.10
	67/116	62/132	64/134	53/67	135/155
Johor	100.00	100.00	100.00	100.00	100.00
	5/5	5/5	10/10	5/5	14/14
Penang	51.92	44.90	77.08	61.54	85.37
	27/52	22/49	37/48	16/26	35/41
Selangor & Malacca	39.42	26.32	78.13	85.71	100.00
	54/137	15/57	25/32	12/14	32/32
Total	49.35	42.80	60.71	76.79	89.26
	153/310	104/243	136/224	86/112	216/242
			2021		
Perak	53.85	34.23	59.82	87.72	79.09
	56/104	38/111	67/112	50/57	87/110
Johor	81.69	40.79	49.38	65.00	91.86
	58/71	31/76	40/81	26/40	79/86
Penang	50.00	37.50	35.29	50.00	71.43
	8/16	6/16	6/17	4/8	15/21
Selangor & Malacca	7.69	54.17	58.33	77.78	90.48
	1/13	13/24	14/24	7/9	19/21
Total	60.29	38.77	54.27	76.32	84.03
	123/204	88/227	127/234	87/114	200/238
Grand total	55.44	40.11	58.08	74.01	87.59
	372/671	286/713	381/656	262/354	642/733

Note: Number of seropositive samples / Total number of samples.

4.3.7 CSF Seroprevalence across Herd Type

According to different age groups in the sample, the herds are divided into two different types: porker herd and breeder herd. Pigs in the age groups of 1-6 weeks, 7-12 weeks, and 13-20 weeks are classified as porker herds, and gilts and sows are classified as breeder herds. It can be seen from Table 4.14 that it is a significant association between the breeder herd and sero-suspected. This indicates that for every 1 unit increase in breeder herd, the number of sero-suspected of samples will decrease by 3.985.

Table 4.14 : Summary of the seroprevalence of porker and breeder herd based on IBM® SPSS Multinomial Logistic Regression in 2019-2021

Seroprevalence of CSF [a]		B	Std. Error	Sig.	Exp(B)
Sero-suspected	Intercept	133.778	4459.355	.976	
	Porker	1.217	101.169	.990	3.377
	Breeder	-3.985	.031	.000	.019
Seronegative	Intercept	130.847	4459.355	.977	
	Porker	1.350	101.169	.989	3.859
	Breeder	-3.954	.000	.	.019

Note:
a. The reference category is: Seropositive
Yellow highlight indicates significant association.

4.3.8 CSF Seroprevalence across Vaccination Protocol and Various Risk Factors

The results obtained through the questionnaire survey showed that there are different vaccination regimens against CSF, and different brands of vaccines and different frequencies of injections were used in Peninsular Malaysia, especially in porker herds. A summary of seropositivity in different vaccination frequencies of different vaccine brands in porker herd is presented in Table 4.15. It can be seen that the seroprevalence of vaccine brand C is the highest,

which indicates that vaccine brand C provides better protection in the farm. Among the mean seroprevalence of different vaccine brands (Table 4.16 & Figure 4.10), and the mean value of vaccine brand C is the highest, which indicates that the farm using vaccine brand C has a higher overall seroprevalence.

Table 4.15 : Summary of number of farms and seroprevalence of swine serum samples based on different vaccine brands and injection frequencies in 2019-2021

Vaccine	Number of farms	Overall Seropositivity	Injections (Number of farm)	Seropositivity in Porker Herd
A	27	59%	Farms with no data× (6)	57%
			1× (9)	45%
			2× (12)	44%
B	4	68%	Farms with no data× (0)	0%
			1× (1)	30%
			2× (3)	66%
C	6	82%	Farms with no data× (3)	70%
			1× (3)	81%
			2× (0)	0%
Total	37			

Table 4.16 : Summary of mean seroprevalence of swine serum samples based on different vaccine brands in 2019-2021

Descriptives				
	Vaccine Brand		Statistic	Std. Error
Seroprevalence	Vaccine A	Mean	57.7828	2.89456
	Vaccine B	Mean	65.7803	6.41088
	Vaccine C	Mean	84.5095	8.04861

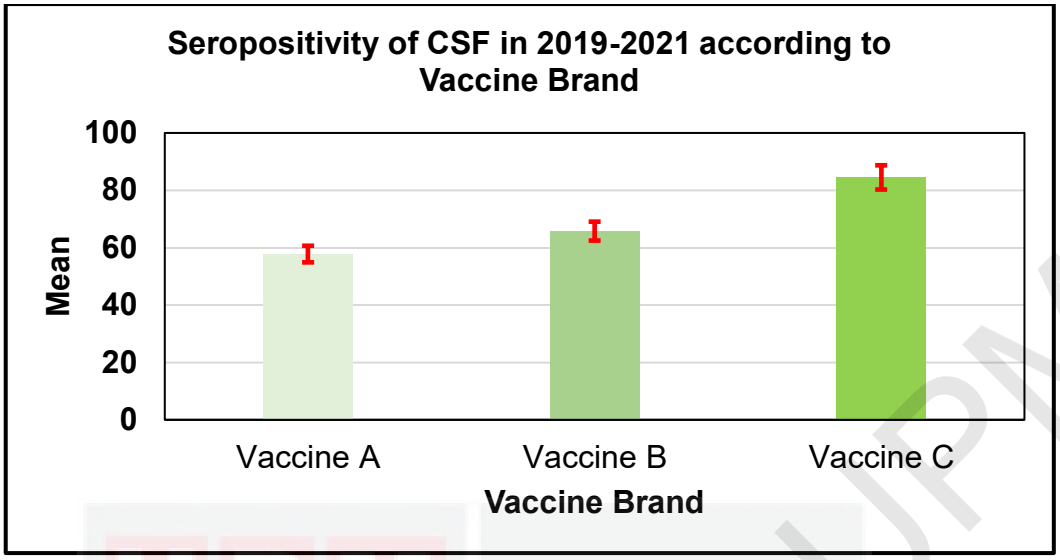

Figure 4.10 : Summary of mean seroprevalence in different vaccine brand among swine serum samples in 2019-2021

There were significant differences in seroprevalence among different vaccine brands (Table 4.17). Significant differences existed between vaccine brand A and vaccine brand C. In pairwise comparison of seroprevalence among different vaccine brand, as shown in Figure 4.11, the average ranking of the seroprevalence in vaccine brand C is the highest. Double vaccination was related to sero-suspected rate of CSF in serum samples (Table 4.18).

