PORCINE CIRCOVIRUS-ASSOCIATED DISEASE (PCVAD) INVESTIGATION IN GOVERNMENT PIG BREEDING FARMS

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ABSTRACT: Porcine circovirus type 2 (PCV2) belonging to genus Circovirus is the primary causative agent of several syndromes collectively known as porcine circovirus-associated disease (PCVAD). An investigative study was conducted on the prevalence of PCVAD following a report of various degrees of morbidity and mortality in targeted government pig farms (NNPBC, Yusipang, NPBC, Wangchutaba & RPPBC, Lingmethang). Screening of sera samples conducted during September 2021 detected seroprevalence of PCV2 of 28/29 (96.5%) at RPPBC Lingmethang, 11/11(100%) at NNPBC Yusipang and 5/5 (100%) at NPBC Wangchutaba. From the same serum samples screened also detected seroprevalence of PRRSV as 7/29(24.1%) at RPBC Lingmethang, 1/11(9%) at NNPBC Yusipang indicating co-infection. However, from the clinical samples of NNPBC, Yusipang 5/10 (50%) tested positive to PCV2 through rtPCR during September 2021, whereas from the clinical samples of outbreak at RRPBC, Lingmethang during September 2022, 11/11(100%) sera tested positive in ELISA to PCV2 antibodies; 11/11(100%) of vaginal swab samples and 5/5(100%) of organ samples tested positive to PCV2 in PCR. All the clinical samples from RRPBC, Lingmethang tested positive to PCV2. As the prevalence of disease is quite evident, a strict biosecurity control measures at the farms should be implemented to stop further spread of the virus. Besides, vaccination of pigs like in other countries may have to be explored and adopted in these government farms if found feasible. Furthermore, a detailed study needs to be conducted in all the government and private pig farms with larger sample size to clearly understand the real status of the disease in the country for devising an appropriate intervention.

Keywords: Porcine circovirus; reproductive; respiratory syndrome virus

1. INTRODUCTION

Porcine circovirus-associated disease (PCVAD) is considered as one of the most economically important emerging disease on the swine industries. Porcine circoviruses (PCV) are considered as one of the most important pathogens for swine industry worldwide (Segales 2005 & 2012) and it belongs to the genus *Circovirus* in the *Circoviridae* family. Porcine circovirus type 2 (PCV2) was reported to be the primary causative agent. The most significant manifestation of PCVAD reported was post-weaning multisystemic wasting syndrome (PMWS) (Savic et al. 2012; Segales et al. 2019), and the clinical manifestation reported in the past were wasting, dyspnoea, enlarged lymph nodes, paleness of skin and diarrhea (Allan and Ellis 2000). As high as 73% PCV2 was detected against the seroprevalence at the government pig farms at Thimphu, Mongar and Sarpang districts (Monger et al 2014) from 2011 to 2012.

Various forms of clinical signs and even mortalities were observed in the pigs at the National Nucleus Pig Breeding Centre

(NNPBC), Yusipang, Thimphu. During the investigation, the animals were observed with clinical manifestations ranging from weakness, skin lesions, lameness, bluish ear, reproductive disorder and anorexia. Two of the animals were detected with antibody against PCV2. This led to further investigation and screening of the animals against the PCV2 in other farms too. In September 2022, sickness and deaths of pigs of various age group was also reported from RRPBC, Lingmethang. Thus, it was imperative to investigate in depth to better understand the prevalence of the disease in the country. Therefore, the main objective of the study was to confirm the cause of pig morbidity and mortality reported from these two farms and devise an appropriate intervention to overcome the situation.

2. MATERIALS & METHOD

2.1 Sample site and collection

The study was conducted at the three Government Pig breeding farms, viz. Regional Pig & Poultry Breeding Centre (RPPBC) in Lingmethang, National Nucleus Pig Breeding Centre (NNPBC), Yusipang and Native Pig Breeding Centre (NPBC), Wangchutaba. The NNPBC, Yusipang established in 2016 reared Landrace and Yorkshire imported from Thailand, and maintains the Great Grand Parents (GGP), Grand Parents (GP) and Parent Stock (PS). RPPBC rears exotic color breeds such as Hampshire and Duroc. NPBC rears native pigs.

Sera samples, oropharyngeal swabs and vagina swabs collected from the clinical cases from the farms were screened at the National Centre for Animal Health (NCAH), Thimphu in November 2021.

In total, 45 sera samples, 15 oropharyngeal swab samples from clinical case; and 11 vaginal swabs were collected and tested. Further, following a report of mortality at the Regional Pig Breeding Centre, Lingmethang, Mongar, additional samples were collected and tested from the clinically affected pigs during September 2022.

