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Serological Investigation of Aujeszky's Disease Between 2019 and 2021 in Peninsular Malaysia

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ABSTRACT

Aujeszky's disease (AD) is a common disease that has spread worldwide. Various porcine viral diseases exist in Malaysia, where AD is the most common viral endemic disease in the country. The serological status of AD in Peninsular Malaysia was reported prior to 2018, but information after that date is very limited. Hence, our study investigated AD's serological status in Peninsular Malaysia pig farms based on commercial samples submitted to the Faculty of Veterinary Medicine, Universiti Putra Malaysia, between 2019 and 2021. In this study, 2,780 serum samples were taken from 61 farms, and an enzyme-linked immunosorbent assay (ELISA) test was performed using the IDEXX Pseudorabies Virus gpl Antibody Test Kit for AD serology diagnosis. The results showed that the overall seropositive rate of Aujeszky disease virus (ADV) was 1.51% (42/2,780), which dropped from 2.62% (23/879) in 2019 to 0.53% (5/937) in 2020 and 1.45% (14/964) in 2021. In addition, 18.03% (11/61) of the 61 farms that submitted samples were infected with AD. The results indicate that AD still exists in Peninsular Malaysia, and some farms are at risk from the disease. Further analysis suggested that the quarterly seroprevalence of ADV may also be related to region. This study provides serological

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data on AD in Peninsular Malaysia, which helps to understand the prevalence and serological status of the disease.

Keywords: Aujeszky's disease, ELISA, Peninsular Malaysia, pig farm, serological status

INTRODUCTION

Aujeszky's disease (AD) is one of the most common pig viral diseases, which causes significant economic losses to the pig industry. AD first appeared in the United States in 1813, and it was first characterized in several animals by scientist Aladar Aujeszky in 1902, so it was named Aujeszky's disease (Aujeszky, 1902). AD, called pseudorabies (PR), is highly infectious. The causative agent of AD is the Aujeszky disease virus (ADV), which is also known as pseudorabies virus (PRV) or Suid herpesvirus 1 (SuHV1) (Freuling et al., 2017). ADV belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, and genus *Varicellovirus* (Davison, 2010). ADV is a double-stranded, enveloped DNA virus, and the total length of the ADV genome is approximately 150 kb. The virus has 11 glycoproteins, namely glycoprotein B (gB), glycoprotein C (gC), glycoprotein D (gD), glycoprotein E (gE), glycoprotein G (gG), glycoprotein H (gH), glycoprotein I (gI), glycoprotein K (gK), glycoprotein L (gL), glycoprotein M (gM), and glycoprotein N (gN) (Mettenleiter, 2000). The gE is also called glycoprotein I (gpI), the latter being very old terminology (Low et al., 2018).

The only reservoirs and natural hosts of ADV are members of the family Suidae (domestic and wild pig), and pigs at every age are susceptible to ADV (Müller et al., 2011). Besides pigs, ADV can also infect many animals, including carnivores, ruminants, and rodents (Konjević et al., 2023). However, only pigs can survive among all animals susceptible to AD (Delva et al., 2020).

AD is mainly transmitted through direct nose-to-nose contact between pigs. In addition, ADV can be transmitted indirectly through aerosols, semen, and fomites (Aznar et al., 2022). The virus can also be transmitted vertically across the placenta (Ukhovskyi et al., 2022). Clinical signs of disease depend on the age of the pig, infection dose, virus strain, and health status (Pomeranz et al., 2005; Szczotka-Bochniarz et al., 2016). Piglets mainly exhibit severe neurological symptoms and fatal encephalitis, with mortality approaching 100%. Fattening pigs' most prominent clinical symptoms are respiratory symptoms, slow growth, and mortality, usually less than 5% (Chen et al., 2022; Nauwynck, 1997). Sows infected with ADV usually have abortions, give birth to mummified fetuses or dead piglets, and are infertile (Baskerville, 1981). Usually, the disease is not fatal in older pigs (Zuckermann, 2000).