Table 4.17 : Summary of seroprevalence in different vaccine brands based on IBM® SPSS Independent Samples-Kruskal Wallis Statistical Analysis in 2019-2021

Dependent Variable	Vaccine Brand		Significance	Adjusted Significance
Seroprevalence	Vaccine A	Vaccine B	.287	.862
	Vaccine A	Vaccine C	.014	.041
	Vaccine B	Vaccine C	.217	.652

Note: Yellow highlight indicates significant difference.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

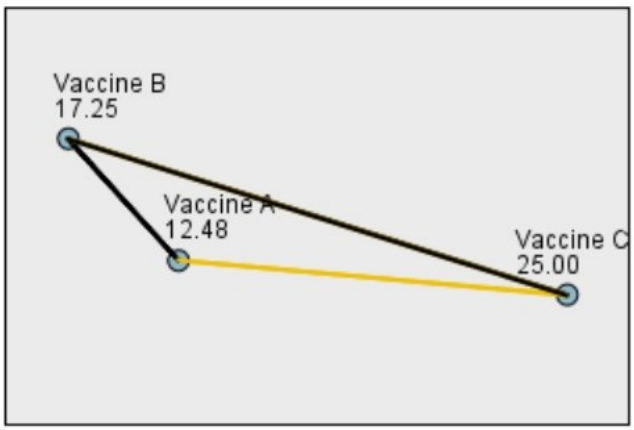

Figure 4.11 : Summary of seroprevalence in different vaccine brands based on IBM® SPSS Pairwise Comparison of Independent Samples-Kruskal Wallis Statistical Analysis in 2019-2021
Note: Each node shows the sample average rank of vaccine brand.
Yellow line indicates significant difference; black line indicates non-significant.

Table 4.18 : Summary of seroprevalence of single and double vaccination based on IBM® SPSS Multinomial Logistic Regression Statistical Analysis in 2019-2021

Seroprevalence of CSF in 2019-2021[a]		B	Std. Error	Sig.	Exp(B)
Sero-Suspected	Intercept	7.000	2.051	.001	
	Single	-.123	.067	.067	.884
	Double	-.161	.076	.034	.851
Seronegative	Intercept	1.846	1.147	.108	
	Single	-.033	.019	.076	.967
	Double	-.009	.023	.698	.991

Note:
a. The reference category is: Seropositive
Yellow highlight indicates significant association.

Table 4.19 shows the seroprevalence of CSF across risk factors, including house type (open house, combined open and closed house, and closed house), site (single sited farm and multi sited farm), vehicle dip, gate & fence, isolation and holding room, foot dip, neighboring farm perimeter. According to data analysis, it was found that combined open and closed house, closed house, and single sited have an impact on seroprevalence of CSF.

Table 4.19 : Summary of seroprevalence of various risk factors in swine serum samples based on IBM® SPSS Linear Regression Statistical Analysis in 2019-2021

Model		Unstandardized Coefficients		Sig.
		B	Std. Error	
1	(Constant)	64.254	4.120	.000
	Open-house	-.315	5.907	.958
1	(Constant)	59.249	2.831	.000
	Combined Open and Closed house	22.439	6.088	.001
1	(Constant)	68.954	3.291	.000
	Closed House	-14.964	5.778	.014
1	(Constant)	53.653	5.632	.000
	Single Sited	13.805	6.474	.040
1	(Constant)	57.357	6.669	.000
	Vehicle Dip	8.317	7.407	.269
1	(Constant)	60.111	4.554	.000
	Isolation and Holding Room	6.710	5.906	.264
1	(Constant)	56.548	6.184	.000
	Foot Dip	9.637	6.985	.176
1	(Constant)	54.458	6.077	.000
	Neighboring Farm Perimeter	12.303	6.864	.082

a. Dependent Variable: Seropositive
Yellow highlight indicates significant relationship.

4.4 Discussion

4.4.1 Indication of ELISA Seroprevalence in Endemic Countries Vaccinated with Modified Live Vaccine (MLV)

In order to control the prevalence and outbreak of CSF, vaccination with MLV is widely used in countries where the disease is endemic. CSF is an endemic viral disease in Malaysia. The Department of Veterinary Services (DVS) in Malaysia made it mandatory for regular vaccination of pig herds through the use of MLV.

Typically, ELISA testing is performed by using the Classical Swine Fever Ab ELISA Test Kit (IDEXX Laboratories, Inc., US). Antibody ELISA is the most widely used, simplest and fastest laboratory diagnostic method for detecting

CSF in serological diagnosis (Wang et al., 2020). The seroprevalence of CSF antibodies can be detected by ELISA test. Serum that tests positive for CSF antibodies can indicate the level of protection of the CSF vaccine in the pig herd. A higher seroprevalence indicates a broader range of antibody protection and fewer pigs are at risk of infection, thus reducing the risk of CSF epidemics or outbreaks. Conversely, serum that is negative in the test for CSF antibodies, which may indicate a lack of immunity against CSF or the presence of a threat of CSF. However, it is worth noting that there are many ways to obtain antibody protection, and the ELISA test cannot distinguish whether the antibodies come from vaccination or infection. Therefore, in order to more accurately understand the status of CSF in pig farms, it is not only necessary to detect CSF antibodies through ELISA tests, but also combined with clinical signs and background investigations of animals.