2.2 Enzyme-linked immunosorbent assay (ELISA)

Sera samples were screened for PCV2 antibodies using ELISA kit (Elab Science, US, Catalogue No: E-AD-E003). The ELISA Microtiter plate pre-coated with recombinant cap protein of porcine circovirus 2 (PCV2), HRP conjugate and other auxiliary reagents applying the principle of enzyme-linked immunoassay (ELISA) to detect porcine circovirus 2 antibody of porcine serum. The test was performed as per the protocol provided by the manufacturer. The absorbance value of each well was measured using a Micro-plate Reader (Heales MB-530) with 450 nm wavelength for the PCV2 antibodies in the samples.

Serum samples were also screened for antibody against porcine reproductive & respiratory syndrome virus (PRRSV) protein using ELISA kit (Elab Science, US, Catalogue No: E-AD-E006). The ELISA Microtiter plate pre-coated with recombinant protein of PRRSV, HRP conjugate and other auxiliary reagents applying the principle of enzyme-linked immunoassay (ELISA) to detect PRRSV antibody of porcine serum. The test was performed as per the protocol provided by the manufacturer. The absorbance value of each well was measured using a Micro-plate Reader (Heales MB-530) with 450 nm wavelength for the PRRSV antibodies in the samples.

2.3 Polymerase chain reaction (PCR)

DNA extraction was done from the oropharyngeal swab samples using Qiagen DNA Mini Kit extraction (Qiagen, Hilden, Germany) and real time PCR were carried out on a QuantStudio 5 thermocycler (Applied Biosystems) with a final volume of 25 µL against PCV2 and porcine reproductive and respiratory syndrome virus

(PRRSV) The PCR kit from applied biosystem (catalogue number: 4387391), primers and probe as described by Zhao et al. 2010 were used for PCV2 reaction with the cycling condition as 95 °C for 10 min and 45 cycles of 95°C for 15 s and 60 °C for 40 s. RNA extraction were also used done from the samples using Qiagen RNA Mini Kit extraction (Qiagen, Hilden, Germany) and real-time (TaqMan) reverse transcriptase (RT)–PCR assays were carried out as described by Kleiboeker for PRRS North American type (NA) and PRRS European type (EU) (Kleiboeker et al. 2005).

2.4 Statistical analysis

Prevalence of each disease was estimated for each disease using simple calculation in Microsoft excel using, number of samples tested positive by number of samples tested.

3. RESULTS & DISCUSSION

Antibody against PCV2 were detected in the proportion of 28/29 (96.5%) pig sera samples from RPPBC Lingmethang, 11/11(100%) samples from NNPBC Yusipang and 5/5 (100%) samples from NPBC Wangchutaba (Table 1).

Table 1: Details of screening of serum samples for PCV2 & PRRS by ELISA

Farm	Serum (n)	+ve to PCV2	+ve to PRRS
RPPBC Lingmethang	29	28/29(96.5%)	7/29(24.1%)
NNPBC Yusipang	11	11/11(100%)	1/11(9%)
NPBC Wangchutaba	5	5/5(100%)	0
Over all	45	44/45(97.7%)	17.7%

The overall seroprevalence of PCV2 appeared very high 44/45(97.7%) in all the three farms ranging from 96.5% to 100%. Similarly, in the earlier study a prevalence of PCV2 as high as 90% was reported from the government pig farms in Thimphu, Mongar and Sarpang districts (Monger 2014). A high prevalence of PCV2 of 85% was reported from the Cambodian farms (Tornimbene et al. 2015) too. A high seropositivity could be due to actual exposure of animals to virus and also assumed to be due to persistence of the antibodies in the host for variable period of time even after elimination of pathogen (Metcalf et al. 2016).

The same sera samples screened for antibodies against PRRSV indicated seropositivity of 7/29 (24.1%) at RPBC, Lingmethang, 1/11(9%) at NNPBC, Yusipang and no antibody was detected at Wangchutaba farm (Table 1). Seroprevalence of 7.6% to 12.2% PRRS were reported from farmed pig population from Hong Kong Special Administrative Region (Flay et al. 2022).

