The epidemiology of pig diseases and the emergence of porcine circovirus type 2 in Papua Province, Indonesia

by

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A thesis submitted for the fulfilment of the

requirements of the Doctor of Philosophy

October 2015

The University of Adelaide

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1. Abstract

Pigs are an important livestock species for the rural community in South East Asia and Pacific, as well as in Papuan community, Indonesia. Despite that importance, the productivity of Papuan piggeries has been very low. Disease and mortality are recognised major constraints for pig production in Papua.

The aim of the thesis was to understand the demographics of pig populations, study the epidemiology of pig diseases in Papuan pigs, including the underdiagnosed PCV2 and to propose locally adapted control approaches to reduce the incidence of selected pathogens. Jayawijaya was chosen as the area of the study because almost one fourth of the pig population in the province can be found in that region.

The study was conducted in four stages: the first was a survey of the demography of the traditional pig farming to gain insights into details of daily husbandry, disease management and the productivity of Papuan pig farms. The second was two case studies that established the prevalence of the selected pig pathogens in dead and healthy pigs. The selected pathogens were Classical Swine Fever (CSF), Porcine circovirus type 2 (PCV2), *Streptococcus suis*, *Streptococcus zooepidemicus*, and five internal parasites i.e. *Trichuris suis,* strongyle parasites, *Strongyloides ransomi, Ascaris suum, and* coccidia. The third study was to confirm the presence or absence of PCV2 virus in Jayawijaya using molecular techniques. The final study was aimed at further characterisation of the complete genome of Papuan PCV2 isolates. Finally, a review was conducted to gather information about the ecology and epidemiology of major pig diseases from Papuan studies and other

places, in order to excerpt available control measures that may be applicable under current Papuan context.

The survey indicated that pig farms in Jayawijaya were of small size. The productivity was low, indicated by small litter size, low annual farrowing frequency and high mortality. Husbandry was of minimal input demonstrated by low housing quality, low feeding input, minimal use of veterinary services and limited awareness of pig diseases and their consequences. CSFV, strongyle parasites, *Trichuris suis* and PCV2 were among the main pig pathogens identified in Papua. An association of the presence of these pathogens with pig mortality might have occured but the sample selection bias may have confounded the results. PCV2 was identified for the first time in Papua in the current study. Analyses of the PCV2 genomes of some of the isolates showed that two different PCV2 genotypes have been circulating in Papuan pigs. In order to control pig diseases in Papua, the role of local government is vital, as many Papuan farmers have very little understanding of the relevant pig diseases and their controls. We propose that confining pigs should be prerequisite before any other control methods are to be introduced. In confined pigs, vaccination against CSFV and regular administration of anthelminthics may be two important and practical control measures that should be introduced in broader areas across Papua, especially in regions with available veterinary offices.

2. Declaration of Originality

I certify that this work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or tertiary institution without the prior approval of the University of Adelaide and, where applicable, any partner institution responsible for the joint award of this degree. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Widi Nugroho

3. Acknowledgements

Firstly, I would like to express my sincere gratitude to the Australian Centre for International Agricultural Research (ACIAR) and the John Allwright Fellowship (JAF) which has given me the opportunity to improve my skills in animal disease investigation through this PhD study.

My sincere thanks goes to Dr. Colin Cargill, who had provided me with an opportunity to join an ACIAR project that he led so I could learn to conduct research into livestock diseases and given me access to compete in the JAF fellowship. Thank too to his soul mate, Dr. Margaret Cargill, for providing me with a warm welcome and a place in their house during my first days in Adelaide.

So much thanks must go to Prof. Michael Reichel, my principal supervisor for the continuous support to my PhD study, for his patience, motivation, bit of challenge and willingness to reply to e-mails quickly, keenness to answer queries of Whatsapp messages promptly day and night, even when he was on a holiday or was being overseas for other works. His guidance and warm friendship has helped me so much during all the time of the research and writing of this thesis and made this journey fun and far less stressful than it otherwise could have been.

I would like to thank my co-authors: AProf. Roy Kirkwood, AProf. Darren Trott, Prof. Isrina Salasia for their insightful comments and encouragement, but also for the hard questions which encouraged me to widen my research from various perspectives.

To I Made Putra DVM., Dewa Ayu Dwita DVM., Werenfridus R. Lestoin S.Si., Merry S. Repasi S.Si., Mitra Slipranata DVM. M.Biotech., and Sidna Artanto DVM. M.Biotech, thanks for your contributions during fields and labworks in Indonesia. I thank colleagues in Roseworthy campus; Dr. Torben Nielsen and Dr. Ryan O'Handley, Dr. Sasha Lanyon, Dr. Caitlin Jenvey, Liza, Ugi, Pardeep, Vivek and Ellison Msimuko for their hands, for the stimulating discussions and for the friendship during this long journey. Also I gratefully thank the pig farmers in Jayawijaya for their enthusiasm during the interview and pig traders in Jibama market for willingness to help me during post mortems.

Last but not the least, I would like to express huge thank to my wife and children who have given me opportunity to take the time that I should have used to look after them, to do my study.

4. Introduction

Village pig farming in Asian countries; practices and productivity

Village pigs play an important role in livestock production worldwide. Around 56% of all pigs produced in the world originate from village farms (Riedel et al. 2012). In developing countries especially, village pigs are an important livestock species that support the livelihood of traditional communities and of people living below the poverty line (Brioudes and Gummow 2015; Huynh et al. 2007; Leslie et al. 2015; Nwanta et al. 2011). In South-East Asia, village pig farms comprise 80% of total pig farms (Huynh et al. 2007; Leslie et al. 2015).

Village pig farms in Asia are characteristically of small size. For example, about 86% farms in North-East India have five pigs or less (Kumaresan et al. 2009), in Sri Lanka, herd sizes of less than 20 heads is a characteristic of 92% village farms (Subalini et al. 2010) and in North Vietnam the average herd size is 12 pigs (n=64) (Lemke and Zárate 2008).

A confinement system has been applied in many village farms in Asia, today. In North-eastern India most pigs are fully confined (Kumaresan et al. 2009; Nath et al. 2013). In Vietnam, since 1950, the majority of village farms have moved from extensive to fully confinement systems (Lemke and Zárate 2008). In Bhutan, national health regulation require farmers to control their pigs either in confinement, by tethering, or in a paddock. Currently, only 13% of Bhutanese farmers rear pigs on free-ranges (Nidup et al. 2011). However, free and semi

scavenging system are reported to be common in village piggeries in Lao PDR and Sri Lanka (Phengsavanh et al. 2010; Subalini et al. 2010).

The feed for Asian village pigs is usually obtained from locally available, traditional sources. The use of household wastes as pig feed is reported from India, Sri Lanka and Bhutan (Kumaresan et al. 2009; Nidup et al. 2011; Subalini et al. 2010). Brewer residues have been used to feed pigs in India, Vietnam and Lao PDR (Kumaresan et al. 2009; Lemke and Zárate 2008; Phengsavanh et al. 2010). Rice byproducts are reported to be used in Lao PDR, North Vietnam and Sri Lanka (Lemke and Zárate 2008; Phengsavanh et al. 2010; Subalini et al. 2010). Other traditional feed sources include maize, grass, coconut cake, cassava, dried cassava, pumpkins, yams, taro, oil cakes, concentrate, soybeans and fish (Kumaresan et al. 2009; Lemke and Zárate 2008; Nidup et al. 2011; Phengsavanh et al. 2010; Subalini et al. 2010). Commercial feed, on other hand, is currently used by more than 40% farmers in Vietnam (Lapar et al. 2012). Feeds are commonly fed twice daily in village farms (Kumaresan et al. 2009; Lemke and Zárate 2008; Phengsavanh et al. 2010).

Common practices of daily pig husbandry in village pig farms in India for example, include vaccination, deworming, the treatment of sick pigs, the washing of the pig house with water biweekly (Kumaresan et al. 2009). Apart from deworming and vaccination, Vietnamese farmers also have used veterinary services daily to treat sick pigs (Lemke and Zárate 2008). In Bhutan, the weaning of piglets is done at 40-55 day of age (Nidup et al. 2011).

Under the aforementioned farming conditions, the average productivity of village pig farms in Asia has remained relatively low. Daily weight gain of village pigs only ranged from 74 to 184 gms/day in North-eastern India, regardless of the breed used (Kumaresan et al. 2009; Nath et al. 2013). In Lao PDR, the average growth rate of village pigs was only 100 to 140 g/day (Phengsavanh et al. 2010). A similar finding was recorded from Vietnam with 65 to 183 gms/day (Lemke and Zárate 2008) and Bhutan with 100-140 grams per day (Nidup et al. 2011). Litter sizes are also small. Average litter sizes of smallholder pig farms in Lao PDR, Vietnam, Sri Lanka and Bhutan were 6.8, 7.2-11.3, 6.44±1.19 and 6.9-9.0, respectively (Lemke and Zárate 2008; Nidup et al. 2011; Phengsavanh et al. 2010; Subalini et al. 2010).

Low productivity of Asian village pigs is also reflected by high piglet mortality. In Lao PDR, where the practice of free scavenging is common, the mortality of piglets owned by different ethnic groups was reported to be ranging from 28 to 45% (Phengsavanh et al. 2010). However, in other areas where confinement systems are common, mortalities were equal to or lower than reported from Lao PDR. In India, for instance, the estimated preweaning mortality (including stillborn piglets) was reported to be 35% (Nath et al. 2013) and in Vietnam 26-32% (Lemke and Zárate 2008). In Sri Lanka, the preweaning mortality of piglings was 20% (Subalini et al. 2010). In other regions of India, the reported total pig mortality was 18% (Kumaresan et al. 2009). In Bhutan, piglet mortality was only reported at 13% (Nidup et al. 2011).

Diseases and mortality in Asian village pigs

Diseases have been reported to be the major causes of village pig mortality in Asia. In Lao PDR, 66% village pig farmers reported that the cause of pig mortality was disease and 34% farmers specifically mentioned diarrhoea as a cause of death (Phengsavanh et al. 2010). In North Vietnam, symptoms of diarrhoea were the most frequent sign observed in diseased pigs, followed by wasting of newborn piglets, with subsequent death (Lemke et al. 2006). In North eastern India, reported major health problems were infection with Classical Swine fever (CSF), swine erysipelas, digestive disorders, nephritis, respiratory disorders and parasitisms. Major parasites reported were *Ascaris suum, Oesophagostomum sp, Metastrongylus apri, Trichuris suis* and Strongyle egged parasites (Kumaresan et al. 2009; Nath et al. 2013). In Sri Lanka, trampling was reported to be the major cause of preweaning mortality (Subalini et al. 2010).

During recent years, a number of outbreaks of endemic and emerging diseases have been reported in Asian pigs. More than 280 CSF outbreaks occurred in 12 provinces and autonomous regions of China in 2011 (Luo et al. 2014). In Nepal, CSF outbreaks were reported in the Makwanpur and Bhaktapur districts during April 2011 and September 2011, respectively (Postel et al. 2013). CSF has been endemic in Vietnam and Laos and sporadic cases were reported in Vietnam during 2010 (Tung et al. 2011) and in India (Sarma et al. 2011).

Highly pathogenic Porcine Reproductive and Respiratory Syndrome (PRRS) was reported to associate with a high mortality outbreak in Lao PDR, in July 2010 (Ni et al. 2012). During August 2010–June 2011, outbreaks of PRRS were reported

in west and north Thailand. This outbreak was suggested to have originated from infected pigs that were transported from Laos to a slaughter house close to the farm where the first outbreak occurred (Nilubol et al. 2012). Additionally, China and Vietnam have been endemically infected with PRRS since 1995 and 2008 respectively (Zhang and Kono 2012).

In the last few years, coronavirus associated porcine epidemic diarrhoea (PED) outbreaks have been reported in several Asian countries, including Thailand, the Philippines and the southern provinces of Vietnam. In October 2010, a large scale outbreak of PED was reported in several provinces in southern China and the virus is now circulating in at least 29 Chinese provinces (Song et al. 2015). In October 2013, Japan reported a PED outbreak after a period of 7 years without an outbreak. In late 2013, PED outbreaks were reported in South Korea and Taiwan (Song et al. 2015).

Foot and Mouth Disease (FMD) outbreaks in pigs were reported in 2010 from China (Zheng et al. 2012), Japan (Muroga et al. 2012) and South Korea (Park et al. 2013). In Cambodia, a study indicated that the incidence and mortality of FMD in pigs in infected villages were 11% (1–41%) and 4% (0–29%), respectively (Bellet et al. 2012). FMD seroprevalence was reported to be low in Bhutanese pigs at 1.9% (95% CI: 0.0, 3.8) (Dukpa et al. 2011). In India, as per the National FMD Sero-surveillance study, the average prevalence of FMD infection in pig was 2.0% (Pattnaik et al. 2012).

Many other diseases have been identified to occur in Asian pigs. Diseases of viral aetiology identified included Porcine circovirus 2 disease (PCVD), Aujeszky's disease, swine influenza, porcine parvovirus (PPV), Japanese B encephalitis (JE), Transmittable gastro-enteritis (TGE), encephalomyocarditis, rotaviral diarrhea, Nipah encephalitis and Swine versicular disease. Among the bacterial diseases reported were streptococcosis, *Escherichia coli* associated oedema disease, brucellosis, leptospirosis, enzootic pneumonia, pasteurellosis, porcine pleuropneumonia, atrophic rhinitis, glassers disease, lungworm, tuberculosis, colibacillosis, exudative epidermitis, salmonellosis, proliferative enteropathy and spirochaetal diarrhoea. Parasitic diseases include toxoplasmosis, trypanosomiasis, coccidiosis and pig-associated zoonoses such as *Taenia solium* and *Trichinella* spp. (Conlan et al. 2011; Fan et al. 2008; Huynh et al. 2007; Kunavongkrit and Heard 2000; Metwally et al. 2010).

Pig farming and diseases in Papua province, Indonesia

Papua province has the fifth largest pig population among provinces in Indonesia. The largest pig populations in Indonesia are found in the provinces of Nusa Tenggara Timur, Sulawesi Selatan, Bali, Sumatra Utara and Papua (Siagiaan 2014).

Papua is the eastern most province of Indonesia and consists of 29 regions (BPS-Papua 2013). Papua has a pig population of 600,000 heads and pigs are raised by 30% of all households in this province (BPS-Papua 2013; BPS-Papua 2014). Pigs are used mainly as cash generator and as offering in socio-cultural activities and religious ceremonies (Mahalaya 2011; Muller 2009).

Within Papua, the Jayawijaya region has had the largest pig population, with 24% of all Papuan pigs (BPS-Papua 2013). Pigs comprise 83% of the domesticated animals in the Jayawijaya region and are an important livestock species for the Papuan tribes who represent more than 80% of the people occupying the region (BPS-Jayawijaya 2011).

Despite the importance of pigs culturally and economically, pig productivity in Papua is reported to be low, reflected by low weight gain, infertility and high mortality (Cargill et al. 2009). Cargill et al. (2009) had conducted several projects aimed at improving pig productivity in the Jayawijaya region. These projects, funded by Australian Centre for International Agricultural Research (ACIAR), included improvements of the housing management by introducing side windows, dunging areas and fenced yards for grazing; feed improvement by ensiling, heat treatment and locally available protein source supplementation; and boar management. These approaches were reported to improve weight gain and reduce mortality under experimental conditions. However, whether the results of these projects have been widely adopted by local farmers and improved the situation of pig production in the region remains open to evaluation.

Furthermore, as a pathway to improve the productivity of Papuan pig farms, an updated knowledge of the characteristics of traditional pig farming and its productivity is important. These characteristics may include traditional housing, feeding, daily husbandry, disease management and boar management. The measurement of productivity may include, daily weight gain, farrowing rate, litter

size and mortality rate. This knowledge may help stakeholders to identify possible underlying factors accompanying the low pig production and to base the future strategies for improving pig production in Papua. A study on this subject is urged to be undertaken, as the knowledge of such characteristics is currently lacking.

Disease and mortality was indicated to be a major problem in Papuan pigs (Mahalaya 2011). An experimental study conducted by Cargill et al. (2009) in Jayawijaya region, Papua, suggested that when helminthiasis was controlled, the pre-weaning mortality could be reduced to as low as 10%. On other hand, an outbreak of CSF was reported in Timika region, Papua, in 2004 and confirmed to spread to Jayapura and Jayawijaya regions (Ministry of Agriculture RI 2006). It indicates that while control of helminthiasis could help to reduce mortality in a Papuan pig farm, other efforts may be needed to control mortality due to other infections, such as CSF.

During the CSF outbreak in Timika, *Streptococcus zooepidemicus* was reported to also be involved (Dinas Peternakan Mimika 2004, unpublished data). *S. zooepidemicus* was reported to cause an outbreak in Sichuan pigs, in China in the summer 1975 and in Bali, Indonesia, 1994 (Fan et al. 2008; Soedarmanto et al. 1996). *Streptococcus zooepidemicus* was also isolated in a PRRS outbreak in Vietnam, in 2007 (Metwally et al. 2010). In addition, *S. zooepidemicus* has been reported as a sporadic zoonosis from contact with infected horses (Villamil et al. 2015). The prevalence of infection with this pathogen in Papuan village pigs, and its role in pig borne zoonotic disease in Papua remains to be determined.

Other pathogens such as *Streptococcus suis* have been isolated in the regions of Timika and Jayawijaya (Salasia et al. 2011; Slipranata et al. 2014), but its role in pig mortality in Papua remains unclear. *S. suis*, however, has been reported to cause significant outbreaks of mortality in pigs and humans in China (Ye et al. 2006).

Pig cysticercosis, a zoonotic potential, have been only studied in Jayawijaya, with 40.5% (n=111) seroprevalence (Assa et al. 2012). The Papuan regions of Paniai, Nabire, Pegunungan Bintang, Puncak Jaya and Manokwari have reported human taeniasis from *T. solium* but the status of pig cysticercosis in these regions is unknown (Margono et al. 2006; Wandra et al. 2013). Cysticercosis has never been reported to involve mortality in pigs, but pig cysticercosis is a serious public health concern (Prasad et al. 2006; Sáenz et al. 2008; Wandra et al. 2013).

A serological survey of some other pig pathogens was conducted in Jayawijaya (n=39). This survey was unable to detect the presence of antibodies against brucella, leptospira (*Leptospira pomona* and *L. tarossovi*), Porcine Parvovirus (PPV), *Mycoplasma hyopneumoniae*, transmissible gastro-enteritis (TGE) virus in the Papuan pigs sampled (Cargill et al. 2009). Methods used in the study however was not presented, so as whether the tests depicted the true prevalence or the inaccuracy of the test interfered the results was unable to be evaluated.

Studies in Indonesia have reported a number of infectious pig diseases from various islands, but their presence in Papua is yet publicly reported. These diseases include porcine circovirus 2 disease (PCVD), colibacillosis, porcine reproductive and respiratory syndrome (PRRS), erysipelosis and brucellosis (Besung 2012; Leslie et

al. 2015; Manokaran et al. 2008; Suartha et al. 2013; Veralyn et al. 2014). Studies are warranted to investigate the impact of these diseases in Papuan pig production.

The studies included in this thesis were aimed at understanding the demographics of pig populations, the epidemiology of pig diseases in Papuan pigs including infection with PCV2 and at proposing locally adapted control approaches to reduce the incidence of selected pathogens. The study was conducted in Jayawijaya region, where, at the time when we designed our study, was reported to have a pig population that comprised 24% of the total pig population in the province.

The thesis organisation

Chapter 5 of this thesis describes an update of our knowledge of the characteristics of traditional pig farming, productivity and the role of mortality as a constraint to pig production in Jayawijaya, Papua. A cross sectional survey was performed and the detail was presented. The results highlighted the traditional farming practices in Jayawijaya and demonstrated the importance of pig mortality as a major constraint to production. This information provides a baseline data for the subsequent studies of the epidemiology of pig diseases in Papua.

Chapter 6 describes the prevalence of selected pathogens which might have had significant contribution to pig disease in Papua. We included in the study pathogens such as CSF virus, a number of selected internal parasites, *S. suis, S. zooepidemicus* and PCV2. PCV2 infection has never been diagnosed in Papua previously. It was included in the current study because during the post mortems a number of pigs showed gross pathology of tan mottled lung and lower score body conditions,

indicative of PCV disease. Due to limited resources we excluded other potentially important diseases from our study, such as cysticercosis, colibacillosis, erysipelosis, brucellosis and PRRS.

The serological survey in the Papuan pig industry indicated that the prevalence of PCV2 was high. In order to confirm the presence of PCV2 virus, detection of the PCV2 partial genome was performed using molecular techniques. The results are presented in the first manuscript in Chapter 7. A further study of the complete PCV2 genome was performed from some local isolates, in order to characterise the Papuan isolates in more details. The results are presented in the second manuscript in Chapter 7.

In Chapter 8, the results from the previous chapters were combined with other studies of selected pathogens, from Papua and other locations, in order to obtain a better understanding of the epidemiology of these pathogens and associated diseases in Papua and to propose possible control measures. In this chapter, various aspects of the epidemiology and control of major pig diseases, including classical swine fever, PCVD, helminthiasis, cysticercosis and Streptococcosis due to *S. suis* and *S. zooepidemicus* in Papua were addressed.

In Chapter 9, a discussion and concluding remarks are presented. These emphasise the key features of pig farming practices in Papua and the major pathogens currently affecting traditional pig farms in the province.

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5. Traditional pig farming practices and productivity in Jayawijaya, Papua, Indonesia

Pig farming has been an important support for the livelihood of local communities in Papua, economically as well as culturally. Knowledge of the basic practices in today's pig farming in Papua is essential to gain insight into the current problem underlying the low productivity in Papuan pig farms, as a base line for future studies and programs to improve Papuan pig performance.

The study was conducted in Jayawijaya, a region with one fourth of pig population in Papua province, to obtain an updated situation of pig farming practice, productivity and some constrains in Papuan pig production.

The paper is published in The Journal of Tropical Animal Health and Production (2015) 47:495–502.

Original article: Traditional pig farming practices and productivity in the Jayawijaya region, Papua Province, Indonesia

Widi Nugroho, Colin Frank Cargill, I Made Putra, Roy Neville Kirkwood, Darren John Trott, Siti Isrina Oktavia Salasia & Michael Philipp Reichel (2015)

Traditional pig farming practices and productivity in the Jayawijaya region, Papua Province, Indonesia

Tropical Animal Health and Production 47: 495–502

Statement of Authorship

Title of Paper	Traditional pig farming practices and productivity in the Jayawijaya Region, Papua Province, Indonesia
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Publication Style
Publication Details	Nugroho, W., Cargill, C. F., Putra, I. M., Kirkwood, R. N., Trott, D. J., Salasia, S. I., and Reichel, M. P., 2015. Traditional pig farming practices and productivity in the Jayawijaya region, Papua Province, Indonesia, Tropical Animal Health and Production, 47: 495-502

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Contribution to the Paper	Designed the survey, designed the questionnaire, collected data, interpreted data and wrote manuscript.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Trop Anim Health Prod (2015) 47:495–502
DOI 10.1007/s11250-014-0748-5

REGULAR ARTICLES

Traditional pig farming practices and productivity in the Jayawijaya region, Papua Province, Indonesia

Widi Nugroho · Colin Frank Cargill · I Made Putra · Roy Neville Kirkwood · Darren John Trott · Siti Isrina Oktavia Salasia · Michael Philipp Reichel

Received: 3 May 2014 / Accepted: 11 December 2014 / Published online: 7 January 2015
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Abstract The objective of the current survey was to provide an update on pig farming practices in the Jayawijaya region, Papua Province, Indonesia. A structured semi-close-ended questionnaire was used to interview 367 farmers across the Jayawijaya region. Results showed that farms, on average, comprised of 8.8 pigs (CI 8.5–9.1). The average litter size was 6.0 (CI 5.7–6.3) piglets, the farrowing frequency was once a year, and the annual mortality rate was 50.2 % (CI 48.4–51.9). On average, 43.4 % farms (CI 36.4–50.7) allowed pigs to roam freely during daylight hours. Farmers used pigs for their own consumption (62.4 %, CI 57.4–67.4), as a gift (56.6 %, CI 51.5–61.7), or for sale (50.7 %, CI 45.6–55.8). Veterinary services were used intensively by just 11.7 % of farmers (CI 8.2–16.5). Furthermore, 34.2 % (CI 29.3–39) of farmers would sell sick pigs, and 63.1 % (CI 58.2–68.1) would slaughter and consume them. It was also recorded that 68.6 % of farmers (CI 63.7–73.4) would eat sick pigs that had died naturally. These findings suggest that traditional pig farms in Jayawijaya are of low productivity. Moreover, the free roaming of pigs and the sale and consumption of sick pigs have the potential to allow pathogens to circulate between pig and human populations.

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Keywords Native pigs · Questionnaire survey · Consumption · Papua

Introduction

Pigs are an important livestock species for the traditional Papuan tribes, who represent more than 80 % of the people occupying the Jayawijaya region, Papua Province of Indonesia (Anonymous 2011). The number of pigs owned is an important determinant of social status and wealth amongst Papuans (Muller 2009). Socio-cultural activities such as weddings and dowry giving, pig feast parties, religious ceremonies, the opening of new gardens, healing rituals, compensation for murders, funerals and many other social conventions all involve pigs as the offering (Muller 2009). Recently, pigs also have been identified as an important source of cash income for the Papuan Dani tribe (Mahalaya 2011). It is, therefore, not surprising that pigs comprised 83 % of domesticated animals in this region (Anonymous 2011).

Problems such as low weight gain, infertility, parasitic diseases and mortality are common on traditional pig farms in Jayawijaya. A project conducted in some villages to improve the efficiency of pig husbandry practices in Dani tribal communities of Papua achieved significant improvements associated with improved feed quality, parasite control and fertility for participating farmers and some of their relatives (Cargill et al. 2009). It is, however, unknown whether results of that project have been adopted widely in order to improve the general situation of pig farming in Jayawijaya.

The present survey was aimed at providing an assessment of the current status of traditional pig farming and husbandry practices in Jayawijaya, Papua, Indonesia. The results will

provide the basis for the development of programs to further improve the productivity of traditional piggeries in this region.

Materials and methods

Ethics approval

This study was approved by the Human Research Ethics Committee of The University of Adelaide (H-2012-092) and the local government of Jayawijaya region. All participants gave informed consent.

Characteristics of the study area

Jayawijaya is a region of Papua Province, Republic of Indonesia. It is situated between 138° 30′–139° 40′ longitude and 3° 45′–4° 20′ latitude; a highland at between 1490–4800 m above sea level (a.s.l). It has an area of 8496 km^2, divided into 11 subdistricts, and contains 290 villages (Anonymous 2011). The climate is of a typical tropical region where rain falls all year round, with the average number of rainy days per month being 24. Monthly rainfall during 2011 ranged from 122.3 to 263.2 mm with an average of 178.4 mm per month. The average humidity was 78 % (range 74–81 %), and the mean ambient temperature 19.3 °C, with an average maximum and minimum of 28.7 and 12.9 °C, respectively (Anonymous 2012b).

Sampling frame

During the study period, the region of Jayawijaya had a population of 196,085 people residing in approximately 47, 245 households, which gave the average household size as 4.2 persons (Anonymous 2011). The subject of this study was the farm, which was defined as a place where a household raised a minimum of one pig. Practically, the farm area consisted of one or two pig houses with or without a yard. Based on local opinion, it was assumed that all households in Jayawijaya were farming. An exception was the district of Wamena where many citizens were not farmers. It was estimated that farmers comprised just 65 % of households in that district.

There were six isolated villages in the south-west area of the district of Wamena which were excluded from the sampling frame. These were located on the other side of Puncak Trikora mountain (3750 m a.s.l.) and required special permission to visit the area. This reduced the size of the sampling frame to 46,018 households in 284 villages. For sampling, a total of 367 farms were selected and distributed proportionally to the size of households in villages of each of 11 districts, for a total of 203 villages. In some cases, there were two or more villages in one district but only one was needed. In these circumstances, the choice was based on convenience. Within a village, farms were chosen by convenience.

Data collections were performed by visiting farmers at their farm and inviting them to participate in the interview process. The geographical positions and the altitudes of sample farms were determined using Garmin GPSmap 60CSX. The survey was conducted for a three and a half month period, from mid-September until late December, 2012.

Questionnaire

Interviews were conducted using a structured semi-close-ended questionnaire. Before the survey was applied, the question structure and wordings were discussed with local staff from the Food Security Body (Badan Ketahanan Pangan) of Jayawijaya region for their clarity and appropriateness to the local situation. If necessary, modifications were made. Interviews were conducted in the Indonesian language, but in some places, questions were also translated into local languages by local personnel who understood the Indonesian language and were hired to accompany the surveyor during interviews. Although not always the case, it was not uncommon for an interview session to be attended by the farmer and several relatives living in the same house, or by neighbours and persons appointed by the village head ("kepala desa"). They cross-checked each other's responses before ending up with one agreed answer.

Briefly, questionnaires covered aspects of the demography of the farming community, i.e. gender, tribe, size of household and main occupation; structure of the farms and stock movements, i.e. farm size, litter size, pig entries and removals; farming practices, i.e. housing, feeding and drinking water provisions, weaning related management; and mortality and disease management, i.e. mortality rate, the treatment of sick and dead pigs on farms. The study investigated the pig farming practices during a period of 1 year, from September 2011-August 2012.