Compared with countries where CSF is endemic, it is easier to diagnose CSF in CSF free countries. Because vaccination may interfere with disease eradication and serological monitoring, vaccination with CSF vaccine is not allowed in CSF free countries such as EU, United States, New Zealand, and Canada. Therefore, as long as positive CSF antibodies are detected, it may be directly considered that the herd has the presence of wild type of CSFV (Low, 2019). In addition, in the EU and other CSF free countries, if CSF is detected as positive, emergency vaccination of pigs is carried out and infected pigs must be slaughtered (Postel et al., 2018).

4.4.2 Justification of Farm Status Classification System for CSF

The results obtained by ELISA testing are difficult to interpret on farm status because vaccines against CSF are usually modified live vaccines rather than DIVA vaccines in Malaysia. Therefore, in order to comprehensively explain the CSF protection status of pig farms, the classification of farm status was modified by referring to the CSF classification method developed by Low (Low, 2019).

Farm status is classified according to CV%, clinical symptoms and seropositivity of each farm. Farm status is divided into ideal, moderate, and less ideal. In addition, the CV% of blocking percentage should be below 45%, and CSF seroprevalence above 75% on the farm is considered ideal according to the recommendations of technical experts in the field of IDEXX.

In order to verify the reliability and accuracy of the developed farm status classification system, statistical analysis was used through the CV% and seropositivity results we obtained. Statistics show that the accuracy of the developed classification system is up to 98.5% in our study (Table 4.5). Therefore, we can classify farms through this classification system. This classification system not only helps farmers better understand farm conditions, but also provides a reference for future researchers studying CSF.

4.4.3 Overall CSF Seroprevalence and Farm Status

In our study of CSF serology, a total of 3127 serum samples were collected from 65 farms in 2019-2021.

Looking at the overall data, the seroprevalence in all samples received from the farm was 63.23% in 2019, 61.45 in 2020, and 61.55% in 2021. The ideal seroprevalence is over 75% on the farm, but the average seroprevalence during the three-year period was below 75%. Furthermore, in terms of farm status, only eight farms (12.31%) were classified as ideal farms. These results suggest that farms may need to take measures to control and prevent CSF, such as strengthening biosecurity measures, restricting the import of pigs from areas where CSF is prevalent, culling infected pigs, and regularly vaccinating and monitoring serological status.

4.4.4 CSF Status across Region

For the Perak region, 1744 serum samples were collected from 36 farms, and the number of farms and samples was the largest in this study. 13.89% of the farms were classified as ideal farm status, and the seroprevalence of samples was 61.53% in this region.

Since the farms from the Perak region are medium or large herd sizes, the less ideal status may be due to the presence of CSF field virus infecting the pig herd, particularly the porker herd. The average seroprevalence in the porker herd is less than 60%, but the average seroprevalence in the breeder herd was above 80%, indicating the presence of the CSF field strain in the porker herd. Normally, porker herds and breeder herds are separated on medium or large herd farms in Malaysia, so breeder herds are not affected by CSF.

In addition, it is known that MDA significantly inhibits the protective immune response induced by vaccines, so this may also interfere with the production of immune antibodies (Ivanyi, 1977). Therefore, pig farms should strengthen feeding management, correctly implement the vaccination system and improve the resistance of pigs.

For the Johor region, the ideal farm status and the overall seropositivity of the samples were the highest among all regions. The reason for the high seroprevalence may be due to antibodies generated by vaccination rather than immunity generated by infected CSF. This may be because samples from most age groups in the region showed higher seropositivity, especially in 1-6 weeks old and sows. The seroprevalence of sows in the Johor region is as high as 95%, which is the highest. A high level of seropositivity indicates a high level of protection from the vaccine and a reduced risk of infection with CSF. The low seronegativity in the Johor region can be attributed to good vaccination regime, husbandry, and biosecurity on farms.

However, the results may be a little biased because only one farm provided serum samples in the region in 2020, and the test results of the serum samples submitted by this farm were all seropositive, which can indicate that the farm took good preventive measures.

For the Penang region, no farms were classified as ideal farm status and the overall seroprevalence of the samples was low. This suggests that farms in the region are lacking in protection for CSF. Although the farms are less ideal,

there have been no reported outbreaks of CSF in the region in recent years.

According to the survey results, it was found that all farms tested in Penang region are small farms. Good herd management is not possible on these farms because of cost constraints, and some farmers lack sufficient awareness of the serological status of CSF, which increase the risk of CSF infection on farms. Therefore, regular serological testing should be carried out on pig farms, and the antibody level and antigen changes of pig herds should be closely monitor to prevent the spread of CSF within the farm.

For the Selangor & Malacca region, the seroprevalence is the lowest, only 58.55%. Based on serological tests, it was observed that the seroprevalence of pigs in all age groups was very low except for sows. In particular, the seroprevalence of weaned piglets aged 7-12 weeks is less than 40%. Internal factors on the farm may be the main cause of low seroprevalence, such as: inadequate preventive measures and lack of farm management. CSFV may be introduced into susceptible herds when weaned piglets are purchased from different farms to fattening herds (Beals et al., 1971). To reduce the risk of transmission of the virus, pigs should be purchased from the ideal herd, and precautions should be taken before pigs enter the farm. Additionally, stable flies (Stomoxys calcitrans) and house flies (Musca domestica) were shown in an experiment to transmit the virus through skin and eye lesions (Dorset et al., 1919; Rout & Saikumar, 2015)

Furthermore, most farms in the region are concentrated in coastal towns and close to water sources, which also creates a humid environment. In general, wet conditions favor longer survival of CSFV and increase the risk of viral infection in herds (Dahle & Liess, 1992; Kramera et al., 2009). Moreover, the density of pig farms in the region is high. Areas with more than 250 pigs/km^2 are considered high density population areas (Fritzemeier et al., 2000). Close proximity and high density can increase the spread of the virus, and in a study, it was found that CSFV could spread from an infected pig farm to neighboring pig farms (Reuss, 1959). In addition, it has been reported that in areas with high pig population densities, humans may be an important factor in the spread of the virus, such as veterinarians, farmers, inseminators, vaccination staff and pig catchers who use contaminated drugs, instruments, or transport equipment to cause further spread of the virus (Terpstra, 1988).