In the 1970–1980s, the first widespread outbreak of ADV occurred in swine herds worldwide (Liu et al., 2022). Although AD has been eradicated in domestic pigs in many countries such as Germany, the United States, New Zealand, Canada, the United Kingdom, and Sweden in the late 1990s, it is still prevalent in Europe, South America,

and Asia (Aznar et al., 2022; Sun et al., 2016). In Malaysia, AD was first reported in 1976 (Lee et al., 1979), and AD was declared endemic in 1984 (Too, 1997). A study in 2016 found that AD field strains still exist in Malaysia (Low et al., 2018). Although AD vaccination is not mandatory in Malaysia's Department of Veterinary Services, it is currently commonly performed in pig farms.

Several modified live vaccines and inactivated vaccines have been approved in Malaysia to prevent and control AD. Most AD vaccines used on farms in the country are gE-deleted vaccines. Such marker vaccines can differentiate between infected and vaccinated animals (DIVA) (Delva et al., 2020; Freuling et al., 2017; Ukhovskyi et al., 2022). Therefore, gE antibodies produced by the AD field virus can be detected in infected animals by the enzyme-linked immunosorbent assay (ELISA) test but not in vaccinated (uninfected) animals (Mettenleiter, 2020; Wang et al., 2019).

Serological surveys were conducted in Peninsular Malaysia in 2016 to better control and prevent AD, but the disease's serological prevalence in recent years remains unknown. Therefore, this study aims to investigate the serological status of AD between 2019 and 2021 in Peninsular Malaysia and detect gE antibodies by ELISA test.

MATERIALS AND METHODS

Sample Collection

Farmers provided verbal informed consent for the collection of serum samples and AD serological diagnosis. Farmers submitted pig serum samples to the Faculty of Veterinary Medicine, Universiti Putra Malaysia. A total of 2,780 serum samples from 61 farms met the criteria of this study from January 2019 to December 2021. In general, at least 20 serum samples were submitted from each farm, comprising 1–6 weeks, 7–12 weeks, 12–20 weeks, gilts, and sows. At least 4 samples were submitted for each listed age group. Each serum sample was collected the day after blood collection, then stored and transported to the laboratory at -80°C. The monthly test results are summarized and sorted by region.

Region Categorization

All farms were categorized according to location into four categories (Perak, Johor, Penang, Selangor, and Malacca regions). Due to the small number of farms and samples in the Selangor and Malacca regions, they are classified as one region.

Serological Detection

Detection of antibodies towards the gE/gpI antigen of ADV in serum samples by ELISA test using the Pseudorabies Virus gpI Antibody Test Kit (IDEXX Laboratories, Inc., USA) according to the manufacturer's instructions. The kit can distinguish infected pigs from vaccinated pigs. The immune response produced by pigs vaccinated with the gE/gpI deletion vaccine does not contain gE/gpI. At the same time, gE/gpI antibodies will be detected in pigs infected with AD field viruses (Low et al., 2018). The gE/gpI deleted vaccine was used to immunize pigs against AD pig farms in Malaysia.

Therefore, when antibodies against gE are detected in the serum of pigs vaccinated with the gE/gpI deleted vaccine, it indicates that the pigs are infected with AD field virus (van Oirschot et al., 1990). The ELISA test is the most widely used diagnostic method to detect the presence of ADV antibodies. The assay can also screen many serum samples in a short time, and it has higher sensitivity and specificity.

The serological status of the sample was determined by the sample-to-negative ratio (S/N) value. S/N ≤ 0.6 is considered positive, which indicates that the sample is infected with ADV field strains. 0.6 < S/N < 0.7 is considered suspect. S/N ≥ 0.7 is considered a negative result for the sample.