Data analysis

Data are presented as means or proportions with their 95 % confidence interval (CI). Differences between groups were analysed using the chi-square test and presented as P values. Calculations were performed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). The spatial representation of sampling locations and spider diagram of the pig trade through Jibama market were produced using ArcGIS 9.3 (Esri Inc., USA).

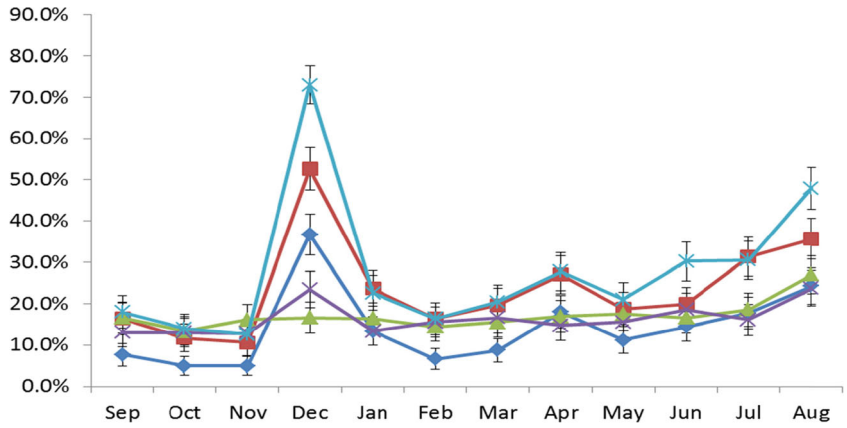

Fig. 1 Pattern of pork consumption amongst farmers in the Jayawijaya region during the 1-year period of September 2011 to August 2012 obtained from a survey of 367 farms conducted by questionnaire. Slaughter their own pigs. Gift from relatives. Eat at tavern. Purchase meat and cook at home. Eat at a tribal ceremony

Results

Demography of the farming community

Interviewees were predominantly men, i.e. heads of households (90.7 %, CI 87.8–93.7). The majority of respondents were from the Dani tribe (74.4 %, CI 69.9–78.9). Other tribes represented were the Lani tribe and other Papuans, Torajanese and Javanese (21.2 %, CI 15.5–27.3; 4.2 %, CI 2.0–8.1; and 0.3 %, CI 0.03–2.6, respectively). The mean size of the household was 9.6 persons (CI 9.3–9.9). Farming was the main occupation for 70.1 % of respondents (CI 65.4–74.8), while others were working as civil servants, employees in private companies or merchants. The majority of farmers lived at an altitude between 1500 and 1800 m a.s.l. (79.0 %, CI 74.8–83.1), while a smaller proportion lived above 1800 m a.s.l. (21.04 %, CI 16.9–25.2).

The structure of the farms and stock movements

The average farm is comprised of 8.8 pigs (CI 8.5–9.1). The number of sows and gilts was 1.7 (CI 1.6–1.9), with 1.3 (CI 1.2–1.4) mature males, 2.2 (CI 2.0–2.3) growers and 3.6 (CI 3.4–3.8) suckling piglets. The mean age of growers was 15.3 months (CI 14.8–15.9) (Table 1). Extrapolating the size of farms to the region, there were approximately 23 pigs per km^2 of the Jayawijaya region.

Black and a mix of at least two colours were the most popular pig types (29.2 %, CI 26.8–31.7 and 29.8 %, CI 26.9–32.8). Other colours were brown, white and grey (23.6 %, CI 21.0–26.4; 12.7 %, CI 10.9–14.7; and 4.8 %, CI 3.7–6.2, respectively).

The mean litter is comprised of 6.0 (CI 5.7–6.3) piglets. The calculated farrowing frequency per year was one. Farmers mainly introduced new pigs from a gift or a purchase (63.8 %, CI 58.8–68.7 and 56.7 %, CI 51.6–61.8, respectively). The hunting for pigs was practised by few farmers (3.8 %, CI 2–5.8), and 19.3 % (CI 15.3–23.3) of farmers did not acquire any new pig during the year (Table 1).

Mostly, farmers used pigs for their own consumption (62.4 %, CI 57.4–67.4) or to present as a gift (56.6 %, CI 51.5–61.7) (Table 1). The peak of pork consumption occurred in December and in August (Fig. 1). The sale of live pigs (50.7 %, CI 45.6–55.8) was less common than slaughter ($P<0.002$), but slaughter was not more common than being given as a gift (62.4 % vs 56.6 %, $P>0.05$). The trade of pigs was centralised at the Jibama market, with more than one third of farmers attending the Jibama market for the purchase and sale of pigs (39.8 %, CI 34.8–44.8 and 36.5 %, CI 31.6–41.4, respectively) (Table 1; Fig. 2). Pig mortality and losses, with some probably due to theft, were relatively common (65.9 %, CI 61.1–70.8 and 29.4 %, CI 24.8–34.1, respectively) (Table 1).

Pig housing

A significant proportion of farmers used wood for flooring and partitions and tin for roofing (19.9 %, CI 15.8–24.0) in pig housing (Table 2). The majority of farmers utilised grass for bedding (79.3 %, CI 75.2–83.4). Only 17.7 % (CI 13.8–21.6 %) of farmers provided natural ventilation via open sidewalls. The remaining pig houses were fully enclosed.

Mostly, farmers allowed their pigs to roam freely during daylight (43.4 %, CI 36.4–50.7). Other farmers either semi-confined the pigs in traditional enclosed yards called “laleken” or mixed the use of the “laleken” with free roaming (10.7 %, CI 6.9–16.0 and 30.1 %, CI 23.8–37.1, respectively). A number of farmers confined their pigs in the house all day (15.8 %, CI 10.6–23.1). The vast majority of farmers weaned the piglets at the age of 2 months or older (96.4 %, CI 94.5–98.3). More than half of farmers mixed weaners from different litters in the same pen (57.2 %, CI 52–62.4) (Table 2).

Feeding regime

Half of the farmers fed their pigs less than twice each day (51.1 %, CI 46–56.2). At least three different feeds were given to pigs by 80.1 % farmers (CI 76.0–84.2). The two most popular daily feeds were sweet potato leaf and tuber

Table 1 Structure of the pig farm and stock movement in Jayawijaya region during a period from September 2011 to August 2012. Result from a survey of 367 farms conducted by questionnaire

Measurement	Categories	Mean/proportion (%)	95 % CI
Mean farm size (n=367)	• Total	8.8[a]	8.5–9.1
	• Sows	1.7[a]	1.6–1.9
	• Mature males	1.3[a]	1.2–1.4
	• Growers	2.2[a]	2.0–2.3
	• Suckers	3.6[a]	3.4–3.8
Mean age in month (n=367)	• Sows	36.9[a]	36.2–37.6
	• Mature males	28.5[a]	27.8–29.2
	• Growers	15.3[a]	14.8–15.9
	• Suckers	2.7[a]	2.5–2.9
Litter size (n=276)		6.0[a]	5.7–6.3
Mortality rate (n=3221)		50.2	48.4–51.9
Ways of obtaining pigs (n=367)	• Gift from relatives	63.8	58.8–68.7
	• Purchase	56.7	51.6–61.8
	• Hunting in the jungle	3.8	2.0–5.7
	• No new pig put in	19.3	15.3–23.3
Ways of removing pigs	• Slaughter and eat (n=367)	62.4	57.4–67.4
	• Present (n=362)	56.6	51.5–61.7
	• Sale (n=367)	50.7	45.6–55.8
	• Stolen/lost (n=367)	29.4	24.8–34.1
	• Died (n=367)	65.9	61.1–70.8
The use of Jibama for pigs trades (n=367)	• Purchase	39.8	34.8–44.8
	• Sale	36.5	31.6–41.4
Proportion of farms with death (n=366)	• None	34.2	28.4–40.4
	• 1–2 heads	13.9	10.1–18.9
	• 3 or more	51.9	45.5–58.2

[a] mean

(93.2 %, CI 90.6–95.8 and 97.5 %, CI 96–99.1, respectively). The other popular daily feed was swill (65.7 %, CI 60.8–70.5). Grass was fed daily by just 15.5 % farmers (CI 11.8–19.2). Other sources of daily feed were vegetable oil, cassava, rice, salt, taro, tofu by-product, betel nut, golden snail and cabbage and were used by only 0.5–8.2 % of farms. Water was provided to the pig house on only 12.0 % farms (CI 8.46-16.81) (Table 2).

Mortality and disease management

The proportion of farms with pig mortality during the 12-month study period was 65.9 % (CI 61.1–70.8). The crude mortality rate was 50.2 % (CI 48.4–51.9) (Table 2). When an animal was sick, 11.7 % of farmers (CI 8.2–16.5) consistently used the services of a veterinarian, while another 19.7 % (CI 15.1–25.2) of farmers used a veterinarian combined with alternative treatments (Table 3). Even if they did not use the veterinary service, 86.6 % farmers (CI 83.2–90.1) believed in the efficacy of veterinary medicine. Other treatments employed were human and traditional medicines (15.0 %, CI 11.4–18.7 and 43.4 %, CI 38.4–48.5, respectively) (Table 3). Amongst traditional means, treatment with the bark of the "Gami/Kami" plant was the most common, practiced by 52.2 % of farmers (CI 44.4–60.0). Some farmers sold sick pigs or left pigs until they died or spontaneously recovered (34.2 %, CI 29.3–39 and 30.3 %, CI 25.6–35, respectively). Regardless of all these treatments, 63.1 % (CI 58.2–68.1) of farmers would slaughter and eat diseased pigs. It was also recorded that 68.6 % of farmers (CI 63.7–73.4) would eat the flesh of sick pigs (Table 3).

Discussion

To our knowledge, this is the first representative study of pig farming practices of the entire Jayawijaya region. In this study, the use of semi-closed questions, the translation into the local language and the discussion amongst farmers before answering may have helped in obtaining more consistent answers. Mantana (1990) suggested that group interviews help to reduce the discrepancies amongst responses. However, Lee et al. (1999) discovered that mistranslation, which could

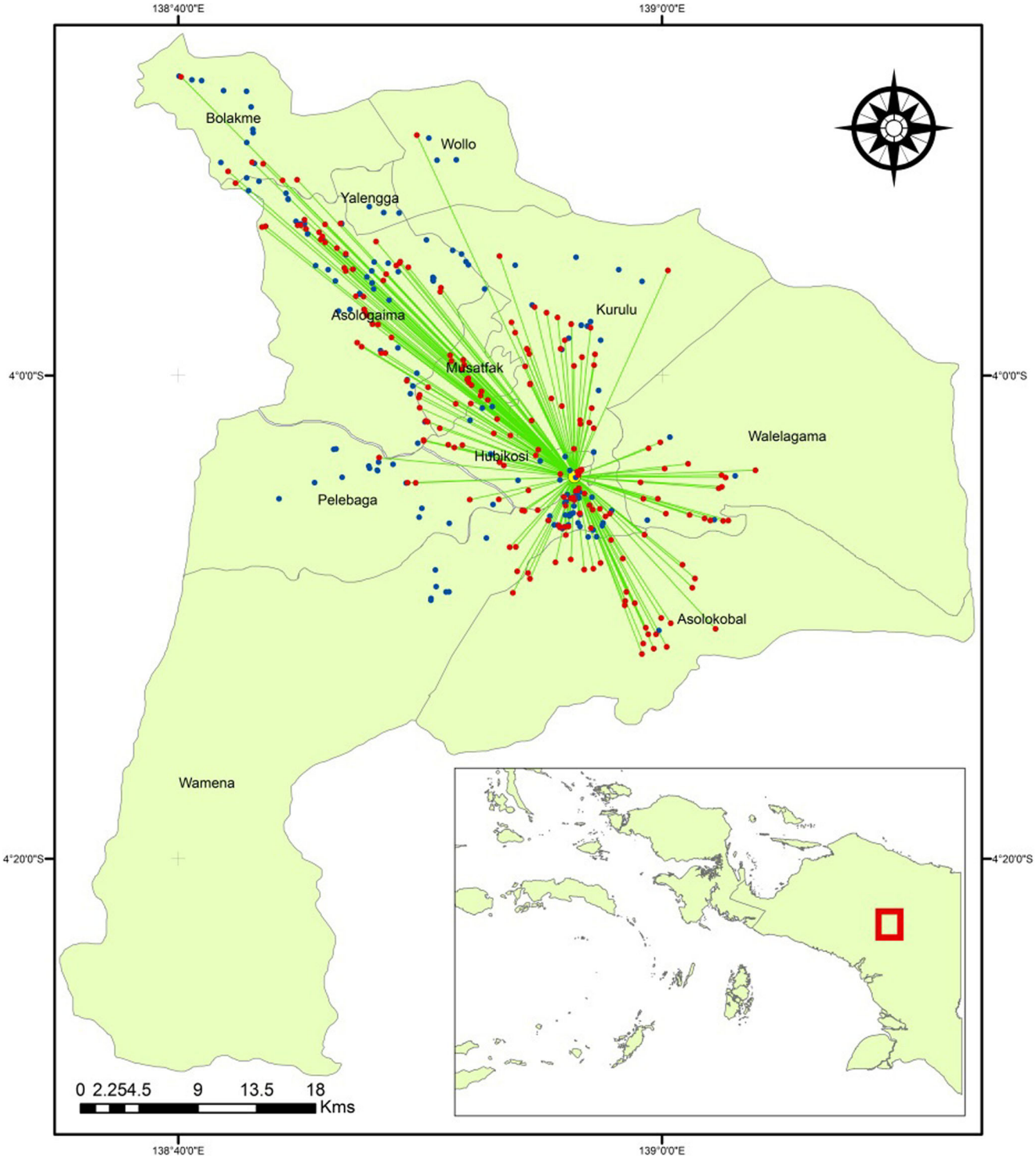

Fig. 2 Geographical spread of farmers using the Jibama market for pig trade. Data was obtained by structured questionnaire during the 1-year period from September 2011 to August 2012 (n=367). Trader farmers. Other farmers. Jibama market. Trade line

contribute to up to 37 % of all questionnaire mistakes, may be unavoidable. Therefore, one should use the result of this cross-cultural survey with caution.

The vast majority of interviewees were men. Interviewing men may help to reduce response biases because men spend approximately 2 hours more a week than women cleaning pig houses, mating and dealing with sick pigs, while women were mainly responsible for daily feeding (Mahalaya 2011). This shared labour suggests an adequate understanding of the interviewees for the subject matter of the questionnaire.

Table 2 Pig husbandry practice in Jayawijaya Region from September 2011 until August 2012. Result from a survey of 367 farms conducted by questionnaire

Husbandry parameters	Categories	Proportion	95 % CI
Material of pig house (floor-partition-roof) (n=367)	• Wood-wood-tin	19.9	15.8–24.0
	• Other combinations	84.2	80.4–87.9
Pig release during daylight (n=366)	• Freely roaming	43.4	36.4–50.7
	• Laleken* and free occasionally	30.1	23.8–37.1
	• Laleken	10.7	6.9–16.0
	• In the pig house all day	15.8	10.6–23.1
Age to wean piglets (n=364)	• 2 months or older	96.4	94.5–98.3
	• Earlier	3.6	1.7–5.5
Putting together weaners from different litter (n=348)	• Yes	57.2	52–62.4
	• No	42.8	37.6–48.0
Frequency of feeding daily (n=362)	• Less than twice	51.1	46–56.2
	• Twice or more	48.9	43.8–54
Number of different feeds fed daily (n=367)	• <Three	19.9	15.3–25.5
	• Three or more	80.1	76.0–84.2
Feeds fed daily (n=367)	• Sweet potato leaf	93.2	90.6–95.8
	• Sweet potato tuber	97.5	96–99
	• Swill	65.7	60.8–70.5
	• Grass	15.5	11.8–19.2
	• Others	0.5–8.2	–
Provision of water for pigs (n=366)	• Yes	12.0	8.7–15.4
	• No	88.0	84.6–91.3

*Laleken is a traditional name for fenced yard

However, we found during the interview process that the mean size of households in the current survey was twice the average size of Jayawijaya households. This interesting result suggests that households that are pig farming are truly larger than non-farmer households, probably because they live with their extended family.

Table 3 The disease and mortality managements on farms in Jayawijaya in 12-month period from September 2011 to August 2012. Survey of 367 farms

Parameter assessed	Proportion (%)	95 % CI
Treatment to diseased pigs (n=366)		
• Leave them without treatment	30.3	25.6–35
• Sell	34.2	29.3–39
• Slaughter and eat	63.1	58.2–68.1
• Traditional medicine	43.4	38.4–48.5
• Human medicine	15.0	11.4–18.7
• Vet medicine only	11.7	8.2–16.5
• Vet medicine and others	19.7	15.1–25.2
Treatment to dead pigs (n=350)		
• Eat	68.6	63.7–73.4
• Dump in river	11.4	8.1–14.8
• Bury	29.7	24.9–34.5
• Burn	29.7	24.9–34.5
• Sell	0.6	0–0.8

Pig farms in Jayawijaya consisted of only 8.8 pigs on average. The pig to person ratio in the current study was similar to findings of previous studies in Jayawijaya (Hide 2003; Mahalaya 2011). This indicates that traditional sizes of pig farms in Jayawijaya over the last 30 years have remained stable. We defined all pigs that have already been weaned but not mated, as growers. Using this definition, the mean age of grower pigs in this study was 15.3 months. Since gilts have the potential to achieve puberty as early as 6–8 months of age (Whittemore and Kyriazakis 2008), the presence of non-mated growers over 1 year of age implies delayed sexual development, possibly resulting from malnutrition. Cargill et al. (2009) reported that pigs raised on traditional feeds in Jayawijaya grew at 18 g/day and took 2.5 years to reach puberty. By doubling the weight of daily feed provided and incorporating extra protein, Cargill, et al. (2009) demonstrated a significant increase of daily gain. However, the impact of improved nutrition on achievement of puberty remains to be determined.

We found that the calculated farrowing frequency was just once a year, which is lower than that reported for intensive farming situations in other regions. A Vietnamese study reported that in demand-driven farming, the farrowing frequency was 1.5 litters/year, while in farms further away from town,

it was 1.1 (Lemke et al. 2006). In industrial piggeries, farrowing frequencies are higher, ranging from 2.2 to 2.5 per year (Anonymous 2012a). Clearly, there is considerable potential for improvements in pig reproduction in Jayawijaya.

Popular daily feeds reported in this study were sweet potato tubers, leaves and swill which were, on 50 % of farms, provided less than twice daily. Swill feeding is a common practice in Papuan piggeries (Iyai 2011), and swill may be an important source of feed in low input farms. Feeding swill from contaminated sources, however, contributed to transmissions of classical swine fever (CSF) virus in Germany (Fritzemeier et al. 2000) and in Southeast Asia (Cameron et al. 1999) and PRRS in Vietnam (Truong and Gummow 2014). The role of swill feeding in pig diseases transmission in Papua warrants further investigation.

Almost half of Jayawijaya farms released pigs to freely roam during daylight. The pigs have an average home range for scavenging 1 km^2 and spend 47 % of their time outside the homestead (Thomas et al. 2013). Free roaming pigs are prone to parasitism (Carstensen et al. 2002; De Fredrick 1977; Hide 2003; Putra et al. 2004; Vermeer et al. 2000), toxicosis (Hide 2003), trauma, starvation and cold (Feenstra 2000) and disease transmission (Thomas et al. 2013). Grass as bedding inside the pig house was provided by the majority of farms but not as deep is recommended by researches. Deep bedding was reported to increase weight gain (Leeb and Baumgartner 2000; Vermeer et al. 2000) and to improve animal welfare (Caldara et al. 2014).

Cargill et al. (2009) reported that in traditional piggeries in Jayawijaya, mortality was 40 % over a 4-month observation period and pre-weaning mortality was higher. In contrast, in Kenya, pre-weaning mortality was as low as 10 % in small holder farms with monthly veterinary service (Wabacha et al. 2004). Average pre-weaning mortality in industrialised countries in 2010 ranged from 8 to 15 % (Anonymous 2012a). While malnutrition may play a part, infectious diseases such as classical swine fever, streptococcal bacteriosis and parasitism appear to be also responsible (Cargill et al. 2009).

Although pig mortality was high and more than 80 % of farmers believe in the efficacy of veterinary medicine, only 11 % of farmers consistently used veterinary services. Factors influencing the demand for veterinary services were not determined in the current study, but others have reported that long distances (Mutambara et al. 2013), small herd size, low farm income, lack of knowledge of husbandry practices, less time spent in husbandry activities (Tambi et al. 1999) and the difference in gender between providers and farmers (Irungu et al. 2006) influenced the demand for veterinary service.

While the use of veterinary services was very limited, the use of the betel nut, a proven efficacious traditional medicine was also lacking. Cargill et al. (2009) introduced the successful treatment of helminthiasis using betel nut and pawpaw in Jayawijaya. Betel nut is plentiful and very popular in Jayawijaya as a human stimulant. Instead of the betel nut, the bark of "gami" plant was very popular as a traditional pig medicine in Jayawijaya. Further investigation is needed to confirm its efficacy and appropriate use.

Pig farmers sell or consume the flesh of sick pigs, and many of them use Jibama market for live pig and pork trade. Our observations at the Jibama market showed that although farmers and traders knew that flesh contained obvious cysticercid cysts or the lung was diffusely haemorrhagic, there was still a market for these unhealthy meats. This finding shows that the Jibama market is an important focal point for pig disease and potential zoonotic spread across Jayawijaya. Festivals at Christmas and August for National Day celebrations are two important times for the risk of disease spread because during these times pork consumption peaks.

Cysticercosis was detected in 50.6 % people and 70.4 % pigs at five villages in Jayawijaya (Subahar et al. 2001) but, in the 30 years since its discovery, the prevalence of human cysticercosis and taeniasis in Papua is said to be unchanged (Salim et al. 2009). *Streptococcus suis* and *S. zooepidemicus*, two potential zoonoses, have been reported in pig mortality cases in Jayawijaya (Cargill et al. 2009). Given the high risk of zoonotic transmission, ongoing public awareness activities need to be developed to raise awareness of and reduce zoonotic risks as might be, for example, a rudimentary meat inspection/grading system.

In conclusion, the productivity of pig farms in Jayawijaya is relatively poor due to low litter size, low farrowing frequency and high annual mortality. Low productivity may be the result of poor farming and husbandry practices such as allowing pigs to roaming freely during daylight hours, feeding a low protein content diet only once daily and the lack of use of the veterinary services. Free roaming and the sale and consumption of sick pigs potentially contribute to the transmission of pig disease and zoonoses. It would be advisable to develop an extension program that introduces the results of previous pig production studies in Jayawijaya.

Acknowledgments We would like to acknowledge personnel in Dinas Perikanan dan Peternakan Kab, Jayawijaya, and Badan Ketahanan Pangan Kab. Jayawijaya for their technical support during the data collection. We would like to thank the pig farmers in Jayawijaya for their kind participation in this study. This study was funded by The Australian Centre for International Agricultural Research (ACIAR) through the project with grant number AH/2007/106 entitled "Improvement and sustainability of sweet potato-pig production systems to support livelihoods in highland Papua and West Papua, Indonesia".

Conflict of interest We confirm that there is no known conflict of interest associated with this publication.

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6. Investigations of selected pathogens among village pigs in Central Papua, Indonesia

Mortality has been known as a major constraint in Papuan pig production. Classical swine fever and helminthiasis were demonstrated to be associated with pig mortality in Papua. However the level of infection with these pathogens is unknown. On other hand, *Streptococcus suis* and *Streptococcus zooepidemicus* have been reported, but only in Timika region. Their presence in regions with high pig populations, such as in Jayawijaya warrants study.

This study investigated the prevalence of selected pathogens in two set of studies; in dead pigs sold in central market in Jayawijaya and in healthy pigs from farms without cases of mortality within three month preceding the study. Pathogens investigated included CSFV, PCV2, *Streptococcus suis*, *Streptococcus zooepidemicus*, and endoparsites; *Trichuris suis*, strongyle parasites, *Strongyloides ransomi*, *Ascaris suum* and coccidian.

The paper was published in the journal of Tropical Animal Health and Production (2016) 48, no. 1: 29-36

Original article: Investigations of selected pathogens among village pigs in Central Papua, Indonesia

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Investigations of selected pathogens among village pigs in Central Papua, Indonesia

Tropical Animal Health and Production 48, no. 1: 29-36

Statement of Authorship

Title of Paper	Investigations of selected pathogens among village pigs in Central Papua, Indonesia
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Publication Style
Publication Details	Nugroho, W., Cargill, C. F., Putra, I. M., Kirkwood, R. N., Trott, D. J., Salasia, S. I., Slipranata M. and Reichel, M. P., 2016. Investigations of selected pathogens among village pigs in Central Papua, Indonesia, Tropical Animal Health and Production, 48: 29-36

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Name of Principal Author (Candidate)	Widi Nugroho		
Contribution to the Paper	Designed the survey and questionnaire, collected samples and data, analysed samples, interpreted data and wrote manuscript.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
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Contribution to the Paper	Supervised development of the study, helped with provision of test kit for viral detection, assisted and directed data interpretation, edited and corrected manuscript, acted as corresponding author		
Signature		Date	07/08/2015

Please cut and paste additional co-author panels here as required.

Trop Anim Health Prod (2016) 48:29–36
DOI 10.1007/s11250-015-0913-5

REGULAR ARTICLES

Investigations of selected pathogens among village pigs in Central Papua, Indonesia

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Received: 25 March 2015 / Accepted: 7 September 2015 / Published online: 17 September 2015
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Summary Village pig husbandry is an important part of livestock production in Papua Province, Eastern Indonesia. However, high level of disease and mortality constrains production. The aim of this study was to investigate the prevalence of the selected pathogens in village pigs in the Jayawijaya Region of Papua Province, Indonesia. Two studies were conducted: Study 1 determined the prevalence of selected pathogens in dead or moribund pigs sent to the main local market for sale. Study 2 recorded the prevalence of the selected pathogens, on pig farms in the Subdistrict of Wamena that had not recorded a case of pig mortality during the duration of Study 1. Blood samples of individuals from both groups were tested for CSF antigen and antibody, as well as antibody against PCV2. Organs with evident pathological changes from Study 1 and tonsilar swabs from Study 2 were subjected to bacteriological culture and identification of *Streptococcus suis* and *Streptococcus zooepidemicus*. Faecal samples from both studies were examined for eggs of strongyle parasites, *Trichuris suis*, *Ascaris suum*, *Strongyloides ransomi* and coccidia. The main infections in both studies were CSF, PCV2 and strongyle parasites, but prevalence was higher in Study 1 ($P<0.05$). *T. suis* and *S. zooepidemicus* were prevalent in pigs in Study 1, but rare in healthy pigs ($P<0.05$). Infections with coccidia, *A. suum* and *S. ransomi* were common but did not differ between groups ($P<0.05$), with *S. suis* infections uncommon in both studies. This suggests that infections with CSF, PCV2, strongyle and *T. suis* are important pathogens in village pig farms in Jayawijaya. Local pig husbandry practices, such as confining pigs and heat-treating pig feeds, may be practical solutions to help minimize infection in village pigs in Jayawijaya.

Keywords Pig · CSF · PCV2 · Endoparasites · *Streptococcus suis* · *Streptococcus zooepidemicus*

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Introduction

Pigs are an important livestock species in the social, cultural and economic settings of communities in South-East Asia (Huynh et al. 2007) and the Pacific (Brioudes and Gummow 2015). More than 80 % of pig farms in these regions are run by smallholders (Huynh et al. 2007, Leslie et al. 2015, Nugroho et al. 2015), and pigs have been important sources of income for people living below the poverty line (Huynh et al. 2007, Leslie et al. 2015). Papua Province, Indonesia, is among the most pig dense regions of Indonesia (Siagiaan 2014), with an estimated total pig population of 0.6 million (BPS 2014). Village pigs are raised by 30 % of total households in this region (BPS-Papua 2013, BPS-Papua 2014). Papuan village pigs provide for multiple purposes: for household consumption, as a cash converter, a gift to relatives (Nugroho et al. 2015), and they are also widely used as the main offerings in cultural ceremonies (Muller 2009). Therefore, low productivity of village pig farms will influence the livelihood of the human population in Papua.

Village pig farms in Papua consist of mixed breeds and have an average size of below nine head (Nugroho et al. 2015). Pigs are confined at night but scavenge freely during daylight hours with low input in feeding or disease management (Nugroho et al. 2015). These low inputs result in an average litter size of just 6 piglets, a farrowing frequency of just once a year and an annual mortality of up to 50 % (Nugroho et al. 2015).

Within Papua, the Jayawijaya Region has the largest pig population, with 24 % of all Papuan pigs (BPS-Papua 2013). Pig trading happens daily in this region, and the main market, Jibama, is used by farmers across the Jayawijaya Region to sell and purchase live pigs and pork, including diseased pigs (Nugroho et al. 2015), thus potentially is a source of a number of important pig pathogens and pig-derived zoonoses.