The results of farms in Selangor & Malacca region only represent sample farms and cannot represent the overall CSF status of the entire region.

In summary, in order to achieve ideal conditions and improve the level of protection of CSF on the farm in Peninsula Malaysia, biosecurity and farm hygiene levels should be strengthened on farms with infection risks. It is recommended that farms use vaccines with good production processes and follow the vaccination regime suggested by the manufacturer. It is also recommended that farms conduct regular serological testing every 4-6 months.

4.4.5 CSF Status across Herd Size

The farms that submitted the most samples were medium-sized farms (50%), with between 500 and 1,000 sows. The number of small-sized farms was the lowest (17.50%), but the seroprevalence of small-sized farms was the highest at 78%. The seroprevalence of medium-sized farms and large-sized farms was 58% and 65%, respectively.

Normally, the seroprevalence of small-sized farms is lower than that of medium- or large--sized farms, but result indicated that small-sized farms have better protection against CSF in our study. On the one hand, this may be due to the smaller number of small herd size farms in this study. On the one hand, medium and large herd size farms purchase a lot of weaners and breeding pigs from different farms, which increases the risk of introducing CSFV virus into the pig farm.

4.4.6 CSF Status across Herd Type

Herds are divided into porker herds and breeder herds according to age groups. Research results show that the seroprevalence rate of breeder herds is significantly higher than that of porker herds. In particular, the seroprevalence rate of sows exceeds 80%. Breeder herds show high seroprevalence because sows are often repeatedly vaccinations with CSF and stay for a longer duration in the farm (Zhao et al., 2022). In addition, the seroprevalence of gilts is lower than that of sows. The gilt is likely to be a new breeding pig introduced to the farm, and they have only been vaccinated with CSF vaccine once or twice. Optimizing vaccination regimens can help prevent

CSFV carriage status, especially in gilts and sows (OIE, 2019a) .

For the porker herd, the seroprevalence of piglets aged 1-6 weeks was less than 60%, which may be due to transmission of CSFV from the environment or naïve pigs to the piglets. In addition, virus-carrying sows and persistently infected piglets may also transmit the disease horizontally to healthy piglets, such as direct contact with colostrum or oronasal spread (Prasath et al., 2023). Although MDA protects piglets from death due to CSF, MDA titers decrease as piglets age. As a result, the protection of piglets by MDA will be reduced (Launais et al., 1978; Terpstra, 1977).

The seroprevalence of weaned piglets aged 7-12 weeks is only about 40%, and the seroprevalence is less than 60% in finishing pigs aged 13-20 weeks in the porker herd. Immune failure may be the main reason. According to the survey obtain, although MLV is commonly used on farms, the vaccination regimen is different. The reasons for immunization failure may be related to vaccination schedule, vaccine quality, and vaccination regime.

4.4.7 CSF Seroprevalence across Vaccination Protocol and Various Risk Factors

Significant differences in the seroprevalence rates of different brands of vaccines in this study, especially between vaccine A and vaccine C. Vaccine C have more seropositive results than vaccine A and vaccine B. This suggests that vaccine C may provide more effective protection. In a survey on the serological status of CSF, it was found that the seroprevalence of CSF was affected by vaccine brand (Low, 2019).

In addition, various risk factors also have different impacts on seroprevalence. Compared with multi-sited farms, single-sited farms are more likely to achieve seroprevalence, possibly because farmers can more easily manage these farms centrally. There may be increased movement of vehicles, farm equipment, veterinarians, farm workers in multi-sited farms, leading to the spread of CSFV on the farm (Douglas, 2002). Farms with a combination of open and closed houses were more likely to obtain seropositive status, while farms with closed houses were less likely to obtain seropositive status.

4.5 Conclusions

Based on the results obtained it was found that the overall seroprevalence of CSF was 62.04%, and the ideal farm status was only 12.31% in Peninsular Malaysia in 2019-2021. The protection status of the farm against CSF is still poor. This also reflects that the preventive measures for CSF need to be improve.

A classification system with 98.5% reliability to classify farm status into ideal, moderate, and less ideal status was used in this study. Ideal farm status and seroprevalence of CSF were highest in farms in Johor region, and seroprevalence of CSF was lowest in farms in Selangor & Malacca region. Furthermore, seroprevalence of CSF is related to herd size, herd type, and animal age. Small-sized farms have better protection against CSF. The results of this study only represent the farms that submitted samples in 2019-2021. Since there is an invasion of ASF on farms in Peninsular Malaysia in 2021, ASF might have an impact on the seroprevalence of CSF in the investigation

of different herd sizes. Breeder herds have higher seroprevalence, especially sows. In addition, vaccine brand, vaccination frequency and biosecurity measures were also associated with seropositivity.

CHAPTER 5

GENERAL DISCUSSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

5.1 General Discussion

AD and CSF are two infectious diseases that cause great economic losses the pig industry worldwide. Both AD and CSF have a long history and have caused great impacted toward the pig farms in Malaysia. This study investigates the serological status of AD and CSF in Peninsular Malaysia. A farm classification system with high accuracy and reliability was applied in serological study of CSF. In addition, some basic information on the farms was also collected in this study through a questionnaire, which adds to a more comprehensive understanding of AD and CSF in Peninsular Malaysia.

Serological diagnostics for both diseases are performed using ELISA kits, but the interpretation of the results obtained for AD and CSF is different. Since AD mostly uses marker vaccines with DIVA function (gE deleted marker vaccines), while CSF mostly uses conventional modified live vaccines, their detect results have different interpretations. Test results for AD are interpreted by the S/N ratio, and the seroprevalence of AD indicates infection status. Test results for CSF are interpreted by blocking percentage, and the seroprevalence of CSF indicates protective status.

