COMMON PATHOGENS DIAGNOSED IN PIG SAMPLES FROM YEAR 2014 TO 2017 BY VETERINARY RESEARCH INSTITUTE

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ABSTRACT. A total of 23,322 specimens collected between 2014 and 2017, from a total of 2,592 cases, were received in Veterinary Research Institute, Ipoh (VRI) from various states in Malaysia and tested for common bacterial, viral, and parasitic diseases in pigs. The highest occurrence of isolated bacteria from 771 samples which tested positive were *Salmonella* (47.38%) and *Escherichia coli* (15.68%), followed by *Staphylococcus (*6.62%*), Streptococcus (*5.57%), *Klebsiella pneumonia (*4.88%*), Pseudomona (*3.38%), *Acinetobacter (*3.14%), *Aeromonas (*2.79%*), Enterobacter (*2.44%), one each of *Bacillus* and *Pasteurella multocida* (1.74%), *Enterococcus (*1.39%) and *Corynebacterium* (1.05%). 1.74% of each bacteria detected were *Moxarella*, *Aspergillus, Burkholderia* and *Chromobacterium*. Positive samples tested by ELISA was Japanese encephalitis virus (JEV) (9.15%), Aujezsky disease virus (ADV) (5.37%), porcine cirvo-virus-2 (PCV2) (5.09%) and porcine reproductive and respiratory syndrome virus (PRRSV) (4.52%). Positive samples tested by the molecular test was PCV2 (1.62%), PRRSV (1.32%) and classical swine fever virus (CSFV) (0.4%). Serology tests were conducted on 11,305 samples and reported positive for *Brucella suis* (15.32%*), Brucella abortus* (0.62%), *Brucella melitensis* (0.85%), and melioidosis (0.05%). Parasitology analyses on 99 samples. revealed presence of 10.1% coccidia and 1% each of helminths and *Sarcocystis*. Within the 4-year period, there were no positive samples for porcine parvovirus (PPV), Nipah virus, swine influenza virus (SIV), and bacteria of Johne's disease and leptospirosis. Continuous assessment is required to establish a comprehensive baseline data of swine diseases in Malaysia.

Keywords: pig diseases, diagnostic information, bacterial, viral, parasitic diseases

INTRODUCTION

Pigs are hosts for diseases that can be transmitted to people and contribute to emerging pathogens that can cause fatal disease outbreaks in humans and animals. Both endemic and emerging viral, bacterial and parasitic diseases are considered to be one of the important impediments for profitable pig production in Malaysia. As reported in Statistik Industri Babi (2014), the pig industry in Malaysia is developing well with the main driving force in the industry is the pork consuming population. Achievements in pig production go hand-in-hand with improved animal health. In Malaysia, almost all pig farms are licensed for operation and the Malaysian Department

of Veterinary Services (DVS) encourages the modern pig farming system (MPF). The pig farms are regularly audited for good animal husbandry practice and certified under the Livestock Farm Accreditation Scheme (SALT) by DVS (Mokthir and Fong, 2014). Even though there have been major achievements in disease control and prevention, the pig production sector continues to be threatened by emerging trans-boundary diseases. The various systems of pig production experience diverse patterns of disease (FAO and OIE, 2010).

A country report by Mokthir *et al*. (2016) from the Pig Unit of Livestock Commodity Development Section of the DVS, reported that pig farmers were grappling with several common diseases and causative agents such as *E. coli*, porcine rotavirus, coccidiosis and coronaviral gastroenteritis (transmissible gastroenteritis, TGE). TGE is the most common cause of pre-weaning diarrhoea. Other concurrent viral infection which may co-exist were porcine cirvo-virus-2 (PCV-2) infection, porcine reproductive and respiratory syndrome (PRRS) or porcine parvovirus (PPV) infection (Mokthir *et al.,* 2016). These are the most important viral diseases of swine worldwide causing porcine reproductive failure and contributes to serious economic losses in the swine industry.