Pig pathogens recognised in various locations in Indonesia include Porcine circovirus 2 (PCV2), *Escherichia coli*, porcine reproductive and respiratory syndrome virus (PRRSv), *Streptococcus equi* subspecies *zooepidemicus (S. zooepidemicus)*, *Erysipelothrix* spp., *Brucella* spp. and classical swine fever virus (CSFv) (Manokaran et al. 2008, Besung 2012, Suartha et al. 2013, Veralyn et al. 2014, Leslie et al. 2015). In Papua province, endoparasite burdens were initially regarded to be a major contributing factor to disease and mortality (Putra et al. 2004). Later, CSFv involvement was confirmed in fatal pig disease outbreaks in Timika, Jayapura and Jayawijaya Regions (Ministry of Agriculture RI 2006). *Streptococcus suis* has been reported in regions of Timika and Jayawijaya (Salasia et al. 2011, Slipranata et al. 2014).

Little is known about the relative prevalence of the various pathogens in village pigs in Papua, but this knowledge may be important for designing cost effective control programmes for the prevention of pig diseases and, to estimate the optimum outcomes from efforts and budgets spent, especially in an area where resources are very limited. Therefore, the aim of this study was to investigate the prevalence of infection of selected pathogens; CSFv, PCV2, *S. suis*, *S. zooepidemicus* and endoparasites (*Trichuris suis*, Strongyle parasites, *Strongyloides ransomi*, *Ascaris suum* and coccidia) in the Jayawijaya. In the current study, two sets of observational case studies were conducted, one at Jibama market to investigate infection with the selected pathogens in dead or moribund pigs and another one in the Subdistrict of Wamena, to understand the level of carriage of the selected pathogens in apparently clinically healthy pigs, from village pig farms with no history of pig mortality during the period of Study 1. These two sites of study were chosen for their close proximity to the local laboratory, to facilitate a more immediate processing of the samples.

Methods

The studies were approved by the University of Adelaide Animal Ethics Committee (S-2012-131) and the local government of the Jayawijaya Region. All participant farmers gave informed consent.

Study 1

This study was performed at Jibama market during a 3-month period in 2013. This market is situated in the center of the Jayawijaya Region and is known as the center of pig trading across the region, with many farmers also selling sick pigs (Nugroho et al. 2015). In the current study, 92 dead or moribund pigs (referred to as dead pigs hereafter) from farmers attending the market were examined by postmortem. Age, sex and subdistrict of origin of the animals were collected using interview and observation. There were no data available regarding the farm where these pigs originated from or the CSF vaccination of this group.

Samples collected included 71 blood samples and organs or tissues with evident pathological changes, i.e. 56 lungs, 49 brains, 13 ascitic fluid samples and six livers. Furthermore, 44 faecal samples were obtained *per rectum* and placed in separate plastic containers. Samples were kept at 4 °C and sent to Badan Lingkungan Hidup Laboratory, Jayawijaya, for processing. Serum preparation, primary bacterial culture and faecal microscopy were performed on the day of sampling.

Study 2

The subdistrict of Wamena is the seat of government of the Jayawijaya Region. The farming community and pig population in this subdistrict are estimated to comprise 9711 households and 85,457 pigs (BPS-Jayawijaya 2011, Nugroho et al. 2015). A total of 103 apparently healthy pigs from 23 village farms were recruited from the Subdistrict of Wamena, of the Jayawijaya Region. The number of sampled pigs give an approximately 10 % precision at the 95 % confidence limit. The farmers were chosen by convenience. Age, sex, CSF vaccination status and husbandry practices were collected during an interview with the owner. To assess CSF and PCV2 status in these animals, 103 blood samples were collected by jugular venipuncture. The tonsils of these pigs were also swabbed, and the swabs stored in transport medium consisting of 2 % tryptone broth supplemented with 0.02 % sodium azide (Breton et al. 1986) until cultured for *S. suis* and *S. zooepidemicus*. Faecal samples were obtained from 102 of these animals to determine endoparasite burden. All samples were stored at 4 °C until processing. Serum preparation, primary bacterial culture and faecal examinations were performed on the day of sampling in Badan Lingkungan Hidup Laboratory, Jayawijaya.

S. suis and *S. zooepidemicus* identifications

Screening for streptococci was done at the Badan Lingkungan Hidup Laboratory, Jayawijaya. Organ samples, blood samples, ascetic fluid and tonsillar swabs were plated onto horse blood agar supplemented with 0.02 % sodium azide and incubated for 18 h at 37 °C in a candle jar. Screening for *S. suis* and *S. zooepidemicus* was based on growth and colony and cell properties (Kilpper-Bälz and Schleifer 1987, Pesavento et al. 2008). Selected streptococci were kept in the refrigerator and recultured fortnightly until the end of the two field studies and subsequently transported to the Laboratory of Veterinary Clinical Pathology, University of Gadjah Mada, Jogyakarta, for further identification.

Identification of *S. zooepidemicus* was performed using the API 20 Strept (Biomeriux SA, France) system. The manufacturer states that for *S. zooepidemicus*, the system has 85 % sensitivity for the Arginine DiHydrolase reaction and up to 100 % for the other tests and 85 % specificity for the Aesculin reaction and up to 100 % for other tests. Identification of *S. suis* was performed using the polymerase chain reaction (PCR) for a defined region of the glutamate dehydrogenase (GDH) gene (Silva et al. 2006). The primer was reported to have 100 % sensitivity and specificity for *S. suis* serotypes 1/2, 1, 2, 7 and 9 (Okwumabua et al. 2003). DNA was extracted using the DNEasy kit (Qiagen, Germany) following the manufacturer's instructions. The PCR reagent was mixed with Maxima kit (Thermo Fisher Scientific). The PCR was run using a thermocycler (Eppendorf AG 22331 Hamburg, Germany), and the results were visualized under ultraviolet light (Uvt-200 Optima, Japan) with the addition of a DNA stain (SYBR safe, Invitrogen, USA).

CSF and PCV2 virus serological testing

Sera obtained were kept in a freezer in the laboratory of Badan Lingkungan Hidup, Jayawijaya, until the end of the two field studies and subsequently transported to the University of Gadjah Mada for further analyses.

IDEXX CSFV Ag (IDEXX, Switzerland) and IDEXX CSFV Ab (IDEXX, Switzerland) ELISA test kits were used following the manufacturer's instructions. Antibody titres against PCV2 virus were measured using SERELISA PCV2 Ab Mono Blocking ELISA (Synbiotics, Delpharm Biotech, France). Results were read using a Biorad 680 Microplate reader at a wavelength of 450 nm (Bio-Rad laboratories, Inc., CA, USA).

The manufacturer states that the IDEXX CSFV Ag has a diagnostic sensitivity of 84 % (n=90) and specificity of 98.5 % in wild boar (n=274) and 100 % in domestic pigs (n=609) (IDEXX, Switzerland). IDEXX CSFV Ab is said to have a specificity of 100 % (n=880). Sensitivity of the PCV2 tests is stated to be 94.1 % and specificity as 100 % (Synbiotics, Delpharm Biotech, France).

Screening for endoparasites

Screening for endoparasites was done at the laboratory of Badan Lingkungan Hidup, Jayawijaya. Five types of endoparasite eggs (strongyles, *S. ransomi*, *T. suis*, *A. suum* and coccidia) were identified using the modified double centrifugation technique (Foreyt 2001). One gramme of faeces was weighed, saturated sucrose solution was used as floating agent and the eggs were identified under the light microscope and counted to obtain the numbers of eggs per gramme (EPG) of faeces. The sensitivity of the test was 1 egg per gramme faeces.

Statistical analysis

Data are presented as the percentage of samples with detectable pathogen. Egg counts of selected parasites were presented as an arithmetic mean and range. Mixed infections were counted using 44 samples of Study 1 and 102 pigs from Study 2, from which the status of all pathogens investigated was able to be determined. A Z-test was performed to measure differences in prevalence of a pathogen between data from the Subdistrict of Wamena and other subdistricts in Study 1, as well as between Study 1 and Study 2. The Mann Whitney U test was employed to examine the difference of EPG between the subdistrict of Wamena and other subdistricts in Study 1, as well as between the two groups. All statistical significance were deemed at the two-sided 0.05 level, using SPSS 21 (IBM SPSS Inc., Chicago, IL).

Results

Demographics of Study 1

A total of 59 pigs were sent to market dead, and 33 were moribund and slaughtered in the market. From those dead pigs, 26 had died for more than 5 h. The majority of sample pigs (78.3 %) came from four of 11 subdistricts, namely Kurulu, Asologaima, Hubikosi and Wamena (23.9, 20.7, 18.5 and 15.2 %, respectively). A further 6.6 % came from four subdistricts, namely Asolokobal, Walelagama, Bolakme and Musatfak (2.2, 2.2, 1.1 and 1.1 %, respectively) and remaining samples (15.9 %) originated from unknown subdistrict in the Jayawijaya. No data were available for these animals as to their prior housing. From 92 pigs sampled, 41 pigs were male. Three pigs were reported to be less than 3 months of age, 32 pigs were between 3–6 months of age and 49 were above 6 months of age. The age of the remaining six pigs was unknown. There were no differences of the prevalence of

selected pathogens or EPG of selected endoparasites, in Study 1, between the Subdistrict of Wamena and other subdistricts ($P>0.05$). This enabled us to compare data from Study 1 with Study 2.

Demographics of Study 2

The study comprised 23 farms and the size of the farms ranged from 1–30 pigs (mean 8.4 head). All farms, except one, used a full confinement pig husbandry system. One farm had confinement but released pigs during the daylight hours. All farms used wood or concrete floors with roofs made of tin. Seventeen farmers reported cooking the pig feed daily before it was fed to pigs. Eight farmers had introduced new pigs onto their farms within 6 months before sampling. The origin of these new pigs were Jayapura (4 farmers), from other farms in Wamena (3 farmers), but also from Jibama market (1 farmer). Vaccination against CSF was reported from 12 farms; eight vaccinated their pigs at more than 9 months prior to sampling, three within 3 months, and one within 3–6 months (Table 1).

All healthy animals, except four, were recruited from fully confined farms. Fifty-six pigs were male. Nine pigs were less than 3 months of age, 50 were between 3 and 6 months old and 41 were older than 6 months. Three pigs were of unknown age. Interviews with farmers indicated that 37 of the 103 healthy animals were vaccinated using a commercial CSF vaccine (Pest-Vac®, Fort Dodge SA, Brazil) by a local veterinarian. From the 37 pigs reported to be vaccinated, 23 were vaccinated at more than 9 months prior to sample collections, 7 were vaccinated within 3 months and 7 pigs were vaccinated within 3 to 6 months.

Table 1 Characteristics of village pig farms ($n=23$) in the Subdistrict of Wamena, Jayawijaya Region without prior history of pig mortality in the 3 months preceding the study

Characteristics	Proportion of farms
Farm characteristics	
Always cook daily pig feeds	17 (73.9 %)
Brought in new pigs in the last 6 months	8 (34.8 %)
○Farms with origin of new pigs:	
●From Jayapura	4 (17.4 %)
●From Wamena	3 (13.0 %)
●From Jibama	1 (4.3 %)
○Time to brought in new pigs:	
●Within 3 months	6 (26.1 %)
●More than 3 months ago	2 (8.7 %)
Floor made of wood/concrete	23 (100 %)
Fully confined	22 (95.7 %)
Vaccination	
○All pigs were vaccinated	5 (21.7 %)
○Some pigs were vaccinated	7 (30.4 %)
○No pig were vaccinated	11 (47.8 %)
Time of the last vaccination (months)	
○<3	3 (13.0 %)
○3–6	1 (4.3 %)
○>6	8 (34.8 %)
Animal characteristics	
Sex ($n=103$)	
○Male	56 (54.4 %)
○Female	47 (45.6 %)
Age ($n=103$)	
○<3 months	9 (8.7 %)
○3–6 months	50 (48.5 %)
○>6 months	41 (39.8 %)
○No response	3 (2.9 %)

S. suis and *S. zooepidemicus*

S. suis was isolated from the organs of only three animals in Study 1, two from the brains and one from a lung (3.3 %). In contrast, *S. zooepidemicus* was isolated from 14 (15.2 %) pigs of Study 1. Isolation of *S. zooepidemicus* from more than one organ of pigs in Study 1 was common. *S. suis* was isolated from the tonsils of 8.7 % of healthy pigs, but *S. zooepidemicus* was not isolated from the tonsils of healthy pigs (Table 2).

CSF and PCV2 viruses

Antigen of CSF virus was detected in 31.0 % of dead pigs, but only in 1.0 % of Study 2. Antibody against CSF virus was detected in 54.9 % of the dead pigs, but only in 33.0 % of healthy pigs. In dead pigs, 72.7 % of the animals that showed detectable antigen also displayed antibody. In healthy pigs, one pig that exhibited detectable antigen also showed antibody. PCV2 antibodies were detected in 59.2 % of dead pigs and in only 28.2 % of healthy pigs. Levels of CSF and PCV2 infections in dead pigs were significantly higher than these from apparently healthy animals ($P<0.05$) (Table 2).

Parasitic infections

Strongyles and *Trichuris* were identified in the majority of dead pigs, 70.5 and 52.3 %, respectively. The prevalence of *Strongyloides*, *Ascaris* and coccidia in dead pigs was 27.3, 9.1 and 38.6 %, respectively. In healthy pigs, the prevalence of strongyles and *T. suis* was 22.5 and 7.8 %, respectively. The prevalence of *Strongyloides*, *Ascaris* and coccidia in healthy pigs was 15.7, 11.8 and 42.2 %, respectively (Table 2). Prevalence and mean of EPG of strongyle parasites and *T. suis* in dead pigs were significantly higher than the levels recorded from healthy pigs ($P<0.05$) (Table 2).

Table 2 Level of infection with selected viruses, endoparasites and bacteria in village pigs detected in two separate studies[a] (Study 1: dead pigs (n=44–92); Study 2: healthy pigs (n=103) from 23 farms) conducted in Jayawijaya, Indonesia

Pathogens examined	Prevalence	
	Study 1	Study 2
Viruses	(n=71)	(n=103)
CSF (antigen)	22 (31.0 %)[a]	1 (1.0 %)
○Ab positive	16 (22.5 %)	1 (1.0 %)
○Ab negative	6 (8.5 %)	0
CSF (antibody)	39 (54.9 %)[b]	34 (33.0 %)
○CSF vaccinated, Ab positive	–	22 (21.4 %)
○CSF vaccinated, Ab negative	–	15 (14.6 %)
PCV2 (antibody)	42 (59.2 %)[b]	29 (28.2 %)
In CSF vaccinated group (n=37)		
○CSF+/PCV2+	–	13 (12.6 %)
○CSF+/PCV2−	–	9 (8.7 %)
○CSF−/PCV2+	–	2 (1.9 %)
○CSF−/PCV2−	–	13 (12.6 %)
Internal parasites	n=44	n=102
○Strongyle	31 (70.5 %)[b] (mean EPG 223.6[b]; range 2–1992)	23 (22.5 %) (7.1; range 1–58)
○*Strongyloides*	12 (27.3 %) (246.6; range 1–2400)	16 (15.7 %) (33.2; range 1–237)
○*Ascaris*	4 (9.1 %) (39.8; range 1–141)	12 (11.8 %) (15.2; range 1–78)
○*Trichuris*	23 (52.3 %)[b] (54.8[b]; range 1–558)	8 (7.8 %) (41.1; range 1–291)
○Coccidia	17 (38.6 %) (142.8; range 1–1569)	43 (42.2 %) (193.6; range 1–4186)
Bacteria	n=92	n=103
○*S. zooepidemicus*	14 (15.4 %)	0 (0.0 %)
○*S. suis*	3 (3.3 %)	9 (8.7 %)

[a] Study 1 was conducted in Jibama Market, with the samples from across the Jayawijaya region, while Study 2 was conducted in the Subdistrict of Wamena, Jayawijaya region

[b] Significantly higher proportion than in the other study population at the same row. Two tailed Z-test at the 0.05 level

Discussion

This study provided the updated information on the agro-ecology of the pig farming in Jayawijaya Region. In Study 1, it described the level of infections with selected pathogens of samples of dead pigs across the Jayawijaya Region. Study 2 described the level of infections with the same pathogens in apparently healthy pigs from the Subdistrict of Wamena. In these two sets of studies, infections with CSF, PCV2 and strongyle parasites were common in village pigs (Table 3).

CSF virus antigen positivity was very prevalent in this study, found in one third of dead pigs. Many of these antigen positive pigs were also antibody positive. Single infection with CSF virus, however, rarely occurred in dead pigs. On the other hand, some healthy pigs that had not been vaccinated were also seropositive. These findings suggest that strains of CSF virus circulating in the Jayawijaya may be of low or medium pathogenicity.

Not all pigs reported to be vaccinated by the local veterinarian showed demonstrable antibodies. While failure due to poor technique cannot be discounted, false reporting of the vaccination status of an individual animal by farmers might cause these seemingly negative results in vaccinated animals. In another study, similar levels of seroconversion were reported when an injectable preparation of a Chinese vaccine strain was used (Malo 2011) or when oral preparations were used for village pigs (Milicevic et al. 2013, Monger et al. 2015). Further study is warranted to assess the efficacy of the Chinese vaccine strain in village pigs.

The prevalence of PCV2 antibodies in dead and healthy animals was relatively high. It may warrant caution when vaccination against CSF is going to be done in the Jayawijaya, as vaccination against CSF in PCV2 infected pigs has been reported to trigger the development of multi-systemic wasting syndrome (PMWS) (Ha et al. 2009). However, we observed that almost half of the CSF vaccinated

Table 3 Mixed and single infections with selected pathogens in village pigs in Jayawijaya detected in two separate studies[a] (Study 1: dead or moribund pigs (n=44[b]); Study 2: healthy pigs (n=102[b]) from 23 farms) conducted in Jayawijaya, Indonesia

Type of infection		Study 1 (n=44)	Study 2 (n=102)
Mixed	CSF-PCV2	5 (11.4 %)[c]	2 (2.0 %)
	S. suis–S. zooepidemicus	0	1 (1.0 %)
	2 or more parasites	4 (9.1 %)[c]	17 (1.7 %)
	Viruses/bacteria	2 (4.5 %)	1 (1.0 %)
	Viruses/parasites	23 (52.3 %)[c]	30 (29.1 %)
	•CSF-parasites	9 (20.5 %)	10 (9.8 %)
	•PCV2-parasites	5 (11.4 %)	4 (3.9 %)
	•CSF-PCV2-parasites	9 (20.5 %)	16 (15.7 %)
	Bacteria/parasites	2 (4.5 %)	3 (2.9 %)
	Viruses/bacteria/parasites	3 (6.8 %)	2 (2.0 %)
Single	CSF	3 (6.8 %)	4 (3.9 %)
	PCV2	2 (4.5 %)	5 (4.9 %)
	S. suis	0	3 (2.9 %)
	S. zooepidemicus	0	0
	Strongyles	0	4 (3.9 %)
	Trichuris suis	0	1 (1.0 %)
	Strongyloides	0	1 (1.0 %)
	Ascaris suum	0	2 (2.0 %)
	Coccidia	0	3 (2.9 %)
No infection		0	23 (22.5 %)[c]

[a] Study 1 was conducted in Jibama Market, with the samples from across Jayawijaya Region, while Study 2 was conducted in the Subdistrict of Wamena, Jayawijaya Region

[b] Analysis was performed on 44 samples of Study 1 and 102 pigs from Study 2, from which the status of all pathogens investigated was able to be determined

[c] Significantly higher proportion than in the other study population at the same row. Two tailed Z-test at the 0.05 level

pigs showed seroconversion to PCV2, but none of them developed clinical PMWS. It is not known whether those pigs acquired PCV2 before or after CSF vaccine administration. A simple test for PCV2 for individual pigs might need to be developed to help to determine the PCV2 status of pigs prior to CSF vaccination.

Mixed infections with viruses and parasites were common both in dead and healthy pigs but were higher in the dead pigs. Moreover, single infections of the selected pathogens were almost absent in dead pigs. These findings highlighted the possible importance of mixed infections in the case of pig mortality in Jayawijaya.

Strongyle parasites and *T. suis* infections were higher in the dead pigs. A study undertaken before the outbreak of CSF in the Jayawijaya reported that endoparasites could have been the most important pig pathogens in the Jayawijaya (Putra et al. 2004). Furthermore, Cargill et al. (2009) reported that, in the Jayawijaya, *Hyostrongylus* infection resulted in higher faecal egg counts per pig than other endoparasites. The higher level parasite infection in the dead pig group might be a result of free scavenging. Anthelmintic treatment of newly introduced pigs, keeping pigs indoors at all times, the use of deep litter or slatted floors and crop rotation, are other ways that have been proposed in order to reduce reinfections (Carstensen et al. 2002).

Although previously 23.7 % of healthy pigs in the Jayawijaya were reported to carry *S. suis* on their tonsils (Slipranata et al. 2014), the carriage rate in healthy pigs was lower in the present study and further, the prevalence in dead pigs was also very low. It is, therefore, unlikely that *S. suis* is a significant pathogen on small-size Papuan village farms. In fact, although several cases of *S. suis* infection in smallholder enterprises have been recorded (Hoa et al. 2013, Goyette-Desjardins et al. 2014), *S. suis* infection has not been recognized as a priority to be controlled in Asian pig production (McOrist et al. 2011). *S. suis* incidence was reported to be higher after infection with PRRS (Hoa et al. 2013). Although PRRS has been reported in Indonesia (Suartha et al. 2013), it remains undiagnosed in Papua. In the present study, prolonged delay between pig death and sampling could influence the survival of targeted bacteria thus lower recovery rate. This caution should be taken into account when using the data from this survey.

S. zooepidemicus was isolated in higher numbers than *S. suis* in the dead pigs of the current study. *S. zooepidemicus* has been isolated previously from a fatal pig disease outbreak in Vietnam, in co-existence with PRRS (Metwally et al. 2010). In healthy pigs, tonsils was known to be a predilection site of *S. zooepidemicus* in carrier pigs (Salasia et al. 2004). However, the tonsillar carriage of *S. zooepidemicus* was not confirmed in apparently healthy pigs in the current study.

In our study, daily pig husbandry practices such as confining pigs at all times, the use of concrete or wooden floor, that could make the floor easy to clean, and the cooking of pig feed were practiced by the majority of farms from which healthy pig samples were obtained and could have contributed to lower prevalence of infections with selected pathogens. Additionally, bringing in pigs from places other than Jibama market, especially only fully confined pigs and from farms without a history of pig mortality over the preceding 3 months, could have helped to reduce the risk of pathogen transmission on a village pig farm. A study at farm level is warranted to confirm this speculation.

Previous surveys reported that farmers commonly sold diseased pigs but rarely sold dead pigs (Nugroho et al. 2015). During the 3 months of the current study, however, at least 92 dead pigs were observed to be sold through the Jibama market. Furthermore, while the samples of dead pigs were obtained from across the Jayawijaya Region, the proportion of dead animals to be sold to Jibama might not reflect the true

population of dead pigs in the Jayawijaya Region as, for example, small dead pigs might not be sent to Jibama for sale due to economic reasons, although their disease status might be worse than that of older dead pigs. On the other hand, homogeneity of data from the Subdistrict of Wamena compared to other subdistricts might be influenced by the small size of sample. Therefore, one should be cautious when trying to extrapolate from the result of this study into broader area.

In the present study, healthy pigs were mainly recruited from fully confined pigs from the Subdistrict of Wamena. A previous study reported that farms with fully confinement systems were only 16 %, while farms without cases of mortality during a year represented 34 % of Jayawijaya farms (Nugroho et al. 2015). Consequently, while samples of healthy pigs from current study could represent fully confined pigs in Jayawijaya, they may not be representative of the situation of healthy pigs of entire region of Jayawijaya.

In conclusion, infections with CSF, PCV2, strongyles and *T. suis* were commonly present in dead village pigs in the Jayawijaya. Preventative health measures such as good husbandry practices, effective vaccinations against CSF, as well as regular anthelmintic treatments seem urgently warranted.

Acknowledgments We gratefully acknowledge the Australian Center for International Agricultural Research (ACIAR) Project (AH/2007/106) for funding this research. We would like to thank IDEXX for the provision of the CSF antigen and antibody test kits. We also acknowledge the pig traders at Jibama market and Jayawijaya farmers for cooperation during postmortem examinations and samplings. Dewa Ayu Dwita, DVM, Werenfridus R. Lestoin S.Si and Merry S. Repasi S.Si are gratefully acknowledged for their assistance during laboratory works in the Jayawijaya. Drs. Torben Nielsen and Ryan O'Handley are gratefully thanked for their comments during manuscript preparation.

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7. Characterization of Papuan Porcine circovirus type 2 isolates

Serological investigations identified PCV2 infection in Papuan pigs. This chapter presents two studies that molecularly detect and characterize genome of Papuan PCV2 isolates. The first paper confirmed the presence of PCV2 virus in Papuan piggeries, indicated an association of PCV2 infection with Porcine Circovirus Disease (PCVD) and characterized Papuan PCV2 isolates based on partial genome sequences. The second study further characterises the Papuan PCV2 isolates using complete genome sequences.

The study of the partial genomes is presented here in publication format. The paper on the complete genome study was accepted in International Journal of Advanced Veterinary Science and Technology 2016, Volume 5, Issue 1, ISSN 2320-3595.

Manuscript: Porcine circovirus type 2 infections in Papuan pigs, Indonesia

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Statement of Authorship

Title of Paper	Porcine circovirus type 2 infections in Papuan pigs, Indonesia
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Publication Style
Publication Details	Nugroho, W., Hemmatzadeh, F., Artanto, S., and Reichel, M. P., 2016. Porcine circovirus type 2 infections in Papuan pigs, Indonesia. Publication style.

Principal Author

Name of Principal Author (Candidate)	Widi Nugroho		
Contribution to the Paper	Collected samples, conducted PCR, analysed the DNA sequences, submitted DNA to GenBank and wrote the manuscript.		
Signature		Date	07/09/2015

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Farhid Hemmatzadeh		
Contribution to the Paper	Designed primers, supervised with method of PCR, helped with method to analysing PCR data and DNA sequences, corrected manuscript.		
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Abstract

Porcine circovirus type 2 (PCV2) is an important pathogen in the pig industry worldwide, but its involvement in pig disease in Papua is unknown. The aim of this study was to describe the PCV2 infections and its role in the course of Papuan pig diseases and to genetically characterise the Papuan PCV2 virus. The samples for this study were obtained from the previous investigation, carried out at a central market in the Jayawijaya Region, Papua Province, Indonesia, in a three month period. Naturally dead or moribund pigs sent to the market for sale were used in the study. A total of 32 samples were selected based on various level of PCV2 antibody detected in the sera for further examination using conventional PCR and sequencing. Ages, sex, subdistricts of origin, body score, gross pathology of lung and gross pathology of lymph glands were recorded. PCVD was indicated in 12.5% of pigs studied based on clinical sign of emaciation, lung lesions and the detectable PCV2 antigen and antibody. Viral genome was detected by PCR in 13 out of the 32 sera examined. BLAST analysis showed that Papuan PCV2 were similar to Chinese PCV2 strains. Phylogenetic analyses of Papuan PCV2 genome fragments showed that the Papuan PCV2 was clustered in two different groups. The 12 samples of 13 Papuan PCV2 genome fragments were closely related to PCV2-IM3 genotype, while one genome fragment was untypeable. This study indicated the contribution of PCVD in Papuan pig diseases and demonstrated that more than one PCV2 genotype has been circulating in Papuan pigs.

Keywords: Porcine circovirus type 2; PCR; IM3 genotype; Papua

Introduction

PCV2 is the cause of a number of clinical syndromes devastating pig industries worldwide (Gillespie et al. 2009; Huynh et al. 2014; Wei et al. 2013). These clinical syndromes are described as PCV Disease (PCVD) in Europe (Segalés 2012) or PCV Associated Disease (PCVAD) in the USA, which includes systemic infection (the category in which Postweaning Multisystemic Wasting Syndrome (PMWS), the former name of PCV2 infection, fits), reproductive disorder, Porcine Dermatitis and Nephropathy Syndrome (PDNS), Porcine Respiratory Disease Complex (PRDC), enteritis (Opriessnig et al. 2007) and Proliferative and Necrotizing Pneumonia (PNP) (Segalés 2012).

Many pigs are infected by PCV2 without being clinically sick (Baekbo et al. 2012) and co-infection with other factor is important for a clinical PCVD to emerge in PCV2 infected animals. Pathogens reported to co-exist with PCV2 in PCVD cases were *Metastrongylus elongates, Mycoplasma hyopneumoniae,* PPV, PRRS, *Salmonella spp.,* or Swine Hepatitis E virus (HEV) (Alarcon et al. 2011; Allan et al. 2000; Ha et al. 2005; Kennedy et al. 2000; Marruchella et al. 2012; Opriessnig et al. 2004; Yang et al. 2015). Co-existence of PCV2 infection with a simple temperature fluctuation was also reported to be capable of triggering growth retardation (Patterson et al. 2015).

In PCVD cases, gross pathology of tan mottled lung was observed in 65% of cases (Segales et al. 2004). Other marked feature of PCVD was a distention and hyperaemic to haemorrhagic discoloration of lymph nodes (Saha 2012). Enlarged

lymph node, the presence of large copy of PCV2 antigens in the pathologic lymph nodes and wasting are used as indications for the PCVD diagnosis (Saha 2012).