For AD research, the field infection rate of AD in 2019-2021 is extremely low, only 1.51%, and 77.05% of farms are not infected. No exposure to AD field strains was detected in Perak region, but Johor region had the most infected

farms and had the highest seroprevalence during the 3 years period. This may be due to cost constraints, unreasonable immunization procedures, inadequate safety prevention and control measures, and feeding conditions that need to be improved. Overall, farm prevention against ADV is very successful in Peninsular Malaysia.

For the study of CSF, the protective status of CSF is not very satisfactory in 2019-2021. Based on the farm status classification system, the percentage of ideal farm status and seroprevalence of CSF were highest in Johor region, no farms were classified as ideal farm status in Penang region. The possible reasons for this result are: (A) the husbandry practice is not good at this region, (B) there are still chronic pig carriers that shed the CSF virus and naïve pigs on the farms. These have a non-negligible impact on the prevention and control of CSF.

At present, serological investigations for AD and CSF are still insufficient in Peninsular Malaysia. This study was conducted from 2019 to 2021 on submitted serum samples from Perak, Johor, Penang, Selangor & Malacca regions for serological testing and analysis of AD and CSF respectively. The purpose is to understand the antibody levels and prevalence status of AD and CSF in farms in Peninsular Malaysia in recent 3 years, and to provide effective data reference and prevention and control suggestions for the comprehensive prevention and control of animal diseases in various regions.

5.2 Conclusions

In summary, the prevention and control effect of AD has remained excellent and stable, while the protection of CSF is not ideal. Therefore, farmers should improve the prevention and control measures against CSF while keeping farms free from AD.

5.3 Recommendations for Future Research and the Industry

In recent years, the prevalence of ADV has decreased significantly in pig farms, and the condition of AD has been controlled in Peninsular Malaysia. It is strongly recommended that the focus of the pig industry changes from controlling AD to eradicating AD in the future.

Cases of ADV infection in humans continue to increase, but the situation is unknown in Malaysia, so it is recommended that researchers investigate whether people are also infected with the disease in the country.

ADV in the latent phase cannot be detected by current diagnostic technologies, so it is particularly important to develop a diagnostic method that can detect latently infected pigs in order to better prevent and eradicate AD.

The prevalence of CSF is high in pig farms, and the farm status is not ideal in Peninsular Malaysia. It is recommended that researchers continuously monitor the serological status of CSF in herds and that farms improve immunization procedures.

The data were analyzed based on samples submitted to VLSU UPM, the data and results obtained are representative of a proportion of Peninsular Malaysia only. It is recommended that future studies be able to collect more samples and involve more farms to better represent the serological status of AD and CSF in Peninsular Malaysia.

The convenience sampling was used instead of prospective sampling due to time and cost limitations in this study. Therefore, it is recommended that prospective sampling be used in future studies to detect viruses in order to obtain more accurate, reliable, and comprehensive results. In addition, it is also recommended to compare the data obtained by prospective sampling with the data obtained by convenience sampling.

In Malaysia, the density of wild boars is high, and domestic pigs can be infected with AD and CSF through wild boars, so it is recommended that researchers carry out epidemiological and serological surveys and risk assessments of ADV and CSFV in wild boars in Malaysia.

For AD and CSF, no effective drugs have been developed to treat these two viral diseases so far, which may pose a potential threat to public health. Therefore, it is recommended that researchers target these two diseases to develop antiviral drugs with good therapeutic effects in future studies, which is very promising in the breeding industry in the future.

Finally, although significant progress has been made with the current development of DIVA vaccines for AD and CSF, difficulties remain with DIVA

serological testing. To test the effectiveness of the vaccine and DIVA serological tests, it is recommended that researchers conduct field tests with the DIVA method.

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APPENDICES

Appendix I

Example of Questionnaire

Questionnaire:

Farm Name: _____
Location: _____

1. **Sow Number:** _____

2. **Farm Type:**
   - □ Open House
   - □ Close House

   Others: _____
   - □ Single Site
   - □ Multi Site

3. **Feed**
   - □ Commercial Feeding：_____
   - □ Home Mix Feeding：_____
   - □ Others：_____

4. **Water Source：(drinking)**
   - □ Pipe Water (government)
   - □ River/Lake Water
   - □ Underground Water
   - □ Others

5. **History (specific issue)**

   _____

6. **Mortality**

Pig Type	Mortality Rate		
	2019	2020	2021
Weaner	□ <10% □ 10%-20% □ >20%	□ <10% □ 10%-20% □ >20%	□ <10% □ 10%-20% □ >20%
Piglet	□ <10% □ 10%-20% □ >20%	□ <10% □ 10%-20% □ >20%	□ <10% □ 10%-20% □ >20%

Vaccination:

Disease targeted	Vaccination	Vaccine Brand	Injection (ml)	Vaccination Regime	
				Breeders	Piglet & weaner
Classical Swine Fever (CSF)	□ Yes □ No				
Aujeszky's Disease (AD)	□ Yes □ No				
Porcine Circovirus Type 2 (PCV2)	□ Yes □ No				
Atrophic rhinitis (AR)	□ Yes □ No				
Porcine Reproductive and Syndrome (PRRS)	□ Yes □ No				
Parvovirus	□ Yes □ No				
Others:	□ Yes □ No				
Others:	□ Yes □ No				

Biosecurity procedures and facilities:

Biosecurity	Vehicle dips	□ Yes □ No
	Gate and fence	□ Yes □ No
	Isolation and Holding room	□ Yes □ No
	Foot dip before enter to farrowing	□ Yes □ No
	Neighboring farm perimeters	□ <1km □ 1km-10km □ >10km