PPV is a DNA virus that causes infertility in pigs, while PCV-2 is a single-stranded DNA virus associated with post-weaning multisystemic wasting syndrome in pigs. PRRS is a viral disease that causes a decrease in reproductive performance in breeding animals and respiratory disease in pigs of any age. In Malaysia, PCV-2 was first identified by the VRI (Hassuzana *et al*., 2004) using restriction fragment length polymorphism (RFLP) methods followed by the first case study of porcine circovirus associated disease (PCVAD) in 2007 based on clinical features, histopathology findings and polymerase chain reaction (PCR) screening (Ooi *et al*., 2007).

To date, there has been no report of highly pathogenic PRRSV in Malaysia however a syndrome very similar to PRRSV has been recorded in various pig farms as early as 1995.

Other viruses which have been identified as causing porcine reproductive and respiratory problems globally are ADV, CSFV (hog cholera virus) and SIV. Aujezsky disease (AD) is a common pig disease that is widespread throughout the world. Despite vaccination, AD outbreaks were reported in different areas of Malaysia in the 1990s (Jasbir, 1998), 1976 (Lee *et al*., 1979) and 1984 (Too, 1997). The first case of CSF disease was reported in the state of Perak, Peninsular Malaysia, in 1895 (Protokol Veterinar Malaysia, 2010). The emergence of this disease at that time was probably due to the importation of pig breeds from overseas. Outbreaks of CSF still occur regularly in most countries and sporadically in Malaysia. Other than causing infectious respiratory disease, the SIV also has effects on herd fertility and abortions in late pregnancy.

Infection in pigs occurs throughout the world. The first isolation of swine influenza H1N1 subtype in Malaysia was made from a pig in Sarawak in 1984 (Lim K.T. *et al*., 1985).

The deadly Nipah virus was first identified in Malaysia in late 1998 during an outbreak among pig farmers and caused

mass culling of nearly one million pigs to control the outbreak. In 2018, it caused havoc in southern India (WHO, Jun 2018). To date, there has been no epidemic reported. A report on the status of Nipah virus in Malaysia from 2001 to 2013 revealed that there has been no positive cases identified in laboratory tests (Naama *et al*., 2013).

The JEV is a vector-borne zoonotic viral disease, prevalent in Asian countries. Pigs play an important role as major amplifying host of JEV exerting a potential health risk to humans (Yamanaka *et al.*, 2010). In Malaysia, major outbreaks of JEV were reported in Pulau Langkawi in 1974 (Fang *et al.*, 1980), Penang in 1998 (Cardosa *et al.,* 1995) and Serian, Sarawak in 1992 (Lam, 1999).

Maintaining a healthy livestock is vital in ensuring safe food supply. The amount of food-borne illnesses attributed to *Salmonella* has remained steady and continues to be one of the top pathogens implicated in food-borne illnesses.

Roseliza *et al*. (2011) reported that the most identified serotypes in pork were *Salmonella typhisuis* (51.2%), followed by *S. typhimurium* (5.8%) and *S. enterica* serovar Weltevreden (1.9%) based on the cases received in VRI in 2009.

Three species of *Sarcocystis* have been recognised in pigs: *S. miescheriana, S. porcifelis* and *S. suihominis*. However, only *S. suihominis* can cause intestinal infection in humans upon consumption of raw pork (Dubey, 2015). In Southeast Asia, the sarcocysts of *S. meischeriana* were reported in pigs in Thailand (Bunyaratvej *et al.* 2007) and the Philippines (Claveria *et al.* 2001). However, there is no report of *S. meischeriana* infection in pigs in Malaysia and other neighbouring countries. There were studies that showed a high prevalence of *S. suihominis* in countries such as India (Saleque and Bhatia, 1991), Japan (Saito *et al.,* 1998), China (Li *et al.,* 2007) and the USA (Dubey & Powell, 1994). To date, no publications have reported *S. porcifelis* and *S. suihominis* infection in pigs in Malaysia or other Southeast Asian countries.

Another parasite usually ingested through consumption of infected meat is *Trichinella spiralis*. A seroprevalence study in VRI showed that 2% of the pigs tested were positive by an ELISA test for antibodies to *Trichinella* (Chandrawathani *et al.*, 2010). This was the first evidence of trichinellosis in pigs in Malaysia.