Serologic test or PCR or combination of both methods solely cannot be used to diagnose PCVD (Saha 2012), but respective tests have been used in surveys to determine the PCV2 status of a population (de Castro et al. 2012; Li et al. 2013; Stephenson et al. 2015; Zhao et al. 2012). Patterson et al. (2011a) reported that under a natural exposure condition, seroconversion and PCV2 genome had been persistently detectable within five months post farrowing, but had started to be only intermittently detectable after this period of time while antibody had been constantly detectable. Another study reported that antibody started to be detected only from day 8 post-infection, while virus genome could be observed in serum as soon as one day after the experimental infection (Patterson et al. 2011b).

Genome of PCV2 have been classified into eight genotypes, i.e. four main genotypes namely PCV2a, PCV2b, PCV2c and PCV2d, and four newly introduced intermediate genotypes 1-4 (IM1-4), using Neighbor-Joining (NJ) method with p-distance model (Grau-Roma et al. 2008; Xiao et al. 2015). PCV2b is by far the most prevalent genotype, followed consecutively by PCV2a, PCV2d, IM1 and rarely isolated PCV2c, IM2, IM3 and IM4 (Xiao et al. 2015).

Papua is one of five provinces in Indonesia where the pig population is high, with village pigs dominating the pig population (Siagiaan 2014). Pigs are important economically and culturally in this region but mortality has been known as one main constraint of pig production (Nugroho et al. 2015) (Cargill et al. 2009; Hide

2003). Report of PCV2 study in Indonesia and Papua has been rare. The first publicly available report was the detectable PCV2 genome in pork exported from the Western part of Indonesia to the Singapore (Manokaran et al. 2008). In this study, analysis of the full genome sequences revealed that the Indonesian isolates belonged to a recombinant cluster of PCV2b, and, interestingly, it was speculated to be the most probable origin of the Chinese and Vietnamese strains (Huynh et al. 2014). The other report was from Papua Province, describing seroprevalence and the co-existence of PCV2 with other pathogens such as CSF, *S. zooepidemicus*, and endoparasites (Nugroho et al. 2016). Despite this study, the role of Papuan PCV2 isolates in PCVD in pigs and characteristics of Papuan PCV2 isolates remains unknown.

The current study was carried out in Jayawijaya region, Papua province, aimed to describe the pathology of naturally diseased pigs with various levels of PCV2 seroconversion and to detect and characterize the Papuan PCV2 genome fragments in these pigs by means of molecular techniques, in order to better understand the epidemiology of PCV2 infection in Papuan pigs. Jayawijaya is a region with 24% pig population across the province (BPS-Papua 2013)

Materials and Methods

The source of samples

Samples used in this study were obtained from the previous study, carried out at a central market in the Jayawijaya Region, Papua Province, Indonesia, in a three month period. The market is used by farmers across the region to trade pigs including sick and dead pigs. Naturally dead or moribund pigs sent to the market which reported to be sick were used in the study (Nugroho et al. 2016). A total of 32 samples were selected based on various level of PCV2 antibody detected in the sera, for further examination using conventional PCR. Ages, sex, subdistricts of origin, body score, gross pathology of lung and gross pathology of lymph glands were recorded. There was no data whether pigs from the same subdistrict were from the same herd.

Primer design

A pair of primers was designed to specifically amplify PCV2 DNA. The genomic DNA sequences of PCV2 (GenBank accession numbers: HM038017, HM038025, HM038030) were aligned using ClustalW and edited by BioEdit version 7.2.5 (Hall 1999) software. Both forward and reverse primers target a highly conserved region of PCV2 genomes and could detect various genotype sequences. The primer pairs were predicted to produce a PCR amplicon of 898 base pairs (bp). The forward primer 5'-AGAATACTGCAGTAAAGAAGGC-3' was located between position 332 and 353, while the reverse primer 5'-CCACTATTGATTACTTCCAACC-3' was located between position 1208 and 1229 of the complete PCV2 genome.

DNA amplification

DNA was extracted using a commercial kit following the manufacturer's instruction (DNeasy Blood & Tissue Kit, Qiagen) and amplified by PCR. The final volume of the PCR mixture was 25 µL, contained 5 µL buffer mix (MyTaq DNA Polymerase, Bioline), 1 µM of each primers, 0.5 U Taq polymerase (MyTaq DNA Polymerase, Bioline) and 3 µL of DNA template. The amplification was run in a thermocycler (Biorad T-100, Bio-Rad) under denaturing conditions at 95^{o}C for 5 min, followed by 45 cycles of 15 s of 95 ^{o}C, 15 s of 52 ^{o}C and 30 s of 72 ^{o}C, and a final elongation for 5 min at 72 ^{o}C. Five microliters of each amplified DNA sample was loaded in 1.5% agarose gel (Agarose Molecular Grade, Bioline) in 1X Tris-Borate-EDTA (TBE Buffer, Thermo Scientific). After electrophoresis at 100 mV and 120 mA for 30 minutes (PowerPac Basic Power Supply, Bio-Rad), DNA was visualised under ultra violet light. The size of the fragment was verified using DNA marker (100 bp DNA ladder, Axygen).

Nucleotide sequencing

PCR products were purified using QIAamp DNA Mini Kit (Qiagen) and sequenced by the Sanger method at the Australian Genome Research Facility (AGRF). The sequencing of each amplified DNA was performed from both the forward and reverse directions using the same primers described above. Only high signals in chromatograms were used for assembling the sequences.

Sequence analyses

Sequences from each sample from both, the forward and reverse directions were assembled to obtain a consensus sequence, using Bioedit (Hall 1999).

Subsequently, a BLAST analysis was performed to confirm the similarity of Papuan PCV2 genome fragments with PCV2 reference genome. Papuan PCV2 DNA fragments were subsequently aligned against selected reference sequences of each of the PCV2 established genotypes obtained from GenBank using ClustalW within Bioedit (Hall 1999). The genetic divergence was calculated between the Papuan PCV2 isolates and the selected reference sequences. Further, an evolutionary analysis was carried out for these reference strains and Papuan PCV2 DNA fragments by the Neighbour Joining (NJ) method based on the Pairwise distance (P-distance) model. For comparison, phylogenetic tree of reference strains were also constructed based on ORF2 region and full genome sequences. 1,000 bootstraps pseudo-replicates were employed to test the phylogeny. All tests were performed in MEGA5 (Tamura et al. 2011).

GenBank Accession numbers

The 13 Papua PCV2 isolates were submitted into GenBank database and can be retrieved with the accession numbers KT026095, KT224514, KT224515, KT224516, KT224517, KT224518, KT224519, KT224520, KT224521, KT224522, KT224523, KT224524 and KT224525.

Results

Animal data

Twenty eight pigs used in current study were from 14 villages in 5/11 subdistricts in Jayawijaya Region, Papua Province. These subdistricts are Wamena, Hubikosi, Kurulu, Asologaima and Bolakme. Samples with detectable PCV2 genomes were from the four subdistricts except Bolakme (Fig 1). Origin of the other four samples was unknown.

Eleven of the 32 pigs were male. Thirteen pigs were less than 6 month age, 10 were between 6-12 month age and nine were older than 12 month age. Body condition was recorder from 21 pigs. Of which, 12 pigs had thin condition and the other nine were normal. Hyperemia of lymph glands was detected in ten of the 13 samples which lymph nodes were observable. Pathologic status of the lung was observed in 23 of 32 cases and different pathologic conditions included normal, atelectasis, edema, multifocal hyperemia and diffuse hyperemia were observed. Co-infections were common with PCV2 and endoparasites, CSF and/or *S. zooepidemicus* (Table 1).

PCV2 viral genomes were detected in 13 of the 32 (41%) samples. All samples with detectable genomes were having antibody at the level of above the threshold of the test (150 ELISA antibody unit). Pigs with detectable genome were mainly at ages of 12 months or younger (92%, 11/13 pigs). Furthermore, in samples with antibody level of higher than 2,400 ELISA units, 35% (6/17) showed detectable viral genome. Viral genomes were mainly detected in samples with antibody levels ranging between 600 - 2,400 ELISA antibody units (60%, 6/10 samples). In

samples with the antibody level of lower than 600 ELISA units, only 20% (1/5) had detectable viral genome (Table 1). Pigs indicative of PCVD were observed in 12.5% (4/32) cases, characterised by the presence of combined conditions of detectable antigen, moderate to high level of PCV2 seroconversion of above 600 ELISA antibody unit, emaciation, multifocal or diffuse hyperemia of lung, with or without apparent co-infection (Table 1).

Sequence and phylogenetic analyses

In this study, sequence assembly resulted in DNA fragments of 791-792 bp length from 12 samples and a 878 bp length fragment from 1 sample. All 13 Papuan PCV2 DNA fragments were confirmed as PCV2 by basic local alignment search tool (BLAST) analysis[1]. Papuan PCV2 DNA fragment KT224525 is most similar to the HM038025 Chinese PCV2 strain in BLAST analysis, while the other 12 Papuan PCV2 DNA fragments were similar to HM776452; the other Chinese PCV2 strain. A further BLAST analyses showed that all samples with 791-792 bp DNA fragments contain a 633 bp partial sequence of the ORF1 gene, a 121 bp partial sequence of the ORF2 gene (complement), a 309 bp complete sequence of the ORF3 gene (complement) and 11 of them contained a 40 bp fragment of non-coding regions, while one DNA fragment contained a 41 bp non coding region. The sample with the 878 bp DNA fragment contains a 652 bp partial sequence of the ORF1 gene, a 186 bp partial sequence of the ORF2 gene (complement), a 312

[1] See: http://www.ncbi.nlm.nih.gov/blast

bp complete sequence of the ORF2 gene (complement), and a 40 bp non coding region.

Pairwise distance analysis showed that 12 of the 13 Papuan PCV2 DNA fragments are highly similar to each other, with the genetic divergence score ranging only from 0.001 to 0.006. These twelve isolates are also closely related to Indian, Croatian and Chinese PCV2-IM3 genotypes with genetic divergence ranging 0.001 - 0.006. One Papuan isolate (30 Papua/KT224525) shows a larger distance (0.028 - 0.030) from the other Papuan isolates, but has low genetic divergence (0.009) from AF055394 PCV2b reference strain (Table 2).

Dendogram using the DNA fragments obtained from current study was compared with dendogram using PCV2 ORF2 fragment or PCV2 complete genome. Using the PCV2 DNA fragment of current study, the topology of PCV2 genotypes PCV2c, PCV2d, PCV2-IM1 and PCV2-IM3 in the tree is conserved. However, the topology of genotypes PCV2a, PCV2b, PCV2-IM2 and PCV2-IM4 is violated (Fig 2).

Phylogenetic analysis further confirmed that the Papuan PCV2 isolates belong to two different clusters (Fig 2). The first cluster involves the most prevalent Papuan PCV2 isolates and strains from China, India and Croatia, belong to PCV2-IM3 genotype. Bootstrap values of 100% confidence support of the topology of the phylogeny tree. The other cluster in the phylogenetic tree which contains one Papuan PCV2 DNA fragment (KT224525), encompasses reference strains of

mixed genotypes of PCV2b, PCV2-IM2 and PCV2-IM4. Therefore the genotype of KT224525 remains unclear.

Discussion

Pig diseases have been known to be one major constraint of Papuan pig production (Nugroho et al. 2016). In the current study we indicated the role of PCV2 in the course of diseases in Papuan pigs. Evidence of wastings, multifocal hyperemia of lungs, detection of PCV2 genomes and high level of PCV2 seroconversion indicated that PCV2 contributed to the disease occurred in Papuan pigs. The pathologic status of lymphoid glands however, was unable to be determined during the current study. It was reported previously that emaciation, multifocal hyperemia in the lung and distended inguinal lymph nodes are a common sign of PCV2 systemic disease (Segalés 2012; Segales et al. 2004).

Concurrent infections with PCV2 and endoparasites, CSF and *S. zooepidemicus* were demonstrated and could have exacerbated the outcome of PCVD in Papuan pigs. On the other hand, low body conditions, detectable antigen and antibody and pathologic lung were also observed in pigs without detectable co-infection with CSF, endoparasite or *S. zooepidemicus*, indicated that co-infection with PCV2 and other factors could have occurred. Arguably, the multifocal hyperemia in lungs and enlarged lymph nodes could be caused by porcine reproductive and respiratory syndrome virus (PRRSv) (Brockmeier et al. 2001). Provided that PRRS has been diagnosed in other part of Indonesia (Suartha et al. 2013), a further study is warranted to confirm the involvement of PRRS in PCVD in Papuan pigs.

We did not detect the virus in the lymphoid tissue or quantify the viral load in blood which are the standard method of PCV2 diagnosis in individual pig (Saha 2012). Either, we did not indicate the increased mortality which has been used as PCV2 diagnosis in the herd level (Saha 2012). However, as the previous study in Jayawijaya indicated that the seroprevalence of PCV2 in dead pigs was higher than that in healthy pigs, the finding in current and previous study indicated that the role of PCV2 in Papuan pig disease could not be ruled out (Nugroho et al. 2016).

Current study indicated that PCVD contribute to 12.5% of pig mortality studied. The PCV2 prevalence showed in the current study, however, may not represent the situation in the Papuan pig population due to bias of sample selection, thus urges further studies to understand the epidemiology of PCVD and its role in pig mortality in Papua. Total pig mortality in Jayawijaya has been reported to be very high at 50% (Cargill et al. 2009; Hide 2003; Nugroho et al. 2015).

Phylogenetic analysis indicated that majority of Papuan PCV2 samples belong to the PCV2-IM3 genotype and an untypeable isolate. This phylogenetic study however, used the DNA fragment other than ORF2 region which is used in the standard method of PCV2 classification (Grau-Roma et al. 2008). As a consequence, one Papuan PCV2 DNA was unable to be correctly classified. The presence of these two clusters amongst Papuan PCV2 isolates, implies that more than one PCV2 genotype is circulating amongst Papuan pigs. The presence of more than one PCV2 genotype in a pig was reported to be common (Zhai et al. 2011).

Papuan PCV2 DNA fragments in present study were similar to Chinese strains based on BLAST analyses. This leads a speculation that the virus was introduced from China recently, provided that pig products from China have been found in Papua these days and might have played some role in the transmission of these PCV2 strains into Papua. However, since pigs have been domesticated in Papua since 3,500 years ago (Muller 2009), the PCV2-IM3 genotype might have been possibly endemic in village pigs in Papua since this long period. A study is warranted to confirm the role of pig transports in the transmission of PCV2 into Papua.

Conclusions

This study confirmed the presence of PCV2 viral genomes in Papuan pigs and indicated the occurrence of PCVD in Papua. This study was indicated that multiple PCV2 genotypes could have been circulating in Papuan pigs. Further study using complete genome sequences or ORF2 region is warranted to determine the genotype of the untypeable PCV2 isolate of current study.

Table 1 Characteristics of diseased Papuan pigs with various level of PCV2 antibody (n=32)

Sample designation	Ages (Month)	Sex	Antibody level (ELISA Unit)[1]	Body score	Lymph glands	Lungs	Co-infection	PCR PCV2	PCVD indicative
J012	12	M	+2484	thin	TB-Hyp	Ed,Atl,DH	P, CSF	-	-
J017	48	F	+2484	thin	normal	Atl,DH	P	-	-
J018	14	F	+2484	ND	normal	Atl,DH	P	-	-
J023	2	M	+2484	thin	ND	ND	P, CSF	+	-
J024	3	M	801.2	thin	ND	ND	P	+	-
J026	24	F	369.6	thin	Ing-Enl	Atl,MH	CSF	-	-
J031	12	F	+2484	thin	Mes,Ing,Man,Enl-Hyp	Normal	P	-	-
J034	12	M	+2484	normal	ND	Atl,DH	-	+	-
J035	14	F	2278.1	ND	ND	DH	-	-	-
J042	5	M	831.9	normal	Mes-Hyp	DH	P	+	-
J044	3	F	<150	normal	Ing-Hyp	Ed,MH,Pry	P, SEZ	-	-
J045	8	F	1439.6	thin	ND	Ed,MH,Pry	P, SEZ	+	+
J046	5	F	882.3	thin	ND	Ed,MH	CSF, SEZ	+	+
J047	6	M	1731	normal	Ing,Mes-Hem	Ed,MH	CSF,SEZ	-	-
J048	7	F	+2484	thin	ND	MH	P	-	-
J054	12	F	+2484	ND	ND	Atl,DH	P, CSF	-	-
J066	24	F	237.6	thin	normal	Atl,DH	-	+	-
J085	3	M	1601	ND	ND	ND	CSF	+	-
J088	4	M	+2484	ND	ND	ND	P	-	-
J091	24	M	+2484	ND	ND	ND	-	-	-
J092	5	M	+2484	ND	ND	ND	P	-	-
J093	6	F	+2484	ND	ND	ND	CSF	-	-
J100	12	F	+2484	normal	ND	DH	ND	+	-
J101	12	F	+2484	normal	ND	MH,Ech	-	+	-
J102	5	M	520.5	ND	ND	ND	P	-	-
J117	24	F	262.8	ND	ND	ND	P, CSF	-	-
J119	36	F	+2484	emaciated	ND	Atl,DH,Pry	-	+	+
J120	5	F	+2484	normal	ND	ND	-	+	-
J129	24	F	900.3	ND	ND	Ed,DH,Pry	-	-	-
J136	1	F	616.6	thin	ND	MH	-	+	+
J149	12	F	952.2	normal	ND	DH,Pry	SEZ	-	-
J157	12	F	+2484	normal	TB-Hem	MH,Pry	P, SEZ	-	-

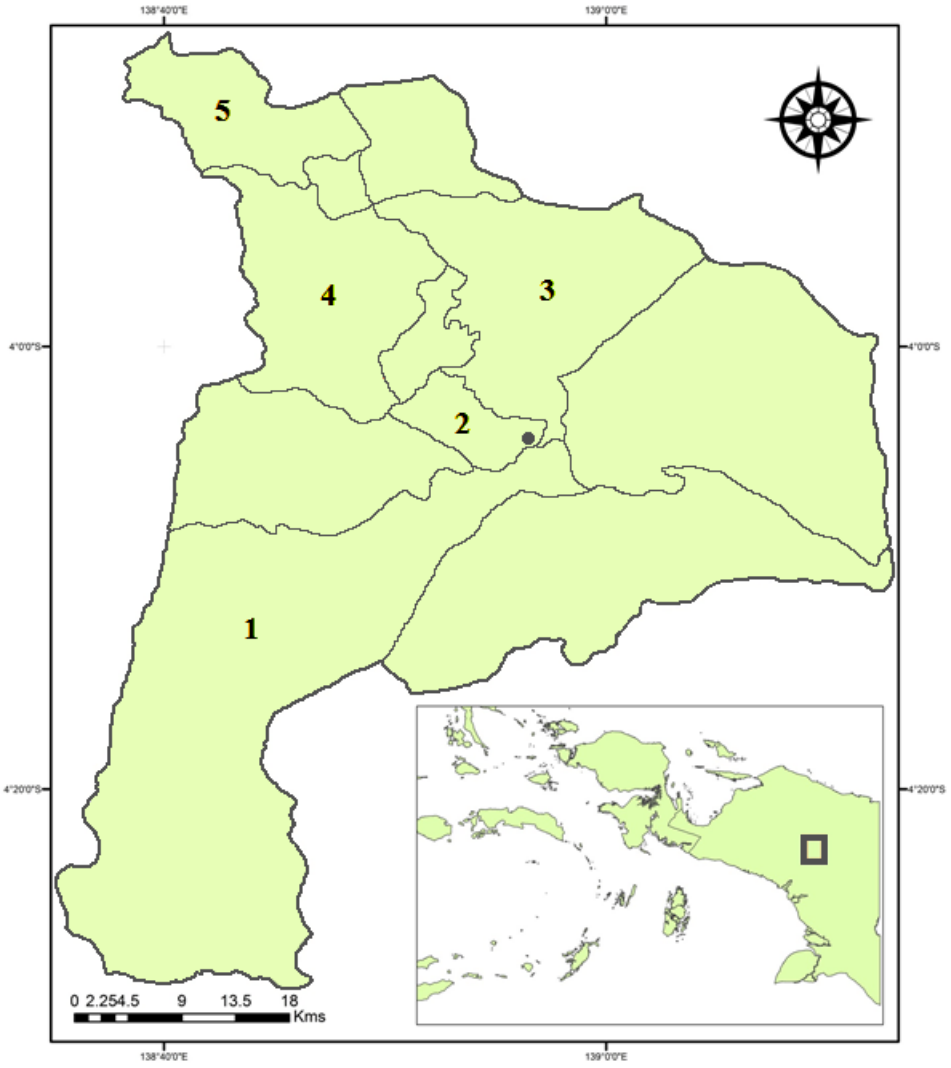
Lymph glands: TB (Tracheo bronchial), Ing (Inguinal), Man (Mandible), Hyp (Hyperemia), Enl (Enlarged), Hem (Hemorrhagy)
Lungs: Ed (Edema), Atl (atelectasis), DH (Diffuse hyperemia), MH (Multifocal hyperemia),Pry (pleurisy), Ech (Ecchymosis)
Co-infection: P (endoparasites), CSF (classical swine fever antigen), SEZ (*Streptococcus zooepidemicus*)
[1]The test has a cut off value of 150 ELISA Unit
ND: Not determined

Table 2

Estimates of Evolutionary Divergence between Sequences. The number of base differences per site from between sequences are shown. There were a total of 789 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

No	DNA samples	1	2	3	4	5	6	7	8	9	10	11	12	13
1	04Papua													
2	05Papua	0.004												
3	08Papua	0.003	0.006											
4	10Papua	0.001	0.003	0.004										
5	12Papua	0.003	0.004	0.005	0.001									
6	13Papua	0.003	0.004	0.005	0.001	0.003								
7	17Papua	0.003	0.004	0.003	0.001	0.003	0.003							
8	18Papua	0.001	0.003	0.004	0.000	0.001	0.001	0.001						
9	23Papua	0.000	0.004	0.003	0.001	0.003	0.003	0.003	0.001					
10	24Papua	0.001	0.005	0.004	0.003	0.004	0.004	0.004	0.003	0.001				
11	27Papua	0.001	0.005	0.004	0.003	0.004	0.004	0.004	0.003	0.001	0.003			
12	28Papua	0.001	0.005	0.001	0.003	0.004	0.004	0.001	0.003	0.001	0.003	0.003		
13	30Papua	0.029	0.030	0.029	0.028	0.029	0.029	0.029	0.028	0.029	0.030	0.030	0.030	
14	AF055392PCV2a	0.022	0.023	0.022	0.020	0.022	0.022	0.022	0.020	0.022	0.023	0.023	0.023	0.015
15	AF154679a	0.028	0.029	0.028	0.027	0.028	0.028	0.028	0.027	0.028	0.029	0.029	0.029	0.020
16	AY099499a	0.020	0.022	0.020	0.019	0.020	0.020	0.020	0.019	0.020	0.022	0.022	0.022	0.016
17	AF055394PCV2b	0.027	0.030	0.027	0.028	0.029	0.029	0.029	0.028	0.027	0.028	0.028	0.028	0.009
18	EF675232b	0.030	0.032	0.030	0.029	0.030	0.030	0.030	0.029	0.030	0.032	0.032	0.032	0.011
19	EU450587b	0.041	0.044	0.041	0.042	0.043	0.043	0.043	0.042	0.041	0.042	0.042	0.042	0.023
20	EU148503PCV2c	0.037	0.038	0.037	0.035	0.037	0.037	0.037	0.035	0.037	0.038	0.038	0.038	0.030
21	EU148504c	0.039	0.041	0.039	0.038	0.039	0.039	0.039	0.038	0.039	0.041	0.041	0.041	0.033
22	EU148505c	0.038	0.037	0.038	0.037	0.038	0.038	0.038	0.037	0.038	0.039	0.039	0.039	0.034
23	AY181946PCV2d	0.033	0.034	0.033	0.032	0.033	0.033	0.033	0.032	0.033	0.034	0.034	0.034	0.025
24	AY181947d	0.028	0.029	0.028	0.027	0.028	0.028	0.028	0.027	0.028	0.029	0.029	0.029	0.025
25	AY484410d	0.029	0.030	0.029	0.028	0.029	0.029	0.029	0.028	0.029	0.030	0.030	0.030	0.024
26	HM776452PCV2-IM3	0.003	0.004	0.005	0.001	0.003	0.003	0.003	0.001	0.003	0.004	0.004	0.004	0.029
27	LC004753IM3	0.003	0.004	0.005	0.001	0.003	0.003	0.003	0.001	0.003	0.004	0.004	0.004	0.029
28	HQ591381IM3	0.004	0.005	0.006	0.003	0.004	0.004	0.004	0.003	0.004	0.005	0.005	0.005	0.028
29	AY035820PCV2-IM1	0.024	0.028	0.024	0.025	0.027	0.027	0.027	0.025	0.024	0.025	0.025	0.025	0.019
30	EU302140IM1	0.027	0.028	0.027	0.025	0.027	0.027	0.027	0.025	0.027	0.028	0.028	0.028	0.019
31	JX506730IM1	0.028	0.029	0.028	0.027	0.028	0.028	0.028	0.027	0.028	0.029	0.029	0.029	0.020
32	KC835189PCV2-IM2	0.022	0.023	0.022	0.020	0.022	0.022	0.022	0.020	0.022	0.023	0.020	0.023	0.015
33	FJ388889IM2	0.025	0.029	0.025	0.027	0.028	0.028	0.028	0.027	0.025	0.027	0.027	0.027	0.016
34	JF317581IM2	0.024	0.025	0.024	0.023	0.024	0.024	0.024	0.023	0.024	0.025	0.025	0.025	0.015
35	AY874167PCV2-IM4	0.030	0.034	0.030	0.032	0.033	0.033	0.033	0.032	0.030	0.032	0.032	0.032	0.013

Figure 1



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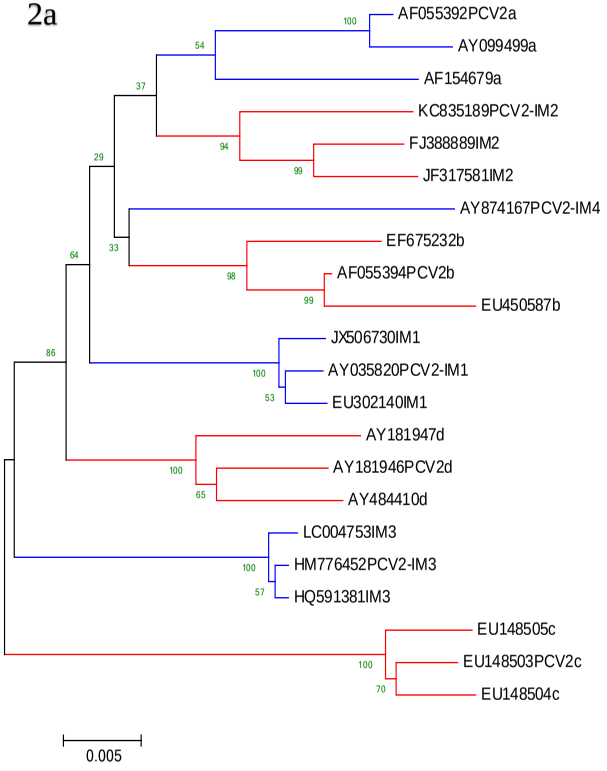
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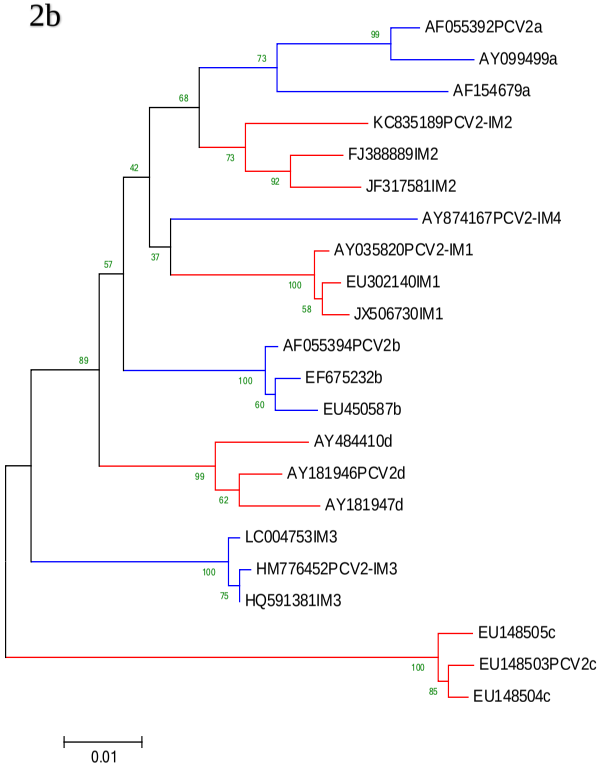
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EU302140IM1
JX506730IM1
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EF675232b
EU450587b
AY484410d
AY181946PCV2d
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0.01

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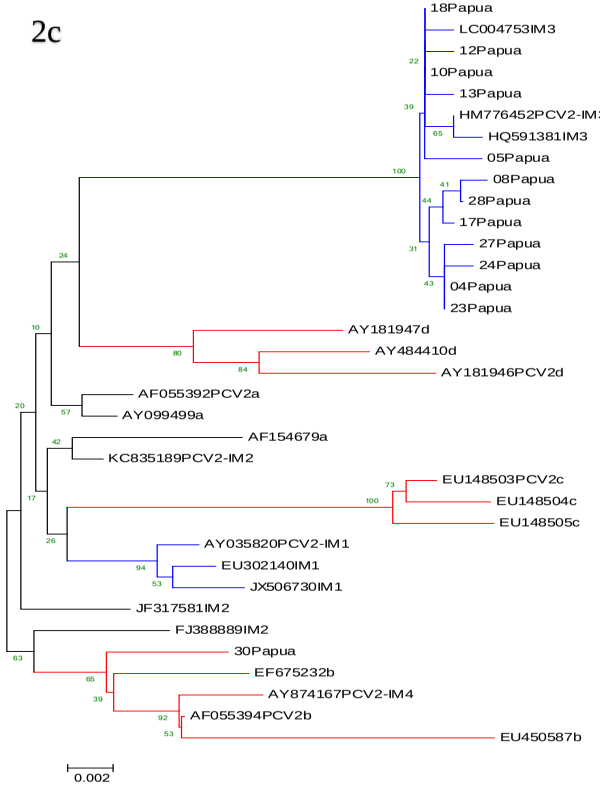
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17Papua
27Papua
24Papua
04Papua
23Papua
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FJ388889IM2
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AY874167PCV2-IM4
AF055394PCV2b
EU450587b
0.002

Figure legends

Figure 1

Subdistricts of origin of pigs used for the study of PCV2 infection in Papua province, Indonesia. 1. Wamena, 2. Hubikosi, 3. Kurulu, 4. Asologaima, 5. Bolakme. The circle in the Hubikosi subdistrict is the local market where the study was conducted. Figure is adapted from Nugroho et al. (2015).

