UNIVERSITI PUTRA MALAYSIA

RETROSPECTIVE STUDY OF PORCINE REPRODUCTIVE AND RESPIRATORY (PRRS) SYNDROME SEROLOGY STATUS AMONG SEROLOGY SAMPLES SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITI PUTRA MALAYSIA (UPM) FROM 2019 TO 2021

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SERDANG SELANGOR
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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Retrospective Study of Porcine Reproductive and Respiratory Syndrome (PRRS) Serology Status Among Serology Samples Submitted to the Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM) from 2019 to 2021", by Amir Shauqi bin Ahmad Sukri and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD4999 – Final Year Project.

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DECLARATION

Declaration by undergraduate student

I hereby confirm that:

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ABSTRACT

Abstract of dissertation presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999- Final Year Project.

RETROSPECTIVE STUDY OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) SEROLOGY STATUS AMONG SEROLOGY SAMPLES SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITI PUTRA MALAYSIA (UPM) FROM 2019 TO 2021

by

Amir Shauqi bin Ahmad Sukri

2022

Supervisor: Dr. Syamira Syazuana Zaini

Co-Supervisor: Associate Professor Dr. Ooi Peck Toung

Porcine Reproductive and Respiratory Syndrome (PRRS) in swine is a viral disease caused by the PRRS virus. PRRS is regarded as a costly disease globally due to its severe impacts, such as reproductive losses, respiratory sickness, decreased growth rate, and increased mortality. PRRSV is classified into two types: PRRSV-1 is prevalent in Europe, and PRRSV-2 is prevalent in the Americas and Asia. In Malaysia, there is a limited study on the current serological status and prevalence of PRRS in swine. Thus, this study aims to determine the PRRS serological status of blood samples

submitted to the Faculty of Veterinary Medicine, UPM, from 2019 to 2021. Blood samples from 101 farms in Johor, Melaka, Selangor, Perak and Penang were used in the study. The samples were divided into 4 to 20-week-old age groups, gilts and sows. Blood was analysed using the IDEXX HerdChek® PRRS X3 anti-PRRSv antibody ELISA test kit and IDEXX xChek Plus® software. The research employed a PRRS status categorisation approach based on sample-to-positive (S/P) ratio and coefficient of variation (CV%) of ELISA data into three categories 'good', 'moderate' and 'poor'. The average seropositive of all farms from 2019 to 2021 was 93.1%, with weaners aged 4 to 8 weeks contributing most of the seronegative among farms. The majority of swine farm status was classified as 'good' (52%). A total of 46.5% and 38.6% were classified as 'good' and 'moderate' in breeding herds, with gilts having better PRRS status than sows. In contrast, most growing herds were classified as 'moderate', accounting for 77.5% of the total farm. However, in growing herds, swine aged 12 to 20 weeks showed a better PRRS status than those aged 4 to 8 weeks. In conclusion, the PRRS serology status obtained from serology samples is similar between states; however, it varies between herds and age groups.

Keywords: coefficient of variation (CV%), ELISA, Porcine Reproductive and Respiratory Syndrome (PRRS), sample-to-positive (S/P) ratio, serological status.

ABSTRAK

Abstrak disertasi yang dikemukakan kepada Fakulti Perubatan Veterinar sebagai memenuhi sebahagian keperluan untuk kursus VPD4999- Projek Ilmiah Tahun Akhir.

KAJIAN RETROSPEKTIF STATUS SEROLOGI SINDROM REPRODUKTIF DAN PERNAFASAN PORSIN (PRRS) DI KALANGAN SAMPEL SEROLOGI YANG DISERAHKAN KEPADA FAKULTI PERUBATAN VETERINAR, UNIVERSITI PUTRA MALAYSIA (UPM) DARI TAHUN 2019 HINGGA 2021

Oleh

Amir Shauqi bin Ahmad Sukri

2022

Penyelia: Dr. Syamira Syazuana Zaini

Penyelia Bersama: Profesor Madya Dr. Ooi Peck Toung

Sindrom Reproduksi dan Pernafasan Porsin (PRRS) dalam babi adalah penyakit virus yang disebabkan oleh virus PRRS. PRRS dianggap sebagai penyakit yang berkos tinggi di seluruh dunia kerana impaknya yang teruk, seperti kehilangan pembiakan, penyakit pernafasan, penurunan kadar pertumbuhan dan peningkatan kematian. PRRSV dikelaskan kepada dua jenis: PRRSV-1 yang berleluasa di Eropah, dan PRRSV-2 yang berleluasa di Amerika dan Asia. Di Malaysia, kajian mengenai status

serologi semasa dan kelaziman PRRS dalam babi adalah terhad. Justeru, kajian ini bertujuan untuk menentukan status serologi PRRS sampel darah yang diserahkan ke Fakulti Perubatan Veterinar, UPM, dari tahun 2019 hingga 2021. Sampel darah dari 101 ladang di Johor, Melaka, Selangor, Perak dan Pulau Pinang digunakan dalam kajian ini. Sampel dibahagikan kepada kumpulan umur 4 hingga 20 minggu, induk dara dan induk betina. Darah dianalisis menggunakan kit ujian ELISA antibodi anti-PRRSv IDEXX HerdChek® PRRS X3 dan perisian IDEXX xChek Plus®. Penyelidikan menggunakan pendekatan pengkategorian status PRRS berdasarkan nisbah sampel kepada positif (S/P) dan variasi koefisien (CV%) data ELISA kepada tiga kategori 'baik', 'sederhana' dan 'lemah'. Purata seropositif semua ladang dari 2019 hingga 2021 ialah 93.1%, dengan babi berumur 4 hingga 8 minggu menyumbang sebahagian besar seronegatif di kalangan ladang. Majoriti status ladang babi diklasifikasikan sebagai 'baik' (52%). Sebanyak 46.5% dan 38.6% diklasifikasikan sebagai 'baik' dan 'sederhana' dalam ternakan pembiakan, dengan induk dara mempunyai status PRRS yang lebih baik daripada induk betina. Sebaliknya, kebanyakan ternakan yang berkembang dikelaskan sebagai 'sederhana', menyumbang 77.5% daripada jumlah ladang. Walau bagaimanapun, dalam ternakan yang semakin meningkat, babi berumur 12 hingga 20 minggu menunjukkan status PRRS yang lebih baik daripada umur 4 hingga 8 minggu. Kesimpulannya, status serologi PRRS yang diperoleh daripada sampel serologi adalah sama antara negeri; walaubagaimanapun, keputusan status serologi berbeza antara kumpulan dan kumpulan umur.

Kata kunci: ELISA, nisbah sample kepada positif (S/P), Sindrom Reproduktif dan Pernafasan Porsin (PRRS), status serologi, variasi koefisien (CV%)

LIST OF ABBREVIATIONS

AASV	American Association of Swine Veterinarians
ADG	Average daily gain
CV%	Coefficient of variation
ELISA	Enzyme-linked immunosorbent assay
FCR	Feed conversion ratio
FPV	Faculty of Veterinary Medicine
HP-PRRSV	Highly Pathogenic Porcine Reproductive and Respiratory Syndrome
IFA	Indirect fluorescent antibody
IPMA	Immunoperoxidase monolayer assay
PAMs	Porcine alveolar macrophages
PCR	Polymerase chain reaction
PEARS	Porcine Epidemic Abortion and Respiratory Syndrome
PRRS	Porcine Reproductive and Respiratory Syndrome
PRRSV	Porcine Reproductive and Respiratory Syndrome Virus
S/P	Sample-to-positive
SIRS	Swine Infertility and Respiratory Syndrome
SVN	Serum virus neutralization
UPM	Universiti Putra Malaysia
VRI	Veterinary Research Institute

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CHAPTER 1

INTRODUCTION

1.1 Background

Porcine Reproductive and Respiratory Syndrome (PRRS) produces severe epidemics in swine characterised by significant reproductive losses, respiratory sickness, decreased growth rate, and increased mortality. In the late 1980s, it was first described as a mystery swine disease in the United States (Zhou *et al*., 2021). By 1991, outbreaks with identical clinical signs were also confirmed in Western European nations. Nevertheless, no relationship was identified between the epidemics (Zimmerman *et al*., 2019). The causative agent of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) was discovered in the Netherlands in 1991 and the United States in 1992, with the strains initially isolated known as Lelystad Virus (the European prototypic strain) and Swine Infertility and Respiratory Syndrome (SIRS) virus, respectively (Guo *et al*., 2018). A previous retrospective study of sera collected between 1978 and 1982 revealed the presence of a PRRS-specific antibody as early as 1979 (Carman *et al*., 1995).

PRRS is caused by a small, encapsulated, positive-sense single-stranded RNA virus that belongs to the family Arteriviridae. Based on genetic characterisation, two closely related but antigenically and genetically distinct major genotypes with over 50% RNA sequence variation (Allende *et al*., 1999). The European strain (EU genotype, Type 1, with Lelystad virus as the prototype) represents the viruses predominantly found in Europe. In contrast, the North American strain (NA genotype,

Type 2, with VR-2332 as the prototype) is predominantly found in North America (Allende *et al*., 1999). Both genotypes have been described as independently evolving in Europe and North America, and the coexistence of both genotypes has become increasingly visible in several nations, including Malaysia, Thailand, Korea, and China (King *et al*., 2016). This virus only infected swine (*Sus scrofa*) and the collared peccary (*Pecari tajacu*), posing little to no risk to human health (Molina *et al*., 2018).

Direct contact between infected and non-infected pigs is the most common mode of PRRSV transmission, and the infection is progressing slowly. The aerosol transmission was previously reported as a crucial method of PRRSV transmission, but this has proven challenging to verify experimentally. A study reported that PRRS could also indirectly spread between infected and non-infected animals via fomites through contaminated needles, gloves and coveralls (Otake *et al*., 2002). Swine are vulnerable to PRRSV via intranasal, intramuscular, oral, intrauterine, and vaginal routes of exposure; however, the likelihood of infection varies by route (Hermann *et al*., 2005). PRRSV has been detected or isolated from serum, semen, saliva, faeces, urine, nasal swabs, oropharyngeal swabs and scrapings (Rossow *et al*., 1998). PRRSV has also been persistent in individual animals for up to 157 days after infection (Cho *et al*., 2006). PRRSV remains one of the biggest global threats to the pig-farming industry, even with vaccines being made available. Even though currently there are around 20 PRRSV vaccines on the market, their availability varies by country (Renukaradhya *et al*., 2015). However, due to the exceptionally high genetic diversity of PRRSV and its ability to regulate the host's immune response, vaccination does not provide complete protection against PRRSV infection (Renukaradhya *et al*., 2015).

1.2 Objectives

This project was conducted to determine the PRRS serological status among serology samples submitted to the Faculty of Veterinary Medicine, Universiti Putra Malaysia, from 2019 to 2021.

i. To evaluate PRRS serological status across selected states in Peninsular Malaysia;
ii. To evaluate PRRS serological status between two major production stages which are breeding and growing herds;
iii. To evaluate PRRS serological status between age groups.

1.3 Justification

Porcine Reproductive and Respiratory Syndrome is considered one of the costly diseases in the swine industry (Lunney, 2010). Despite the higher cost and severity of the disease, there is a lack of studies published related to PRRS disease status in Malaysia. This research was conducted to evaluate the PRRS serological status of blood samples submitted between 2019 to 2021 to the Faculty of Veterinary Medicine (FPV), Universiti Putra Malaysia (UPM). From 2019 to 2021, the UPM swine laboratory accumulated an extensive database of PRRS enzyme-linked immunosorbent assay (ELISA) samples from several swine farms in Peninsular Malaysia. To provide insight into the PRRS situation in Malaysia, this research was intended to make the most use of the available data.

The PRRS status classification approach proposed in this study intends to categorise Malaysian farms as “good,” “moderate” or “poor” by the IDEXX suggested PRRS classification system of ELISA data. By utilising a standardised classification approach to assess the PRRS status of individual farms, we could facilitate communication among swine industry stakeholders, especially swine farmers and farm veterinarians. As a result, the existing PRRS disease management and prevention efforts can progress more systematically. This approach uses ELISA data and accounts for sample-to-positive (S/P) ratios and coefficient of variation (CV%). Swine farmers may be motivated to comply with this PRRS control measures initiative due to the affordable cost of ELISA testing and the clarity of the categorisation system. Besides, farmers may benefit from this categorisation system by using it to determine the production stages most affected by PRRS and then perform a SWOT analysis on their current management practices, including their PRRS vaccination plan. Strong preventive measures might be enforced throughout key manufacturing phases to reduce PRRS morbidity and death.