Pasteurellosis is sporadic in Malaysia and the distribution of serogroups were diverse in all species of animals with no definitive host, pigs being not excluded (Khoo *et al.,* 2017). Nafiza *et al*. (2014) reported that 28.8% of *Pasteurella* spp. were isolated from various hosts such as porcine, rabbit, cervin and canine based on pasteurellosis cases tested in VRI from 2009 to 2013.

Although several important viral, bacterial and parasitic diseases were reported in all Asian countries, those from Malaysia have been few. Therefore, this study is crucial in contributing information and to update the current situation of disease status in pig farms which could then be used for disease control and monitoring planning, improving diagnostic techniques, vaccination development and surveillance structure. It could also be used to develop a continuous study in the economic impact of veterinary studies related to the pig industry.

MATERIALS AND METHOD

Between year 2014 and 2017, a total of 22,322 samples, were received by VRI for various purposes such as diagnostic cases, surveillance programmes for herds, monitoring, disease control programmes, quality control, permits, and as references. All data were obtained from the laboratories: virology, bacteriology, parasitology and serology. The laboratories tests conducted were: viral isolation, PCR, reverse transcriptase-polymerase chain reaction (RT-PCR), bacterial isolation and identification of pathogens, ELISA test, complement fixation test (CFT), rose bengal plate test (RBPT), microscopic agglutination test (MAT) and serum agglutination test (SAT). Tests were carried out for all pig samples with a turnaround time of 3 to 30 days for a full diagnostic procedure and confirmation of a particular disease or condition. Data was generated from a laboratory information management system (LIMS) and analysed using Microsoft Excel™ (version 2013) spreadsheet. The percentage was calculated by dividing the number of positive samples with the total number of sample tested.

Bacteriology

All samples submitted to VRI were subjected to physical or post-mortem examinations prior to being sent for further tests in the laboratories including the bacteriology laboratory. A total of 935 samples received were subjected to bacterial isolation in blood and McConkey agar. Suspect colonies were further subjected to subculture and biochemical tests for confirmation. Upon confirmation of bacteria, serotyping analysis was carried out to identify the pathogenic strain. The diagnostic methods used were in accordance with standard protocols of conventional techniques (Quinn *et al*., 1994) and OIE standards. Of all the samples received, 411 were *Salmonella* isolates from various regional veterinary laboratories (RVL) and veterinary public health laboratory (VPHL) of the DVS between 2014 and 2017. These isolates were from various states in Malaysia, i.e. from pig farms, meat and the environment including slaughter houses. The isolates were confirmed biochemically as *Salmonella* spp. at RVLs before serotyping at VRI on nutrient agar slant. Serotyping was conducted according to the Kauffmann-White classification scheme using a battery of somatic and flagellar antisera. (OIE Terrestrial Manual, 2008).

Virology

A total of 3,022 pig samples were received from state DVS and RVLs between 2014 and 2017. The diagnoses of specimens were by viral isolation (OIE Manual, 2004), serology and molecular detection. PCR and RT-PCR assays were performed using an extraction kit as described by the manufacturer's protocol and published primers. The amplified genomic sequence were carried out using PCR/RT-PCR system (Promega Corporation, Madison, USA). The final PCR product was detected by gel electrophoresis, health view staining, and UV light transillumination.

Parasitology

99 samples collected between 2014 and 2017, including meat, organ, faecal, blood and worm, from pigs were submitted to the parasitology unit, VRI, for diagnoses of parasitic infection. Faecal samples received were subjected to McMaster's and floatation methods while thin blood smear examination was conducted on blood samples as described by Christopher *et al.* (1992). All results were recorded as the number of eggs per gram (epg). The presence of helminth and coccidia oocysts was considered to be a positive examination. Meat and organ samples were examined by digestion and impression smear (DOB smear) methods (Christopher *et al.*, 1992) and stained by Giemsa staining and observed microscopically for *Sarcocystis* bradyzoites (Gut, 1982; Fazly Ann *et al.*, 2014).