Figure 2

Phylogenetic tree of Papuan PCV2 genome and PCV2 reference genotypes. The evolutionary history was inferred using the Neighbor-Joining method and p-distance model, with 1000 bootstraps pseudo replicates. Bootstraps values are shown next to the branches. 2a. The analysis involved 22 complete genome of PCV2 reference strains (1763 nucleotides); 2b. The phylogenetic tree of 22 ORF2 regions of PCV2 reference strains (698 nucleotides); 2c. The analysis of PCV2 genome fragments of 13 Papuan PCV2 samples and 22 PCV2 reference strains (789 nucleotides). Evolutionary analyses were conducted in MEGA5.

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Original article: Complete Genome Characteristics of Porcine circovirus Type 2 (PCV2) Isolates from Papuan Pigs, Indonesia

W. Nugroho, F. Hemmatzadeh, S. Artanto and M. P. Reichel (2016)

Complete Genome Characteristics of Porcine circovirus Type 2 (PCV2) Isolates from Papuan Pigs, Indonesia (2016)

Accepted in International Journal of Advanced Veterinary Science and Technology, Volume 5, Issue 1, ISSN 2320-3595

Statement of Authorship

Title of Paper	Complete Genome Characteristics of Porcine circovirus Type 2 (PCV2) Isolates from Papuan Pigs, Indonesia
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Publication Style
Publication Details	Widi Nugroho, Farhid Hemmatzadeh, Sidna Artanto and Michael P. Reichel, 2016. Complete Genome Characteristics of Porcine circovirus Type 2 (PCV2) Isolates from Papuan Pigs, Indonesia, International Journal of Advanced Veterinary Science and Technology, 5: 239-247

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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Helped with design of the study, edited and corrected manuscript, acted as corresponding author		
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Cloud Publications
International Journal of Advanced Veterinary Science and Technology
2016, Volume 5, Issue 1, pp. 239-247, Article ID Sci-377
ISSN 2320-3595

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Research Article **Open Access**

Complete Genome Characteristics of Porcine circovirus Type 2 (PCV2) Isolates from Papuan Pigs, Indonesia

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Publication Date: 21 April 2016

Article Link: http://scientific.cloud-journals.com/index.php/IJAVST/article/view/Sci-377

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Abstract Porcine circovirus type 2 (PCV2) has been recognised as an important pathogen in the pig industry world-wide. The virus was recently identified in Papuan pigs, yet information about the characteristics of Papuan PCV2 isolates is limited. The aim of the present study was to characterise the complete genome of Papuan PCV2 isolates. Viral DNA was isolated from 13 blood samples of village pigs from the Jayawijaya region. Four of the PCR positive samples were selected for full genome sequencing. Neighbour Joining phylogeny with P-distance model showed that the four Papuan PCV2 isolates belong to genotype PCV2-IM3. Sequence identity analysis of the Papuan PCV2 genomes further showed 99.5% to 99.6% similarity with a Chinese PCV2-IM3 reference strain. The current study revealed that genotyping based on the ORF2 sequence resulted in a substantially different characterisation of PCV2 genotypes compared to classification based on the complete genome sequences. Furthermore, the study showed that the topology of the PCV2 phylogeny based on the ORF2 sequence was different from the topology based on capsid proteins. Genotyping using the complete genome, ORF2 region or capsid protein sequences resulted in substantial variance in the classification of the PCV2 isolates. The clinical consequence of these different genotyping methods needs further study.
Keywords *Papua; Pigs; ORF2; Neighbour Joining; PCV2-IM3 Genotype*

1. Introduction

Porcine circovirus type 2 is an emerging pathogen causing economic loss in the pig industries worldwide. Infection with PCV2 manifests in various clinical outcomes, termed porcine circovirus associated diseases (PCVD) and characterized by a respiratory disease complex, dermatitis and nephropathy syndrome or post weaning multisystemic wasting syndrome (PMWS). In order for PCVD to be produced, co-infection with PCV2 and other factors is required (Baekbo et al., 2012). Reported PCVD co-factors included porcine reproductive and respiratory syndrome (PRRS), porcine parvovirus (PPV), Swine Hepatitis E virus (HEV), *Mycoplasma hyopneumoniae, Salmonella spp.* Or *Metastrongylus elongatus* (Allan et al., 2000; Ha et al., 2005; Kennedy et al., 2000; Marruchella et al.,

2012; Opriessnig T. et al., 2004; Yang et al., 2015) as well as environmental stressors, such as changes in temperature and high stocking density (Patterson et al., 2015).

Genotype variations of PCV2 virus have been observed; one widely recognised method for the classification of PCV2 genotypes is the Neighbour Joining (NJ) phylogenetic tree approach with pairwise proportional difference of nucleotides (P-distance) model, using the ORF2 gene region or the complete genome as target sequences. The cut off value of proportional nucleotide diversity for genotype demarcation is 0.035 in ORF2 based analysis or 0.02 when the complete genome is used (Segales et al., 2008).

Using the NJ method with P-distance model, four major genotypes of PCV2 have been established, namely PCV2a, PCV2b, PCV2c and PCV2d. Further, a few intermediate (IM) groups; IM1-IM4 have also been reported (Xiao et al., 2015). The most prevalent circulating genotype in farmed pigs has been PCV2b, followed by PCV2a. Genotype PCV2d may be a new emerging genotype in farmed pigs and recently has been identified amongst herds with vaccination failure in USA, Korea and Brazil (Segales, 2015; Xiao et al., 2015). Genotype PCV2c consists so far of only four strains (Franzo et al., 2015). Intermediate clades have consisted of less than fifty strains (Xiao et al., 2015).

Commercial vaccines against PCV2 have been developed based on the capsid protein of ORF2 genes of the PCV2a genotype and known to be protective against infections with PCV2a and PCV2b (Segales, 2015). However, the observation that PCV2d can be retrieved from vaccinated pigs invites a discussion on the cross protection provided by commercial vaccines for PCV2d (Segales, 2015; Xiao et al., 2015). Furthermore, the level of cross protection of available vaccines against PCV2c and intermediate groups is unknown.

To date, only two papers have been published on the topic of PCV2 from Indonesia, one from Western Indonesia (Manokaran et al., 2008) and the other one was from Papua (Nugroho et al., 2015). Papua province has the fifth highest pig population in Indonesia (Siagiaan, 2014) and village pigs are important livelihood assets for the Papuan community (Nugroho et al., 2015). The aim of the present study was to characterise the Papuan PCV2 isolates based on either the complete genome sequences, ORF2 region and capsid protein using NJ method. This information will contribute to a better knowledge of the epidemiology of PCV2 infections in Papua, Indonesia.

2. Materials and Methods

2.1. PCR and Complete Genomes Assembly

Total DNA from thirteen serologically PCV2 positive blood samples was used in present study. The samples were retrieved from village pigs from Jayawijaya Region, Papua, Indonesia. The two pairs of primers used in the current full genome sequencing study were 20 base pair (bp) length respectively, reported previously (Fenaux et al., 2000). The first pair of primers, CV1 and CV2, amplified a 989bp fragment. The CV1 primer is 5'-AGGGCTGTGGCCTTTGTTAC-3', situated at position 1336-1355 in PCV2 genome and CV2 is 5'-TCTTCCAATCACGCTTCTGC-3' located at position 536-556 of PCV2 genome. Second set of primers, CV3 and CV4, amplified a 1,092-bp fragment. The sequence of CV3 and CV4 were 5'-TGGTGACCGTTGCAGAGCAG-3' and 5'-TGGGCGGTGGACATGATGAG-3' respectively, positioned at 452-471 and 1525-1544 in PCV2 genome. The PCR products of PCV2 DNA resulting from amplification using these two pairs of primers overlap at positions 452-536 and 1355-1544 in PCV2 genome.

Amplification of PCV2 genomes was conducted using conventional PCR. Each of the PCR mix contained 2.5 µL of 10X buffer, 0.5 µL of 10 mM dNTP, 1 µL of 50 mM $MgSO_4$, 1 µL of each of primers, 0.2 µL Platinum *Taq*DNA Polymerase High Fidelity (Invitrogen, USA) and 3 µL DNA. The

PCR cycle was done using Biorad T-100 thermocycler (Bio-Rad, USA) and programs for both primer pairs consisted of an initial denaturation of 94^{o}C for 2 min, followed by 35 cycles of consecutive denaturation at 94^{o}C for 30 sec, annealing at 55^{o}C for 30 sec and extension at 68^{o}C for 1 min. The PCR was completed at a final extension of 68^{o}C for 5 min. Only PCR products that showed clear target bands with minimal additional lower bands were selected for further sequencing.

The selected sets of PCR products were sequenced using the Sanger method at the Australian Genome Research Facility (AGRF, Adelaide, Australia). The sequences were assembled based on the overlapping sequences for each sample compared to a few selected sequences available at NCBI (http://www.ncbi.nlm.nih.gov/).

2.2 Genotyping and Capsid Proteins Analyses

In order to infer the genotype of the Papuan PCV2 isolates, the selected Papuan complete genomes, as well as their ORF2 genes were aligned with 1390 PCV2 sequences obtained from GenBank, using ClustalX 2.1 (Larkin et al., 2007). Phylogenetic trees were subsequently constructed based on the Neighbour Joining method and P-distance model with 1000 bootstraps pseudo replication, in *MEGA* 5 software (Tamura et al., 2011). The phylogenetic tree was generated using FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and genotyping using the full genome sequence was compared with genotyping using ORF2 sequence. For the analysis of the capsid protein, amino acids coded by the ORF2 genes of the Papuan isolates and 1390 PCV2 sequences obtained from GenBank were translated into Bioedit (Hall, 1999) and aligned in ClustalX 2.1 (Larkin et al., 2007). NJ P-distance phylogenetic trees were then constructed to compare the groups of capsid proteins amongst PCV2 strains.

Furthermore, the P-distance matrix of the Papuan isolates and selected reference strains of different genotypes were calculated in MEGA 5 (Tamura et al., 2011). P-distance analyses were performed at the level of the complete genome, ORF2 genes as well as at the predicted amino acid sequences of the capsid proteins. Additionally, a sequence identity matrix (SIM) and classical dendograms of complete genomes was constructed for the Papuan PCV2 isolates and a few selected reference strains of PCV2-IM3, PCV2a, PCV2b, PCV2c and PCV2d genotypes.

3. Results

3.1. Complete Genomes Description and Genotyping

Four out of positive 13 samples that showed clear PCR products on agarose gel were selected for complete genome sequencing. The full genome of the four PCV2 isolates comprised of 1,767 bp length. The nucleotide divergence of the Papuan isolates ranged from 0.002-0.004. The size of the ORF1 sequence was 945 bp, situated at nucleotide position 51-995, encoding a protein of 314 amino acids (aa) size. The size of the ORF2 sequence was 705 bp, at position 1734-1030, encoding a protein of a size of 234 aa.

The complete genomic phylogenetic tree suggested that the Papuan PCV2 isolates are grouped together with strains belong to the PCV2-IM3 genotype. The members of this genotype, apart from the Papuan isolates are Brazilian isolates (KJ094602, KJ094605, KJ094606), the Croatian isolate (HQ591381), the Indian isolate (LC004753) and a Chinese isolate (HM776452) (Figure 1a), with a mean pairwise genetic distance of 0.018 (SE: 0.002, range: 0.001-0.035). Genotyping based on the ORF2 gene however, excluded the Brazilian strains (KJ094602, KJ094605, KJ094606) from the genotype, but included a further 39 Chinese isolates and retained the Croatian (HQ591381), Indian (LC004753) and Chinese (HM776452) isolates in the IM3 genotype. This comparison resulted in a larger sized group that comprised 46 strains (Figure 1b) with a genetic distance of 0.033 (SE: 0.003,

range: 0.001-0.088). The P-distance of the Papuan strains and IM3 genotypes, based on the complete genome and ORF2 genes, was 0.004 and 0.007 respectively, lower than the threshold of 0.02 and 0.035, respectively supporting the position of Papuan PCV2 isolates in the IM3 genotype (Table 1). SIM (Table 2) and classical dendogram (Figure 2) further confirmed that the Papuan isolates belong to the PCV2-IM3 genotype. Additionally, variation within the Papuan isolates occurs in the intergenic region at position 42, in the ORF1 gene at positions 131, 389, 405, 604, 910, and in the ORF2 gene zat positions 1558, 1561, 1591 and 1619.

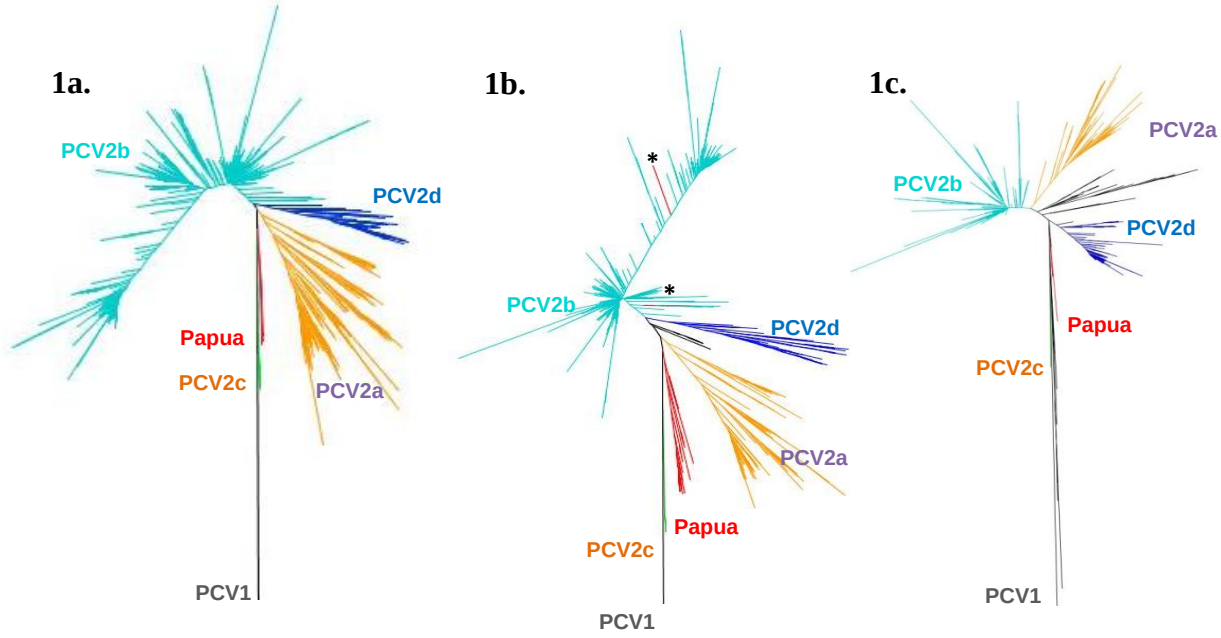

Figure 1: *Evolutionary relationships of the Papuan PCV2 isolates and other genotype strains. 1a. Phylogenetic tree based on complete genome (1682 nt), 1b. Phylogenetic tree based on ORF2 gene (664 nt) and 1c. Phylogenetic tree based on Capsid protein (222 aa). The analysis involved 1,395 nucleotide sequences. PCV1 was included as an outgroup. Evolutionary analyses were conducted in MEGA5. Color explanation: Orange, PCV2a; Light blue, PCV2b; light green, PCV2c; deep blue, PCV2d; red, Papuan and IM3 genotypes; black, PCV1.* (asterix) shows the position of Brazilian strains KJ094602, KJ094605 and KJ094606 which move from the Papuan group in Figure 1a. to PCV2 b genotype group in Figure 1b.*

Table 1: *Genetic divergence of Papuan isolates and different PCV2 genotypes, calculated using Pairwise distance method with 1,000 bootstrap pseudo replication. Analyses involved complete genomes, ORF1 genes, ORF2 genes and amino acid of capsid protein sequences. Papuan isolates show lowest genetic divergence from the PCV2-IM3 reference strains compared to its genetic divergence with other genotypes*

Region of DNA sequence	Within Papuan isolates (SE) n=4	Between Papuan isolates and other genotypes				
		PCV2-IM3	PCV2a	PCV2b	PCV2c	PCV2d
		HM776452	AF055392	AF055394	EF524532	AY181946
Complete genome (1735 nt)	0.003 (0.001)	0.004	0.04	0.036	0.047	0.041
ORF1 gene (933 nt)	0.004 (0.001)	0.002	0.014	0.022	0.02	0.028
ORF2 gene (691 nt)	0.002 (0.001)	0.007	0.081	0.058	0.086	0.063
Capsid protein (231 aa)	0.002 (0.002)	0.01	0.083	0.057	0.096	0.049

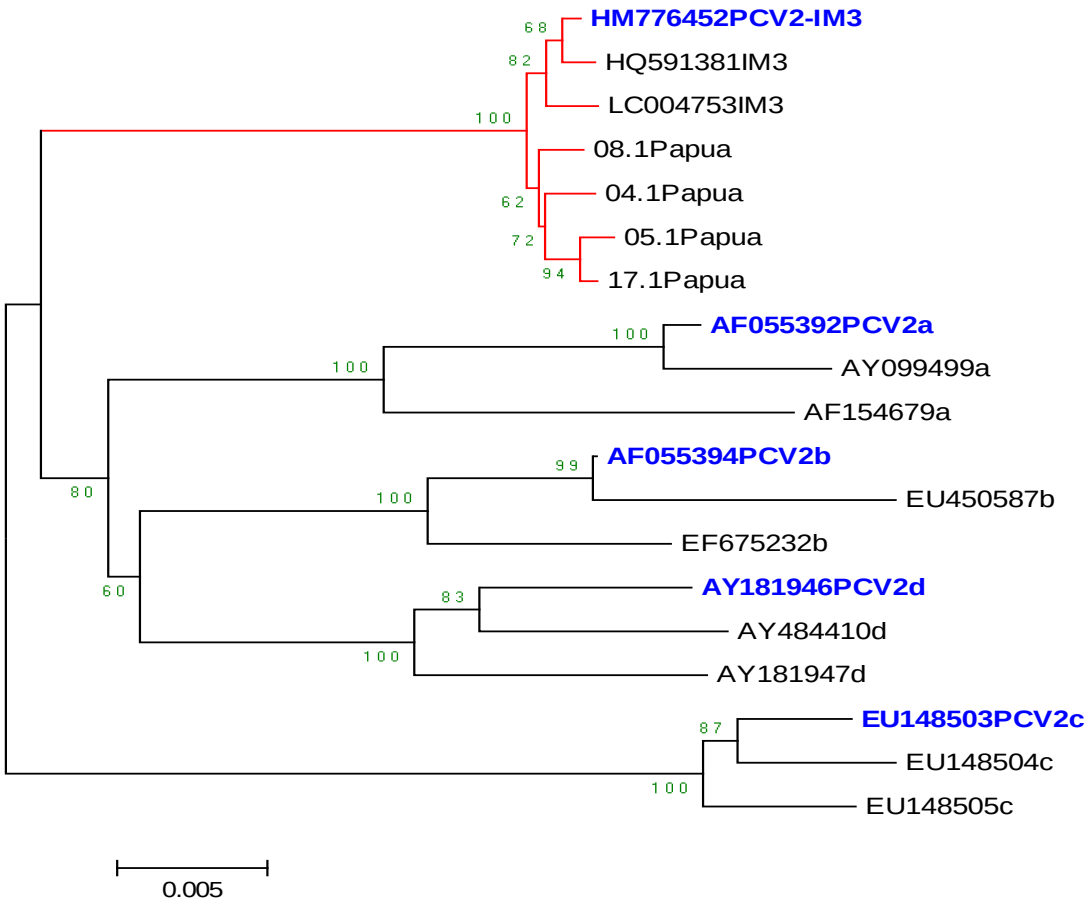

Figure 2: *Dendogram of Papuan PCV2 isolates and selected reference strains from different genotypes. The analysis involved 19 nucleotide sequences. There were a total of 1765 positions in the final dataset. Evolutionary analyses were conducted in MEGA5. Papuan PCV2 isolates are grouped in the clade of PCV2-IM3 genotype*

Table 2: *Sequence identity matrix (SIM) of complete genomes of Papuan PCV2 isolates and selected reference strains from different genotypes. Papuan isolates show high similarity of 99.5-99.6% with PCV2-IM3 genotype reference strain*

PCV2 strains	04.1Papua	05.1Papua	08.1Papua	17.1Papua	PCV2IM3	PCV2a	PCV2b	PCV2c	PCV2d
					HM776452	AF055392	AF055394	EU148503	AY181946
04.1Papua									
05.1Papua	99.6%								
08.1Papua	99.6%	99.6%							
17.1Papua	99.6%	99.8%	99.6%						
HM776452PCV2-IM3	99.6%	99.5%	99.6%	99.6%					
AF055392PCV2a	95.7%	95.7%	95.7%	95.7%	95.9%				
AF055394PCV2b	96.2%	96.2%	96.4%	96.2%	96.3%	96.2%			
EU148503PCV2c	95.0%	94.9%	95.1%	95.0%	95.0%	94.6%	95.3%		
AY181946PCV2d	95.8%	95.8%	95.8%	95.8%	95.9%	95.8%	96.8%	95.1%	
AY193712PCV1	76.6%	76.6%	76.6%	76.5%	76.4%	76.1%	76.5%	76.2%	75.7%

3.2. Predicted Capsid Protein Analyses

The topology of PCV2 phylogeny based on capsid proteins was slightly different from the topology of the PCV2 phylogeny based on the ORF2 gene. In the capsid proteins based phylogeny, PCV2b was located closer to PCV2a, while when analysis was based on the ORF2 gene, PCV2b is the sister clade of PCV2d. The capsid proteins of the Papuan isolates and PCV2-IM3, however remain in consistent topology as immediate descendants of PCV2c (Figure 1c). The amino acid divergence of the capsid protein of Papuan PCV2 isolates was lowest with PCV2d compared to their amino acid divergence with other major PCV2 genotypes. The amino acid sequences of the capsid proteins of the Papuan isolates differed only at position 39 (Arg39Lys).

3.3. GenBank Accession Number

The complete genomes of the Papuan PCV2 isolates used in this study can be retrieved from GenBank with the accession numbers KT369067, KT369068, KT369069 and KT369070.

4. Discussion

PCV2 is an important disease in the pig industry world-wide, causing significant economic loss (López-Soria et al., 2014). In Eastern Indonesia, where traditional pig husbandry practices are predominant, study of the infection with this pathogen is rare. In our current study, we characterized the complete genome of four Papuan PCV2 isolates obtained from village pigs. The Papuan PCV2 isolates in the current study can be grouped with the PCV2-IM3 genotype using NJ phylogeny with P-distance model.

Strains belonging to the PCV2-IM3 genotype have been isolated from pigs across Brazil, China, Croatia and India. In a Brazilian study (Franzo et al., 2015) and our current investigation strains were retrieved from feral pigs. There is no information available as to the host characteristics of the isolates from China and Croatia. PCV2-IM3 genotype might be actually more prevalent in feral pigs rather than in modernly farmed pigs.

The current study demonstrated that there were substantial differences between the results of genotyping based on the ORF2 region and the classification using complete genome sequences. Analysis using the complete genome showed that PCV2-IM3 has lower genetic diversity compared with genotyping using the ORF2 gene. Furthermore, in the current analysis, three Brazilian reference isolates, which belong to the PCV2-IM3 genotype in the complete genome, based analysis grouped with PCV2b when the ORF2 gene was used for genotyping. ORF2 was perceived as the region's representative of the complete genome variation and it has been suggested by others to use either the ORF2 region or full genome sequences in PCV2 genotyping (Segales et al., 2008). We suggest that complete genome sequences should always be used for genotyping of PCV2, as the methods to obtain complete genome of this small virus have largely been available.

A previous study reported that the three Brazilian reference isolates belong to the PCV2d genotype when the analysis involved only a small number of samples consisting of just 36 complete genome sequences (Franzo et al., 2015). Similarly, a study indicated that a phylogenetic analysis using just 48 strains resulted in a topology different from an analysis using a large (n=1, 680) set of PCV2 reference strains, noting the importance of large sample size when determining the classification of PCV2 (Xiao et al., 2015). Not only a different number of samples, but also a different set of sequences within the same number of samples used in the construction of phylogeny tree may produce a different topology. A guideline to genotyping PCV2 using a smaller number of samples may require further study.

Monovalent vaccines based on the capsid protein of either PCV2a or PCV2b genotype have been efficacious to prevent infections with either PCV2a, PCV2b or their mixed infections (Jeong et al., 2015; Opriessnig et al., 2013; Segales 2015). The PCV2d strains on other hand, were isolated from herds that had been vaccinated with PCV2a vaccines (Segales, 2015). Our current study showed that ORF2 of PCV2d was more similar to PCV2b than PCV2a, while ORF2 of PCV2a was highly similar to PCV2b compared to other genotypes. This might explain partly the cross reaction of PCV2a and PCV2b vaccines, and the failure of PCV2a vaccine to protect infection with PCV2d genotype. In the case of Papua PCV2 isolates, the similarity of their ORF2 genes with PCV2b is comparable to with PCV2a. However, the capsid protein of Papuan PCV2 isolates was more similar to PCV2d. The implication of these phenomena on the efficacy of commercial vaccines against Papuan PCV2 isolates requires further study.

5. Conclusion

In conclusion, the present study showed that Papuan PCV2 isolates belong to the PCV2-IM3 genotype. Distribution of this genotype currently encompasses China, Croatia, India and Indonesia. Genotyping using complete genome, ORF2 region or capsid protein sequences resulted in substantial difference in PCV2 strains classification. The clinical implication of these different genotyping methods requires further investigation.

Acknowledgements

This study was funded by The Australian Center for International Agricultural Research (ACIAR) through the project (AH/2007/106) entitled “Improvement and sustainability of sweet potato-pig production systems to support livelihoods in highland Papua and West Papua, Indonesia.”

Conflicts of Interest

All authors declare no conflict of interest.

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8. Pig diseases in Papua Province, Indonesia; aetiology, eco-epidemiology and control options

Mortality has been known to constrain production of Papuan pigs and a few diseases have been recognised to burden Papuan pigs. The paper presented the updated knowledge of pig farming and further discussed the diseases constrained pig production in Papua. In advance, it reviewed the ecology, epidemiology and controls of pig diseases from other places worldwide and proposed possible approaches to control the burdens of major diseases in Papuan pigs. The manuscript was accepted in Springer Science Review.

Original article: Pig diseases in Papua Province, Indonesia; aetiology, eco-epidemiology and control options

Widi Nugroho, Roy Neville Kirkwood and Michael Philipp Reichel (2016)

Pig diseases in Papua Province, Indonesia; aetiology, eco-epidemiology and control options

Accepted in Springer Science Review

Statement of Authorship

Title of Paper	Pig diseases in Papua Province, Indonesia; Aetiology, Eco-Epidemiology and Control Options
Publication Status	☐ Published ☑ Accepted for Publication ☐ Submitted for Publication ☐ Publication Style
Publication Details	Nugroho, W., Kirkwood, R. N. and Reichel, M. P., 2016. Pig diseases in Papua Province, Indonesia; Aetiology, Eco-Epidemiology and Control Options, Accepted in Springer Science Reviews

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Contribution to the Paper	Reviewed papers, books and documents, wrote the manuscript.		
Signature		Date	07/09/2015

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.
iv.