RESULTS AND DISCUSSION

In this study, 2,780 serum samples were submitted from four regions in Peninsular Malaysia, including 879 samples in 2019, 937 samples in 2020, and 964 samples in 2021. The results of the ELISA test show that the overall positive seroprevalence of samples was 1.51% during 2019–2021. The seroprevalence of samples from 2019 to 2021 was 2.62% (23/879), 0.53% (5/937), and 1.45% (14/964), respectively. In addition, different regions have different seroprevalences of samples in 2019–2021. None of the samples tested positive in the Perak region. In contrast, the seroprevalence of samples was 5.92% in the Johor region, 0.27% in the Penang region, and 3.77% in the Selangor and Malacca regions (Table 1).

These serum samples were collected from 61 farms, including 20 in 2019, 22 in 2020, and 19 in 2021. The percentage of infected farms is not the same from 2019 to 2021. Eleven (18.03%) farms had positive serum samples detected in these three years. Six (30%) farms were infected with AD in 2019, two (9.09%) farms in 2020, and three (15.79%) farms in 2021. In addition, the percentage of infected farms in different

Table 1
Percentage of seropositive samples in different regions across years between 2019 and 2021 in Peninsular Malaysia

Region	Percentage of seropositive samples (%)			
	2019	2020	2021	Overall
Perak	0	0	0	0
	0/527	0/568	0/466	0/1,561
Johor	16.90	10	2.89	5.92
	12/71	4/40	9/311	25/422
Penang	0	0	1.28	0.27
	0/107	0/188	1/78	1/373
Selangor and Malacca	6.32	0.71	3.67	3.77
	11/174	1/141	4/109	16/424
Total	2.62	0.53	1.45	1.51
	23/879	5/937	14/964	42/2,780

Note. The number of seropositive samples/The number of total samples

regions also varied in 2019–2021. No farms were infected with AD in the Perak region, while pig farms are highly affected by the disease in the Selangor and Malacca regions, pig farms are moderately affected in the Johor region, and pig farms are lowly affected in the Penang region (Table 2).

The seroprevalence also varies among different age groups. The seroprevalence of samples was 2.37% in piglets aged 1–6 weeks, 0.47% in weaned piglets aged 7–12 weeks, 0.34% in fattening pigs aged 13–20 weeks, 0.88% in gilts, and 3.16% in sows (Table 3). Based on this study, it was observed that at 1–6 weeks of age, gilts, and sow herds were most likely to be seropositive. For breeding herds (gilts and sows), high seroprevalence may be

Table 2
Percentage of infected farms in different regions across years between 2019 and 2021 in Peninsular Malaysia

Region	Percentage of infected farms (%)			
	2019	2020	2021	Overall
Perak	0	0	0	0
	0/10	0/13	0/10	0/33
Johor	100	100	20	50
	2/2	1/1	1/5	4/8
Penang	0	0	50	10
	0/3	0/5	1/2	1/10
Selangor and Malacca	80	33.33	50	60
	4/5	1/3	1/2	6/10
Total	30	9.09	15.79	18.03
	6/20	2/22	3/19	11/61

Note. The number of infected farms/The number of total farms

Table 3
Percentage of seropositive samples in different regions across age groups between 2019 and 2021 in Peninsular Malaysia

Region	Percentage of seropositive samples (%)				
	1–6 weeks	7–12 weeks	13–20 weeks	Gilts	Sows
Perak	0	0	0	0	0
	0/289	0/63	0/339	0/187	0/383
Johor	7.23	3.23	1.15	0	14.56
	6/83	3/93	1/87	0/56	15/103
Penang	0	0	1.20	0	0
	0/82	0/82	1/83	0/44	0/82
Selangor and Malacca	7.45	0	0	5.77	6.25
	7/94	0/97	0/85	3/52	6/96
Total	2.37	0.47	0.34	0.88	3.16
	13/548	3/635	2/594	3/339	21/664

Note. The number of seropositive samples/The number of total samples

caused by the introduction of pigs from AD-infected herds or the failure of vaccine immunization (Siegel & Weigel, 1999). The higher seroprevalence in piglets aged 1–6 weeks is most likely caused by the influence of maternally derived antibodies (MDA). When MDA levels rise during vaccination, immunization effectiveness declines, and the development of active immunity is also interfered with by MDA (Stegeman, 1995). Another reason might be that sows are infected with ADV during pregnancy, causing newborn piglets to be infected with ADV from the placenta (Laval & Enquist, 2020).