1.4 Hypotheses

This study evaluated three hypotheses about swine serology samples taken from several swine farms in Peninsular Malaysia and submitted between 2019 and 2021 to the Faculty of Veterinary Medicine, Universiti Putra Malaysia.

i. PRRS serological status varies across the states in Peninsular Malaysia;

ii. PRRS serological status varies between growing and breeding herds;

iii. PRRS serological status varies between age groups.

CHAPTER 2

LITERATURE REVIEW

2.1 History, Aetiology and Epidemiology

The Porcine Reproductive and Respiratory Syndrome (PRRS) was first discovered in the United States in 1987 with clinical manifestations of severe reproductive losses in late gestational sows, increases in the number of weak live-born pigs, severe pneumonia in neonatal and nursery pigs, reductions in growth performances, and increased mortality (Hill, 1990). By 1990, the disease had been diagnosed in 11 states in the United States and two Canadian provinces (Hill, 1990). The disease also showed up in Germany in 1990 with a similar clinical syndrome and expanded rapidly through Western Europe. It was previously known as mysterious swine sickness due to the inability to identify the causative agent. PRRS was also referred to as Blue Ear Disease, Swine Infertility and Respiratory Syndrome (SIRS), or Pig Epidemic Abortion and Respiratory Syndrome (PEARS). The causative agent was then discovered as a virus and named the Lelystad virus since it was initially isolated at the Central Veterinary Institute in Lelystad (Wensvoort *et al*., 1991).

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is classified in the order Nidovirales, in the family Arterividae, from the genus Arterivirus RNA viruses. PRRSV is an encapsulated, single-stranded, positive-sense RNA virus ranging in size between 50 and 65 nm diameter. PRRSV has two primary prototypes: the European isolate (Lelystad virus, LV) and the North American isolate (VR-2332), representing two genotypes with antigenic differences—type 1 and type 2, respectively (Nelson *et al*., 1993). Both strains have a worldwide distribution, with PRRSV-1

predominant in Europe and PRRSV-2 predominant in the America and Asia (Zimmerman *et al.*, 2019). PRRSV-1 has been subdivided into four subtypes. PRRSV-1 subtype 1 (PRRSV-1.1) is prevalent across Europe, but PRRSV-1.2, 1.3, and 1.4 are mostly seen in Eastern Europe (Stadejek, 2013). The infection caused by PRRSV-1.3 is characterised by a higher body temperature, more severe clinical signs, and lung pathology, causing it even more pathogenic than PRRSV-1.1 (Morgan, 2013).

In China, the highly pathogenic PRRSV (HP-PRRSV) strains with higher pathogenicity were initially discovered in 2006 after an epidemic in which 2 million pigs were infected and 400,000 died (Tian, 2007). It is known that HP-PRRSV was derived from a type 2 (North American) PRRSV strain that was already circulating in China. This highly virulent strain of PRRSV rapidly spread to Southeast Asian countries such as Vietnam, Thailand, Laos, and Malaysia (OIE, 2009).

2.2 Situation in Malaysia

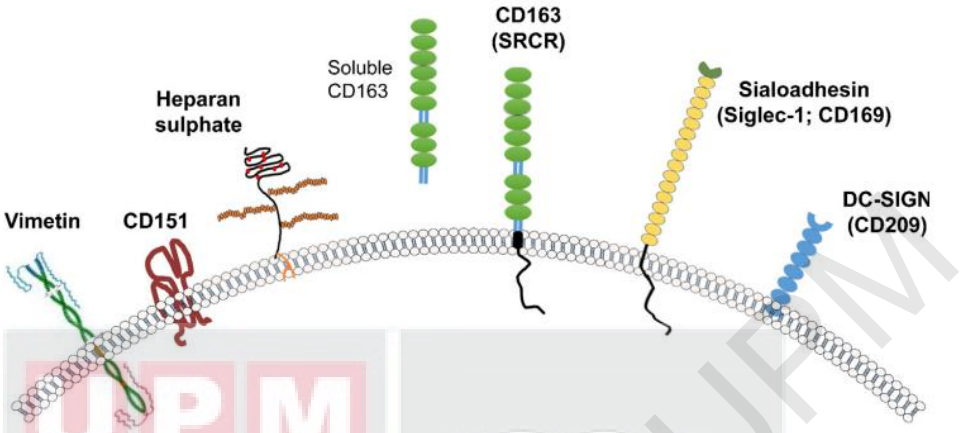
As early as 1995, a condition remarkably similar to PRRSV was seen in numerous pig farms in Malaysia (Too, 1995). PRRSV was found in 12 of the 27 tissue samples taken from 11 farms. The PRRSV genomes isolated from the 12 PRRSV-positive tissue homogenates were all Type 2 PRRSV, with no Type 1PRRSV found (Ling, 2012). However, a study in 2013 involving 16 Malaysian farms revealed the presence of positive of both Type 1 PRRSV and Type 2PRRSV (Jaganathan *et al.*, 2013). According to the findings of the study, the selected pig farms have a higher prevalence of Type 2 PRRSV, which is a US strain (Jaganathan *et al.*, 2013). Furthermore, according to research that was conducted utilising the ELISA test and molecular detection method, positive PRRSV was discovered in swine samples

submitted to Veterinary Research Institute (VRI) from 2014 to 2017 (Aisya *et al*., 2018). Similarly, a serology study done in a few swine farms across regions in Malaysia reported high seroprevalence with 98%. A high seroprevalence for PRRSV may indicate an endemic status of PRRSV in domestic pigs in Malaysia (Yee *et al*., 2018).

2.3 Pathogenesis of PRRS

Entry of the virus into its host cell is the first essential stage in the infection process. PRRSV has a very narrow cell tropism, with porcine alveolar macrophages (PAMs) as its primary target cells for PRRSV infection (Duan *et al*., 1997). This restricted cell tropism of a virus is caused by the presence of specific entry mediators in the target cells. As shown in Figure 1, there are at least six molecules described as potential cellular receptors for PRRSV that includes heparan sulphate, vimentin, CD 151, CD 163, sialoadhesin (CD 169) and DC-SIGN (CD 209) (Zhang, 2015). Among these 6 PRRSV molecular receptors, CD 163 and sialoadhesin have been widely studied. Many findings suggest that PRRSV only replicates in a subset of monocytes or macrophages. This can be supported by an in-vitro study conducted by Duan *et al*. (1997) indicated that PRSV replication was present in freshly isolated porcine alveolar macrophages, but there was no detection of PRRSV replication in porcine peritoneal macrophages or porcine blood monocytes. Furthermore, it is also known that immature macrophage and macrophage progenitor stem cells are not susceptible to PRRSV infection. This was especially evident in another study by Duan *et al*. (1997), where replication of PRRSV could not be seen in the bone marrow cells of PRRSV-infected pigs.

Figure 1. 6 molecular receptors that have been reported as cellular receptor for PRRSV which are heparan sulphate, vimentin, CD 151, CD 163, sialoadhesin (CD 169) and DC-SIGN (CD 209) (Zhang, 2015).



Heparan glycosaminoglycans sulphate act as a PRRSV attachment factor crucial in the virus initial binding on the macrophage surface. The previous study stated that this heparan sulphate does not involve in any virus internalization but concentrates virus particles at the surface for subsequent binding to one or more receptors involved in virus internalization (Bernfiel *et al*., 1999). This is followed by the gradual increase in the interaction with sialoadhesin (Sn). It has been hypothesized that this sialoadhesin (Sn) protein is exclusive to specific macrophages and functions as a receptor for the PRRSV internalisation process in both European and American strains (Vanderheijden *et al*., 2003). This can be supported by research conducted in which it is reported that porcine sialoadhesin (pSn) specific antibodies were previously demonstrated to be internalised by primary macrophages, demonstrating that sialoadhesin is an internalization-capable receptor (Vanderheijden *et al*., 2003). The next phase involves the receptor CD 163, which is the most specific receptor for PRRSV entrance and infection. During this phase, the viral glycoproteins designated GP2 and GP4 will bind to CD163. Transfection with CD163 renders non-permissive cells vulnerable to PRRSV infection, hence demonstrating CD163 important function

(Calvert *et al*., 2007). The viral genome must be released into the cytoplasm of the target cell once it has been internalised in order for productive infection to occur (Van *et al*., 2010).

2.4 Clinical Symptoms of PRRS

PRRS infection's predominant symptoms are reproductive failures, such as third-trimester abortions, preterm parturition, increased foetal loss rates, diminished growth performance, and increased death due to respiratory illness (Cho *et al*., 2006). However, the severity of the disease tends to vary across isolates, and experimentally infected animals have shown diversity in the pathogenicity of PRRSV virulence. As per studies, pigs experimentally infected with nine distinct isolates of PRRSV from the United States exhibited significant variations in clinical illness, rectal temperatures, and gross and histological lung lesions. In these experiments, low-virulent animals infected with isolates exhibited temporary fever, dyspnea, and tachypnea. Infection with highly virulent isolates resulted in laboured breathing, fever, lethargy, and anorexia. In addition, research has shown that the effect on reproductive performance may vary by isolate (Cho *et al*., 2006). Lastly, the severity of clinical PRRS may be connected to increased viral levels in the blood and tissues due to the highly virulent isolate enhanced capacity to reproduce in the host (Johnson *et al*., 2004).

Other studies concluded that infection of vulnerable pigs with highly virulent isolates of PRRSV led to more extended periods of viremia, increased severity of clinical symptoms and mortality. Furthermore, it caused markedly increased viral loads in blood and tissues than infection with mildly virulent isolates or cell culture-adapted (Johnson *et al*., 2004). Other variables, including the age of the animal and

bacterial co-infection, might impact viral replication and clinical manifestations. Younger animals (4–8 weeks) infected with PRRSV showed more prolonged viremia and higher replication rates in macrophages than older pigs (16–24 weeks). Furthermore, several bacterial agents, including Bordetella bronchiseptica and Mycoplasma hyopneumoniae, seemed to increase the duration and severity of pneumonia and lung lesions caused by PRRSV. In addition, PRRSV infection increased pig susceptibility to Streptococcus suis type 2 infection and exacerbated Salmonella choleraesuis infection (Cho *et al*., 2006).

2.5 Transmission of PRRS

Swine are the only known hosts of PRRSV and are vulnerable to infection. Parenteral, intranasal, intramuscular, oral, intrauterine, and vaginal exposure have all been recognised as possible routes of PRRSV transmission (Benfield *et al*., 1999). Research revealed that the minimal infectious dose for each transmission mode varies depending on anatomical location (Yoon *et al*., 1999). Parental exposure on a farm includes regular husbandry operations such as ear notching, tail docking, teeth trimming, and administering medications and immunizations through needles. Furthermore, since PRRSV persists in oropharyngeal secretions for weeks after infection, fighting amongst pigs may play a role in PRRSV transmission across herds (Zimmerman *et al*., 2019). PRRSV may also be transferred vertically from viraemic mothers to foetuses, leading to foetal mortality or the delivery of piglets that are either weak or seem healthy (Christianson *et al*., 1992). PRRSV may be spread indirectly via equipment, clothing, food, and drink. After infection in a herd, PRRSV-infected pigs will shed the virus through saliva, nasal secretions, urine, sperm, breast fluids, and faeces. According to studies, the importation of PRRSV-positive animals and sperm

for artificial insemination is a substantial risk factor for PRRSV infection on farms (Dee *et al*., 2022). Regional swine density and herd size are other risk factors that have led to PRRS outbreaks. It is hypothesised that high-density areas are more likely to be exposed to and infected with PRRSV by airborne transmission from surrounding affected areas (Velasova *et al*., 2012).

2.6 Diagnostic Test of PRRS by ELISA

Any herd with reproductive illness in breeding swine or respiratory disease in pigs of any age is suspected of having PRRSV infection. Some PRRSV strain infections have also been linked to neurological symptoms such as shivering, lameness, and muscular spasms. Additionally, breeding herds with clinically active PRRSV may have more stillbirths, preterm births, early farrowing, and abortions. However, the absence of clinical symptoms does not indicate that a community is PRRSV-free. Laboratory tests can be used to provide the additional assurance that is required (Zimmerman *et al*., 2019).