Immunoassay

A total of 7,958 samples of pig cases were received by the immunoassay laboratory either for national surveillance or applicaiton of permits in importation and exportation of pigs. Nipah samples were tested using Nipah in-house ELISA test for IgG antibodies detection according to Veterinary Test Method (VTM001) of the Immunoassay Unit, modified from Muniandy (1999), White *et al., (*2000) and Jamal (1999). For other disease antibodies, detection tests were performed and interpreted using a commercial ELISA kit as described by manufacturer's protocols and accordance to the OIE manual.

Serology

Between 2014 and 2017, a total of 11,308 pig sera samples were received by the Serology Unit, requesting for diagnoses of brucellosis (11,188), Johne's disease (1), leptospirosis (77) and melioidosis (39) in pigs. For brucellosis, tests conducted for *Brucella abortus, Brucella suis* and *Brucella melitensis* were CFT and brucella rose bengal plate test (RBPT) using the method specified by OIE Manual (2009). For Johne's (OIE Manual, 2014) and melioidosis (OIE Manual, 2009), the sera samples were tested for antibody detection using CFT and for leptospirosis using MAT (OIE Manual, 2014).

RESULTS AND DISCUSSION

All the samples received from various states in Malaysia from 2014 to 2017 comprised various types of samples such as sera, blood, organ, swab, faeces, enviromental swab and water. Different diagnostic tests were conducted: 935 samples in the bacteriology laboratory, 7,958 samples in the immunoassay laboratory, 99 samples in the parasitology laboratory, 11,308 in the serology laboratory and 3,022 in the virology laboratory (Figure 1). The highest number of samples received was from Perak (5,892), followed by Selangor (5,372) and Sarawak (3,586) (Figure 2).

A total of 771 positive samples were identified for bacterial diseases and of these 47.38% were *Salmonella*, 15.68% *Escherichia coli,* 6.62% *Staphylococcus,* 5.57% *Streptococcus,* 4.88% *Klebsiella pneumonia,* 3.38% *Pseudomonas*, 3.14% *Acinetobacter*, 2.79% *Aeromonas,* 2.44% *Enterobacter,*

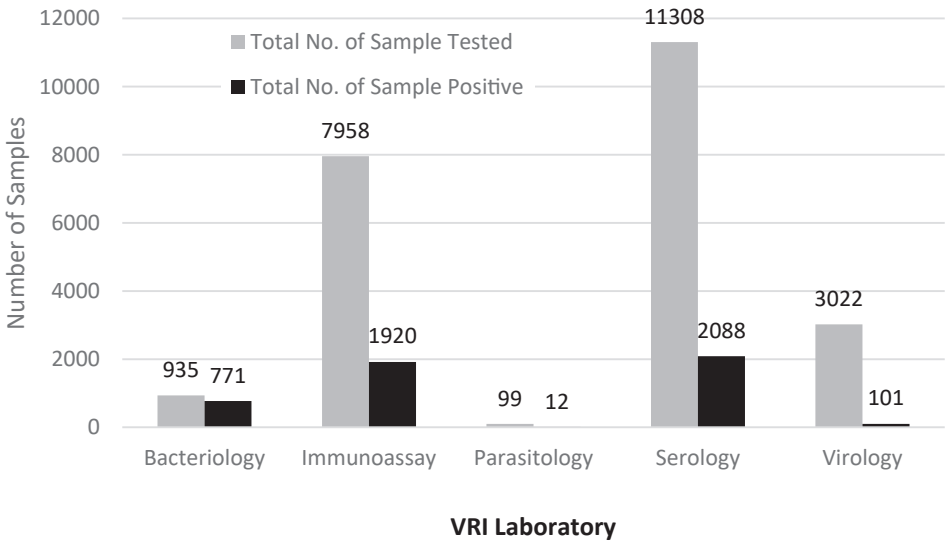

Figure 1. The Total Number of Pig Samples Diagnosed in VRI from 2014 to 2017.