Name of Principal Author (Candidate)	Roy N. Kirkwood		
Contribution to the Paper	Edited and corrected manuscript		
Overall percentage (%)			
Signature		Date	07/09/2015

Name of Co-Author	Michael P. Reichel		
Contribution to the Paper	Edited and corrected manuscript extensively, acted as corresponding author		
Signature		Date	04/09/2015

Springer Science Reviews
DOI 10.1007/s40362-016-0039-9

NARRATIVE STUDENT REVIEW

Pig Diseases in Papua Province, Indonesia: Aetiology, Eco-epidemiology and Control Options

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Received: 21 September 2015 / Revised: 10 April 2016 / Accepted: 4 May 2016
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Abstract Pigs are an important commodity for Papuans, culturally and economically, but diseases and high pig mortality hamper production. The purpose of this review is to describe the ecology and epidemiology of pig diseases prevalent in Papua and to propose control options that may be suitable for the Papuan situation. The review was conducted using published papers on pig production and diseases in Papua, government documentation and published papers on related diseases from other locations. We determined that the major pig pathogens in Papua are Classical Swine fever (CSF), porcine circovirus 2 (PCV2), *Trichuris suis*, strongyle parasites and *Streptococcus zooepidemicus*. Farmers' knowledge of pig diseases is low; hence the role of local government in control measures is pivotal. Control approaches should involve pig confinement as a prerequisite. Vaccination against CSF and parasite control, when indicated, should be part of routine control measures for confined pigs. Education of farmers is an important part of any control program and needs to focus on good farming practices such as the aforementioned confinement, appropriate feeds and feeding, sanitation, recognition of the clinical signs and major pathology of pig diseases, and the reporting of disease to local veterinary services. The ecology and epidemiology of pig diseases in Papua are still largely not understood. Future studies should be aimed at the evaluation of the proposed methods of disease control, an understanding of the impact of PCV2 infection on pig production in Papua and the role of the movement of pig products into and among regions in Papua in regard to CSF and PCV2 viral transmission as well as investigations of other underdiagnosed yet important pig diseases, such as PRRS, H1N1 influenza and toxoplasmosis.

Keywords Pig diseases · Ecology · Epidemiology · Control · Papua · Indonesia

Introduction

Pigs are a major livestock of social, cultural and economic importance in South-East Asia and Pacific areas [27, 69] including Papua province (referred to as Papua hereafter), Indonesia [98]. For centuries, Papuan pigs have been used as an economic commodity, as offerings in traditional events, as gifts for relatives and for family consumption [107, 119, 125]. Cash income generated from pigs in 2006 by traditional farmers in Jayawijaya region, Papua comprised 67–86 % of total family income, with actual figures of 14–16.5 million IDR (~1400 USD at an exchange rate of 1 USD equal to 10,000 IDR). Family in the study was defined as the traditional *sili*, which comprised, on average, 13 persons [98]. Apart from the high dependency of pig farmers on pigs as a source of cash, the number of pig farmers in Papua was also high; 196,724 households [24], or approximately 30 % of all total 658,794 household in Papua [23]. This highlights the importance of pig production for the Papuan economy. A recent survey

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suggested that pig consumption peaks during December and August, which may reflect the more intensive use of pigs and the pig trade on special occasions, such as Christmas and National Day celebrations [125].

The latest agricultural census in 2013 recorded the pig population in Papua as 1,346,800 heads [24]. Papua, the eastern most province of Indonesia has the fifth highest pig population in the country, after Nusa Tenggara Timur (NTT), Bali, North Sumatra and South Sulawesi [162]. Among 29 regions in the province, the Jayawijaya region has the highest pig population, comprising 24.1 % of the total pig population [23]. Pig density in Jayawijaya was estimated to be 23 heads per km^2 [125]. Pig density in Papua Province is illustrated in Fig. 1. It shows that besides that in Jayawijaya, high pig densities are also found in other regions, such as Pegunungan Bintang, Lanny Jaya, Paniai, Jayapura Kota, Yahukimo, Yalimo, Tolikara and Mamberamo Raya.

Domestic Papuan pig production has doubled in the period from 2001 to 2013 (Fig. 2), but the production has only been for local consumption, none of it for export. There are no specific data regarding pig/pork imports for Papua but imports of combined food stock and life animals was reported to have reached 39,000 metric tonnes in 2012, while no food stock/live animal was exported from Papua [23]. At the national level, the export of life pigs was recorded as reaching 32,000 metric tonnes, while import

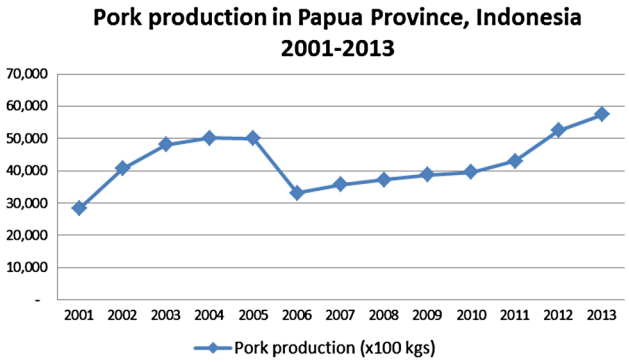

Fig. 2 Total pork production (kg) during 2001–2013 in Papua Province, Indonesia (adapted from [23])

was negligible. However, the import of pork was reported to be 765.5 metric tonnes, while the export was recorded far lower, at 68 metric tonnes [112]. Some of the imported pork can be seen in Papuan markets.

While Papuan pig production has been increasing, pig farm productivity in Papua has remained low [33, 125]. Studies in Jayawijaya revealed that pig disease and mortality have been major constraints to pig farming [98, 125]. Not only are these conditions economically devastating, a few identified pig diseases in Papua also have zoonotic potential. The potentially zoonotic pathogens *Streptococcus suis* and *S. zooepidemicus* were recently isolated from

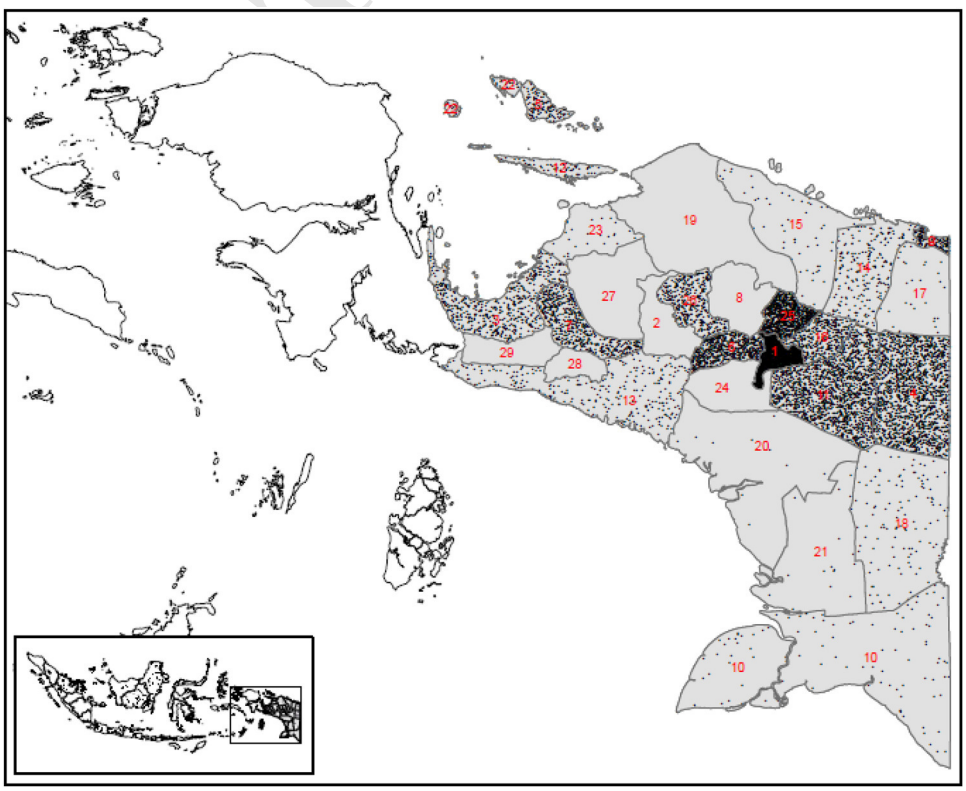

Fig. 1 Density of pig populations in regions of Papua Province, Indonesia (*one dot* represents 50 pigs). Names of regions: 1. Jayawijaya, 2. Puncak, 3. Nabire, 4. Pegunungan Bintang, 5. Biak Numfor, 6. Lanny Jaya, 7. Paniai, 8. Mamberamo Tengah, 9. Kota Jayapura, 10. Merauke, 11. Yahukimo, 12. Kepulauan Yapen, 13. Mimika, 14. Jayapura, 15. Sarmi, 16. Yalimo, 17. Keerom, 18. Boven Digoel, 19. Mamberamo Raya, 20. Asmat, 21. Mappi, 22. Supiori, 23. Waropen, 24. Nduga, 25. Tolikara, 26. Puncak Jaya, 27. Intan Jaya, 28. Deiyai, 29. Dogiyai (adapted from [23])

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cases of mortality in Papuan pigs and their involvement in human diseases needs further studies [126]. Cysticercosis due to *T. solium* is known as an important pig-associated zoonosis in Papua. Wandra et al. [185] indicated that cysticercosis was associated with incidences of epileptic seizure in humans in Jayawijaya. Further studies reported human taeniasis from *T. solium* in regions of the Jayawijaya, Paniai, Nabire, Pegunungan Bintang, Puncak Jaya and Manokwari [102, 150, 184]. A further recent study reported the seroprevalence of pig cysticercosis (PCC) in Jayawijaya as high, at 40.5 % [9].

The purpose of this review is to describe the ecology and epidemiology of pig diseases in Papua and to propose an approach that could be applicable for and transferable to Papua, based on existing scientific information, in order to alleviate the problems of pig diseases and zoonoses in the province. To this effect, we reviewed papers on pig production and diseases in Papua from peer-reviewed journal articles, government documents, conference papers, books, theses and unpublished works. Some pictures from our experience during the conduct of studies in the Jayawijaya Region are also presented to assist with an understanding of pig farming and some pig diseases in Papua. With the relatively small number of relevant references related to the topic from Papuan studies, the review was expanded to include publications from other locations studying these diseases of interest.

We organised this review by firstly describing the traditional pig production system, followed by a description of the diseases affecting pigs in Papua, and then discussing the ecology, epidemiology and control of each selected disease under the subheadings of: prevalence, impact on pig performance, co-infection, pathology, risk factors and available control measures. Finally, we propose steps to be taken in disease control, appropriate in the context of Papuan pig farming.

A Concise Overview of Pig Production in Papua, Indonesia

Pig farms in Papua are relatively small. Using either household or the *sili* (defined as several closely related family groups living in one enclosure) as the unit of observation, the average pig farm in Jayawijaya comprises 8–13 head of pigs, with the ratio of humans to pigs being one [98, 125]. The average numbers of boars, sows, growers and sucker piglets in household farms are 1.3, 1.7, 2.2 and 3.6, respectively [125].

Pig movements onto a farm as a gift or by purchase are common, while hunting for pigs in the bush is rarely carried out. Pig housing is largely traditional; most pig housing is without ventilation, uses bare earth as the floor, has thatched roofs, and commonly uses grass bedding [125]. Thatched roofs were reported to provide for lower temperature fluctuations compared to tin-roofed pig housing or direct exposure to ambient temperature, therefore facilitating a better environment for pig production [33]. Despite the provision of pig housing, most farmers allow pigs to scavenge outside during the day and only 16 % of farms fully confine their pigs. The vast majority of farmers weaned pigs at 2 months of age and more than half of them mixed weaners from different litters [125]. Figure 3 depicts the local pig breed, daily scavenging, and traditional pig housing in Jayawijaya.

Farrowing rates and the litter size of Papuan pigs are low; Cargill et al. [33] reported that sows produced just 0.7 litter/year. The size of the litter ranged from 4.4 to 6 piglets per litter [33, 98, 125]. Traditional pig feeds are sweet potato tuber and vine, and domestic swill. Half of the farmers fed their pigs twice a day, or more frequently. Water provision for pigs in the pen was uncommon [125]. One study reported low bodyweight gains of just 18 g/day in Papuan pigs that were fed with uncooked sweet potato tuber and vines. However, when fed with boiled sweet potato tuber and vines, healthy pigs grew at 160–220 g/day, while the same breed fed with cooked feed with a higher level of protein could grow as fast as 300 g/day [33]. Heat treatment of sweet potato reduces the toxic HCN content, increases ileal digestibility [124] by reducing trypsin inhibitors and improving starch digestibility [50]. Many farmers with full confinement systems reported cooking the feed daily for their pigs [126], although many other farmers also fed pigs uncooked feeds [33].

Furthermore, total pig mortality rate is high in Papua. Pig mortality rates on traditional piggeries may be as high as 40–50 % [33, 125]. Farmers do realise that pig disease and mortality act as a major constraint to production in Papua [98]. However, extensive use of veterinary services is rare and many farmers leave diseased pigs without any attempts to treat them. On the other hand, two-third of farmers reported that they consumed sick pigs or those that had died from natural causes [125], rather major concerns about foodborne infections and intoxications.

Infrastructure to support pig production is available in Papua. Local livestock offices are available in all 29 regional governments in Papua to assist with local livestock production. However, only six regions; Merauke, Puncak Jaya, Nabire, Paniai, Timika and Supiori have specialised offices for livestock, while other regions mix the livestock offices with fisheries, horticulture or field crops [112]. Some of them; Timika, Nabire, Jayapura, Sentani and Jayawijaya employ veterinarians but other regional offices have not hired veterinary staff. A few regional livestock bureaus have simple laboratories,

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Fig. 3 Traditional pig farming system in Papua Province, Indonesia. Pigs are confined in a fenced yard called "laleken/lakenma/enggenma" and allowed to scavenge freely during the day. During the night, pigs are confined to the traditional pig house constructed with wood partitions and thatched roof and a floor consisting generally of bare earth

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capable of examining faeces microscopically, and able to store vaccines and tissue samples. This situation may be a reflection of different priorities for the livestock sector in the regions.

To cope with animal diseases, the central government has developed a diagnostic laboratory in Maros, South Sulawesi province and conducts annual animal disease surveillance in 10 provinces in eastern Indonesia, including Papua. Four quarantine offices have operated in Papua aimed at protecting Papua from exotic disease [109]. In the legislation aspect, the Ministry of Agriculture has declared 22 animal diseases of national priority, which for pigs have included Brucellosis (*Brucella suis*), CSF, cysticercosis, helminthiasis, H1N1 influenza, PRRS and toxoplasmosis [110].

Aetiology of Pig Diseases in Papua

Pig diseases can impact the performance of Papuan pigs in a number of ways, such as by causing low daily weight gain, low annual farrowing rate, low litter size and high mortality. Several pathogens have been identified as major causes of disease in Papuan pigs. Details of studies on the investigation of pig pathogens and zoonoses is presented in Tables 1, 2. It shows that pathogens such as Classical swine fever (CSF) virus, porcine circovirus type 2 (PCV2) virus, *S. zooepidemicus*, *S. suis*, *Taenia solium* and endoparasites, especially *Trichuris suis* and strongyle parasites have been the major pathogens of pigs in Papua.

Among the zoonotic diseases, cysticercosis is a well-known endemic in Papuan pigs [169]. Cargill et al. [33] demonstrated the presence of two other potential zoonotic diseases serologically from pigs in Jayawijaya; Trichinosis and Toxoplasmosis. Further studies are required to confirm the presence of these diseases in Papuan pigs. Clinical Japanese encephalitis (JE) was reported in humans in Timika and Jayapura regions of Papua by serology [139, 165, 166]. Pigs are known to be capable of acting as amplifier hosts for JE virus and transmission to humans from pigs may occur via *Culex* mosquitoes acting as vectors [40]. While the pig population in Papua is high, the role of pigs in the transmission of JE to humans in Papua has never been investigated.

A pig disease serological survey was performed in Jayawijaya region, Papua in 2002 looking at the presence of porcine brucellosis (*B. suis*), leptospirosis (*Leptospira pomona* and *L. tarossovi*), porcine parvovirus (PPV) and Mycoplasmosis (*Mycoplasma hyopneumoniae*). Thirty-nine sera taken from 10 villages failed to demonstrate indications of the presence of these diseases in Papuan pigs [33]. Other important pig diseases, such as colibacillosis and porcine reproductive and respiratory syndrome (PRRS) have been reported from pigs in Bali Island [18, 168] but have as yet not been identified in Papua. The following sections will review the ecology, epidemiology and control of the abovementioned major pig pathogens identified in Papua.

Porcine Circovirus Type 2 Disease (PCVD)

PCV2 is one of the smallest known viruses, with a non-enveloped virion particle of 12–23 nm in diameter. PCV2 has a circular, covalently closed, single-stranded DNA that contains 1767–1768 nucleotides. It belongs to the family Circoviridae, genus *Circovirus* [158]. Four major genotypes of PCV2 have been established based on ORF2 region or full genome sequencing, namely PCV2a, PCV2b, PCV2c and PCV2d. Later on, four different intermediate (IM) clades have also been proposed [191]. PCV2a, PCV2b and PCV2d have been reported to be equally pathogenic. PCV2b is the most prevalent genotype in farmed pigs, followed by PCV2a and PCV2d [156, 191]. By far, the virulence of PCV2c and intermediate clades are unknown.

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Table 1 Pathogens of pigs and zoonoses of viral and bacterial origin studied in Papua, Indonesia

Organism	Methods	Prevalence in pigs, % (*n*)	Prevalence in humans, % (*n*)	Regions of the study	References
Viruses					
Classical swine fever	ELISA antigen	1 (103)	–	Jayawijaya	[126]
	ELISA antibody	33 (103)	–	Jayawijaya	[126]
Porcine circovirus type 2 (PCV2)	ELISA antibody	28 (103)	–	Jayawijaya	[126]
	PCR	41 (32)*	–	Jayawijaya	(Nugroho et al., 2015, unpublished thesis)
Transmissible gastroenteritis (TGE)	Serology	Nil (39)	–	Jayawijaya	[33]
Porcine parvovirus (PPV)	Serology	Nil (39)	–	Jayawijaya	[33]
Japanese encephalitis (JE)	ELISA	ND	1 (226)**	Jayapura	[139]
	ELISA	ND	9 (96)	Timika	[166]
Pseudorabies virus (Aujeszkey's disease)	Serology	13 (39)	ND	Jayawijaya	[33]
Bacteria					
Streptococcus suis	Isolation-PCR	9 (103)	ND	Jayawijaya	[126]
	Dot-Blot	11(67)	ND	Timika	[149]
Streptococcus zooepidemicus	Isolation-API 20 Strep	Nil***	ND	Jayawijaya	[126]
Leptopsira pomona	Serology	Nil (39)	ND	Jayawijaya	[33]
Leptospira tarossovi	Serology	Nil (39)	ND	Jayawijaya	[33]
Brucella suis	Serology	Nil (39)	ND	Jayawijaya	[33]
Mycoplasma hyopneumoniae	Serology	Nil (39)	–	Jayawijaya	[33]

* From PCV2 seropositive samples

** Samples were non-malaria febril patients

*** 15 % ($n = 92$) prevalence was reported using isolation in pig mortality cases [125]

A study has identified genotype PCV2b and PCV2-IM3 in Jayawijaya with PCV2-IM3 having a higher prevalence (Nugroho et al. 2015, unpublished thesis).

Prevalence

PCV2 is a ubiquitous virus present in domestic as well as in feral pigs worldwide [12, 55]. In Jayawijaya Region, Papua, PCV2 was detected in 59 % ($n = 71$) of dead pigs and in 28.2 % ($n = 103$) of healthy pigs [125]). For comparison, in Chinese farms the reported prevalence ranged from 36.3 to 64.2 % [83, 196], was 22 % in Brazilian pig herds [45], and 63 % in Hawaiian feral pigs [167].

Impact on Pig Performance

PCV2 is known to contribute to various pathologic conditions, collectively called Porcine Circovirus Diseases (PCVD) [12]. The most well-known clinical feature of PCVD is post-weaning multi-systemic wasting syndrome (PMWS), which causes significant pig mortality [80, 187]. More chronic PCV2 infections have been known to result in stunting, reduced weight gain and reproductive failure including return-to-oestrus, late abortion, mummified fetuses, stillbirths and non-viable live-born piglets [156]. There has been a shift in PMWS manifestation in Europe and North America from a fatal to a more chronic and subclinical outcome [12] but mortality is still reported from China [193]. There is currently no indication or knowledge of impact of PCV2 infection on pig performance in Papua.

Co-infections

In Papua, co-infection of pigs with PCV2, CSF virus and endoparasites is common. Specifically, infection with both, PCV2 and CSF virus was more common in dead pigs when compared to healthy pigs [126]. Co-infection of pigs with PCV2 and various pathogens has been reported to increase the severity of PMWS. Pathogens reported to co-infect with PCV2 include PRRS, PPV, Swine Hepatitis E virus (HEV), *M. hyopneumoniae*, *Salmonella* spp., or *Metastrongylus elongatus* [4, 5, 64, 76, 128, 193]. Recently, a simple temperature fluctuation and high stocking density without involvement of any other pathogen was shown to be capable of triggering clinical manifestations of PMWS [131].

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Table 2 Internal parasitic pig pathogens and zoonoses studied in Papua, Indonesia

Organism	Methods	Prevalence in pigs, % (*n*)	Prevalence in humans, % (*n*)	Regions of the study	References
Internal parasites					
Cysticercus cellulosae	ELISA, Serology[1]	41 (111)	8 (109)[1]	Jayawijaya	[9], Swastika in [184] [1]
	Immunoblotting	ND	29 (633)	Paniai	[150]
	Serology	ND	9 (105)	Nabire	(Wandra et al. 2007)
	Immunoblotting	ND	2 (654)	Puncak Jaya	[150]
	Immunoblotting	ND	3 (391)	Pegunungan Bintang	[150]
Toxoplasma gondii	Serology	18 (39)	ND	Jayawijaya	[33]
Trichinella spiralis	Serology	13 (39)****	ND	Jayawijaya	[33]
Trichuris suis	Faecal examination	8 (102)	–	Jayawijaya	[126]
Strongyloides ransomi	Faecal examination	16 (102)	–	Jayawijaya	[126]
Ascaris suum	Faecal examination	12 (102)	–	Jayawijaya	[126]
Hyostrongylus rubidus	Faecal examination	10 (10)	–	Jayawijaya	[134]
Globocephalus urosubulatus	Faecal examination	80 (10)	–	Jayawijaya	[63]
Macracanthorhyncus hirudinaceus	Faecal examination	50 (10)	–	Jayawijaya	[63]
Ascarop strongylina	Faecal examination	ND	–	Jayawijaya	[33]
Physocephalus sexalatus	Faecal examination	ND	–	Jayawijaya	[33]
Metastrongylus spp.	Faecal examination	ND	–	Jayawijaya	[33]
Oesophagostomum spp.	Faecal examination	ND	–	Jayawijaya	[33]
Gnathostoma hispidum	Faecal examination	ND	–	Jayawijaya	[134]
Eimeria debliecki	Faecal examination	ND	–	Jayawijaya	[33]
Eimeria scabra	Faecal examination	ND	–	Jayawijaya	[33]
Eimeria suis	Faecal examination	ND	–	Jayawijaya	[33]
Balatidium coli	Faecal examination	ND	–	Jayawijaya	[33]
Entamoeba sp.	Faecal examination	ND	–	Jayawijaya	[33]
Jodamoeba sp.	Faecal examination	ND	–	Jayawijaya	[33]

[1] Indicating a corresponding data

**** The cyst has never been described

The odds of PMWS decrease when vaccination against atrophic rhinitis [91] or *Escherichia coli* is administered [146]. In contrast, PMWS has been reported to emerge after vaccination against PRRS [51, 91, 146, 187]. Emergence of PMWS was also reported when PCV2 infected animals were vaccinated against CSF virus [65]. These findings suggest that vaccination could otherwise risk the PMWS, therefore the implementation of vaccination to prevent PMWS needs to consider the health and likely infection status of an animal.

Transmission

International transmission, transmission among local herds and rapid viral evolution were thought to contribute to the spread of PCV2 [56]. The role of pork imports on the course of PCV2 infection in Papua is unknown, but imported pork products from several countries have been available in Papua.

Transmission among pig herds in Papua may be facilitated by direct contact between pigs as most farms leave their pigs scavenging during daylight [125]. Other potential modes of transmissions for PCV2 have never been studied in Papua, but have been identified in other countries. Semen from infected boars was reported as a source of infection [115, 146]. Transmission by humans as a mechanical vector has been suspected and one study has suggested that humans should have no pig contact for at least 2 days prior to visiting a farm [4]. *Culex* mosquitoes and *Musca* flies living on pig farms may carry PCV2 [19, 192]. Other insects or external parasites that live on pig body surfaces may act as mechanical vectors for the virus and could partly explain why regular treatment against external parasites was found to reduce the risk of PMWS

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[146]. Potential airborne transmission has been suggested and interestingly the level of air contamination was indicated to be independent of stocking density [179]. In the external environment, viable PCV2 was isolated from pig manure [180] leading to speculation that contaminated water might be another vector for PCV2 transmission. In addition, the role of other species such as calves and rodents as biological vectors have been demonstrated [66, 82, 88, 92].

Pathology

PCV2 infections manifest in various forms, either as systemic disease, respiratory, enteric disease, dermatitis and nephropathy syndrome or reproductive diseases. Enlarged superficial inguinal lymph nodes are a common *ante-mortem* sign of PCV2 systemic disease. Other signs may include rough hair coat, emaciation, irregular red-to-purple skin macules and papules, locally subcutaneous haemorrhages and oedema, and cutaneous scars in cases that have recovered from the acute phase [157].

During *post-mortems*, lungs may show a tan-mottled surface and a lack of collapse [157]. This pathology occurred in approximately 65 % of diseases associated with PCV2 infection [159]. Lesions in the alimentary tract show catarrhal enteritis with or without mesenteric oedema, thickened mucosa and enlargement of mesenteric lymph nodes. Moreover, bilateral renal enlargement with small cortical petechiae or whitish spots, and oedema of the renal pelvis may be observed. Lesions in other organs include occasional splenic infarcts, atrophic-discoloured liver and slightly rough hepatic surface [157].

As a reproductive disease, PCV2 infections were reported to cause mummification or oedema of aborted fetuses. Fetal livers were enlarged and congested and fetal hearts showed hypertrophy with multifocal discoloured areas of myocardium. Additionally, ascites, hydrothorax and hydropericardium of the fetuses were detected [157]. There is no detailed study of PCV2 pathology in Papua, but gross lesions of non-collapsed tan-mottled lungs were found in a dead pig in which PCV2 genetic material was detected (Fig. 4). It indicated that in Papua PCV2 might associate with respiratory disease.

Risk Factors

In healthy Jayawijayan pigs, where the prevalence of PCV2-CSF co-infection is lower than in dead pigs, farms were characterised as fully confined, used cooked feeds, had floors made of concrete or wood and were relatively isolated from contact with other pigs [126]. In contrast, on the majority of Papuan pig farms where dead pigs originated from, pigs were raised on bare-earth floors and were scavenging during the daylight [125]. It seemed that the locally adopted confinement system was beneficial in reducing the risk of PCV2 infection in Papua compared to a scavenging system.

Studies on the role of confinement in PCV2 infection have produced conflicting results. A European study on wild boar suggested that intensively managed wild boar had a higher prevalence of PCV2 [181]. However, studies in Hawaii and Brazil reported that the prevalence of PCV2 in wild boar can be higher than in domesticated pigs [45, 167]. This suggests that housing and stocking density may be only among other factors sufficient to induce the infection with PCV2. In Papua, while confinement seemed to reduce the risk of PCV2 infection, the level of subclinical PCV2 infection in healthy confined pigs in Jayawijaya was still high, approaching 30 % of the animals studied [126]. The significance of subclinical PCV2 infection for performance parameters of Papuan confined pigs, such as a possible reduced daily weight gain and reproductive failure, remains open for investigation.

In intensive piggeries, management factors may be important for reducing clinical manifestation of PMWS. A European case study of a PMWS outbreak indicated that after a reduction in stocking density, segregation of batches of pigs, cleaning and disinfection of the housing and a strict application of an all-in/all-out pig flow, mortality dropped from 12 % to 6 % [95]. Leaving farrowing and weaning pens empty for 5 days could reduce the risk of PMWS [146]. In contrast, an increased severity of PMWS was reported to be associated with rearing growers indoors with a density of more than 1 pig per m^2 [4] and with having poorly isolated hospital pens, indicating a role for higher density as a risk factor and the hospital pen as a source of infection for other pens [146].

Age at infection with PCV2 may be important in the development of PMWS. Suckling piglets were more likely to exhibit PMWS if they were weaned before 21 days and infected with PCV2 before 7 weeks of age [91, 145]. An increased severity of PMWS was associated with a high level of cross-fostering during the first 24 h of life, which might be due to increased risk of early PCV2 transmission from different sows to newborn piglets [146]. In Papua, the average farmer weaned their piglets at 2 months of age and cross fostering was not common [125]. Therefore, infection resulting from such intensive newborn rearing strategies is unlikely in Papua.