ELISA is the most common method used for the serological detection of anti-PRRSV antibodies. It is a quick and affordable way to identify and monitor the status of a herd PRRS virus infection. PRRSV antibodies can be found in the pig's serum and oral fluid samples. Many ELISAs have recently been created, with the majority of them detecting antibodies against both PRRSV types 1 and 2 such as iNgezim PRRS 2.0, Priocheck® PRRSV Ab porcine and CIVTEST suis PRRS E/S PLUS (Sattler *et al*., 2014). However, certain ELISAs are designed to be able to identify type 1 antibodies from type 2 antibodies (Sattler *et al*., 2014).

This study collected data from samples tested with the IDEXX PRRS X3 Ab Test (IDEXX, Westbrook, USA). The sensitivity and specificity of the IDEXX PRRS X3 Ab Test (IDEXX, Westbrook, USA) are 98.8% and 99.9%, respectively, according to the manufacturer. It is the test that receives the most citations and is widely regarded as the gold standard among ELISAs for the discovery of antibodies against PRRSV (Sattler *et al*., 2014).

ELISA does, however, have several drawbacks. The antibody preparation is time-consuming and costly because it demands a sophisticated technique and expensive culture media to produce a particular antibody (Sakamoto *et al*., 2018). If the surface of the microtiter plate that is immobilised with the antigen is not sufficiently blocked, there is also a considerable risk of receiving erroneous positive or negative results. Additionally, as the antibody is a protein, its instability requires proper refrigerated transport and storage (Sakamoto *et al*., 2018). Other tests used to detect antibodies to PRRSV are immunoperoxidase monolayer assay (IPMA), indirect fluorescent antibody (IFA), and serum virus neutralization (SVN).

2.7 Economic Impact of PRRS

PRRS affects both breeding and growing herd populations on a farm, causing a decrease in reproductive health, growth rate and an increase in mortality. These implications are estimated to result in an annual economic impact of $66.75 million on breeding herds and $493.57 million on growing pig populations (Neumann *et al*., 2015). This results in an annual financial burden for swine farmers in the United States of around $560.32 million (Neumann *et al*., 2015). For the breeding herd, it is hypothesised that PRRS-affected herds have a higher rate of abortion and an increased number of weak live-born pigs. This decreases the number of litters per sow every year

and creates additional labour and veterinary costs (Nathues *et al*., 2017). This increased expense accounts for any further artificial inseminations that are required.

Furthermore, PRRS potentially decreases the average weight of piglets weaned per year and subsequently decreases the revenue for weaners sold. Likewise, in growing herds, a percentage of clinically affected PRRS swine will die of the disease, increasing overall mortality. As a result, the number of swine produced on the farm will decrease and subsequently decrease farm revenue. Affected herds will have extra labour costs to care for ill pigs and additional veterinary expenses due to increased consultative visits by the veterinarian and treatment to treat secondary infections. Furthermore, affected pigs have increased feed conversion ratio (FCR) and decreased average weight gain (ADG) (Nathues *et al*., 2017). Hence, more days on the farm are required to achieve the necessary live weight for sale.

2.8 PRRS Herd Classification System

The PRRS herd classification system was established in 2010 by the American Association of Swine Veterinarians (AASV) to ease communication between veterinarians, swine producers, and others in the swine industry. According to Holtkamp *et al* (2011), this PRRS herd classification method was constructed primarily on the basis of two criteria: viral shedding and prior exposure. A few numbers of tests, such as polymerase chain reaction (PCR) or viral isolation, were used to evaluate virus shedding. Nevertheless, PCR is the test of choice. There are three types of PRRS viral shedding: negative, positive, and unclear. Positive and negative viral shedding, on the other hand, represent the presence and absence of viral shedding in the herd, respectively. Positive shedding status was also assumed if the data on the shedding status was unavailable. In contrast, an uncertain shedding status is indicated

when diagnostic results in tested herds suggest a negative shedding status without sufficient confidence to support a negative shedding status. In order to determine viral exposure, an ELISA test was used. Virus exposure was categorised as either positive or negative. Positive exposure indicates prior exposure to PRRSV, whereas negative exposure shows a lack of past PRRSV exposure.

Table 1 illustrates the PRRS herd classification system comprised of four categories: Positive Unstable (I), Positive Stable (II), Provisional Negative (III), and Negative (IV). Category II was further divided into II-A for herds not undergoing elimination and II-B for those undergoing elimination. Classification of positive unstable herds (Category I) when both their shedding and exposure statuses are positive. Herds demonstrating a clinical PRRS outbreak and recurrent viral shedding will likewise be classified as Category I. Positive stable (Category II) have uncertain viral shedding and positive exposure status. The positive stable is established when there are no clinical symptoms and no detectable viraemia in weaning-age pigs for at least 90 days. Weaning age-pigs must be sampled every thirty days and have at least four consecutively negative PCR herd tests. Exposure status remains positive possibility due to animals still infected and later shedding the virus cannot be ruled out. Category II-A herds are not going elimination while II-b herds undergo elimination. The elimination method begins when the final seropositive breeding replacements are introduced or when the last intentional exposure to any live PRRSV, wild-type, or vaccinations occurs (live or killed or both). Provisional negative (Category III) herds are characterised as having a negative shedding status. The sustained introduction of negative breeding replacements without seroconversion to the PRRSV is necessary for a herd to be classed as Category III. The absence of seroconversion in imported animals is a sufficient indication that PRRSV is no longer

being spread in the herd. Negative breeding replacements must have had exposure to previously positive animals and must remain seronegative by ELISA for at least 60 days after entering the breeding herd. For category IV, the shedding status and exposure must be negative. Category IV also includes new premises startups populated with negative breeding replacements or premises that were totally depopulated and repopulated with seronegative negative breeding replacements. To determine the negative exposure status of the herd, a negative ELISA herd test in adult breeding animals is necessary at least 30 days after the premises are populated. Hence, a standardised approach to the PRRS herd classification system based on a set of relevant criteria is required to implement regional and national PRRSV management and eradication.

Table 1. PRRS Herd Classification System based on Shedding and Exposure Status (Holtkamp *et al*, 2011).

Herd category		Shedding status	Exposure status
Positive unstable	I	Positive	Positive
Positive stable	II-A	Uncertain	Positive
	II-B	Uncertain	Positive
Provisional negative	III	Negative	Positive
Negative	IV	Negative	Negative

CHAPTER 3

MATERIALS AND METHODS

3.1 Data Collection

The data for this retrospective study was sourced from pooled porcine blood samples collected from swine farms in Peninsular Malaysia which were submitted to the Faculty of Veterinary Medicine, Universiti Putra Malaysia for PRRS diagnosis. The sample size of this study was recorded at 101 farms with 3689 samples from 2019 to 2021. The samples were tested using a commercial IDEXX Herdchek®PRRS X3 PRRSv antibody test kit at the time of receiving. The ELISA report was then evaluated using the IDEXX xChek Plus® programme (IDEXX Laboratories Inc., Maine, USA).

The dataset was organised into five states that were located in Peninsular Malaysia which are as follows:

i. Johor;
ii. Melaka;
iii. Selangor;
iv. Perak;
v. Penang.

The dataset was then divided into two broad production steps and further sub-divided as follows:

i. Growing herd
    a. Sows;
    b. Gilts.

ii. Breeding herd

a. 4 weeks;

b. 8 weeks;

c. 12 weeks;

d. 16 weeks;

e. 20 weeks.

Two features of ELISA interpretation were noted down from the ELISA reports produced using IDEXX xChek Plus® software (IDEXX Laboratories Inc., Maine, USA) which were sample-to-positive (S/P) ratio and coefficient variance (CV%) values. There is a copy of the ELISA report in *Appendix 1*.

3.2 IDEXX Suggested PRRS Classification System

As indicated in Table 1, a PRRS status classification system was developed by IDEXX based on the sample-to-positive ratio and percentage of coefficient variance values. As illustrated by IDEXX, both breeding and growing herds have different range of status classifications.

Table 2. IDEXX Suggested PRRS Status Classification System for Herds Based on Sample-to-Positive Ratio and Coefficient Variance Values.

Breeding Herd		**Status**	**Growing Herd**	
S/P Ratio	**CV (%)**		**S/P Ratio**	**CV (%)**
1.0 – 2.4	< 40.0	Good	0.4 – 2.4	< 40.0
<1.0, or >2.4	< 40.0	Moderate	<0.4, or >2.4	< 40.0
1.0 – 2.4	> 40.0	Moderate	0.4 – 2.4	> 40.0
<1.0, or >2.4	>40.0	Poor	<0.4, or >2.4	>40.0

Based on their S/P ratio means and CV%, the production stages were categorised as "good", "moderate" or "poor" As suggested by IDEXX a herd must meet the S/P ratio and CV% requirements to be rated "good". If one of the two conditions is met it is classified as "moderate". If a herd fails to meet both criteria, it will be classified as "poor". As illustrated in Table 2, the example of application of IDEXX suggested PRRS classification system. For example, for JHR01 breeding farm, the S/P ratio is 0.850 which is not between the range of 1.0 to 2.4 and the coefficient variation percentage is less than 40%. Thus, the PRRS status of the breeding herd is classified as "moderate". In contrast to the growing herd, the S/P ratio is 0.820 which is between the range of 0.4 to 2.4 and the coefficient of variation percentage is less than 40%, thus the PRRS status of JHR 01 growing herd is classified as "good".

Table 3. Example of Application of IDEXX Suggested PRRS Classification System.

Farm ID		JHR01	MLK02	SEL01
Growing Herd	**S/P Ratio**	0.850	2.038	1.780
	CV%	22.8	26.8	46.9
	Status	Moderate	Good	Moderate
Breeding Herd	**S/P Ratio**	0.820	2.971	2.256
	CV%	30.6	60.4	41.9
	Status	Good	Poor	Moderate

An overall PRRS status of a farm was derived after obtaining the PRRS status of both breeding and growing herds. As indicated in Table 3, IDEXX suggested overall PRRS status of a farm will based on the PRRS status of breeding and growing herds. An example of the application of the overall PRRS status of a farm is illustrated as follows in Table 4. As indicated in Table 5, in which farm ID JHR 01 was classified as "moderate" in breeding herd while good in growing herds. Thus, based on the IDEXX overall PRRS status classification system of a farm, JHR 01 was classified as "good".

Table 4. IDEXX Suggested Overall Farms PRRS Status Classification System Based on Production Stage of PRRS Status.

PRRS Status		
Growing Herd	**Breeding Herd**	**Overall Farm**
Good	Good	Good
Good	Moderate	Good
Moderate	Good	Good
Good	Poor	Moderate
Poor	Good	Moderate
Moderate	Moderate	Moderate
Moderate	Poor	Moderate
Poor	Moderate	Moderate
Poor	Poor	Poor

Table 5. Example of Application of IDEXX Suggested Overall Farms PRRS Status Classification System.

Farm ID		JHR01	MLK02	SEL01
Growing Herd	**S/P Ratio**	0.850	2.038	1.780
	CV%	22.8	26.8	46.9
	Status	Moderate	Good	Moderate
Breeding Herd	**S/P Ratio**	0.820	2.971	2.256
	CV%	30.6	60.4	41.9
	Status	Good	Poor	Moderate
Overall Farm Status		Good	Moderate	Moderate

3.3 Statistical Analysis

The statistical programme IBM®SPSS Statistics 27 was used to analyse data for this research. Chi-squared test were used to analyse the PRRS status between states, herds and age groups independently. $P < 0.05$ and a 95% confidence interval were used to determine the statistical significance of each test.

CHAPTER 4

RESULTS

4.1 Farms and Samples Distribution

The distribution of farms and samples was evenly spread from 2019 to 2021, as shown in Table 6. 101 farms in total, accounting for 34 farms, 36 farms, and 32 farms in the years 2019, 2020, and 2021, respectively, were contributed to this study. The distribution of samples for the year 2019, 2020, and 2021 was likewise equal, with 1132, 1335, and 1222 samples, respectively. These represent the 3689 total samples employed in this study.

Table 6. Number of Farms and Samples Distribution across the Year among Serology Samples Submitted to FPV, UPM in 2019 to 2021.