Appendix II

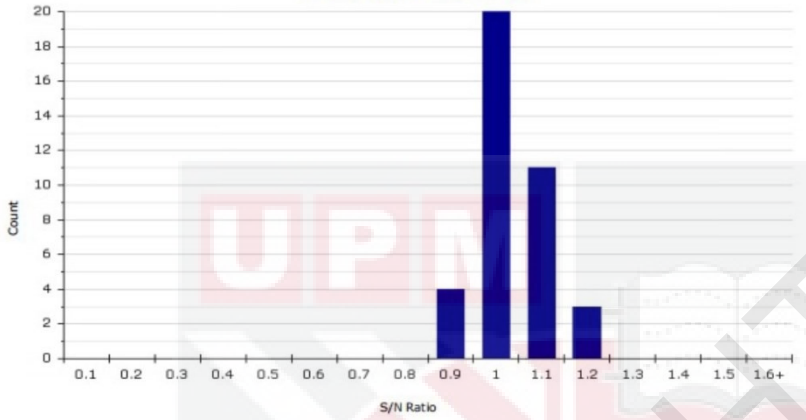
Sample IDEXX xCheck Plus® ELISA Report for AD

IDEXX Laboratories, Inc.
One IDEXX Drive
Westbrook, ME 04092
USA
4/28/2022

Test With Confidence™ IDEXX

Analyze Case Report

SOON WANG (27 SEPT. 2021)-PRVgpI



Count	38
GMean	0.986
Mean	0.988
SD	0.071
% CV	7.2
Min	0.818
Max	1.143
Tech	Vyn
Date	10/6/2021

Case SOON WANG (27 SEPT. 2021) - PRV gI (21st) 10/6/2021-001 [S

PRVgpI - 10/6/2021 - Vyn

	Well	O.D.	Tube #	Animal ID1	Animal ID2	S/N	Result
Neg	A1	1.198					
Neg	B1	1.146					
Neg	C1	1.164					
Pos	D1	0.236					
Pos	E1	0.245					
1	F1	1.214	1	SW_1w	A	1.038	Neg
2	G1	1.337	2	SW_1w	B	1.143	Neg
3	H1	1.311	3	SW_1w	C	1.121	Neg
4	A2	1.037	4	SW_1w	D	0.887	Neg
5	B2	1.144	5	SW_4w	A	0.978	Neg
6	C2	1.164	6	SW_4w	B	0.995	Neg
7	D2	1.089	7	SW_4w	C	0.931	Neg
8	E2	1.161	8	SW_4w	D	0.993	Neg
9	F2	1.253	9	SW_8w	A	1.072	Neg
10	G2	1.224	10	SW_8w	B	1.047	Neg
11	H2	1.159	11	SW_8w	C	0.991	Neg
12	A3	1.107	12	SW_8w	D	0.947	Neg
13	B3	1.180	13	SW_12w	A	1.009	Neg
14	C3	1.154	14	SW_12w	B	0.987	Neg
15	D3	1.059	15	SW_12w	C	0.906	Neg
16	E3	1.165	16	SW_12w	D	0.996	Neg
17	F3	1.128	17	SW_16w	A	0.965	Neg
18	G3	1.147	18	SW_16w	B	0.981	Neg
19	H3	1.218	19	SW_16w	C	1.042	Neg
20	A4	1.260	20	SW_16w	D	1.078	Neg
21	B4	1.157	21	SW_20w	A	0.989	Neg
22	C4	1.156	22	SW_20w	B	0.989	Neg
23	D4	1.192	23	SW_20w	C	1.019	Neg
24	E4	1.092	24	SW_20w	D	0.934	Neg
25	F4	1.043	25	SW_G	A	0.892	Neg
26	G4	1.097	26	SW_G	B	0.938	Neg
27	H4	1.241	27	SW_G	C	1.061	Neg
28	A5	1.171	28	SW_G	D	1.001	Neg
29	B5	1.257	29	SW_P2	A	1.075	Neg
30	C5	1.017	30	SW_P2	B	0.870	Neg
31	D5	1.297	31	SW_P3	A	1.109	Neg
32	E5	0.957	32	SW_P3	B	0.818	Neg
33	F5	1.058	33	SW_P4	A	0.905	Neg
34	G5	1.136	34	SW_P4	B	0.971	Neg
35	H5	1.219	35	SW_P5	A	1.042	Neg
36	A6	1.138	36	SW_P5	B	0.973	Neg
37	B6	1.092	37	SW_P9		0.934	Neg
38	C6	1.092	38	SW_P10		0.934	Neg

Appendix III

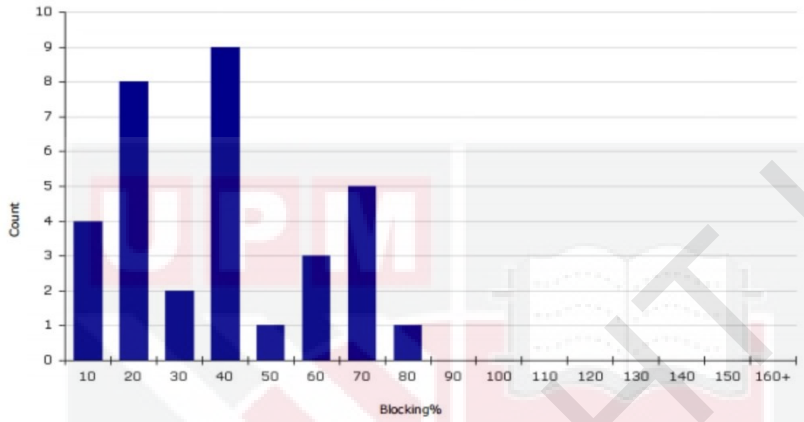
Sample IDEXX xCheck Plus® ELISA Report for CSF

IDEXX Laboratories, Inc.
One IDEXX Drive
Westbrook, ME 04092
USA
4/28/2022

Test With Confidence™ IDEXX

Analyze Case Report

HING LOONG FATT_PORKER (16 JUL 2021)-CSFVAb



Count	33
GMean	24.271
Mean	33.170
SD	21.078
% CV	63.5
Min	1.872
Max	77.734
Tech	Vyn
Date	8/9/2021