Feeding regimes may be another important preventive factor of PMWS. A feeding frequency of more than twice daily for weaners until they reach 14 weeks of age reduced the risk of PMWS [4]. Feeding twice daily may reduce the risk of scavenging by pigs and may reduce the risk of disease transmission, including that of PCV2 infection. A feeding regimen of twice daily or more was practiced by

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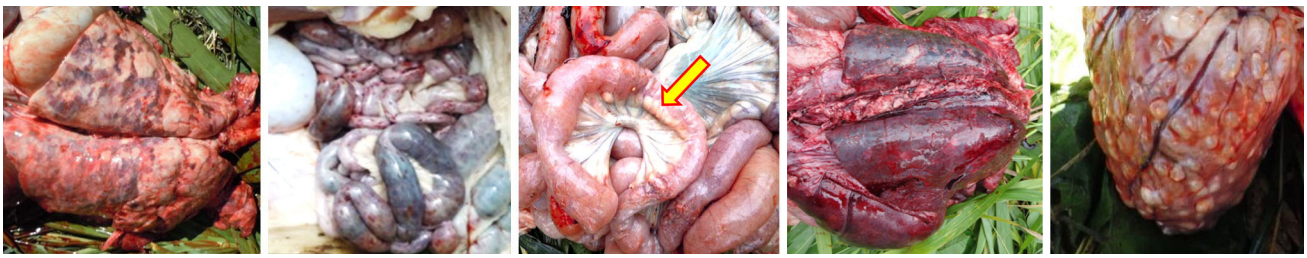

Fig. 4 Examples of the gross pathology of pig diseases in Papua: A non-collapsed tan-mottled lung due to severe PCV2 infection, ecchymotic haemorrhage of the intestine caused by classical swine fever (CSF) virus infection, nodules in the serous layer of the colon due to *Oesophagostomum* infestation (*arrow*), diffuse haemorrhagic pleuropneumonia from an acute *Streptococccus zooepidemicus* infection and heavy infestation with cysticeri of the heart (from *left to right*) (Nugroho, unpublished data)

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almost half of farmers in the Jayawijaya region [125]. A study is warranted to understand whether feeding twice daily is beneficial in reducing the risk of PCV2 infection and its clinical manifestation under Papuan piggeries settings.

Control Measures

Vaccination has been widely used in many countries as an effective tool for the control of PCV2 disease [41]. Available commercial vaccines have been developed based on the PCV2a genotype. Such vaccines have been reported to be efficacious against PCV2a and PCV2b genotypes but the discussion is still open on their effectiveness against PCV2d genotypes [156]. Further, some of the Papuan PCV2 strains belong to the IM3 genotype, which was only recently recognised and there has not been study on the efficacy of commercial vaccines against this particular PCV2 genotype. The presence of PCV2b genotype in Papua has also been demonstrated (Nugroho et al. 2015; unpublished thesis). However, as there is no information as to the prevalence of PCV2b and PCV2 IM3 genotypes in Papua, or any knowledge of heterologous protection of commercial vaccines for the IM3 genotype, the effectiveness of any vaccination program for Papuan farms using commercial vaccines cannot be predicted.

Classical Swine Fever (CSF)

The virus causing CSF (CSFv) belongs to the genus Pestivirus. It consists of a single-stranded, positive sense, 12.3 kb RNA genome, enclosed in a 45 nm in diameter hexagonally shaped envelope. Based on 190 nt of the E2 envelope glycoprotein gene, CSFv can be divided into three genotypes with three or four sub-genotypes: 1.1, 1.2, 1.3; 2.1, 2.2, 2.3; and 3.1, 3.2, 3.3, 3.4. Highly virulent CSFv strains and the vaccine strains belong to genotype 1. Genotypes 2 and 3 are moderately virulent. All of the genotypes have been found in Asia, but genotype 1 is mainly prevalent in South and Central America, genotype 2 in the European Union and genotype 3 in Asia [36]. Subgenotype 2.2 has been reported in Java, Indonesia [137], but the genotypes circulating in Papua have not yet to be determined.

Prevalence

Recently in Jayawijaya, CSF viral antigen was detected in 31 % ($n = 71$) of dead pigs. In addition, antibody testing demonstrated that 55 % ($n = 71$) of dead pigs were seropositive. In healthy animals, the seroprevalence was just 33 % ($n = 103$) [126]. CSF is absent from North America, Australia, New Zealand, and most of Western Europe but remains a challenge in Asia, South America, Eastern Europe and parts of the former Soviet Union [72]. In a few endemic areas in Asia the seroprevalence of CSF in nonvaccinated pigs are varied. In Nusa Tenggara Timur, Eastern Indonesia, CSF seroprevalence in unvaccinated domesticated pigs was estimated at 13 % ($n = 883$) [154]. In Timor Leste, the seroprevalence in non-vaccinated pigs is 25 % ($n = 468$) [153]. Prevalence of CSF infection has been reported to be <1 % in non-vaccinated domestic pigs on Jeju Island, South Korea ($n = 22\,601$) [164]. A study in Karnataka, a state in the South western region of India, demonstrated a range in seroprevalence from 61 to 21 % in regions with intensive to primitive pig farming without vaccination ($n = 218$) [38].

Impact on Pig Performance

After its first report in Timika Papua in 2004, outbreaks of CSF causing pig mortality have rapidly spread to four other regions, namely Jayapura, Puncak Jaya and Jayawijaya [108]. CSF causes economic loss on pig farms due to high mortality associated with highly virulent strains. Lower virulence strains cause milder clinical signs and less mortality, while avirulent strains may only produce fever without further consequence [16]. Infection occurring during gestation can result in abortion, mummification, stillbirths, or persistently infected (PI) piglets [49, 177].

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Co-infection

An infection with high or moderately pathogenic strains of CSFv is capable of producing severe clinical disease [89]. However, co-infection with pathogens may be important for lesser pathogenic strains to be able to produce clinical signs. Failure of CSF vaccination in PCV2 infected animals may be an example of how, with a co-factor, a non-pathogenic CSF could become pathogenic [65].

Co-occurrence of CSF and endoparasitic helminths was observed in 41 % of dead pigs [126]. The same study reported that mixed infection of CSF and PCV2 occurred in 11 % of cases of dead pigs, which was more than five times higher than that of healthy pigs. These data indicate that co-infection of CSF and endoparasitic helminths or PCV2 could play a significant role in cases of pig mortality in Jayawijaya. In contrast, a survey in Guangxi China reported co-infection of CSFv with swine influenza virus (SIV), PRRSV and PCV2 in less than 6 % of animals [140, 190].

Pathology

Pathological changes in Classical Swine Fever include conjunctivitis, petechiation of skin and ear necrosis, while, during *post-mortem* examination, petechial to ecchymotic haemorrhages of various organs have been the most common findings [52, 114]. Petechiae were observed during *post-mortem* examination of dead pigs in Papua and they might be the simplest gross pathology that farmers can recognise and report to local veterinarians (Fig. 4). Diagnosis of CSF using gross pathology of petechial or ecchymotic haemorrhages of bladder, kidney, stomach, lung or skin will result in 86.3–97.9 % specificity but a sensitivity of only 14.4–40.4 % [52]. However, gross lesions may be useful in locating hot spots in outbreaks involving large numbers of pigs.

Within the thoracic cavity, various pathological conditions may be observable including pulmonary oedema, pneumonia, pleuritis and chronic bronchitis, as well as chronic pericarditis, hydropericardium and hydrothorax. The pathology of the alimentary tract involves fibrin formation, chronic gastric ulceration, a hyperaemic intestinal tract, watery contents of jejunum and colon, oedema of the mesocolon and dry faecal contents in the colon. Renal cysts, renal enlargement and degeneration, liver and splenic enlargement have been reported in a few cases [52].

Transmission

Direct contact, horizontally and vertically, is the most efficient way to transmit CSFv [143] and may be the most effective transmission route in Papua. Various indirect transmission routes through a vector have also been proposed from a number of studies elsewhere. Wild boars are known to be an important biological vector [133, 143]. Mechanical vectors include contaminated feeds, vehicles, personnel, and infected semen used for artificial insemination. Additionally, airborne spread over short distances may be possible [143].

Risk Factors

Some risk factors for CSF infection have been identified. In a central market in Jayawijaya, CSFv was identified among pigs being traded, making this market a potential reservoir for CSFv and other pathogens for connected areas [126]. Pigs or pig feeds originating from, or people recently visiting, this market, may act as vectors of CSFv onto a farm.

Feeding vegetables harvested from areas with infected pigs, feeding offal from wild boar or feeding, swill feeding, contact with a neighbour's pigs, and artificial insemination have all been reported to increase the risk of CSFv transmission [115, 143]. Frequent shipments of pigs were also anticipated to increase the risk of CSFv transmission through contact with contaminated trucks [47, 104].

A study in the Eastern Cape Province of South Africa indicated that the risk of contracting CSF was lower if pigs were kept indoors but the risk was increased when farmers lived away from their farms or were uneducated [96]. Another study in Bulgaria suggested that areas, which were economically deprived were more likely to have a higher number of CSF [104]. These studies indicated that the risk of contracting CSF maybe linked to poverty in pig farmer communities. Inadequate knowledge or resources and thus not practicing general hygiene in underdeveloped regions may attribute to disease spread. The level of poverty in Papua is the highest among Indonesian provinces [11] and it may make the control of CSF in Papua more challenging.

Control Measures

Control efforts against CSF in Papua have not been successful to date. Vaccination against CSF was conducted by regional governments in Jayawijaya, Timika and Jayapura using injectable C-strain vaccine preparations, but the effectiveness remains unknown. Attempts to control CSF in other parts of Indonesia and in other countries will be discussed below to become a reference point for the design of comprehensive CSF control efforts in Papua in the future.

On Alor Island, NTT Province, a project to control CSF was conducted by the Australian Centre for International Agricultural Research. The activities included surveillance for the disease, education of students and farmers, vaccinations and the regulation of pig transports. Vaccination of 43 % of the pig population along with these other efforts was said to be sufficient to reduce the incidence of CSF

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infection. However, eradication of CSF in Alor may not be feasible because a large population of wild boar makes vaccination of all pigs essentially impossible [144]. In contrast, West Sumatra Province was declared free of CSF in 2014, after previously being infected [111]. Control of CSF in West Sumatra had relied upon surveillance and elimination of serological reactors without vaccination.

In the Netherlands, where a policy of "no vaccination" was chosen after CSF had been eradicated from the country, separation of trucks used for national and international transport of pigs was thought to be the most cost-effective approach to prevent the risk of reintroduction of CSF into the country [47]. Information concerning the frequency of the importation of pigs or pork into Papua is unavailable publicly, but imported pig products have found their way into Papuan markets. The risk of CSF transmission in Papua from imported pork is unknown.

Vaccination has been used as the main strategy to control CSF in many endemic countries where a test and cull approach was not possible. The injectable C-strain vaccine was capable of producing complete protection in just 7 days after a single vaccination and protected pigs from horizontal infection [176]. Vertical transmission of CSFv from carrier sows can produce immunotolerance and persistently infected piglets [177], but oral vaccination of pregnant sow at 5 weeks after insemination was reported to be capable of protecting piglets from vertical transmission [73]. After vaccination, piglets older than 5 weeks developed a higher immune response than 3 week old pigs, indicating that a booster may be needed when young piglets are given a vaccine [170]. The health status of an animal may also be important for successful vaccination; CSF immunisation during an acute phase of PRRSv infection resulted in vaccination failure [171]. Further, vaccination against CSFv in PCV2 infected pigs resulted in the development of PMWS [65].

Infected boars were able to transmit virus to a sow through insemination and produce embryonic loss [97]. In Papua, where the CSF status of boars used for insemination is difficult to determine due to lack of diagnostic tools, it may be appropriate to vaccinate females against CSF 7 days before insemination to protect the sows and stop vertical transmission to the developing litter. However, further study is needed to confirm this option.

Early post-natal infection of piglets born from naïve sows also produces PI piglets. Such piglets neither produce neutralising antibody nor respond to vaccination [120, 121]. However, this scenario may be prevented by vaccinating pregnant sows.

Apart from the use of injectable vaccines, the efficacy of oral vaccination has been evaluated. In Serbia, a commercial oral vaccine (RIEMSER®) resulted in 73 % ($n = 41$) of pigs older than 12 weeks age being immune and 64 % ($n = 44$) of pigs being immune at 28 days post-vaccination [106]. In Bhutan, the same vaccine resulted in a mean of 60 % ($n = 193$) pigs of all ages and breeds being immune, and local farmers welcomed such an approach because of the ease of administration [116]. In light of the unsuccessful attempts to deliver an injectable vaccine across Papua, the efficacy and practicability of the use of an oral vaccine warrants a field trial.

When a CSF outbreak occurs, vaccination around the focus of the outbreak (ring vaccination) can limit the size and spread of the epidemic and thus reduce mortality [133]. C-vaccine is the vaccine of choice for this purpose rather than the oral vaccine, as it induces complete immunity after 7 days [176]. Theoretically, with ring vaccination the success in controlling the spread of CSFv is determined by whether viral transmission is able to reach the edge of the 'ring' pig population after the ring has been formed. Currently, there is no local strategy proposed for emergency vaccination in Papua.

When an outbreak of CSF occurs in a region, the consequences may be reduced if the disease can be recognised early [79]. In the Netherlands, farmers were encouraged to call a veterinarian when they observed mortality on their farms, after which further diagnostics were performed and the CSF status of the herd established [79]. Syndromic reporting to local government veterinary clinics was encouraged in NTT [144] and has actually been practiced by farmers in some regions in Papua. Education of farmers concerning clinical signs of diseases has been initiated in Jayawijaya but the results have not been evaluated [33]. Continuation and improvement of this initiative could assist with better records of CSF and other diseases in the future and support a better design of outbreak preparedness and surveillance programs.

The successful prevention of spread of CSFv in an epidemic area, through the isolation of affected herds, destruction of pigs and disinfections has been reported [79]. In Jayawijaya, pig movement onto farms through purchasing and as gifts was found to be very common and closely related to the local culture [125]. Therefore, prohibition of pig transport from and to an outbreak area seems currently not feasible.

The feasibility of eradication of infected pigs from an endemic area through a test and cull program in Papua is unknown, because there is no information on important aspects such as the availability of sufficient resources for compensation, the availability of rapid testing and preparedness of trained personnel. The lack of an adequate compensation scheme is known to have caused reluctance in farmers in Africa to follow such an approach though they realised CSF was a devastating disease [96]. Farmers hid piglets indoors or away in the bush when the government officers came to cull infected pigs [96, 133].

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Wild boar may act as an important reservoir of the CSF virus [133, 143]. Control of CSFv infection in wild boar has been achieved through vaccination and a reduction of the population by various means such as shooting, trapping, fertility control or poisoning [31]. The status of CSFv in the Papuan wild boar population and its role in CSF transmission to farmed pigs is unknown and should be the subject of future study since many domesticated pigs are scavenging freely during the day and may come in contact with wild pigs.

Taenia solium—Cysticercosis

Prevalence

A recent study reported the seroprevalence of pig cysticercosis (PCC) in Jayawijaya at 40.5 % [9]. In other regions in Papua, although reported to be endemic for taeniasis due to *T. solium*, there is no publicly available information as to the prevalence of pig cysticercoses. Paniai, Nabire, Pegunungan Bintang, Puncak Jaya and Manokwari regions have reported human taeniasis from *T. solium*. A human case was once reported in the Merauke region but thought to be an infection acquired from other region since there has been no evidence of *T. solium* contamination in the region [102, 184]. In other Indonesian provinces, *T. solium* was only reported in Balinese people and serologically in Balinese pigs in Karangasem, with the seroprevalence at 15.8 % [184]. Additionally, the prevalence in dogs, a natural intermediate host of *T. solium* in Jayawijaya, tested by an immunoblotting technique was 11 % ($n = 64$) [71].

Impact on Pig Performance

Carcass condemnation is the main impact of PCC on pig performance. Excessive salivation, excessive blinking and tearing with or without subconjunctival nodules was reported in all pig samples with neurocysticercosis ($n = 18$), but not in neurocyst-free pigs ($n = 12$), indicating the association of neurocysticercosis with the abovementioned clinical signs [138]. Restlessness, due to these clinical signs presumably also has contributed to the reduced growth rate. However, pigs can be infected with more than a hundred cysts in the brain without being clinically affected [147]. However, the highest concern of pig cysticercosis is its public health consequence.

Co-infection

No data is available on PCC and co-infection and its clinical consequences for pigs.

Pathology

In heavy infections, lesion can spread throughout the muscles of the body. However, kidney, spleen, liver and lung were likely unaffected even in heavy infections with 80,000 cysts, and oesophagus was least affected [20]. On the other hand, in very light infected 2-month-old pig, showing only a single cyst, the liver was reported to be the only organ affected [155]. In naturally infected pigs with relatively light number of cysts (less than 80 vesicles), cysts were absent from the tongue, which may compromise the accuracy of diagnosis based on tongue inspection [84, 155]. In an experimental infection with 100,000 viable eggs, vesicles in the tongue were palpable 30 days post-infection. In this experiment, cyst in the pigs' brains remained vesicular and infective 350 days post-infection, but in the muscle they degenerated into caseous forms over the same period of time [44]. Cysts began to be infective approximately 45 days post-infection [85].

Transmission

Pigs acquire cysticercosis after ingestion of *T. solium* eggs. In the pig's alimentary tract, the eggs hatch and develop into onchospheres, which subsequently migrate to the muscle and encyst. Dogs are reported to be a natural intermediate host in Jayawijaya and could act as a pathway to pig cysticercosis in this area [71]. The life cycle of the encysted larvae can be completed when humans eat raw or undercooked pork or dog meat contaminated with the cyst. Under natural condition, humans are by far the only definitive host of *T. solium* [57]. *Chinchilla laniger*, immunosuppressed with methyl prednisolone acetate (MPA), was experimentally the only rodent able to act as a definitive host [10, 53, 101]. Hamsters treated with MPA would allow cysts to develop into mature proglotids but the proglotid was incapable of producing eggs [53]. Rat infected with *T. solium* experimentally, activated oncospheres intracranially and immunosuppressed mice experimentally infected subcutaneously were shown to be capable of developing cysticercosis [70, 178]. However, the role of rodents in the transmission of *T. solium* in the field is not apparent.

The beetle *Ammophorus rubripes* may carry *T. solium* eggs in its alimentary tract and the 40 % of eggs may remain viable at 24 days [59]. The role of insects in *T. solium* transmission has, however not been established in Papua. Human cysticercosis and taeniasis remain an important parasitic zoonosis in Papua [184].

Risk Factor

A cross sectional survey in Jayawijaya reported that free roaming and feeding uncooked feed could be risk factors

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for pig cysticercosis [9]. In other study, confinement was also shown to prevent new exposure to *T. solium* to pigs in Jayawijaya as shown by serologic testing [3].

Pig owners not using a latrine was shown to be risk for pig cysticercosis in Tanzania [26], but not detected as a risk factor in Jayawijaya [9]. As pigs may roam in a radius of 1 km^2 [174], and a square km of land may be occupied by four to five families in Jayawijaya (BPS [25], free scavenging pigs may access other human faeces although the owners use a latrine in their own home, thus explaining the non- significant role of a latrine in pig cysticercosis infections in Jayawijaya.

Poverty, with an unawareness of personal hygiene and the exposure to risky animals is thought to correlate with the high prevalence of human cysticercosis [62]. Poor personal hygiene might also be a risk for transmission of *T. solium* to pigs. Contaminated feedstuff, even when boiled was thought to contribute to pig cysticercosis in Tanzania [26].

Control Option

A few options for the control of cysticercosis/taeniasis have been described. These include anthelminthic mass medication, vaccination, public education or combinations of any two of those options [85]. If anthelminthic treatment for pigs is chosen, using Oxfendazole at the dose of 30 mg/kg is one choice. All cyst were destroyed from tissue 12 weeks post-treatment [173]. A coverage of 75 % of the population and several rounds of drug administration over a period of several years (e.g. twice a year for 5 years) is likely to be required to have a sustained effect on the prevalence of *T. solium* [173]. In Papua, while 86 % of pig farmers in Jayawijaya trust in the efficacy of modern (western) medicine, only 12 % of them use modern medicine consistently [125]. The reasons that only these 12 % of farmers use modern medicine are unknown but it may hamper achieving the 75 % coverage required if anthelminthic treatments are to be effectively performed. This gap in knowledge may require further study before effective anthelminthic treatment can be achieved.

Efficacious, double-dosing vaccines against *Cysticercus cellulosae* or its oncospheres have been available [60, 117]. A study proposed a combination of chemotherapy and vaccination twice, using the TSOL18 vaccine in 4-month intervals to effectively eradicate *T. solium* in pigs in a population. The scenario assumes that pigs will be slaughtered at 12 months of age [85]. While this could be an excellent scenario under suitable conditions, approximately 50 % of Papuan pigs died during the first 4 months of age and Papuans would have slaughtered and consumed the meat [33, 125] and thus pigs would not get the second vaccination needed for full protection. If the vaccine could be modified so that it can be protective in a single dose this might be an excellent tool to combat porcine cysticercosis and, in turn, human taeniasis.

A control effort based solely on public education in Peru has seen an increase in the use of confinement pig husbandry systems, from 7 to 96 % in 42 months after initiation [85, 152]. In Mexico, education, in combination with vaccination programs, was reported. A pamphlet was delivered to a third of the target population, while one tenth of the population attended 219 oral presentations. 250 video copies were also delivered. The campaign reported to have increased the level of pig confinement from 36 % ($n = 220$) to 63 % ($n = 213$) within a period of 3 years, as well as increased the use of latrines by and the provision of potable water to the community [43].

Only 16 % of farmers confine pigs in Jayawijaya Papua, an area with the highest prevalence of cysticercosis [125]. Another study reported that properly confining pigs was the concern of only 1.7–4.3 % ($n = 228$) of farmers in that region [98]. Problems that may hamper a campaign aimed at confining pigs in Papua may be lack of resources to build a pig house and, in the long term, the ability to provide feed. A study in Jayawijaya, however reported that 48 % of farmers planted sweet potato with the purpose of feeding pigs. Moreover, when a project conducted by ACIAR (Australian Centre for International Agricultural Research) introduced feed processing technology that included ensiling, feed enrichment using fish protein, and heat treatment, 29 % of farmers would have adopted at least one the feed technologies introduced to them [33, 98]. Only 14 % of farmers, however, perceived that quality feed was important for pigs [98].

In Papua, where some tribes eat dog meat [71], eradicating human cysticercosis might be hampered in these communities. An assessment aimed at estimating the risk of acquiring pig and human cysticercosis and taeniasis, which is posed by consuming dogs is required.

Endoparasitosis

Prevalence

A study of endoparasite infections performed in the Jayawijaya Region of Papua, by faecal examination, found strongyles and *T. suis* were among important species detected. The prevalence of strongyle parasites in dead pigs was high at 70.5 % ($n = 44$), while in healthy, fully confined pigs it was much lower at 22.5 % ($n = 102$) [126]. Four strongyle parasites were identified in Papua; the stomach worm *Hyostrongylus rubidus* with a prevalence of 10 % ($n = 10$) [134], the small intestinal worm *Globocephalus urosubulatus* at 80 % ($n = 10$) [63], the colon worm *Oesophagostomum* spp. and the lung worm

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Metastrongylus spp. [33]; the prevalence of the latter two is unknown. *T. suis* was present in 55 % ($n = 44$) of dead pigs in Jayawijaya, while in healthy pigs raised in concrete floored confinement the prevalence was low, at 8 % ($n = 102$) [126]. For comparison, in Denpasar, Bali, the overall prevalence of *T. suis* in confined pigs was 33 % ($n = 300$), with the prevalence in pigs confined on bare earthed floors at 52.7 % ($n = 74$), while the prevalence in pigs raised in concrete floored pig housing was 26.1 % ($n = 226$) [172]. Anthelminthic usage in the farms was not described in these two papers. However, it has been mentioned above that only 12 % ($n = 366$) of Papuan farmers use modern medicine [125]. In Bali, it was indicated that commercial anthelmintics were too expensive for local village farmers or they might just have been reluctant to purchase them [8].

Impact on Pig Performance

The most commonly expected outcome of endoparasite burden in pigs is reduced weight gain [78]. However, *T. suis* infection was reported to be associated with severe and persistent diarrhoea, growth retardation, emaciation and/or anaemia in a significant number of gilts and in fattening pigs [34]. Further, Cargill et al. [33] reported that parasitism was among the most important pathogens to cause pig mortality in Papua.

Co-infections

In cases of mortality studied in Jayawijaya recently, parasitism was found to be co-existing with either other parasites or with other pathogens, whereas in healthy pigs single infection of different endoparasites occurred at 1–4 % prevalence for each parasite investigated [126]. This indicated a possible need for concurrent infection with endoparasites and other pathogens to trigger clinical consequences. Experimentally, *T. suis* was reported to exacerbate the frequency and severity of diarrhoea and the severity of pathology of *Campylobacter jejuni*, while single infections of either pathogen caused only mild symptoms [100].

In Papua, concurrent burdens involving the gastric endoparasites *H. rubidus*, *Gnathostoma hispidum*, *Physocephalus sexalatus*, and *Ascarops strongylina* have been identified [33, 134]. Further, in the pig's small intestine a few parasites such as *Strongyloides ransomi*, *Ascaris suum*, *Macracanthorhyncus hirudinaceus* and *G. urosubulatus* were identified [63]. Additionally, the lung worm *Metastrongylus* spp. has also been identified [33]. These findings imply that a more complex mixed parasite burden may occur in Papuan pigs. However, competition among parasites could also occur in the alimentary tract of the host and at some levels of infestation. Stunted adult parasites were observed that could limit the overall endoparasitic load on the host [7]. An example of a negative interaction has been between *T. suis* and *O. dentatum* with *T. suis* domination [135].

Transmission

Transmission of *T. suis* and the four strongyle parasites is through the ingestion of eggs or larvae and the source of eggs and infective larvae may be contaminated soil or feed [123]. *T. suis* has a pre-patent period of 6 weeks, *O. dentatum* of approximate 5–6 weeks and *H. rubidus* of 3 weeks [54]. Temperatures of 6–26 °C and moisture in the soil are needed for the eggs to hatch and grow into infective larvae [7]. These suitable conditions occur in tropical Papua all year round [23] and may facilitate the continuous survival of parasite eggs and larvae in the ground. Indeed, parasite loads were found to remain relatively high throughout the seasons in free scavenging pigs in Jayawijaya [3].

Pathology

H. rubidus is a gastric parasite and, after ingestion, larvae penetrate the epithelial folds of the gastric mucosa, grow in the submucosal layer and result in the destruction of the epithelium and the formation of lentil-sized nodules and ulcers; adult worms produce a chronic catarrhal gastritis leading to the formation of a diphtheritic membrane as well as ulceration [7]. *G. urosubulatus* is not highly pathogenic, with young pigs more likely to become anaemic than older pigs [197].

T. suis and *Oesphagostomum* spp. are endoparasites of the caecum and colon of pigs. *T. suis* larvae penetrate the epithelial lining and the crypts of Lieberkühn and return to the lumen when mature. Lesions of *Oesphagostomum* spp. in pigs are most obvious in the caecum and are first observed 48 h post-infection. *T. suis* and strongyles may cause the formation of nodules and ulcers in the caecum and mid-colon within a few days post-infection [7]. Anaemia resulting from infections with *T. suis* was observed [34]. Nodules of *Oesphagostomum* spp. may be easily recognised by farmers as this lesion is quite visible (Fig. 4).

Risk Factors

Housing may be an important husbandry practice that could assist in reducing parasitism in Papua. A 15-month prospective observational study in Jayawijaya showed the effectiveness of confinement in reducing the prevalence of endoparasites in pigs [3]. In this study, sharp declines in the

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prevalence of *T. suis*, strongyle parasites, *A. suum*, *Physocephalus* spp. and *Metastrongylus apri* occurred in confined pigs, whereas in the scavenging pigs the prevalence of all species increased. Another study supported this finding, showing that without anthelmintics, indoor housed pigs were likely to have lower burdens of *T. suis*, *A. suum* and *Oesophagostomum* spp. when compared to pigs with outdoor access [123]. Additionally, a lower burden of endoparasites in free range pigs was reported to be associated with the provision of night housing [75].

In pigs raised indoors with a higher level of hygiene, the level of infections of *T. suis* and *Oesophagostomum* spp. was negligible while *A. suum* remained but at a lower level compared to conventional indoor pigs [123]. A lack of bedding increased the risk of parasitism and the use of deep litter or slatted floors have been advised [35, 74].

The provision of low quality feeds was found to be significantly related to a high prevalence of *Oesophagostomum* spp. and *T. suis* [74]. In particular, feed rich in lignin and non-starch polysaccharides was shown to assist the establishment of *Oesophagostomum* spp. [136]. In this regard, cooking pig feeds as practiced by Papuan farmers [126] may be useful in increasing the digestibility of the feed and in reducing the chance of the establishment of parasite burdens. This could be incorporated into a program of parasite control in Papua.