Year	Number of Farms	Number of Samples
2019	34	1132
2020	36	1335
2021	32	1222
Total	101	3689

4.2 Seroprevalence

Overall, as illustrated in Figure 2, 93.1% of all 101 farms examined between 2019 and 2021 were seropositive, whereas just 7% were seronegative. Figure 3 depicts the seropositive rate exceeding 90% across the year. As indicated in Figure 4, the seropositive rates in all states remained over 90%, with Selangor having the highest seropositive (97%), followed by Penang (96%), Melaka (94%), Johor (91%), and Perak (91%).

Figure 2. PRSS Seroprevalence of Serology Samples Submitted to FPV, UPM between 2019 to 2021.

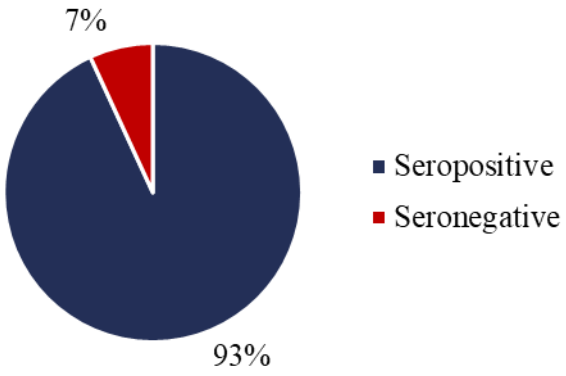


Figure 3. PRRS Seroprevalence of Serology Samples Across the Year among Serology Samples Submitted to FPV, UPM in 2019 to 2021.

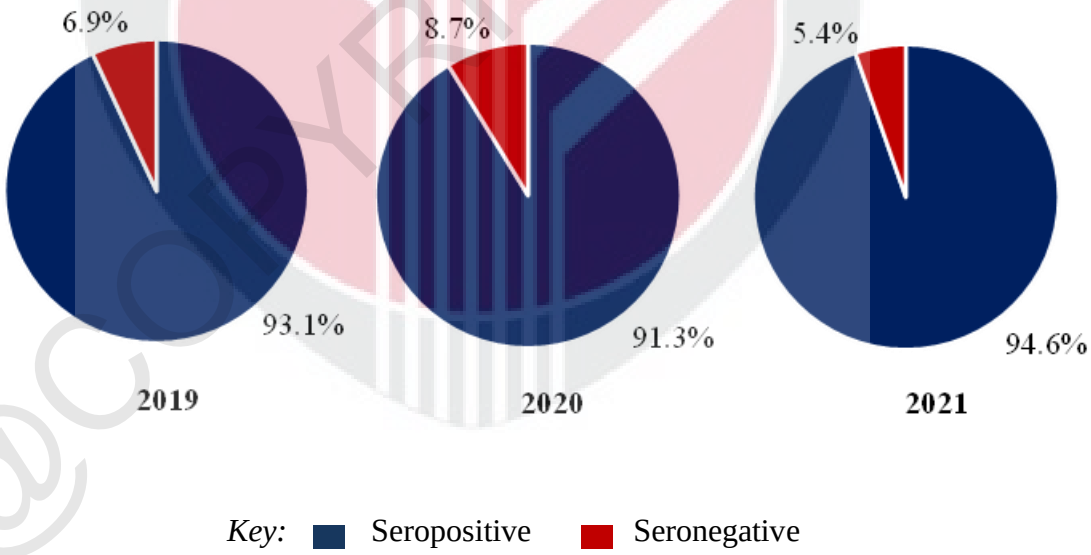
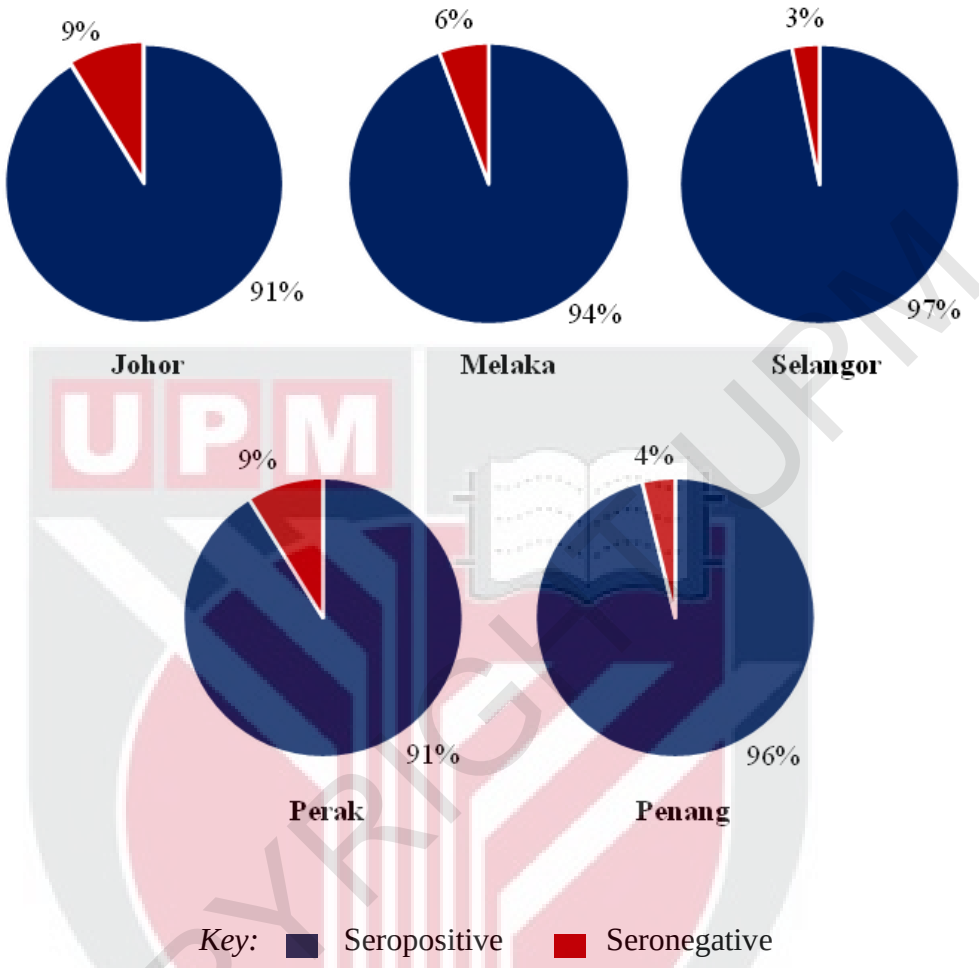


Figure 4. PRRS Seroprevalence of Serology Samples Across States among Serology Samples Submitted to FPV, UPM in 2019 to 2021.



4.3 PRRS Status Classification

Considering the S/P ratio and CV% values, each production stage (breeding and growing herd) was classified as “good”, “moderate” and “poor”. Then, the farm classification will be based on both breeding herds and growing herds as illustrated in Table 7. This would provide a gross picture of the PRRS serological status on a farm. To provide more insight, further PRRS classification was done on the age group in each farm as illustrated in Table 8.

Table 7. Summary of PRRS Status of Farms among Serology Samples Submitted to FPV, UPM in 2019 to 2021.

State	Farm Id	Herd type		
		Farm	Breeding	Growing
		2019		
Johor	J1	Mod	Poor	Mod
	J2	Good	Good	Mod
	J3	Mod	Mod	Mod
Malacca	M1	Mod	Mod	Mod
Penang	PG1	Mod	Mod	Mod
	PG2	Good	Good	Mod
	PG3	Good	Good	Mod
	PG4	Good	Good	Mod
	PG5	Good	Good	Good
Perak	PK1	Good	Good	Good
	PK2	Mod	Mod	Mod
	PK3	Good	Good	Mod
	PK4	Mod	Mod	Mod
	PK5	Good	Good	Good
	PK6	Mod	Poor	Mod
	PK7	Mod	Mod	Mod
	PK8	Mod	Mod	Mod
	PK9	Good	Good	Mod
	PK10	Mod	Mod	Mod
	PK11	Poor	Poor	Poor
	PK12	Mod	Mod	Mod
	PK13	Good	Good	Mod
	PK14	Good	Good	Mod
	PK15	Good	Good	Good
	PK16	Good	Good	Good
	PK17	Good	Good	Good
Selangor	S1	Good	Good	Mod

	S2	Mod	Mod	Mod
	S3	Good	Good	Mod
	S4	Mod	Mod	Mod
	S5	Good	Good	Good
	S6	Good	Good	Mod
	S7	Good	Good	Good
2020				
Johor	J4	Good	Good	Mod
	J5	Mod	Mod	Mod
Malacca	M2	Mod	Mod	Mod
	M3	Mod	Mod	Mod
Penang	PG6	Mod	Poor	Mod
	PG7	Good	Mod	Good
	PG8	Mod	Mod	Mod
	PG9	Good	Good	Mod
	PG10	Mod	Mod	Mod
	PG11	Mod	Mod	Mod
	PG12	Mod	Mod	Mod
	PG13	Good	Good	Mod
Perak	PK18	Mod	Poor	Good
	PK19	Good	Mod	Good
	PK20	Mod	Poor	Mod
	PK21	Mod	Poor	Mod
	PK22	Mod	Mod	Mod
	PK23	Mod	Mod	Mod
	PK24	Good	Good	Mod
	PK25	Good	Good	Mod
	PK26	Good	Good	Mod
	PK27	Good	Good	Mod
	PK28	Mod	Mod	Mod
	PK29	Mod	Mod	Mod
	PK30	Good	Mod	Good
	PK31	Good	Good	Mod
	PK32	Mod	Poor	Mod
	PK33	Mod	Mod	Mod
	PK34	Mod	Mod	Mod
	PK35	Mod	Poor	Mod
	PK36	Good	Good	Good
Selangor	S8	Good	Good	Mod
	S9	Good	Good	Mod
	S10	Good	Good	Mod
	S11	Good	Good	Mod
	S12	Mod	Mod	Mod
2021				
Johor	J6	Good	Good	Good

	J7	Mod	Mod	Mod
	J8	Mod	Mod	Mod
	J9	Mod	Poor	Mod
	J10	Mod	Mod	Mod
	J11	Good	Good	Mod
	J12	Good	Mod	Good
	J13	Mod	Mod	Mod
Malacca	M4	Good	Good	Mod
	M5	Good	Good	Mod
Penang	PG14	Mod	Poor	Mod
	PG15	Good	Good	Mod
	PG16	Mod	Mod	Mod
	PG17	Good	Good	Good
	PG18	Good	Good	Mod
Perak	PK37	Mod	Poor	Mod
	PK38	Mod	Poor	Mod
	PK39	Mod	Mod	Mod
	PK40	Good	Mod	Good
	PK41	Mod	Mod	Mod
	PK42	Mod	Mod	Mod
	PK43	Good	Good	Mod
	PK44	Good	Good	Mod
	PK45	Good	Good	Mod
	PK46	Good	Good	Mod
	PK47	Good	Good	Good
	PK48	Good	Mod	Good
	PK49	Mod	Poor	Mod
	PK50	Mod	Poor	Mod
Selangor	S13	Good	Good	Good
	S14	Good	Good	Good
	S15	Good	Good	Mod

Key: Good Moderate Poor

Table 8. Summary of PRRS Status of Age Groups among Serology Samples Submitted to FPV, UPM in 2019 to 2021.