This study's overall positive rate of ADV gE antibodies dropped from 2.62% in 2019 to 0.53% in 2020 and 1.45% in 2021. This result was lower than 4.25% (49/1154) in 2016 (Low et al., 2018). Moreover, the percentage of AD-infected farms dropped from 30% in 2019 to 9.09% in 2020 and 15.79% in 2021. Although the seroprevalence rates in different years are irregular, the overall data shows that the field infections of AD are on a downward trend in Peninsular Malaysia. It may be related to the coronavirus disease (COVID-19) outbreaks in 2020 and African swine fever (ASF) in 2021 in Malaysia. Farmers have started to practice restricted movement by strengthening biosecurity measures and feeding management to prevent COVID-19 and ASF from invading the farm (Khoo et al., 2021).

The obtained data indicated that the seroprevalence rate of ADV infection in pigs in different regions is different in Peninsular Malaysia, which may be due to different factors such as farm setup, husbandry practice, vaccination and concurrent infections in this serological survey: Perak region had the largest number of samples and farms, no seropositive samples were detected, and no farms were infected with the AD field virus during these three years. It may be attributed to good husbandry and biosecurity practices on farms, as well as regular vaccination of pig herds with highly effective AD vaccines. Although no samples were found to be seropositive for field-type AD infection in this investigation, it does not mean that the region is completely free of the threat of AD. Therefore, farmers should also remain concerned about this disease.

Compared to other regions, the Johor region has the highest overall seropositivity rate, and half of farms are infected with the AD field virus. However, with only a limited number of farms and samples collected from this region, it was not able to represent the whole situation in the region. This region had high levels of seroprevalence in 2019, which may be largely due to internal factors on farms causing AD infections. Different factors, such as farm husbandry, vaccinations, and biosecurity measures, all contributed to farm disease conditions (Ukhovskyi et al., 2022). Although the seroprevalence of AD was very high in 2019, the situation has improved greatly in 2020 and 2021.

The AD field infection challenge for the Penang region is very low, and only one farm was affected. It indicates that AD was stable and did not challenge the farms. According to the results, there was no AD field virus

infection in the Penang region in 2019 and 2020, while only one farm was infected by ADV in 2021. Overall, the spread of AD field viruses in the Penang region was relatively low, consistent with the results of the serological survey on AD in the Penang region in 2016 (Low et al., 2018).

The number of ADV-infected farms is the highest in the Selangor and Malacca regions. The AD field virus is regional and common in this region, most likely due to the high pig farm density in these two regions. ADV can also spread through the air over long distances between farms, more than 10 km (Casal et al., 1997). Moreover, according to serology results in the Selangor and Malacca regions, antibodies against the AD field virus were detected in piglets, gilts, and sows. The external introduction of ADV, which carries breeding pigs (sows and gilts) and semen, is the main source of farm infection with AD (Song et al., 2017). Infected sows may continue to spread and excrete ADV on the farm, which may expose naive porkers or sows to ADV infection. Therefore, to reduce the high seroprevalence of AD and prevent farms from being affected by the disease, farmers must strictly implement biosecurity measures and strengthen the management of farm husbandry. Further investigation found that farms with biosecurity measures such as vehicle dip, isolation and holding room, and foot dip have a reduced risk of ADV infection. It was also observed that open-type farms and farms surrounded by neighboring farms are more susceptible to ADV challenges.