Control Measures

It has been suggested that the overall pig mortality in Jayawijaya could be reduced from 48 to 10 %, by regularly treating with anthelminthic [33]. The effectiveness of the anthelmintic betel nut (*Areca catecu*) and papaw fruit (*Carica papaya*) has been examined. At a dose of 20 mg/50 kg body weight, a single dose of dried betel nut was capable of eradicating *T. suis*, *Strongyle* spp., *S. ransomi* and *A. suum* from the pig alimentary tract. With papaw, although it showed comparable efficacy, the high dose rate required of 1 kg/10 kg body weight makes this impractical for farmers [32]. However, another study reported that a single dose of 450 µmol cystein proteinase extracted from *C. papaya* provided good efficacy against *T. suis* infections in pigs [81].

Oxfendazole administered orally to naturally parasitised piglets at a single dose of 30 mg/kg was safe and highly efficacious against the adult stages of *A. suum*, *Oesophagostomum* spp., *T. suis* and *Metastrongylus* spp. [6, 113]. Experimentally, both Ivermectin and Abamectin administered orally for a period of seven consecutive days at a daily dosage of 100 µg/kg were highly effective against *H. rubidus*, *S. ransomi*, *A. suum* and *M. salmi* [90]. However, the need for prolonged treatment with these anthelmintics may constrain their use by Papuan farmers.

The timing of the administration of anthelmintics may be critical. It was recommended that farmers gave anthelmintic treatment to newly introduced pigs before being mixed with other pigs on a farm [35]. The rainy season might be a suitable time for antiparasitic treatment since the prevalence of nematodes was found to be positively correlated with the amount of rainfall [75]. In Jayawijaya, however, burdens of pig parasites in traditional scavenging systems remain high throughout the year and do not seem to follow the rainfall pattern [3]. Therefore, seasonality may not be relevant for anthelmintic treatment in Papua.

S. zooepidemicus and S. suis

Prevalence

S. zooepidemicus was isolated in 15 % of cases of pig mortality in Jayawijaya but tonsillar carriers in healthy pigs were not detected. In contrast, *S. suis* was isolated in only 2 % of cases of pig mortality in Jayawijaya but the tonsillar carrier rate in healthy pigs was 8 % [126]. Slipranata et al. [163] reported a 24 % cumulative prevalence of *S. suis* in a 15-month longitudinal study, while Salasia et al. [149] reported an 11 % seroprevalence of *S. suis* in a cross sectional survey in Timika using muramidase released protein monoclonal antibody dot blot.

Impact on Pig Performance

S. zooepidemicus caused a fatal outbreak resulting in significant economic losses and remains a threat to the Chinese swine industry [94]. Sporadic zoonotic infections have also been reported from contact with infected horses [182] but zoonotic infection from pigs may be underdiagnosed. *S. suis*, on other hand, is known to be an important disease in modern pig industries and was associated with a fatal zoonotic outbreak in China in 2005 [194]. In pigs, *S. suis* caused ongoing weekly mortalities of 10–20 % of weaners and retarded the growth of affected piglets [183]. However, a study reported that infection of *S. zooepidemicus* and *S. suis* in dead pigs in Jayawijaya was 15 and 2 %, respectively [126] implying that in economic terms, *S. zooepidemicus* could be more important while *S. suis* may not be a major problem for Papuan farmers.

Co-infections

In pigs, fatal infection with *S. zooepidemicus* alone was rare but co-infections with either endoparasites, PCV2, or both, were more common [126]. Co-infection with *S. zooepidemicus* and non-hemolytic *E. coli*, PCV2, or PRRSV was reported in an outbreak in pigs in Vietnam [105].

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Streptococcus suis occurs in co-infections with a broad range of pathogens including viruses such as PCV2, PRRS and SIV, and bacteria such as *M. hyopneumoniae*, *Pasteurella multocida* and *Haemophilus parasuis* [22]. The combination of PRRSV, PCV2 and *S. suis* was reported to be common in China [196]. Furthermore, co-infections among different serotypes of *S. suis* are common [39, 183]. It is interesting that a study of mixed infection suggested that *S. suis* serotype 9 partially suppressed the severity of infection with *S. suis* serotype 2 [132].

Transmission

Multiple species including pigs, monkeys, sheep, cows, goats, foxes, birds, rabbits, guinea pigs, dogs and horses potentially act as biological reservoirs for *S. zooepidemicus* [2, 148]. Information of other modes of *S. zooepidemicus* transmission is lacking.

Transmission of *S. suis* infection has been shown to occur effectively through direct nose to nose contact with diseased pigs [127]. Contaminated food has been suspected as a mechanical vector [99]. Airborne transmission of *S. suis* in confinement has been shown to be possible [17, 21]. In the Papua setting where pigs and humans live in close proximity [119], reverse transmission from humans to confined pigs might be possible, as human carriers of *S. suis* have been reported elsewhere [21].

Pathology

Clinically, *S. zooepidemicus* infection in pigs was reported to result in swelling of the joints, respiratory distress and diarrhoea, with most of the pigs dead within a few days. The post-mortem findings were polyarthritis, bronchopneumonia, pleuritis, epicarditis, endocarditis, and meningitis [148] and diffuse haemorrhagic pneumonia (Fig. 4).

Neurologic signs may be the most distinguishable in *S. suis* infection [61]. However, the most common clinical signs of *S. suis* infection were reported to be varying levels of coughing and sneezing, and ill thrift, with neurologic signs including lateral recumbency, paddling, ataxia and sudden death being less common [142]. The most common pathology identified during post-mortem was suppurative bronchopneumonia, usually secondary to enzootic pneumonia, and pleuropneumonia. Other gross lesions observed less commonly include valvular endocarditis, arthritis, vaginitis and abortion [1, 151].

Risk Factors

Factors contributing to the emergence of *S. zooepidemicus* in pigs are poorly understood. [126] reported the carriage of *S. zooepidemicus* in confined pigs to be negligible. This suggested that confinement might be preventative for *S. zooepidemicus* infection in pigs.

In contrast, studies of risk factors for *S. suis* infection are abundant. A study in Jayawijaya suggested that carriage of *S. suis* may be more constant in confined pigs than in scavenging pigs [163]. Full confinement of pigs is practiced by only 16 % of farmers in Jayawijaya [125] and this low proportion of confinement likely explains the very low prevalence of *S. suis* in cases of pig mortality. Factors in confinement that allow establishment of *S. suis* in Papuan pigs are unknown.

Accumulation of *S. suis* serotype 2 has been reported to be at a higher level over a long period in confined pigs suffering *S. suis* clinical cases compared to those pigs confined without *S. suis* cases [21]. Airborne transmission of *S. suis* in confinement was demonstrated [17], implying that closed buildings could play a role as a niche for aerosolisation of *S. suis*.

The role of effective ventilation has not been studied in infection with *S. suis*. However, one study suggested that maintaining effective air flow inside pig buildings could reduce respiratory infections [161], which comprised half of the manifestations of *S. suis* infections [1, 142, 151]. The majority of traditional pig farms in Papua, however, are fully closed without even a simple open sided window [125]. Social factors in Papua such as a high occurrence of theft might hamper implementation of ventilation in pig buildings.

Herd size may not be a risk for *S. suis* infection. Among herds within sizes of 14 head to thousands of pigs, the number of groups infected and the total morbidity in the herd were not significantly different [141, 183]. Likewise, pig density may not be consistent with the carrier rate of *S. suis*, for example in a German National Park where the pig density is very low, the carrier rate of *S. suis* of various serotypes was as high as 92 % [15]. This contrasts with Papuan data showing low level of *S. suis* infection in free ranging pigs with low stocking density [126, 163]. The reason for this discrepancy is unknown.

Serotype 2 has been recognised as a dominant cause of clinical *S. suis* infections both in pigs and humans worldwide [61]. Serotype 2 was dominant among strains recovered from diseased pigs in a Chinese study [186] but the carrier rate of serotype 2 in healthy domestic pigs in China was as low as 3 % [195]. In wild pig populations where the pig density is very low, serotype 2 carriage can vary from as much as 58 % in one place to nil in other places [15]. Other studies suggested that herd size influences the carrier rate of serotype 2 [118, 130]. These phenomena imply that the burden of specifically serotype 2 rather than any *S. suis* in general could better express the health status of a herd. The prevalence of *S. suis* type 2 in Papua, however, remains unknown.

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Some particular daily farm managements may play some role for prevention of *S. suis* infection. A retrospective study reported that mortality in nursery farms decreased from 20 to 3 % when a regimen of a more constant number of pigs weaned weekly was applied (reflecting less fluctuation in weekly stocking density). Increasing weaning age and weight, lowering pig density, controlling temperature fluctuation, and improvement of sanitation were not correlated with a reduction of pig mortality [183]. Another study suggested that mixing pigs from different litters after weaning increased the risk of *S. suis* serotype 2 infection [118].

Control Measures

Antibiotic use for *S. zooepidemicus* and *S. suis* has been intensively studied. However, susceptibility of Papuan *S. zooepidemicus* strains to antibiotics has not been studied. Reports of antibiotic susceptibility of pig *S. zooepidemicus* strains from other locations are also lacking. Ceftiofur, ticarcillin, trimethoprim-sulfamethoxazole, cephalexin, amoxicillin, ampicillin, penicillin, enrofloxacin and doxycycline, have all been reported to be efficacious against *S. zooepidemicus* in dogs and equines [29, 42, 93].

In contrast, antibiotic efficacies against *S. suis* isolated from pigs have been extensively evaluated. In Europe, susceptibility of *S. suis* to amoxicillin/clavulanic acid, ceftiofur, enrofloxacin, florfenicol and trimethoprim/sulfamethoxazole is reported for 91 % to 100 % of isolates [46]. Resistance was very high for tetracycline, lincomycin, tilmicosin, erythromycin and tylosin [30]. A similar pattern of resistance was reported in China, with the addition that the antibiotic resistance rate among *S. suis* isolates was very low, below 1 % for cefaclor and ceftriaxone, but high for azithromycin and clindamycin at around 67 % of isolates studied [37]. Intravenous benzylpenicillin and gentamicin followed by oral amoxicillin was successful for the treatment of human case of *S. suis* [175] but their benefit in controlling outbreaks in pig herds has not been apparent [67, 183].

Vaccines against *S. zooepidemicus* infection have been developed only recently. A recombinant vaccine based on the M-like protein was able to protect 70 % of mice [86]. A combined recombinant vaccine against *S. zooepidemicus* and PCV2, based on an M-like protein of *S. zooepidemicus* and a capsid protein of PCV2 was able to protect 87 % of immunised Bama mini-pigs from a dual challenge [87]. However, to date no commercial vaccine is available for *S. zooepidemicus* in pigs.

Vaccine development based on killed organisms (bacterin), subunit protein or live attenuated bacteria has been widely studied for *S. suis*, with varied results. Experimentally, vaccination with *S. suis* serotype 2 bacterins resulted in levels of protection against homologous challenge of 100 % protection from morbidity [189], to 49–71 % for reduced mortality [14, 129], depending on the strain used and the route of vaccination. Experimental vaccination of sows with *S. suis* type 2 bacterin resulted in passive immunity of their piglets, which protected 67 % from morbidity following a homologous challenge at 6 weeks of life [13]. Subunit vaccines using Murein Associated Protein only protected 12 % pigs from mortality after a homologous challenge [14]. Mice immunised with substrate binding protein (Sbp) of *S. suis* type 2 had a 70 % increase in survival from homologous challenge; detailed morbidity however, was not reported (Zhou et al. 2015). In a mice model, surface-anchored DNA-nuclease (SsnA) of *S. suis* serotype 2 adjuvanted in aluminium hydroxide protected 100 % against mortality after a homologous challenge, with only 33 % of mice suffering mild septicaemic signs (rough coat, moderately swollen eyes, or depression), which subsided after 2 days post-challenge [58].A mutant strain ΔSsPep/ΔSsPspC of *S. suis* serotype 2 was reported to protect 90 % of mice from mortality against lethal challenge. However, morbidity was not clearly reported [68].

While many experiments have been conducted for *S. suis* type 2 with some promising results, a serotype 9 bacterin completely failed to protect pigs against homologous challenge [48]. Also, serotype 2 bacterin was reported to only cross-protect 33 % of pigs against mortality following infection with serotype 9 [14]. The lack of cross protection was also reported from different strains within serotype 9 [28].

In nature, different strains of *S. suis* can be isolated from a herd or from an animal at the same time [39]. Furthermore, different serotypes can cause clinical disease consecutively, such as in a *S. suis* serotype 7 outbreak. Three months after the introduction of a homologous vaccine subsequent cases of mortality were caused by mixed serotype 4/8 infections [183]. This may hamper the use of *S. suis* vaccines against natural challenges as it may be unknown what level cross protection against different circulating serotypes these vaccines will be able to provide. Practically, effective vaccines against *S. suis* are currently lacking [160]. For Papuan pigs vaccination against *S. suis* may not be warranted since the contribution of *S. suis* to mortalities and its prevalence in healthy pigs was found to be very low [126].

Designing Locally Adapted Controls of Pig Diseases in Papua

Before possible control techniques are introduced to local farmers, it is important to bear in mind that many Papuan farmers have very little knowledge as to what should be

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done to reduce the incidence of disease or even what diseases are present or absent from their farms [125]. Therefore, the involvement of the local veterinary authority is vital in introducing farmers to suitable methods of biosecurity, such as a basic knowledge of good farming practices, disease recognition, vaccine application and anthelmintic treatment. Control programs may be introduced firstly to regions with high pig density and regions with available veterinary staff. When a program has achieved observable success in those regions, it may be disseminated to the remaining areas.

In all control approaches, the confinement of pigs should be a prerequisite and accompany any disease control program. Confinement has been shown to reduce the risk of parasitism, CSF, infection with *S. zooepidemicus* as well as pig cysticercosis in Papua [3, 9, 126]. Furthermore, without proper confinement, other interventions such as vaccination may be more difficult to assess; pigs may have an increased chance to be infected with other pathogens just after the vaccine administration and the owner could believe that the sickness or death which occurs post-immunisation is due to vaccination. Confinement was reported to increase the risk of PCV2 infection [181] and *S. suis* infection elsewhere [17]. However, the confinement prevented pigs from being exposed to co-factors of PCV2 infection such as CSFv and *Metastrongylus elongates*, which are necessary for PVCD to emerge [103, 126]. In the case of the small scale pig farms in Papua, the positive effects of confinement for pig health maybe outweigh its potentially negative impacts.

In addition to the pig confinement campaign, farms can be expected to be an open population where new pigs move in and out. Therefore, any new incoming pig may need to be quarantined and monitored for signs of sickness and for prior treatments such as vaccination and anthelmintic treatment before being allowed to be mixed with other animals in the farm. Further, applied feed processing technologies should be implemented on pig farms to improve feed quality in confinement pig farms to reduce the problem of feed provision in confined pig farming systems.

Control of classical swine fever should be the first priority after confinement is initiated. Classical swine fever is clearly the most economically important pig disease in Papua currently. The history of big outbreaks in three regions in Papua shows how devastating this pathogen is while other diseases are presenting more subclinically or with lower prevalence. Vaccination against CSFv would be an important option, potentially helping to improve overall pig performance in Papua. Vaccination of sows a week before insemination, using an injectable preparation and their piglets at 5 weeks of age may be conducted year round to achieve complete protection of a farm.

Vaccinations have been conducted in regions such as Timika and Jayapura. While it seemed to have become routine in the city, its delivery in the rural areas apparently faces logistical problems that affect proper field transport of the vaccines during mass administration, where vaccines sometimes were seen to be kept in a box with warm temperature. The temperature of vaccine during storage and transport is a critical factor in vaccine quality maintenance. However, recent studies indicated that suboptimal temperature of vaccine during storage and field transports were likely a problem in hot climates of tropical areas. In India, at all storage and transport levels, up to 18 % of the total time vaccines were kept in suboptimal condition, either subzero or above 8 °C, with up to 88 % of total boxes observed affected. Temperatures above 8 °C were more common [122]. Another study in New Guinea reported suboptimal vaccine temperature with freezing as a more common finding [188]. In Papua, even in the city area, there has not been an evaluation of the cold chain for the CSF vaccine.

Annual surveillance needs to be undertaken in monitoring the effectiveness of CSF vaccination. In this regard, the Disease Investigation Centre (DIC) Maros, the central laboratory at Sulawesi which covers animal disease control in Papua, should prioritise regions in Papua with high pig density in their survey and share the result of a survey promptly with local veterinary authorities so as action plan for the control strategies in the coming year can be based on the most updated evidence. Furthermore, updated scientific information on pig diseases, diagnoses and control methods should be disseminated by the central laboratory to local veterinarians regularly. Prioritisation of particular regions for disease control is important for DIC Maros, as this diagnostic laboratory covers a large area of 10 provinces in Eastern Indonesia.

It is difficult to assess the subclinical infection status of a pig prior to CSF vaccination and a clinical sickness could occur post-vaccination. While confinement reduces the risk of exposure of animals to pathogens post-vaccination, there is still a risk of vaccine failure or post-vaccinal adverse effects due to a subclinical infection prior to vaccination. Informed consent prior to vaccination and good communication between the veterinary authority and farmers is crucial, especially when a case of post-vaccinal adverse effects occur.

Eradication of CSFv through a test and cull strategy is currently impossible in Papua for several reasons: CSFv is currently widespread in the province and might have crossed provincial borders; there are currently no regulations in place to control transport of pigs or to isolate an endemic location from other areas; the population of wild pigs and their distribution is unknown; seroprevalence is greater than 50 % making the cost of culling prohibitive; the majority of domesticated pigs scavenge freely, facilitating transmission among pigs through direct contact;

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currently there is not available a simple rapid diagnostic tool; the capability of local government to pay and manage compensation is also doubtful given that even less expensive program; vaccination, have not been run properly and the willingness of farmers to cooperate in letting their pigs be slaughtered is unknown.

A series of studies to answer abovementioned gaps in knowledge are needed before we can construct a pathway towards an effective test and cull policy to eradicate CSFv from Papua. Vaccination, therefore, is currently a more reasonable approach than test and cull, as the cost per animal is much lower and vaccination provides insurance from economic loss.

Culling may be still important for CSF-PI pigs which continuously shed virus. A PI-culling program should include educating farmers on the clinical signs of suspected PI pigs which are usually stunted, and the role of PI pigs in CSF transmission. Voluntary culling by knowledgeable farmers should be prioritised, rather than implementing a government-subsidised forced test and cull.

AQ1 Even if pigs are confined and CSF is controlled, parasitism would still be a problem, especially species with a direct life cycle such as *T. suis* and strongyle parasites. It has been documented that the prevalence of *T. suis* and strongyle parasites in fully confined pigs were 8 and 22.5 %, respectively [126]. Anthelmintic treatment is still needed, especially for newly introduced pigs during the quarantine period. In addition, it was recommended to treat sows and gilts at breeding and just before farrows and their progeny twice during the weaning and fattening period (Roepstorff and Nansen 1998). This regime, however, may be too much for most Papuan farmers who are not well educated and may have limited access to veterinary services. A simpler anthelminthic regimen needs to be set up following a governmental program of monitoring of the level of parasitism in confined pig systems. The frequency of monitoring can be adjusted to the available budget but an initial arbitrary frequency of twice a year can be used. Veterinary preparations of Oxfendazole should be made available for farmers when needed.

For bacterial infections, as farm size is small, the use of antibiotics to treat sporadic cases may be useful after a diagnosis of *S. zooepidemicus* or *S. suis* has been made by a veterinarian [77, 175]. Suitable antibiotics for both pathogens, such as Amoxicillin/clavulanic acid, enrofloxacin, or trimethoprim/sulfamethoxazole may be used [29, 42, 46, 93].

Education, although there have been ongoing debate on its effectiveness in pig disease control such as that in cysticercosis control [85], was indicated to increase the rate of pig confinement in Peru and Mexico [43, 152]. A training program was also reported to be success in disseminating feed processing technologies in Jayawijaya [98]. Therefore, education on good farming practice should accompany any other control methods. It is always important to build a good relationship with elders of a community in rural area before a program started. Once a program is approved by the elders, it may be intensified by developing a systematic pig farming educational program that builds proper understanding by local farmers on what they should do to reduce pig disease and mortality.

Continuous education programs for farmers should at least include emphasising the benefits of confinement and applied feed processing technologies such as heat treatment and ensiling. Other subjects include increasing the frequency of feeding, regular cleaning of pig pens, anthelmintic treatments, CSF vaccination, the identification of clinical signs of various diseases, recognition of gross pathology and the reporting mortality cases. The culling of suspected CSF PI piglets may also be an important subject to address. An evaluation is needed on how to improve the quality of training methods in times to come.

Knowledge of the ecology and epidemiology of pig diseases in Papua is still largely lacking. Future studies should be aimed at field trials and evaluation of the proposed methods of disease control in Papua. Suitable programs to educate farmers on good pig husbandry as a prerequisite for other control measures warrants development. Further study is needed to adjust the timing of vaccination using oral preparations, as protection will only be complete after 28 days post-vaccination [116]. This knowledge is important as the implementation of oral vaccination will minimise the need to train vaccinators. The impact of subclinical PCV2 infection on weight gain and reproductive performance of confined pigs needs further investigation. At the regional level, investigation is needed of the role of pig products imported into Papua in transmission of CSF and PCV2. Further, information on pig movements among the regions of Papua would assist in determining priority regions for future surveillance programs for CSF and PCV2. In addition, the epidemiology of pig diseases identified as of national priority in Papua, such as PRRS, H1N1 influenza and toxoplasmosis warrants further study.

Conclusions

Pig production is important for traditional communities in Papua, but high levels of pig disease and mortality constrain production and may also impact human health. A few major diseases and pathogens have been identified, namely CSF, cysticercosis, PCV2 infection, parasitism from *T. suis*, strongyle parasites and *S. zooepidemicus*. CSF, cysticercosis and helminthiasis are the high priority diseases to control. Using the three elements of

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confinement, CSF vaccination and regular anthelmintic treatment would reduce natural infections. PCV2 infections, however, may remain as high as 30 %. Future studies should be aimed at a field trial of the proposed methods of disease control in Papua, an improved understanding of the effect of PCV2 infection in confined pigs as well as an understanding of the effects of the movement of pig products into and amongst the regions in Papua.

Acknowledgments This manuscript is part of a PhD study of the first author, funded by a John Allwright Fellowship/Australian Centre for International Agricultural Research. We thank Dr. Margaret Cargill for corrections during the preparation of this manuscript.

Compliance with Ethical Standards

Conflict of interest None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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9. Discussion and Conclusion

Pigs are an important livestock species for the livelihood of rural communities in South East Asia and the Pacific. In the Papua, Indonesia, pigs have been contributing to local communities in multiple ways, such as for daily consumption, a gift to relatives, economic commodity and offering in cultural events. Despite this importance attached to them, the productivity of Papuan piggeries has been very low, with disease and mortality recognised as major constraints. The aim of the thesis was to study the characteristics of local pig farming, the diagnosis, epidemiology and control options for the major pig pathogens observed on traditional pig farms in Papua.

The study was conducted in four stages. The first study presented in this thesis described the demographics of the pig farming industry in Papua (Chapter 5). It suggested that farm size in Papua was small comprised of less than ten pigs with majority applied scavenging systems. Intensive veterinary services were used by just 12% of farmers and majority farmers would just consume or sell pigs when the animals were sick or naturally dead. Under the existing farming systems, Papuan pig farms was shown to remain in low productivity indicated by high mortality, low litter size and low farrowing frequency. These figures in Papua were similar to these in average Asian village pig farms.

The second stage of study further explored the prevalence of selected pathogens previously believed to be major cause of pig diseases in Jayawijaya region of the province (Chapter 6). Current study suggested that CSF, *Trichuris suis*, strongyle

parasites and PCV2 were among most prevalent pathogens, with respective levels of more than 50% in dead pigs. Classical swine fever may be the most important pathogen for the pig industry in Papua presently. Apart from the high prevalence of CSF in dead and healthy pigs, the presence of persistently infected (PI) animals was also indicated. These PI pigs may have played an important role in CSF infection endemicity in Papuan pigs.

It was apparent that prevalence of pathogens observed was higher in dead pigs compared to those in healthy pigs. The two groups of dead and healthy pigs were in the same proportion of sex. However the groups were not equivalent in terms of age, area of sample collection and the type of farming system. Pigs in the healthy group were fully confined, originated from one district and majority were at 3 to 6 month of age. In the group of dead pigs, the farming system was unknown, pigs were from eight subdistricts and majority were older than 6 month of age.

Wamena subdistrict, where samples of healthy pigs were taken, is the central of pig trade in the region. Therefore it likely acts as the reservoir area of pathogens across the region so that the prevalence of pig pathogens in the subdistrict could be expected to be high. The low level of pathogens in heathy pigs in Wamena revealed in the study and the high level of those in dead pigs therefore might indicate that pathogens investigated were attributable to mortality. Age however, could confound the result. Moreover, older and bigger dead pigs tended to be delivered to market for sale than smaller pigs, thus could not represent the true age structure of pig mortality in the region. Farming system might confound the result as confined pigs could get a lesser chance of exposure to pathogens. These selection

biases limited the power of the study to demonstrate the association between the pathogens in pig mortality in Papua.

In the third stage of the study, the presence of PCV2 infection and its involvement in Papuan pig diseases were indicated for the first time in Papua (Chapter 7). Full genome analysis demonstrated that one marked characteristic of some Papuan PCV2 isolates was that they belong to the PCV2 IM3 genotype; a genotype different from the major PCV2a or PCV2b groups causing PMWS in the modern pig industry. As the prevalence of PCV2 in Papuan pigs is high, studies in chapter 8 highlighted the importance of further investigation of the epidemiology and control of the PCV2 IM3 genotype in Papuan pigs.

Chapter 8 is the review of the available information, including our own studies, of the ecology and epidemiology of selected pig diseases in Papua, and proposes locally adapted control measures of the major Papuan pig diseases. It is indicated in the review that the role of local government may be pivotal in the development of a pig disease control program, as many farmers had very little understanding of the role of relevant pathogens in pig diseases and their control. Pig confinement was proposed to be a prerequisite before any other control methods be introduced. Vaccination against CSF and anthelminthic administrations were suggested to be two priorities and practical control measures to be disseminated in confined pigs across Papua, especially in regions where veterinary offices are present.

Moreover, the review indicated that knowledgeable farmers were a key to the success of further disease controls, therefore it was anticipated in the review that

methods to educate farmers on good farming practice need a development in the future. It was further suggested that future studies of the control of pig diseases in Papua should be aim at assessing suitable approaches to disseminate the use of CSF vaccine across Papua Province, adjustment of the timing of the use of oral vaccine and the comparative field trial of oral versus injectable vaccination. The other factors speculated in the review to contribute to the pig disease persistence in Papua thus require further study were the role of imported pig products in pig disease transmission into Papua and the pattern of pig movements across Papua. Overall, an action research may be needed to assess the effectivity of this proposal and identify other problems in Papuan pig diseases which have not been captured in current study.

In conclusion, this thesis highlights that pig production in Papua remains of low productivity, with disease and mortality being among the main concerns. Pathogens of CSF, PCV2 and internal parasites are among the major causes of diseases that should be controlled in Papuan pigs. The role of local government is pivotal in introducing control methods for pig diseases and in educating farmers of good farming practice. While control methods are available for CSF and helminthiasis in Papuan pigs, further studies are needed to better understand the epidemiology of PCV2 in Papuan pigs.

10. Appendix: Supporting Publication – Conference Paper

W. Nugroho, C. F. Cargill, I. M. Putra, R. N. Kirkwood, D. J. Trott, S. I. O. Salasia and M. P. Reichel (2014) Pig Production and Food Safety in Papua, Indonesia. Epidemiology Chapter, Australian and New Zealand College of Veterinary Scientists Science Week Scientific Conference, Gold Coast, July 10-12, 2014

Traditional Pig Production and Food safety in the Jayawijaya Region, Papua Province, Indonesia

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The purpose of current survey was to provide an update on the animal husbandry practices of traditional pig farms in the Jayawijaya region, Papua Province, Indonesia. A structured semi close-ended questionnaire was used to interview 367 farmers across the Jayawijaya region.

Results showed that farms, on average comprised of 8.8 pigs (CI: 8.5-9.1). Mean litter size was 6.0 (CI: 5.7-6.3), the farrowing frequency was once a year and the annual mortality rate 50.2% (CI: 48.4-51.9). As many as 43.4% farms (CI: 36.4-50.7) allowed pigs to roam freely during daylight. In general, farmers used pigs for their own consumption (62.4%, CI: 57.4-67.4) or to present as a gift (56.6%, CI: 51.5-61.7), as well as for sale (50.7%, CI: 45.6-55.8).

Veterinary service was used intensively by just 11.7% of farmers (CI: 8.2-16.5). As many as 34.2% (CI: 29.3-39) of farmers would sell or slaughter and consume sick pigs (34.2%, CI: 29.3-39 and 63.1%, CI: 58.2-68.1). It was also observed that 68.6% of farmers (CI: 63.7-73.4) would eat sick pigs that had died naturally.

These findings suggest that traditional pig farms in Jayawijaya are of low productivity. Moreover, the free roaming, the selling and the consumption of sick pigs have the potential to allow pathogens and zoonoses to circulate in the pig and human populations.