State	Farm Id	Age Group Status						
		Gilt	P1P5	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks
2019								
Johor	J1	Mod	Mod	Mod	Mod	Good	Good	Mod
	J2	Good	Mod	Poor	Poor	Good	Good	Good
	J3	Good	Mod	Good	Mod	Mod	Mod	Good
Malacca	M1	Good	Mod	Good	Good	Mod	Good	Good
Penang	PG1	Good	Mod	Mod	Good	Good	Good	Good
	PG2	Good	Good	Mod	Good	Good	Good	Good
	PG3	Good	Good	Mod	Poor	Poor	Good	Good
	PG4	Good	Good	Good	Mod	Good	Mod	Good
	PG5	Good	Good	Good	Good	Mod	Good	Good
Perak	PK1	Good	Good	Good	Good	Good	Good	Good
	PK2	Good	Mod	Mod	Mod	Good	Good	Good
	PK3	Mod	Good	Mod	Good	Good	Mod	Good
	PK4	Good	Poor	Mod	Poor	Good	Good	Good
	PK5	Good	Good	Mod	Good	Good	Good	Good
	PK6	Poor	Poor	Mod	Mod	Good	Good	Good
	PK7	Mod	Mod	Mod	Mod	Good	Good	Good
	PK8	Mod	Mod	Poor	Good	Good	Good	Good
	PK9	Mod	Good	Poor	Mod	Good	Good	Good
	PK10	Good	Poor	Mod	Good	Good	Mod	Good
	PK11	Mod	Poor	Poor	Mod	Poor	Poor	Mod
	PK12	Good	Mod	Mod	Good	Good	Good	Good
	PK13	Good	Good	Mod	Mod	Good	Good	Good
	PK14	Good	Mod	Mod	Poor	Good	Good	Good
	PK15	Good	Good	Good	Good	Good	Good	Good
	PK16	Good	Mod	Good	Good	Good	Good	Good
	PK17	Good	Good	Mod	Good	Good	Good	Good
Selangor	S1	Good	Good	Good	Mod	Good	Mod	Mod
	S2	Good	Good	Mod	Mod	Mod	Good	Mod
	S3	Mod	Good	Mod	Mod	Good	Good	Good
	S4	Mod	Poor	Mod	Poor	Good	Good	Mod
	S5	Good	Mod	Mod	Good	Good	Good	Good
	S6	Good	Good	Mod	Mod	Good	Good	Good
	S7	Good	Good	Mod	Good	Good	Good	Good
2020								
Johor	J4	Good	Mod	Poor	Poor	Good	Mod	Good
	J5	Good	Mod	Mod	Mod	Good	Good	Good
Malacca	M2	Good	Mod	Mod	Poor	Mod	Mod	Mod
	M3	Mod	Mod	Mod	Mod	Good	Good	Mod
Penang	PG6	Good	Poor	Mod	Mod	Good	Good	Good
	PG7	Mod	Mod	Mod	Good	Good	Good	Good

	PG8	Good	Poor	Mod	Good	Good	Good	Good
	PG9	Good	Good	Mod	Mod	Good	Good	Good
	PG10	Good	Poor	Mod	Mod	Good	Good	Good
	PG11	Good	Mod	Mod	Good	Mod	Mod	Mod
	PG12	Mod	Mod	Mod	Mod	Good	Good	Mod
	PG13	Good	Good	Mod	Mod	Good	Good	Good
Perak	PK18	Mod	Poor	Good	Good	Mod	Good	Good
	PK19	Mod	Mod	Good	Good	Good	Good	Good
	PK20	Poor	Poor	Mod	Good	Good	Good	Good
	PK21	Mod	Poor	Mod	Mod	Good	Good	Good
	PK22	Mod	Mod	Good	Good	Mod	Good	Good
	PK23	Good	Mod	Poor	Mod	Mod	Good	Good
	PK24	Good	Mod	Good	Mod	Good	Good	Good
	PK25	Good	Mod	Poor	Poor	Good	Good	Good
	PK26	Good	Good	Mod	Good	Good	Good	Mod
	PK27	Good	Good	Mod	Good	Mod	Good	Mod
	PK28	Mod	Mod	Poor	Mod	Good	Good	Good
	PK29	Good	Poor	Poor	Mod	Mod	Good	Good
	PK30	Good	Mod	Mod	Good	Good	Good	Good
	PK31	Good	Good	Mod	Mod	Mod	Mod	Good
	PK32	Good	Poor	Mod	Poor	Poor	Poor	Good
	PK33	Mod	Good	Mod	Poor	Poor	Poor	Good
	PK34	Poor	Good	Mod	Mod	Mod	Good	Mod
	PK35	Poor	Poor	Poor	Poor	Good	Good	Mod
	PK36	Good	Mod	Mod	Good	Good	Good	Good
Selangor	S8	Good	Good	Mod	Mod	Good	Good	Good
	S9	Good	Good	Mod	Mod	Good	Good	Good
	S10	Good	Good	Mod	Mod	Good	Good	Good
	S11	Good	Mod	Mod	Mod	Good	Mod	Good
	S12	Good	Poor	Poor	Poor	Poor	Good	Good
2021								
Johor	J6	Good	Mod	Mod	Good	Mod	Good	Mod
	J7	Good	Mod	Mod	Good	Good	Mod	Good
	J8	Good	Mod	Mod	Poor	Good	Mod	Good
	J9	Poor	Poor	Mod	Poor	Mod	Good	Poor
	J10	Good	Mod	Mod	Good	Good	Good	Good
	J11	Good	Good	Poor	Good	Good	Good	Good
	J12	Good	Mod	Mod	Mod	Good	Good	Good
	J13	Poor	Mod	Mod	Mod	Good	Good	Good
Malacca	M4	Good	Good	Poor	Mod	Good	Good	Good
	M5	Good	Good	Good	Good	Good	Good	Good
Penang	PG14	Mod	Poor	Mod	Good	Mod	Good	Good
	PG15	Mod	Good	Mod	Good	Good	Good	Mod
	PG16	Good	Mod	Mod	Mod	Good	Good	Mod
	PG17	Good	Mod	Mod	Good	Mod	Good	Good

	PG18	Good	Good	Good	Good	Good	Good	Good
Perak	PK37	Good	Poor	Poor	Mod	Good	Mod	Good
	PK38	Mod	Poor	Mod	Good	Good	Good	Good
	PK39	Good	Mod	Mod	Good	Good	Good	Mod
	PK40	Mod	Mod	Mod	Good	Good	Good	Good
	PK41	Poor	Good	Mod	Good	Good	Good	Good
	PK42	Mod	Good	Mod	Good	Good	Good	Mod
	PK43	Good	Good	Mod	Poor	Good	Good	Good
	PK44	Good	Good	Mod	Good	Good	Good	Good
	PK45	Good	Good	Mod	Mod	Good	Good	Good
	PK46	Good	Good	Mod	Good	Mod	Good	Good
	PK47	Good	Good	Good	Good	Good	Mod	Good
	PK48	Mod	Good	Mod	Good	Good	Good	Good
	PK49	Good	Mod	Mod	Poor	Good	Mod	Good
	PK50	Good	Poor	Good	Mod	Mod	Good	Mod
Selangor	S13	Mod	Good	Mod	Good	Good	Good	Good
	S14	Good	Good	Mod	Good	Mod	Good	Mod
	S15	Good	Good	Good	Mod	Good	Good	Good

Key: Good Moderate Poor

4.4 PRRS Status Between Age Groups

The age group of pigs in every farm was divided based on the production stage. The breeding herd was divided into gilts and sows while the growing herd was divided into 4 weeks, 8 weeks, 12 weeks, 16 weeks and 20 weeks age group. When comparing the overall PRRS status of age groups, gilts provide better PRRS status than sows in the breeding herd as shown in Figure 5. Furthermore, sows show the most "poor" PRRS status overall as high as 20%. Weaners aged 4 to 8 weeks have poorer status in the growing herd than weaners aged 12 to 20 weeks as shown in Figure 5.

Figure 5. Overall PRRS Status between Age Groups among Serology Samples Submitted to FPV, UPM in 2019 to 2021.

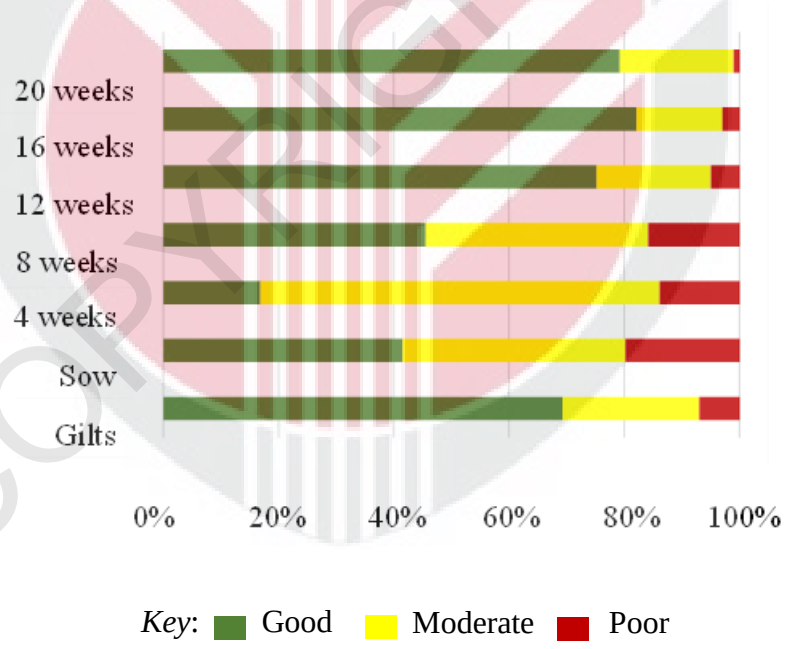
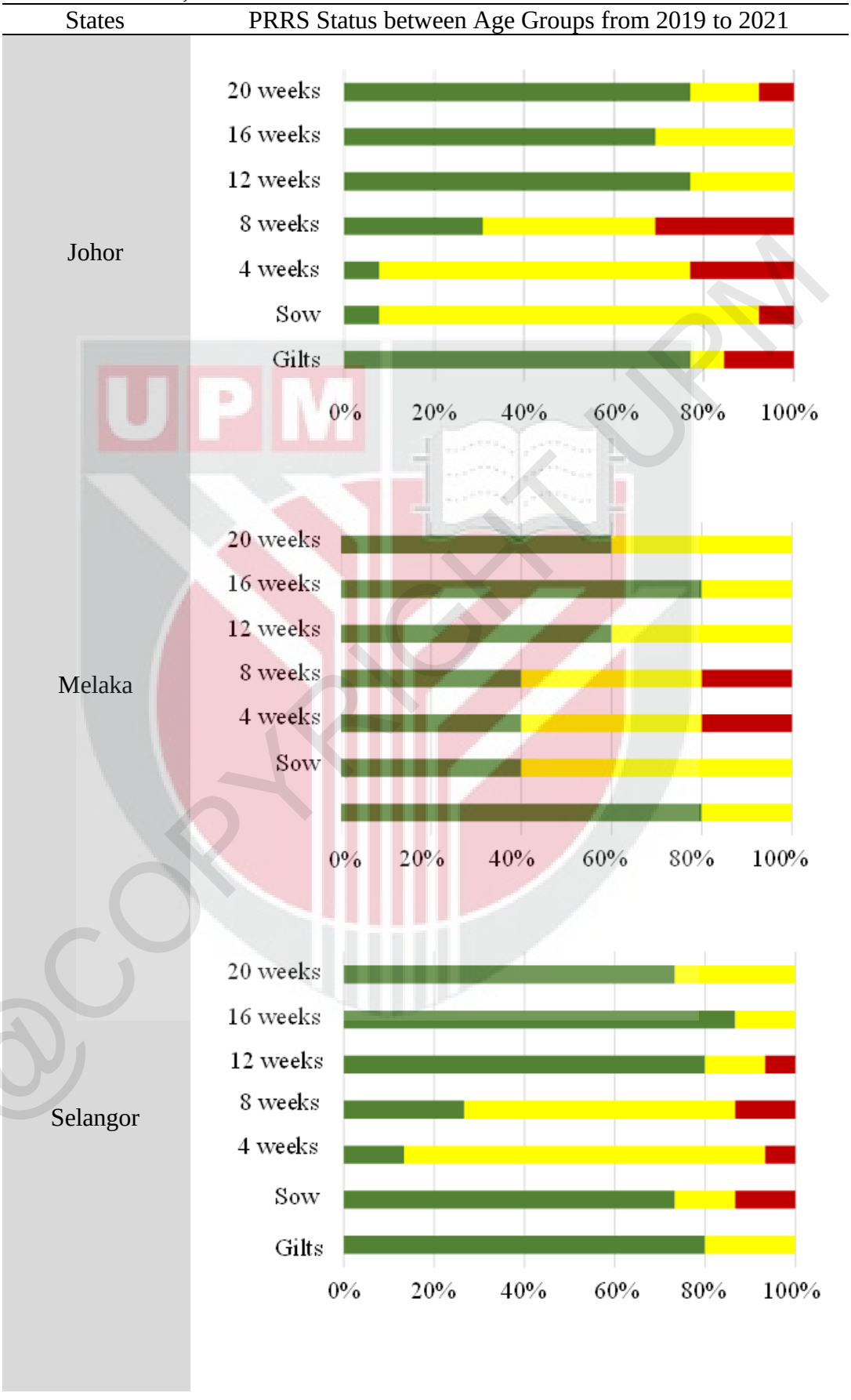
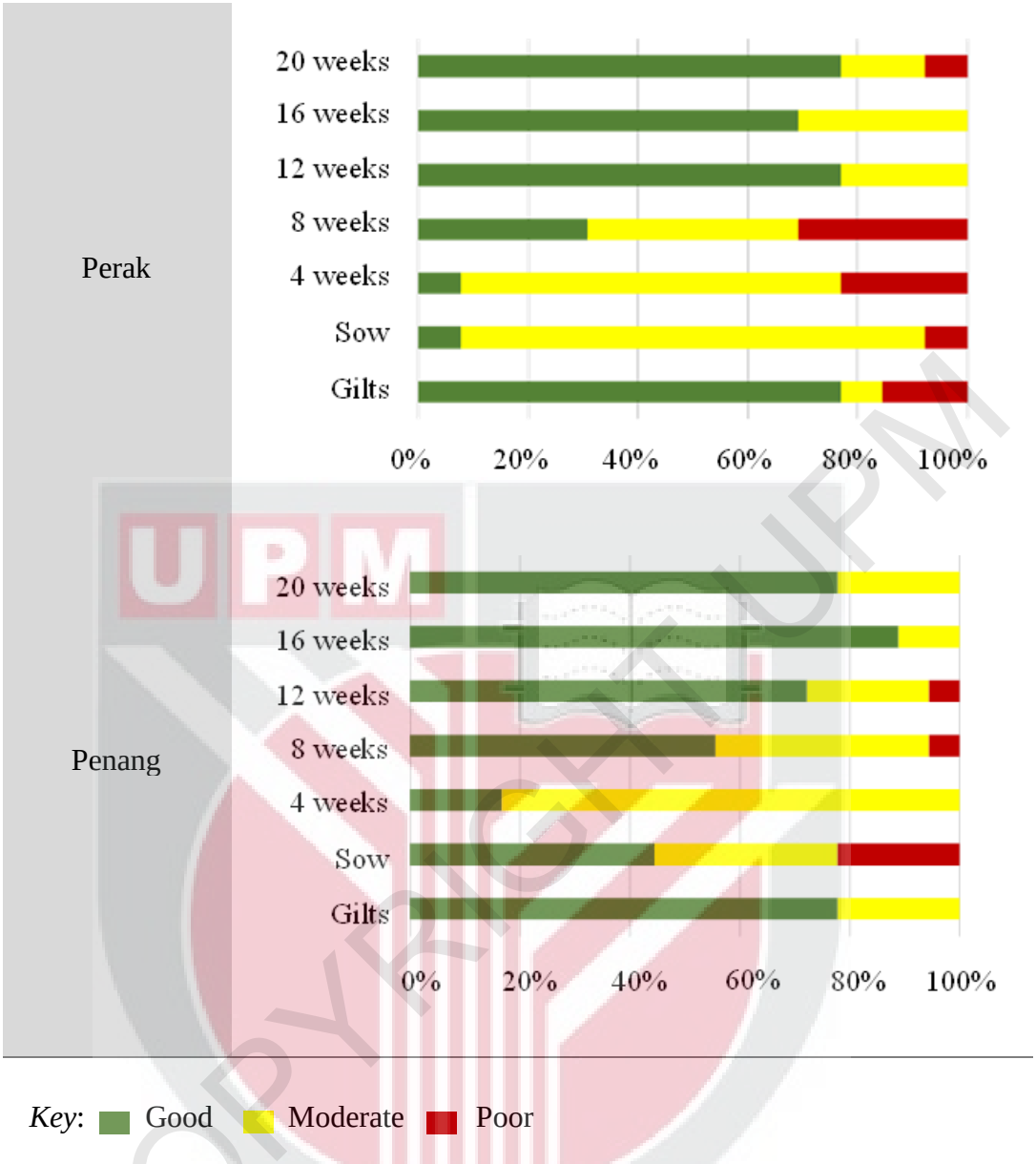