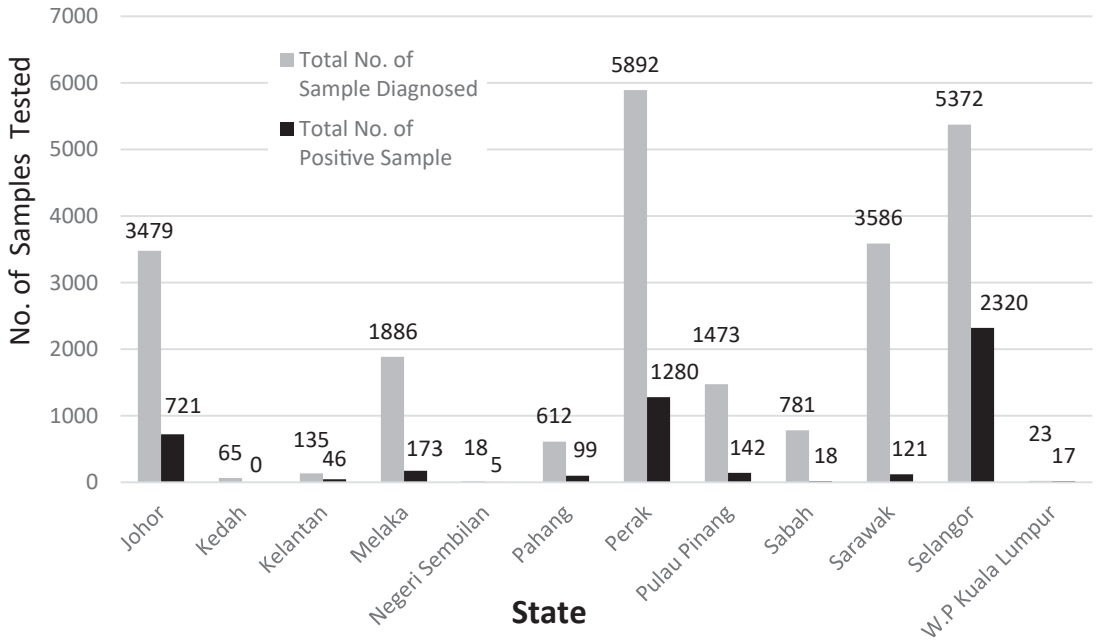

Figure 2. The total number of pig samples diagnosed from each state of Malaysia in 2014 to 2017.

1.74% each of *Bacillus* and *Pasteurella multocida,* 1.39% *Enterococcus* and 1.05% *Corynebacterium*. 1.74% each of bacteria were *Moxarella*, *Aspergillus, Burkholderia* and *Chromobacterium* as shown in Figure 3.

The results shown in Table 2 is of bacterial diseases diagnosed serologically from a total of 11,305 pig sera samples received by the serology unit. From these samples, 1,732 (15.32%) were tested positive for B*rucella suis* with increasing trend from 2014 to 2017 (4.78%, 4.85%, 23.73% and 56.38% progressively), 184 (1.63%) were tested positive for *Brucella* spp., 96 (0.85%) for *Brucella melitensis*, 70 (0.62%) for *Brucella abortus*, and 6 (0.05%) for melioidosis.

No case of Johne's disease and leptospirosis were diagnosed within the 4-year period.

Upon evaluation, the bacteria that affected pigs were diverse and vast, and can be grouped by area of primary lesion such as digestive system (commonly associated with *Salmonella, E. coli*, *Bacillus, Enterococcus* and etc.*),* respiratory system (caused by S*treptococcus, Pasteurella multocida* and *Pseudomonas)*, cutaneous i.e. skin (caused by *Staphylococcus)* and reproductive system (associated with bacteria such as *Brucella* species) (Rokayya *et al.*, 2017).