From the oropharyngeal swab samples of clinical cases at NNPBC, Yusipang, 5/10 (50%) were positive to PCV2 in molecular test (Table 2) whereas, from the clinical samples of RRPBC, Lingmethang, 11/11(100%) sera tested positive in ELISA to PCV2 antibodies; 11/11(100%) of vaginal swab samples and 5/5(100%) of organ samples tested positive to PCV2 in PCR (Table 3). In earlier genetic study of virus in pigs in Bhutan, the PCV2 was detected in as high as 47% (16/34) from the tissue samples (Monger 2017). Detection of as high as 28.2% was also reported from pigs in the North-eastern India through PCR (Bhattacharjee et al. 2021).

There is possibility of getting the infection in the farm from the source through the importation of these pigs. Further, some of the farm area like NNPBC Yusipang is also surrounded by orchards and the forest hence,

Table 2: Molecular test for oropharyngeal swab of NNPBC Yusipang

Diseases	Nos. tested(n)	Nos. positive	Percentage
PCV2	10	5	50%
PRRS	10	0	0

probability of getting infection from the wild boar is also quite high. Detection of PCV2 in the wild boar population serving as the reservoir for infection for the nearby pig farms have been reported in other countries like Romania, Poland and Serbia (Turcitu et al. 2011; Fabisiak et al. 2012; Nisavic et al. 2022). However, in this case, it could be confirmed only after testing the wild boars for PCV2 in the surrounding forest.

Table 3: Clinical samples tested for PCV2 and PRRS from RRPBC, Lingmethang

Sample type	Nos.	Test Type		
		ELISA PCV2	rtPCR PCV2	rtPCR PRRS
Blood (serum)	11	11 +ve		
Vaginal swab	11		11 +ve	All -ve
Oropharyngeal swab	5		5 +ve	All -ve

The PCVAD is due to multifactorial causes and occurs as co-infection of PCV2 with other pathogens and immune inflection of host (Tsai et al 2019). Co-infection with other viral and bacterial pathogens increase the incidence of the disease and also more severe clinical form; among the viral, PRRSV, porcine parvo virus (PPV) and *Mycoplasma hyopneumonae* were observed. However, at the NNPBC Yusipang, the co-infection with PRRSV and *Mycoplasma hyopneumonae* was not detected in earlier investigation during 2021 (Thapa et al. 2021).

Co-infection of the farmed pig population with PRRS and PCV2 was reported earlier at the Hong Kong Special Administrative Region (Flay et al. 2022). Due to lack of kit for *Mycoplasma hyopneumonae*, the samples could not be screened against the infection.

During the investigation, the animals were observed with clinical manifestations ranging from weakness, bluish ear, dermatic lesions, lameness, reproductive disorder and anorexia. According to the American Association of Swine Veterinarians (AASV), PCVAD can be subclinical or include one or more clinical manifestations including multisystemic disease with weight loss and high mortality, respiratory disease, porcine dermatologic and nephropathy syndrome, enteric signs including diarrhea, and reproductive disorders on an individual or herd basis (AASV 2017). In this case, lameness and bluish colour of ear tip could be due to other conditions.

Virus transmission occurs in several ways and the most common is the oro-nasal contact through contaminated feces (Seagles et al. 2005). The virus is shed through respiratory secretions, oral secretions, urine and feces in both with clinical signs as well as sub clinically affected pigs. Being viral infection, there is no specific treatment however, symptomatic treatment is usually recommended. Prevention of PCV2 is often difficult and the infection have been detected even in the well managed farms with strict isolation practices (Gillespie et al.2009). However, the trivalent vaccine containing PCV2a/b and *M. hyopneumoniae* evaluated in the field trials elicited protective immunity against PCV2d and *M. hyopneumoniae* (Um et al. 2021). Routine disinfection and also disinfection before addition of every batch of pigs in the herd and regular monitoring needs to be carried out in the farm.

4. CONCLUSION & RECOMMENDATIONS

The seropositivity of PCV2 was detected in the target government pig farms. It indicates that either the animals were exposed at the country of origin or might have been exposed in the farm through transfer of animal attendants or other sources like feed trucks or other materials like feeder and waterers. A further study could be conducted in all the government farms, and private farms where feasible, to better understand the status of the disease in the country. PCV2 a primary agent for PCVAD has economic impact in the pig farms and many of the syndromes are associated with other infectious agents like *Mycoplasma* and Porcine Reproductive and Respiratory

Syndrome (PRRS). Seropositivity of PRRS was detected in two government pig farms in Bhutan. The study concludes that a strict biosecurity measure must be put in place at the farms to stop further spread of the virus. Besides, vaccination of pigs against PCV2 must be explored and adopted in these government farms. Additionally, there is need to conduct a detailed study in both government and private pig farms with larger sample size to draw inferences on real disease status, its implications in the piggery sector and formulate an appropriate intervention.

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