Figure 6 depicts a similar pattern of PRRS status across age groups among states, with Melaka giving the most consistent result. The PRRS status of gilts is much better than that of sows in all states except Selangor, where the difference is quite marginal.

Figure 6. PRRS Status between Age Groups across States among Serology Samples Submitted to FPV, UPM in 2019 to 2021.





To analyze the variation of PRRS status between age groups, a Chi-square analysis test was done. As proven in Table 9, the *P*-value is less than 0.05 which indicates that the variation in PRRS status between age groups is significant.

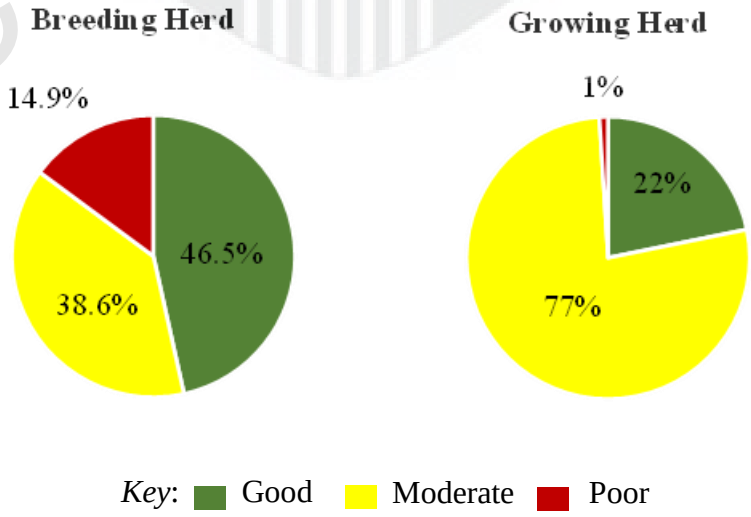
Table 9. Summary of IBM® SPSS Chi-square test statistical analysis for PRRS status between age group among serology samples submitted to FPV, UPM in 2019 to 2021.

Factors	Dependent Variable	Significant
Age Group	PRRS Status	0.000

4.5 PRRS Status Between Herds

The data on PRRS status in age group were gathered into breeding and growing herds to analyse the variation of PRRS status between the two production stages. As illustrated in Figure 7, when the breeding herd and the growing herd are compared, the breeding herd is found to have a higher percentage that fall into the "good" category (46.5%) compared to the growing herd (22%). However, in breeding herd, the fraction of "poor" status which covers 14.9% is markedly higher than growing herd which only covers 1%.

Figure 7. Overall PRRS Status between Herds among Serology Samples Submitted to FPV, UPM in 2019 to 2021.



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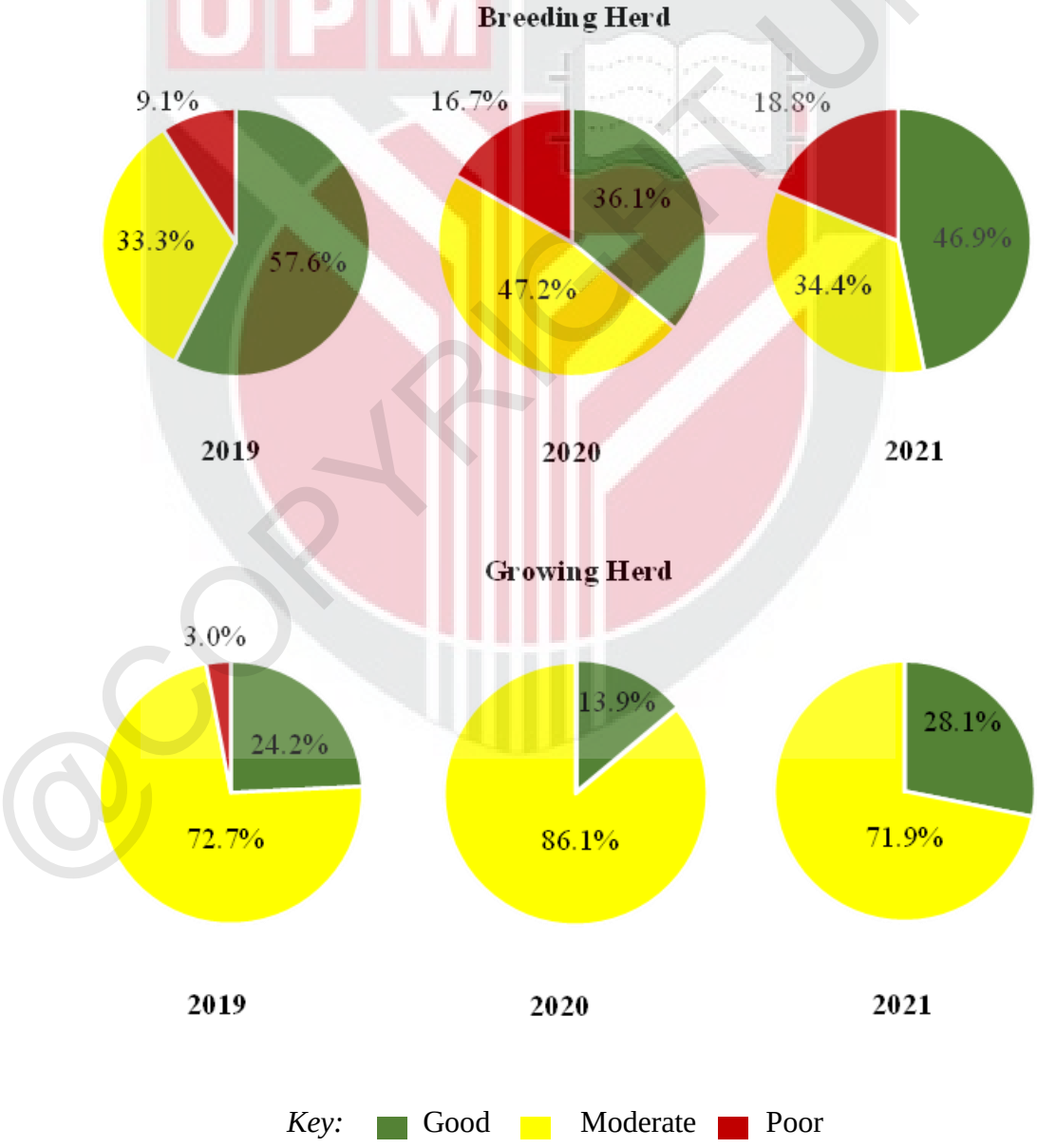
In comparison between the years, the PRRS status in both breeding and growing herds is the poorest in 2020, as compared to 2019 and 2021. Throughout the year, as illustrated in Figure 8, the “poor” status in breeding herd is increasing gradually. In contrast, growing herd shows improvement as more were classified as good and lesser were classified as poor as the year goes by.

Figure 8. PRRS Status between Herds by Year among Serology Samples Submitted to FPV, UPM in 2019 to 2021.



As illustrated in Figure 9, by comparing the PRRS status of the herds in different states, Perak state's shows "poor" status in both breeding and growing herd, whereas in Johor and Penang state, only breeding herds have "poor" status. Melaka and Selangor show stable PRRS serology results. Prove of PRRS status variation between breeding and growing herd can be seen in Chi-Square test showing there is significant difference as the *P*-values is less than 0.05 as shown in Table 10.

Figure 9. PRRS Status between Herds by States among Serology Samples Submitted to FPV, UPM in 2019 to 2021.