Case HING LOONG FATT_PORKER (16 JUL 2021) - CSF (24th) 8/9/202

CSFVAb - 8/9/2021 - Vyn

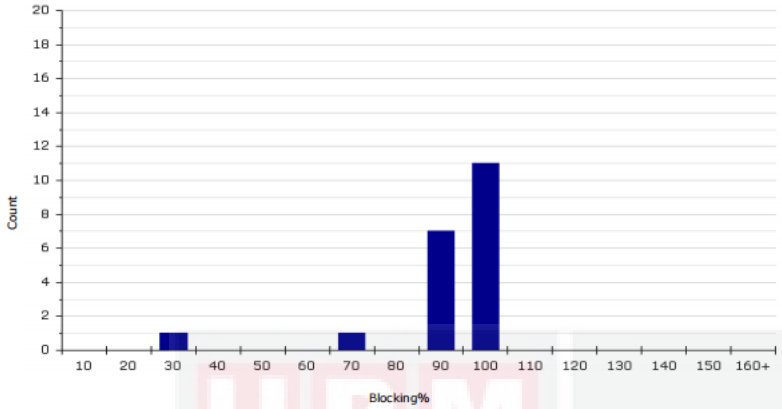
	Well	O.D.	Tube #	Animal ID1	Animal ID2	Blocking%	Result
Neg	A1	1.542					
Neg	B1	1.503					
Pos	C1	0.121					
Pos	D1	0.110					
1	E1	1.033	1	HLF_1w	A	32.151	Sus
2	F1	0.640	2	HLF_1w	B	57.964	Pos
3	G1	0.927	3	HLF_1w	C	39.113	Sus
4	H1	0.582	4	HLF_1w	D	61.773	Pos
5	A2	0.566	5	HLF_1w	E	62.824	Pos
6	B2	1.011	6	HLF_4w	A	33.596	Sus
7	C2	0.983	7	HLF_4w	B	35.435	Sus
8	D2	1.494	8	HLF_4w	C	1.872	Neg
9	E2	1.207	9	HLF_4w	D	20.722	Neg
10	F2	1.377	10	HLF_4w	E	9.557	Neg
11	G2	1.300	11	HLF_4wS		14.614	Neg
12	H2	1.345	12	HLF_8w	A	11.658	Neg
13	A3	1.045	13	HLF_8w	B	31.363	Sus
14	B3	1.053	14	HLF_8w	C	30.837	Sus
15	C3	1.348	15	HLF_8w	D	11.461	Neg
16	D3	1.250	16	HLF_8w	E	17.898	Neg
17	E3	0.972	17	HLF_12w	A	36.158	Sus
18	F3	0.998	18	HLF_12w	B	34.450	Sus
19	G3	0.546	19	HLF_12w	C	64.138	Pos
20	H3	1.462	20	HLF_12w	D	3.974	Neg
21	A4	1.295	21	HLF_12w	E	14.943	Neg
22	B4	1.115	22	HLF_16w	A	26.765	Neg
23	C4	1.308	23	HLF_16w	B	14.089	Neg
24	D4	0.824	24	HLF_16w	C	45.878	Pos
25	E4	0.559	25	HLF_16w	D	63.284	Pos
26	F4	0.695	26	HLF_16w	E	54.351	Pos
27	G4	0.988	27	HLF_20w	A	35.107	Sus
28	H4	1.493	28	HLF_20w	B	1.938	Neg
29	A5	1.281	29	HLF_20w	C	15.862	Neg
30	B5	0.339	30	HLF_20w	D	77.734	Pos
31	C5	1.299	31	HLF_20w	E	14.680	Neg
32	D5	0.541	Duplicate	HLF_1w	B	64.466	Pos
33	E5	0.701	Duplicate	HLF_1w	E	53.957	Pos

IDEXX Laboratories, Inc.
One IDEXX Drive
Westbrook, ME 04092
USA
4/28/2022

Test With Confidence™ IDEXX

Analyze Case Report

KULAI_BREEDER (21 JUL. 2021)-CSFVAb



Count	20
GMean	84.480
Mean	87.400
SD	16.138
% CV	18.5
Min	22.574
Max	96.442
Tech	Vyn
Date	8/9/2021

Case KULAI_BREEDER (21 JUL. 2021) - CSF (24th) 8/9/2021-002 [KU
CSFVAb - 8/9/2021 - Vyn

	Well	O.D.	Tube #	Animal ID1	Animal ID2	Blocking%	Result
Neg	A1	1.567					
Neg	B1	1.525					
Pos	C1	0.110					
Pos	D1	0.111					
1	E5	0.200	1	KUL_G	2415	87.063	Pos
2	F5	0.058	2	KUL_G	2416	96.248	Pos
3	G5	0.074	3	KUL_G	2418	95.213	Pos
4	H5	0.174	4	KUL_G	2419	88.745	Pos
5	A6	0.484	5	KUL_G	2421	68.693	Pos
6	B6	1.197	6	KUL_G-P1	2358	22.574	Neg
7	C6	0.163	7	KUL_G-P2	2145	89.457	Pos
8	D6	0.088	8	KUL_G-P3	1870	94.308	Pos
9	E6	0.084	9	KUL_G-P4	1551	94.567	Pos
10	F6	0.055	10	KUL_G-P4	1557	96.442	Pos
11	G6	0.102	11	KUL_P1	2244	93.402	Pos
12	H6	0.074	12	KUL_P1	2257	95.213	Pos
13	A7	0.059	13	KUL_P2	1908	96.184	Pos
14	B7	0.191	14	KUL_P2	1993	87.646	Pos
15	C7	0.090	15	KUL_P3	1584	94.179	Pos
16	D7	0.237	16	KUL_P3	1692	84.670	Pos
17	E7	0.097	17	KUL_P4	1414	93.726	Pos
18	F7	0.084	18	KUL_P4	1485	94.567	Pos
19	G7	0.229	19	KUL_P5	1133	85.188	Pos
20	H7	0.156	20	KUL_P5	1173	89.909	Pos