Between 2014 and 2017, 10,980 samples were received for different tests

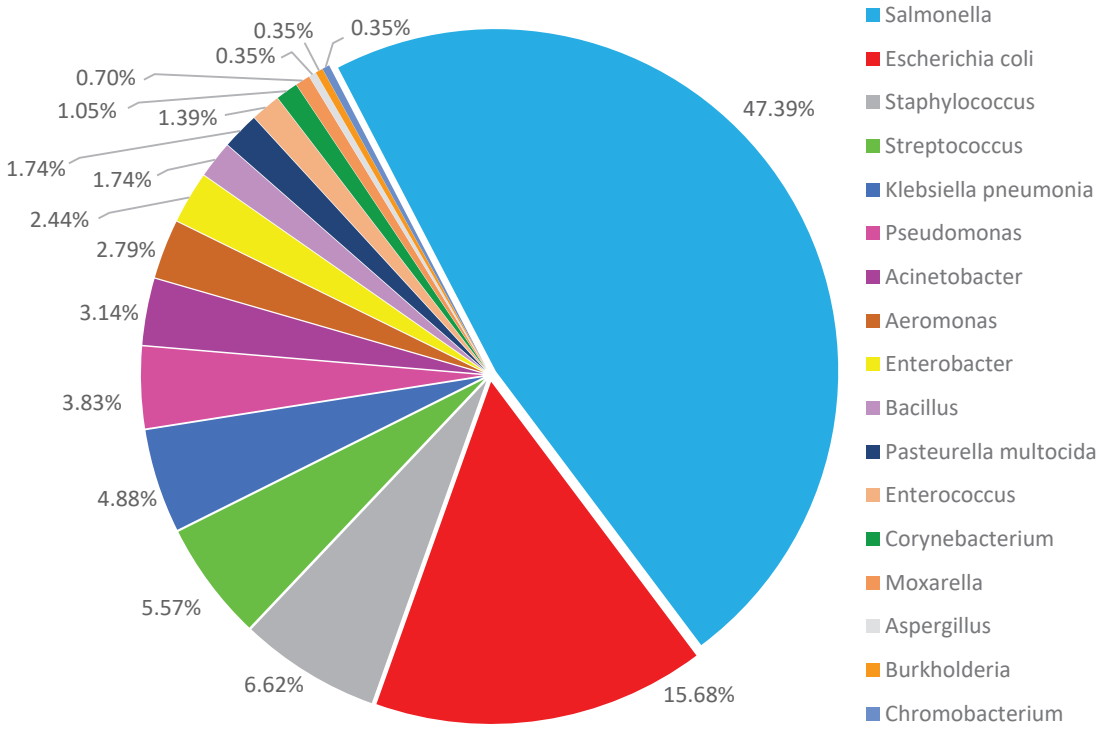

Figure 3. Percentage of Bacteria Identified from Pig Samples Tested in VRI from 2014 to 2017.

using ELISA (7,958) and molecular analyses (3,022) for the detection of viral diseases.

Out of this, generally, the highest percentage of positive samples tested by ELISA was JEV (9.15%), followed by ADV (5.37%), PCV2 (5,09%) and PRRSV (4.52%).

Using molecular analyses, the highest percentage was PCV2 (1.62%), followed by PRRSV (1.32%) and CSFV (0.4%). No positive

Table 1. Total number of positive samples diagnosed in VRI for viral diseases from 2014 to 2017.

	Positive samples/ total samples			
Viral Diseases	**2014**	**2015**	**2016**	**2017**
ELISA test				
Aujeszky's disease (AD)	-	427/523	-	-
Japanese encephalitis (JE)	69/106	640/1121	19/30	-
Nipah virus	0/1172	0/1231	0/816	0/2139
Porcine circovirus type 2 (PCV2)	-	405/440	-	-
Porcine reproductive & respiratory syndrome (PRRS)	-	360/380	-	-
Molecular Detection				
Aujeszky's disease (AD)	0/91	0/7	0/11	0/1
Classical swine fever (CSF)	0/234	0/211	0/186	12/103
Japanese encephalitis (JE)	0/803	0/310	0/123	0/30
Nipah virus	-	-	-	0/1
Porcine parvovirus (PPV)	-	-	-	0/2
Porcine circovirus type 2 (PCV2)	16/86	33/44	0/12	0/4
Porcine reproductive & respiratory syndrome (PRRS)	0/86	30/481	10/11	0/2
Swine influenza A (SIV)	0/39	-	-	0/15

Table 2. Total number of positive samples diagnosed for serology in VRI from 2014 to 2017.