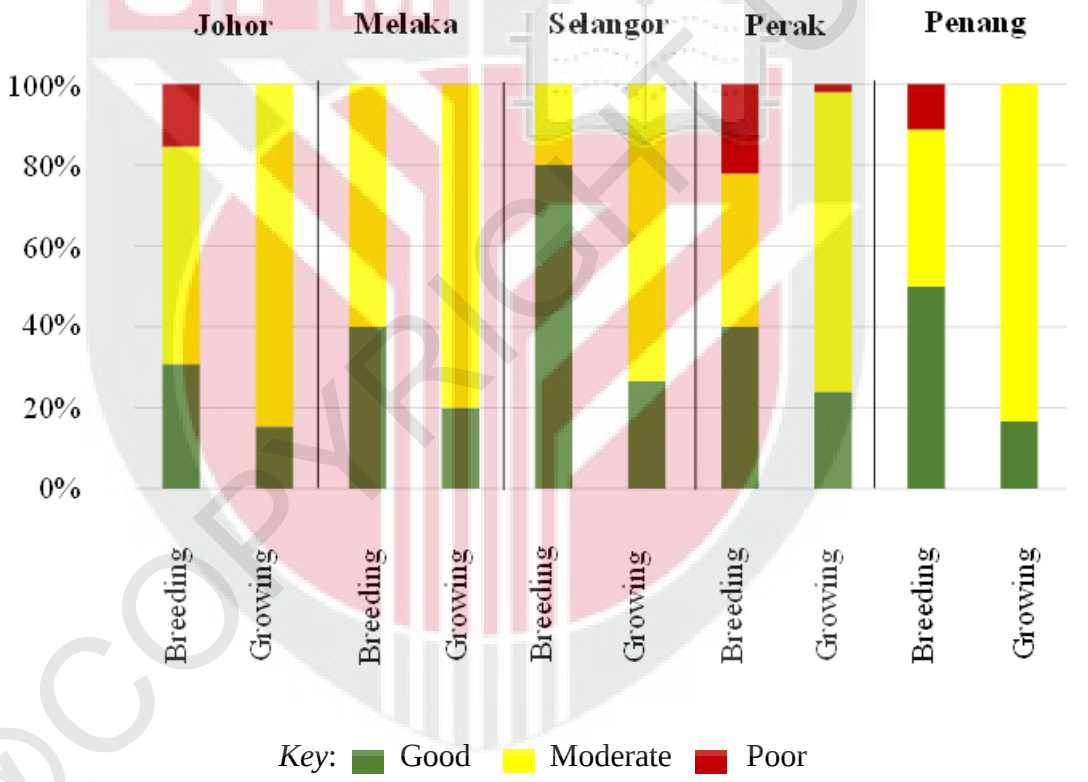


Table 10. Summary of IBM® SPSS Chi-square test statistical analysis for PRRS status between herds among serology samples submitted to FPV, UPM in 2019 to 2021.

Factors	Dependent Variable	Significant
Production Stages	PRRS Status	0.000

4.6 PRRS Status Between States

According to the overall PRRS classification between states in Figure 10, the majority of farms in Johor, Melaka, Selangor, Perak, and Penang are classified as "good" and moderate, with just 2% classified as "poor" in Perak. The apparent lack of change in PRRS status between states showed a consistent distribution pattern. However, there was no statistical difference noted as proven in Chi-Square test in Table 11 as the *P*-value is 0.506 which is more than 0.05.

Figure 10. Overall PRRS Status between States among Serology Samples Submitted to FPV, UPM in 2019 to 2021.

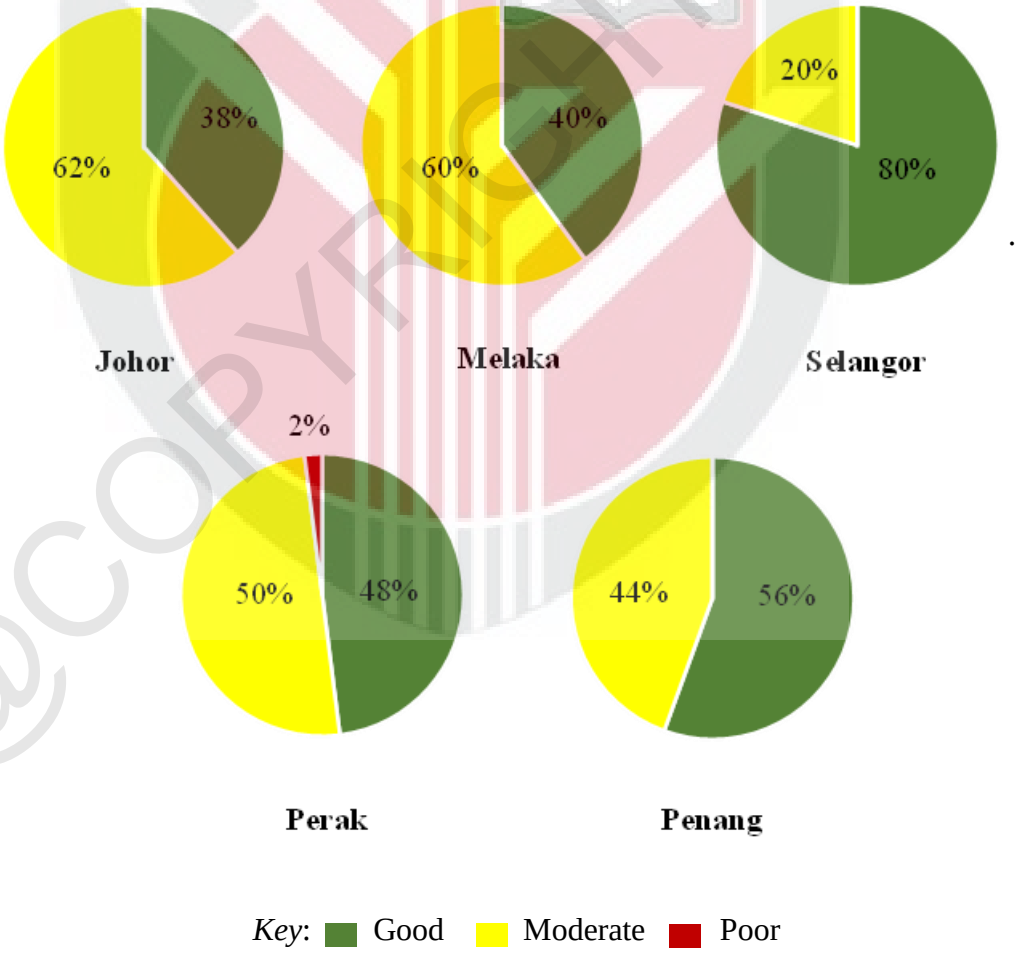


Table 11. Summary of IBM® SPSS Chi-square test statistical analysis for PRRS status between states among serology samples submitted to FPV, UPM in 2019 to 2021.

Factors	Dependent Variable	Significant
State	PRRS Status	0.506

4.7 Overall PRRS Status

Throughout the year, there is little variation in PRRS status. On the other side, we can see that the year 2021 is comparatively better than the others as "poor" status was reduced to zero percent as seen in Figure 11. Altogether in Peninsular Malaysia from 2019 to 2021, PRRS status are mostly ranged from "good" to "moderate" as shown in Figure 12.

Figure 11. Overall PRRS Status by Year among Serology Samples Submitted to FPV, UPM in 2019 to 2021.

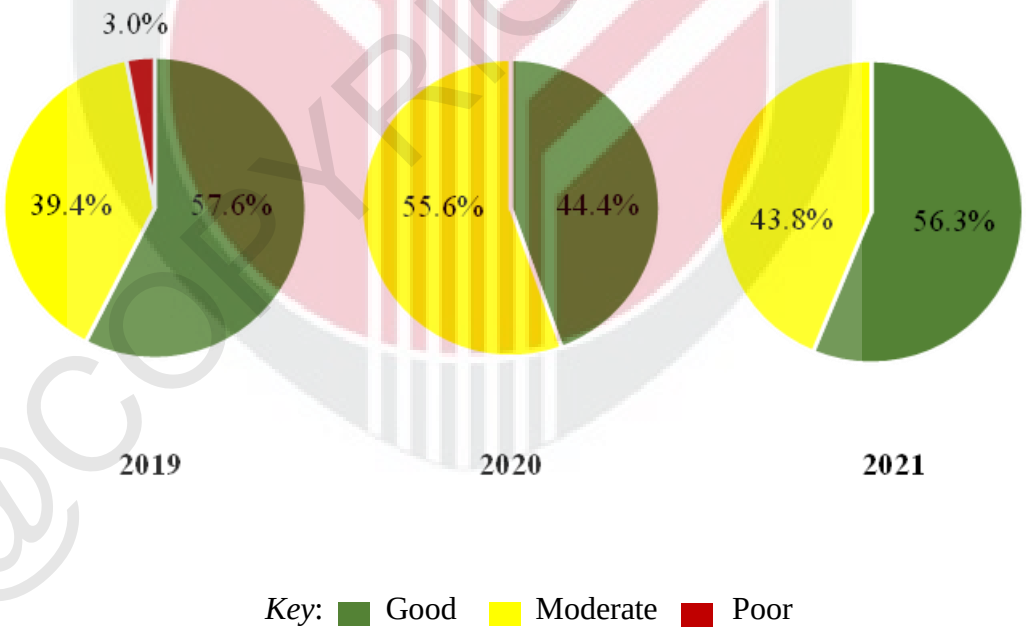
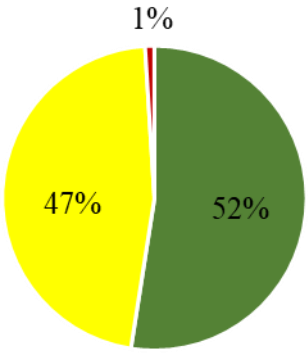


Figure 12. Overall PRRS Status among Serology Samples Submitted to FPV, UPM in 2019 to 2021.



CHAPTER 5

DISCUSSION

5.1 Seropositivity

In this study, IDEXX Herdchek®PRRS X3 PRRSv antibody test kit was used to detect antibody PRSSV antibody. IDEXX PRRS X3 known to be gold standard for ELISA as it has higher sensitivity and specificity. A study reported in the comparison of three ELISA kits, it is found that IDEXX PRRS X3 has the highest sensitivity (97.4%) and specificity (98.1%) compared to Qiagen and iNgezim kits (Sapundžić *et al*., 2020). In this ELISA test, seropositivity is indicated when the S/P value is more than 0.4.

Current findings (see Figure 3 and Figure *4*) showed that there was high seropositive which was more than 90% throughout the years in all states and this may suggests the endemicity status of the PRRS in the farm. However, findings may also postulate that high seropositive could be due to vaccinations regime in the farm. But, there was no data on the current status of vaccination regime of farms were provided to confirm the above assumption. A study proven that IDEXX PRRS X3 ELISA was not able to detect antibody in serum samples of piglets that vaccinated (Sattler *et al*., 2016). Hence, no evidence to support that high seropositive might due to vaccinations.

5.2 PRRS Status between Age Groups

From the result illustrated in Figure 5, gilts have better status than sows in breeding herds. However, in growing herds, weaners aged 12 to 20 weeks have better status compared to weaners 4 to 8 weeks. This consistent trend can be observed in all five selected states as illustrated in Figure 6. It is postulated gilts have more "good" status as high-quality breeding gilts were usually purchased for long-term productivity. Furthermore, routine vaccination of gilts was done prior introduction to the breeding herd. For example, in PRRS MLV vaccine by Ingelvac®, vaccination schedule of gilts was done 30 days before entry into the breeding herds (Cheah *et al.*, 2017). Vaccination of purchased breeding gilts is crucial because it reduces the risk of pathogen transmission that will cause disease and subsequently decrease productivity performance (Bernaerdt *et al.*, 2021). In contrast, sows had many "poor" statuses possibly due to an outdated vaccination regime. Due to the lack of data on the immunisation schedule, it is likely that sows have an out-of-date vaccination schedule as a result of the necessity for regular vaccination in sows. For example, Ingelvac® PRRS MLV routine mass vaccination in sows were done 4 times a year (Cheah *et al.*, 2017). This may be inefficient especially for small farm due to labor intensive and cost constraint, some farms might only be able to vaccinate their sow two times a year.

For growing herd, weaners aged 12 to 20 weeks have better PRRS status compared to weaners 4 to 8 weeks. Weaners aged 4 to 8 weeks may have contributed more to the "poor" status since they may not vaccinate or very recently got their vaccines as vaccine were given to weaners between 14-days old to 45-days old depending on vaccines (Zhao *et al.*, 2012). Amervac® is one of the commercial PRRS vaccines available in Malaysia that is administered to weaners as early as 3 to 4 weeks of age (Cheah *et al.*, 2017). Furthermore, MLV PRRS vaccine-induced antibodies will

take around 3 to 4 weeks after vaccination to reach detectable levels (Meier, 2003; Piontkowski, 2016).