Appendix IV

Disease Status and Farm Questionnaire Information among the Surveyed Farms

Farm status	ID	Sow Number	Farm Type		CSF Vaccination Protocol							Biosecurity					
					Vaccine			Breeders			Piglet & Weaner						
								Gilt		Sow			Vehicle Dips	Gate & Fence	Isolation & Holding Room	Foot Dip	Neighboring Farm Perimeter
			House	Site	Brand	Inj. vol. (cc/pig)	Injections	Duration			Injections	Duration					
Less Ideal	P3	1200	close	multi	A	2	2x			mass vac	2x	4w	y	y	y	y	n
Moderate	P7	900	Open	Single	A	2	-	-		-	1x	4w	Y	Y	N		
Less Ideal	P9	600	open	single	A	2	2x			mas vac	2x	4w	Y	Y	N	Y	Y
Less Ideal	P10	4000	close	multi	A	2	2x			mass vac	2x	4w	Y	Y	Y	Y	N
Ideal	P11	650	Close + Open	Single	A	2	1x	Before Mating		3 days After Weaning	1x	6w	Y	Y	Y	Y	1
Less Ideal	J01	700	Open	Single	A	2				3x Mass Vaccination	1x	6w	N	Y	N	N	2
Less Ideal	PG01	400	open	single	A					mas cavination			Y	Y	N	N	2
Less Ideal	SM03	200	opne	single	A	2	2x			b4 weaning			N	Y	N	Y	3
Less Ideal	P12	800	close	single	A	2							Y	Y	Y	Y	2
Less Ideal	P16	700	close	single	A	2							Y	Y	Y	Y	1
Less Ideal	P17	680	Close	Single	A	2	2x	6 month old + 1 month before mating		2 days Before Weaning	1x	6w	Y	Y	Y	Y	2
Less Ideal	P20	900	Open	Single	A	2	-	-		-	1x	4w	Y	Y	N		
Less Ideal	P21	600	open	single	A	2	2x			mas vac	2x	4w	Y	Y	N	Y	Y
Less Ideal	P22	600	open	single	A	2	2x			mas vac	2x	4w	Y	Y	N	Y	Y
Less Ideal	PG03	400	open	single	A					mas cavination			Y	Y	N	N	2
Less Ideal	P26	1200	close	multi	A	2	2x			mass vac	2x	4w	y	y	y	y	n
Less Ideal	P27	1200	close	multi	A	2	2x			mass vac	2x	4w	y	y	y	y	n
Less Ideal	P28	1200	close	multi	A	2	2x			mas vac	2x	4w	y	y	y	y	n
Less Ideal	P29	1200	close	multi	A	2	2x			mass vac	2x	4w	y	y	y	y	n
Less Ideal	P35	900	Open	Single	A	2	-	-		-	1x	4w	Y	Y	N		
Less Ideal	J05	700	Open	Single	A	2				3x Mass Vaccination	1x	6w	N	Y	N	N	2
Less Ideal	J06	700	Open	Single	A	2				3x Mass Vaccination	1x	6w	N	Y	N	N	2
Less Ideal	J07	700	Open	Single	A	2				3x Mass Vaccination	1x	6w	N	Y	N	N	2
Less Ideal	J09	1700	Open	Single	A	2				3x Mass Vaccination	3x	1d, 6w & 9w	Y	Y	N	Y	1
Less Ideal	PG09	400	open	single	A					mas cavination			Y	Y	N	N	2
Less Ideal	SM01	1000	Close	Multi	B	2	1x	Before Mating		After Weaning	1x	5 - 6w	Y	Y	N	Y	1
Ideal	SM02	>1200	Open	Single	B	2	2x	2 months interval		Weaning	2x	5w & 9 w	Y	Y	Y	Y	1
Less Ideal	SM07	>1200	Open	Single	B	2	2x	2 months interval		Weaning	2x	5w & 9 w	Y	Y	Y	Y	1
Less Ideal	SM09	>1200	Open	Single	B	2	2x	2 months interval		Weaning	2x	5w & 9 w	Y	Y	Y	Y	1
Less Ideal	P5	900	Close + Open	Single	C	2	2x	4 months interval		3x Mass Vaccination	-	-	Y	Y	Y	Y	3
Less Ideal	J02	200	Close + Open	Single	C	2	1x	After mating		3 -4x Mass Vaccination	1x	5 - 6w	Y	Y	Y	Y	3
Ideal	P15	900	Close + Open	Single	C	2	2x	4 months interval		3x Mass Vaccination	-	-	Y	Y	Y	Y	3
Ideal	J03	200	Close + Open	Single	C	2	1x	After mating		3 -4x Mass Vaccination	1x	5 - 6w	Y	Y	Y	Y	3
Ideal	P30	900	Close + Open	Single	C	2	2x	4 months interval		3x Mass Vaccination	-	-	Y	Y	Y	Y	3
Less Ideal	J04	200	Close + Open	Single	C	2	1x	After mating		3 -4x Mass Vaccination	1x	5 - 6w	Y	Y	Y	Y	3

BIODATA OF STUDENT

The student Li Hongxia, was born on 31st October 1995 in Hebei, China. She received her primary education at Nangongyou School, followed by her secondary and higher secondary education at Yingcaiyuan School in Gu'an County, Langfang City, Hebei Province, China. From 2014 to 2017, she studied veterinary medicine at Hebei North University, Zhangjiakou City, Hebei Province, China. In 2019, she obtained her Bachelor of Veterinary Medicine degree from the Faculty of Veterinary Medicine and the Faculty of Traditional Chinese Veterinary Medicine at Hebei Agricultural University, Baoding City, Hebei Province, China. In 2021, she received an offer from the Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, to pursue a master's degree in virology. She is very interested in research on swine viral diseases in Malaysia.

PUBLICATION

Li, H. X., Fong, M. W. C., Rahaman, N. Y. A., Low, S. E., Lee, J. X., Cheah, E. H. C., ... & Hui, R. L. Serological Investigation of Aujeszky's Disease Between 2019 and 2021 in Peninsular Malaysia.