	Positive samples/ total samples tested			
Serological	**2014**	**2015**	**2016**	**2017**
Brucella spp.	40/396	144/1835	-	-
Brucella abortus	-	0/284	0/10	70/2320
Brucella melitensis	-	-	-	96/96
Brucella suis	59/1234	74/1526	308/1197	1291/2290
Johne's	-	0/1	-	-
Leptospirosis	0/31	0/43	-	0/3
Melioidosis	5/31	0/1	1/7	-

Table 3: Total number of positive samples diagnosed in VRI for parasitic diseases from 2014 to 2017.

	Positive samples/ total samples			
Parasitic Diseases	**2014**	**2015**	**2016**	**2017**
Blood Protozoa	0/29	0/10	-	0/2
Coccidiosis	3/3	-	-	7/24
Helminthiasis	1/1	-	-	-
Sarcocystis	-	1/30	-	-

samples for PPV, ADV, JEV, Nipah virus, and SIV were found for the 4-year period.

As mentioned in Table 1, in 2014, 69 (65.09%) out of 106 samples were tested positive for JEV using ELISA. The same test was carried out between 2015 to 2017 and a change in pattern of the number of samples were found. 640 (57.09%) out of 1,121 samples in 2015, and 19 (63.33%) out of 30 samples in 2016 were reported positive for JEV. No test was conducted in 2017. No positive samples were detected molecularly for the 4-year period.

Other than JEV, only PCV2 (18.60%) was reported in 2014, and PRRSV (90.91%) was reported in 2016.

Many samples were received and tested positive for viral diseases in 2015. This may be due to the Malaysian national surveillance and control programmes of 2015 by DVS to achieve the objectives to determine the prevalence of porcine brucellosis, Japanese encephalitis (JE), AD, PRRS and PVC2 infection in pigs (Jabatan Perkhidmatan Veterinar Malaysia, 2015). The percentage of positive samples tested for ADV was 81.64%, JEV 57%, PCV2 92.04% and PRRSV 94.74% using the ELISA test. PCV2 22.92% and PRRSV 6.24% by molecular detection. Between 2014 and 2017, CSF (11.65%) was only detected positive in 2017 by molecular detection. ELISA test and molecular detection showed no positive samples in pigs for Nipah virus during the 4-year period.

A total 99 samples from pigs were submitted to the parasitology laboratory between 2014 and 2017 as shown in Table 3. 10 (10.1%) tested positive for coccidia and one (1%) each of helminth and *Sarcocystis*. There were no case of blood protozoa reported. There were no case submitted for parasitic diseases in year 2016.

CONCLUSION

As these results were analyses from retrospective data, further analyses are needed to collate information pertaining to the various diseases as well as to elucidate the reason for the number of positive tests for each of the pathogens. The main information showed the common pathogens diagnosed in the samples from pigs submitted to VRI. It indicates occurrences of important diseases in local pigs and indicates the need for concerted efforts to treat and control the diseases which are responsible for huge economic losses to the pig producers affected by the mortality and cost

of medication associated with the diseases. Diagnosis of these zoonotic diseases is essential in giving an early warning sign so that control measures can be taken.

The results indicate that some of the important bacterial diseases recorded in pigs include salmonellosis (47.39%) and colibacillosis (15.68%) by isolation and identification method and *Brucella suis* (15.32%) by serology test method. Among the viral diseases, JE (9.15%) showed the highest percentage of occurrence followed by AD (5.37%), PCV2 (5,09%) and PRRS (4.52%) using the ELISA test. National surveillance and control programmes by the DVS to screen all pig farms in Malaysia and animals to be exported and imported against Nipah virus antibodies has so far been successful. There is a need to strengthen existing surveillance systems so that changes in the incidence of known diseases are routinely reported and information on the emergence of new or unusual diseases is readily available to the ministries in other nations.

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