The data show that the seroprevalence of ADV may be correlated with quarter and region. Due to the impact of COVID-19 and ASF, staff access to farms has been restricted, and many farms have closed down, making it difficult to collect samples for this study. Therefore, in some quarters, we did not collect samples. However, it can be seen from the limited data that the seroprevalence rate was 5.11% in the third quarter (Q3) and 2.01% in the second quarter (Q2) of 2019. In 2020, the seroprevalence rate in the third quarter (Q3) was 0.95%. The seroprevalence rate in the fourth quarter (Q4) was 6.78%, and it was the highest in 2021, followed by 2.87% and 0.58% in the first (Q1) and third quarters (Q3), respectively (Table 4). The overall data results indicate that all cases will increase in the fourth quarter (Q4) of 2021. From 2019 to 2021, no samples were detected positive for seropositivity in the Perak region. In the Johor region, the seroprevalence rate was the highest in the third quarter (Q3), especially in 2019, with a seroprevalence rate of 16.90%. No serum samples were detected positive in the Penang region from the first quarter (Q1) of 2019 to the third quarter (Q3) of 2021, but 2.50% of the serum samples were positive until the fourth quarter (Q4) of 2021. In the Selangor and Malacca regions, the seroprevalence of ADV is irregular. There was a clear upward trend in the fourth quarter (Q4) of 2021, but it is worth noting that the serum samples in the first quarter (Q1) from 2019 to 2021 were seronegative (Figure 1). It can be found that the Perak region has not been affected by

ADV during the three-year period, while the seroprevalence in the Johor, Selangor, and Malacca regions is higher. There are some differences in seroprevalence rates among regions in different quarters. ADV infection rates vary by quarter and region, consistent with previous studies (Zheng et al., 2021). It shows that although the seroprevalence of ADV is very low, it is still difficult to eradicate the disease in Peninsular Malaysia. In addition, the seroprevalence rate of pigs infected with ADV is on the rise in the fourth quarter (Q4) of 2021. Therefore, pig farms also need to take preventive measures against the disease.

Many cases of human infection with ADV have been reported in recent years, especially in China. Furthermore, it has been demonstrated that ADV may invade the human central nervous system (CNS) and cause endophthalmitis and encephalitis in humans when infecting them through the eyes (Ai et al., 2018) and wounds (Yang et al., 2019). Although human cases of ADV infection occurred in China, its potential harm to human public health worldwide cannot be ignored. Through communication with farmers, it could be learned that in Malaysia, to prevent human infection with ADV, unauthorized people are required to stay away from the farm, and staff allowed to enter the farm are required to decontaminate and disinfect their hands, shoes, and clothing before leaving the farm. In addition, people who work with pigs (e.g., pig farmers, veterinarians, and slaughterers) are advised to protect themselves while working, avoid exposure to wounds, and avoid direct contact with infected pigs or their fluids.