5.3 PRRS Status between Herds

Breeding and growing herd have different S/P range for PRRS status classification according to IDEXX. As illustrated in table 2, the S/P range for breeding herd is between 1.0 to 2.4 whereas growing herd is between 0.4 to 2.4. In comparison to the growing herd, the breeding herd was given a more restricted range. This is due to the breeding herd being kept longer in the same enclosure and being subjected to a more stricter vaccination routine. The IDEXX suggested that the antibody titre range for breeding herds is between 1.0 and 2.4. This range must be met in order to produce a sufficient immune response within herd. A high antibody titre might be a sign of current PRRS infection. On the other hand, a low antibody titre that falls within a range may suggest an inadequate antibody response to the PRRS virus, increasing the risk of infection even after vaccination. Growing herds, on the other hand, were given a broader range due to their short stay on the farm. This is due to the fact that once the pigs reach selling weight, they will be slaughtered. Furthermore, in order to maximise revenues, the vaccination routine is not as frequent as the breeding herd. This influences the herd's immunological response to PRRSV. Overall, based on the findings in Figure 7, breeding herds have a better status than growing herds may due to frequent immunisation routine.

5.4 PRRS Status between States

As seen in Figure 10, the PRRS status showed lack of variation between states, with the majority of states having “good” or “moderate” status. This indicates a consistent states distribution of PRRSV in Peninsular Malaysia. The endemicity of PRRSV is one of the factors that has led to the consistent states’ distribution of PRRS status in Malaysia. This is supported by high seroprevalence in all states, as indicated in Figure 4. Besides as illustrated in Figure 10, more farms are classified as “good” and “moderate” in all states can also be due to good vaccination practices in every state. Furthermore, each farm may implement strict biosecurity that controls transmission of PRRSV throughout the country. On the other hand, the government's movement control order issued in response to the Covid-19 outbreak had an influence on the movement of pigs into and out of Malaysia. As a result, the transmission of PRRSV from other countries is reduced, resulting in a stable PRRS status in all states in Peninsular Malaysia. Without information on vaccination schedules and animal movement, it can only be assumed that this study population practises vaccination programmes, heightened biosecurity, and decreased pig movement in and out, resulting in a lack of variation across states.

CHAPTER 6

CONCLUSION AND FUTURE RECOMMENDATION

6.1 Conclusion

Of the three hypotheses tested in this study, only one was nullified. In conclusion, among the serology samples submitted to the Faculty of Veterinary Medicine, Universiti Putra Malaysia from 2019 to 2021:

i. PRRS serology status is the same across the states in Peninsular Malaysia;
ii. PRRS serology status varies between breeding herds and growing herds;
iii. PRRS serology status varies between age groups of 4 weeks, 8 weeks, 12 weeks, 16 weeks and 20 weeks.

6.2 Recommendation

This PRRS farm status classification system method that only utilizes ELISA interpretations has great room for improvement. The sample size from each farm may be increased to improve the sensitivity of this approach in categorising each farm amongst categories (i.e. good, moderate, and poor). Given that the seroprevalence of PRRS in Malaysia is more than 30%, a sample size of 10 animals per test group is adequate at 95% confidence. However, in order to increase sample sensitivity, 30 breeding animals may need to be bled (Dee and Joo, 1996). Besides, the population coverage can be widened by recruiting more farms per region and to include Sabah and Sarawak. This helps in understanding more on the current PRRS situation in Malaysia.

Farm status classification was solely based on ELISA results and can be misleading. Thus acquiring data on vaccination regimes and health status of pigs (e.g. clinical symptoms) are required for improving the accuracy of data analysis. Plus, collaboration with swine farmers to offer more real-time data would be beneficial. By obtaining data on the vaccination regime, clinical symptoms and productivity performance of a farm, the correlation with serological data could be studied to increase the relevance and reliability of this study.

The farm's PRRS status classification system used in this study is devised based on the ELISA interpretation of recommendations from the United State of America, which were modified slightly to perceive the current scenario of Malaysian swine farming. Future studies could consider developing a customised categorization system more relevant to Malaysia's situation provided the data pool is vast and thorough enough with little bias.

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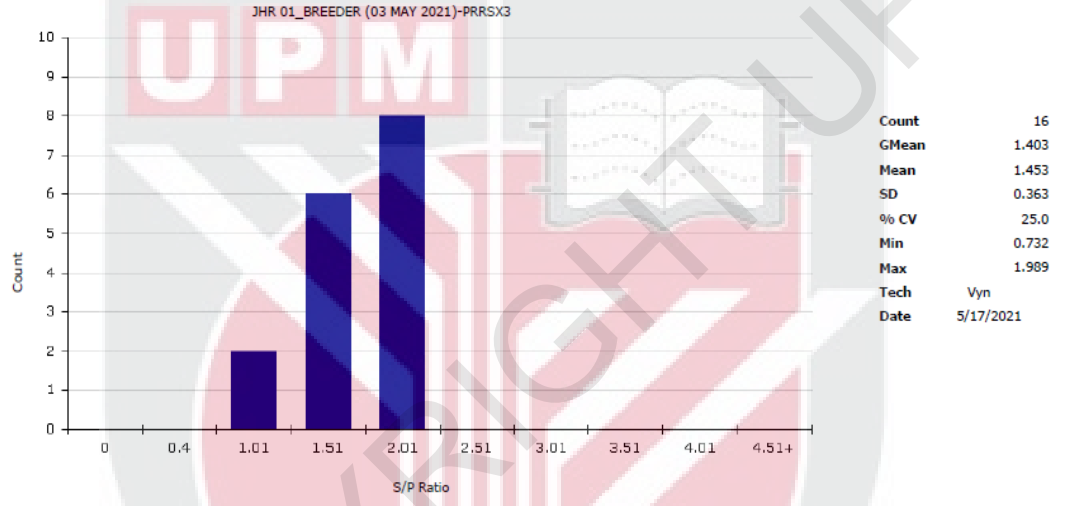
APPENDICES

Appendix 1. Example of IDEXX xChek Plus ELISA Report of Breeding and Growing Farms.

IDEXX Laboratories, Inc.
One IDEXX Drive
Westbrook, ME 04092
USA
9/6/2022

Test With Confidence™ IDEXX

Analyze Case Report



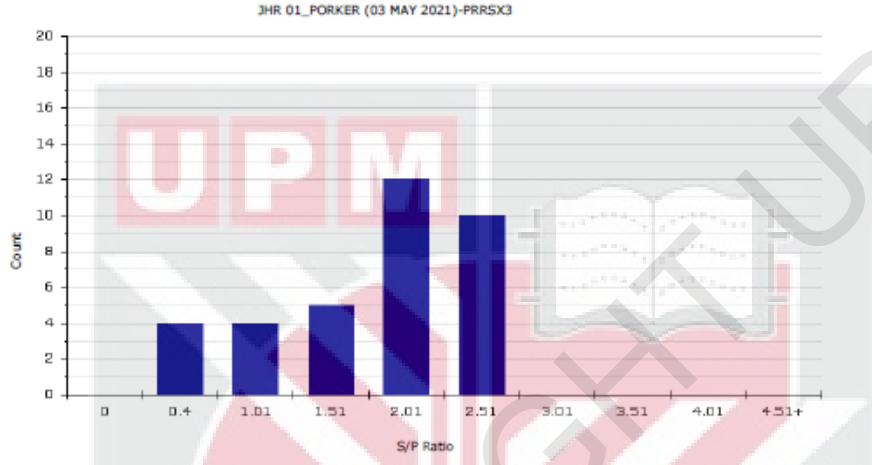
Case JHR 01_BREEDER (03 MAY 2021) - PRRS (23rd)
5/17/2021-002 [PRRSX3 - 5/17/2021 - Vyn

	Well	O.D.	Tube #	Animal ID1	Animal ID2	S/P	Result
Neg	A1	0.051					
Neg	B1	0.054					
Pos	C1	0.799					
Pos	D1	0.793					
1	H5	1.356	1	LVS_BR	G1	1.753	Pos
2	A6	0.912	2	LVS_BR	G2	1.156	Pos
3	B6	1.294	3	LVS_BR	G3	1.670	Pos
4	C6	1.531	4	LVS_BR	G4	1.989	Pos
5	D6	1.389	5	LVS_BR	G5	1.798	Pos
6	E6	1.353	6	LVS_BR/B1	P1/6838	1.749	Pos
7	F6	0.597	7	LVS_BR/B1	P2/7613	0.732	Pos
8	G6	0.772	8	LVS_BR/B1	P3/7532	0.968	Pos
9	H6	0.917	9	LVS_BR/B1	P4	1.163	Pos
10	A7	0.932	10	LVS_BR/B1	P5/7294	1.183	Pos
11	B7	1.021	11	LVS_BR/B2	P1	1.303	Pos
12	C7	1.407	12	LVS_BR/B2	P2/6977	1.822	Pos
13	D7	1.016	13	LVS_BR/B2	P3	1.296	Pos
14	E7	0.924	14	LVS_BR/B2	P4	1.172	Pos
15	F7	1.244	15	LVS_BR/B2	P5	1.603	Pos
16	G7	1.455	Duplicate	LVS_BR	G4	1.886	Pos

IDEXX Laboratories, Inc.
One IDEXX Drive
Westbrook, ME 04092
USA
9/6/2022

Test With Confidence™ IDEXX

Analyze Case Report



Count	35
GMean	1.208
Mean	1.522
SD	0.706
% CV	46.4
Min	0.096
Max	2.473
Tech	Vyn
Date	5/17/2021

Case JHR 01_PORKER (03 MAY 2021) - PRRS (23rd) 5/17/2021-002

[PRRSX3 - 5/17/2021 - Vyn

	Well	O.D.	Tube #	Animal ID1	Animal ID2	S/P	Result
Neg	A1	0.051					
Neg	B1	0.054					
Pos	C1	0.799					
Pos	D1	0.793					
1	E1	1.496	1	LVS_BR/B1	1wA/6838	1.941	Pos
2	F1	1.191	2	LVS_BR/B1	1wB/7613	1.531	Pos
3	G1	1.132	3	LVS_BR/B1	1wC/7294	1.452	Pos
4	H1	0.857	4	LVS_BR/B1	1wC/7532	1.082	Pos
5	A2	1.430	5	LVS_BR/B2	1wA/6977	1.853	Pos
6	B2	0.338	6	LVS_BR/B2	1wB	0.384	Neg
7	C2	0.476	7	LVS_BR/B1	4wA	0.570	Pos
8	D2	0.732	8	LVS_BR/B1	4wB	0.914	Pos
9	E2	0.189	9	LVS_BR/B1	4wC	0.184	Neg
10	F2	1.362	10	LVS_BR/B2	4wA	1.761	Pos
11	G2	0.891	11	LVS_BR/B2	4wB	1.128	Pos
12	H2	0.134	12	LVS_BR/B2	4wC	0.110	Neg
13	A3	1.393	13	LVS_BR/B1	8wA	1.803	Pos
14	B3	1.500	14	LVS_BR/B1	8wB	1.947	Pos
15	C3	1.735	15	LVS_BR/B1	8wC	2.263	Pos
16	D3	1.516	16	LVS_BR/B2	8wA	1.968	Pos
17	E3	1.745	17	LVS_BR/B2	8wB	2.276	Pos
18	F3	1.609	18	LVS_BR/B2	8wC	2.093	Pos
19	G3	0.124	19	LVS_BR/B1	12wA	0.096	Neg
20	H3	1.765	20	LVS_BR/B1	12wB	2.303	Pos
21	A4	1.776	21	LVS_BR/B1	12wC	2.318	Pos
22	B4	1.891	22	LVS_BR/B2	12wA	2.473	Pos
23	C4	1.626	23	LVS_BR/B2	12wB	2.116	Pos
24	D4	1.422	24	LVS_BR/B2	12wC	1.842	Pos
25	E4	1.402	25	LVS_BR	16wA	1.815	Pos
26	F4	1.728	26	LVS_BR	16wB	2.254	Pos
27	G4	1.266	27	LVS_BR	16wC	1.632	Pos
28	H4	0.396	28	LVS_BR	16wD	0.462	Pos
29	A5	1.048	29	LVS_BR	16wE	1.339	Pos
30	B5	1.294	30	LVS_BR	20wA	1.670	Pos