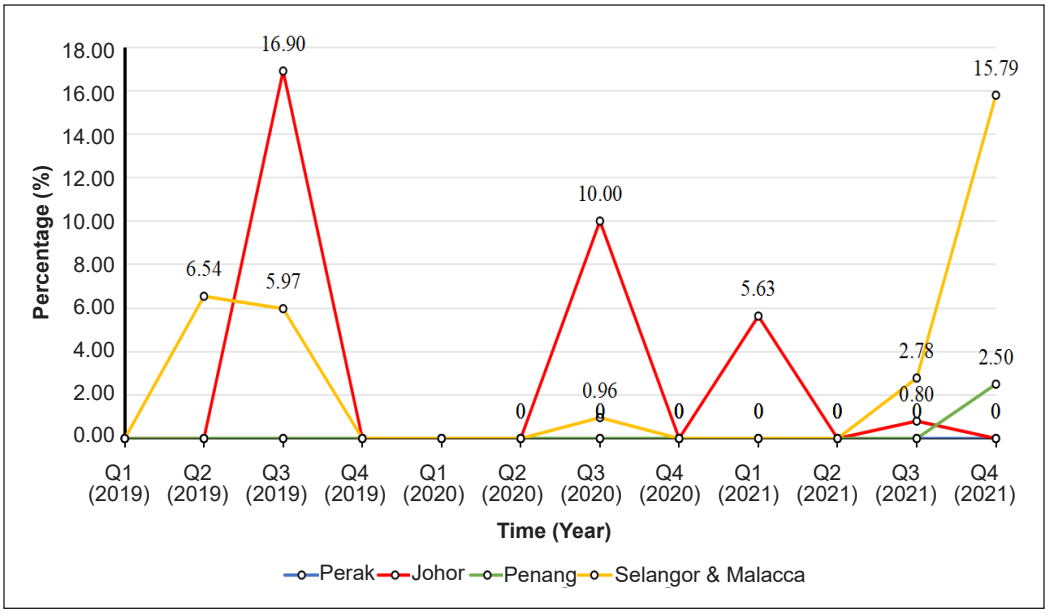

Figure 1. Percentage of seropositive samples in different quarters across regions
Note. Q1 = First quarter (from January to March); Q2 = Second quarter (from April to June); Q3 = Third quarter (from July to September); Q4 = Fourth quarter (from October month to December)

Table 4
Percentage of seropositive samples in different quarters across regions between 2019 and 2021 in Peninsular Malaysia

Time	The regions of Peninsular Malaysia				Total
	Perak	Johor	Penang	Selangor and Malacca	
January–March (Q1) 2019	NA	NA	0.00% 0/47	NA	0.00% 0/47
April–June (Q2) 2019	0.00% 0/242	NA	NA	6.54% 7/107	2.01% 7/349
July–September (Q3) 2019	0.00% 0/115	16.90% 12/71	0.00% 0/60	5.97% 4/67	5.11% 16/313
October–December (Q4) 2019	0.00% 0/170	NA	NA	NA	0.00% 0/170
Total 2019	0.00% 0/527	16.90% 12/71	0.00% 0/107	6.32% 11/174	13.22% 23/879
January–March (Q1) 2020	0.00% 0/95	NA	0.00% 0/34	0.00% 0/37	0.00% 0/166
April–June (Q2) 2020	0.00% 0/131	NA	0.00% 0/34	NA	0.00% 0/165
July–September (Q3) 2020	0.00% 0/302	10.00% 4/40	0.00% 0/82	0.96% 1/104	0.95% 5/528
October–December (Q4) 2020	0.00% 0/40	NA	0.00% 0/38	NA	0.00% 0/78
Total 2020	0.00% 0/568	10.00% 4/40	0.00% 0/188	0.71% 1/141	0.53% 5/937
January–March (Q1) 2021	0.00% 0/83	5.63% 8/142	NA	0.00% 0/54	2.87% 8/279
April–June (Q2) 2021	0.00% 0/237	0.00% 0/44	NA	NA	0.00% 0/281
July–September (Q3) 2021	0.00% 0/146	0.80% 1/125	0.00% 0/38	2.78% 1/36	0.58% 2/345
October–December (Q4) 2021	NA	NA	2.50% 1/40	15.79% 3/19	6.78% 4/59
Total 2021	0.00% 0/466	2.89% 9/311	1.28% 1/78	3.67% 4/109	1.45% 14/964
Grand Total (2019–2021)	0.00% 0/1561	5.92% 25/422	0.27% 1/373	3.77% 16/424	1.51% 42/2,780

Note. NA = Not applicable as no samples were submitted during that period; Q = Quarters

CONCLUSION

This study investigated the serological prevalence of AD in Peninsular Malaysia during 2019–2021. The seroprevalence of AD varies between different regions. The Perak region is free of AD field virus infections. The seroprevalence of AD is the highest in the Johor region but might be biased due to sample size. The seroprevalence of AD in the Penang

region is relatively low, and the number of farms infected with AD is also small. The Selangor and Malacca regions have the highest number of AD-infected farms. In addition, the seroprevalence of ADV is also related to the quarter, with pigs being more susceptible to the disease in the third quarter (Q3). Overall, the exposure level to AD field viruses is low from 2019 to 2021. With the widespread use of highly effective AD vaccines, AD has been well controlled in pig farms in Peninsular Malaysia, so the next step should be to eradicate the disease in the country. This study contributes to a better understanding of the serological Investigation of AD in Peninsular Malaysia and provides basic information for the disease's prevention, control, and eradication.

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