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Seroprevalence of PCV2 in north eastern hill states of India

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ABSTRACT

This study describe the seroprevalence of porcine circovirus type 2 (PCV2) in north eastern hill (NEH) states of India. Information on PCV2 from NEH states is lacking. Serum samples (306) were collected from the pigs and different epidemiological parameters like age, sex, and litter size of drift or sounder, system of rearing and different clinical symptoms from November 2017 to May 2018. Samples were screened for PCV2 infection by commercially available ELISA kit (INGEZIM PCV IgG and INGEZIM PCV IgM, Ingenasa, Madrid, Spain). Out of 306 serum samples tested, 151 samples were positive for PCV2 infection. An overall PCV2 prevalence of 49.35% was recorded in NEH states of India. Highest prevalence was found in Sikkim (94%) and lowest prevalence in Nagaland (4%). This study revealed that PCV2 is prevalent in all the north eastern states of India which necessitates the need for implementation of prevention and control measures.

Key words: ELISA, NEH, PCV2, Pig, Seroprevalence

The pig population in India is 8.8 million, which is 1.09% of the world's pig population of 967.38 million (FAOSTAT 2017). Pig population contributed by the north eastern hill (NEH) states are Manipur (2.69%), Mizoram (2.38%), Meghalaya (5.27%), Nagaland (4.89%), Tripura (3.52%) and Sikkim (0.29%) (19th Livestock Census 2012). The smallholder pig farming system is always facing challenges and thus offering ample opportunities for its improvement for better livelihood (Haldar *et al*. 2017).

Porcine circovirus disease (PCVD) is one of the most significant diseases that affect industrial pig production worldwide (Allan and Ellis 2000). Porcine circovirus type 1 (PCV1) is non-pathogenic whereas porcine circovirus type 2 (PCV2) is pathogenic in nature. PCV1 and PCV2 belong to the *Circoviridae* family and *Circovirus* genus. The members of *Circoviridae* family are small (17 nm), non-enveloped, icosahedron shaped viruses with a single-stranded covalently closed circular negative stranded deoxyribonucleic acid (DNA) molecule. Pigs are susceptible to PCV2 infection which leads to morbidity and mortality resulting in considerable economic losses to pig industry. A PCV2 prevalence study in NEH states is lacking. In India, only scanty studies are being conducted to track the epidemiological pattern of PCV2. Hence, an epidemiological investigation is necessary. The aim of this study was to investigate the seroprevalence of PCV2 in all NEH states and to understand the epidemiology of PCV2 infection which will ultimately help in devising control strategy against the disease. The NEH states are sharing boarders with Tibet, China, Bhutan, Myanmar, Bangladesh and Nepal. Thus, prevalence studies of major viral diseases of swine in these states are important.

MATERIALS AND METHODS

Population screened: The study was conducted in Mizoram, Manipur, Meghalaya, Nagaland, Tripura, Arunachal Pradesh and Sikkim which are having sub-tropical climate and the study period was from November 2017 to May 2018. Blood samples without an anticoagulant were randomly collected from a representative subset of the pig population and data were categorized under sex, age, size of drift or sounder, management system followed. Blood samples were transported to laboratory and serum was separated by centrifuging at 2,500 rpm for 5 min and kept at -20ºC until use.

Laboratory testing of the samples: All the serum samples were screened for PCV2 infection by using commercially

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available ELISA kit (INGEZIM PCV IgG and INGEZIM PCV IgM, Ingenasa, Madrid, Spain) as per manufacturer's instruction. Results were recorded as IgM positive or IgG positive or both IgM and IgG positive.

Seroprevalence study: Seroprevalence under different category were calculated using the formula:

$$\text{Prevalence (P)} = \frac{\text{No. of animals found positive for either IgM or IgG or both IgM and IgG}}{\text{Total number of animals tested in that category}} \times 100$$

Statistical analysis: Statistical analysis was done using Statistical Package for the Social Sciences (SPSS version 20, 2011). Different groups were analysed using Chi-square test (χ^2 test). Recorded Chi square statistic and significance noted at 5% level.

RESULTS AND DISCUSSION

The present study revealed that seroprevalence of PCV2 in NEH states was 49.35%. Previous studies reported the prevalence of PCV2 in some of the NEH states. Kikon *et al*. (2017) had reported 51.57% seroprevalence of PCV2 in Nagaland from 223 samples tested whereas Mukherjee *et al*. (2018) had reported 96.2% seroprevalence of PCV2 in Meghalaya from 1,899 samples tested. The high prevalence rate of PCV2 may be due to the stable nature of virus that can be shed through oro-nasal secretions, faeces, urine, colostrum, milk and semen (Patterson *et al*. 2011). Disease outcome is related to immunological status, stressors, co-infection and virus load (Duy *et al*. 2015).

State-wise distribution: Among individual states screened, the highest seroprevalence (94%) was recorded in Sikkim from 50 samples tested and the lowest seroprevalence (4%) was recorded in Nagaland (Table 1) from 25 samples tested. In Sikkim, the unhygienic conditions of the building predispose pigs to diseases (Nath *et al*. 2013). Further, they had reported that the main diseases in that area included swine fever, diarrhoea, worm infections, respiratory problems, and mange, all of which have been reported to be common in free-range pigs and some symptoms of these maladies are similar to PCV2 infection. Another factor is the availability of local pigs is gradually decreasing due to their replacement by crossbred pigs. Indigenous pigs are better adapted to local climatic conditions in terms of health and disease resistance (Hadem *et al*. 2016). The low prevalence rate in Nagaland is in contrast to the earlier observations (Kikon *et al*. 2017). It may be due to low sample size in the present study and difference in area from where samples were collected. In another study, Pegu *et al*. (2017) had reported 15% seroprevalence of PCV2 in Nagaland from 112 samples. There may be a decreasing trend in infection rate of PCV2 in Nagaland. The prevalence rate reported in Tripura (35.71%) and Meghalaya (19.05%) were more or less in agreement with Pegu *et al*. (2017), who reported 28.57% in Tripura and 21.43% in Meghalaya. The seroprevalence rate at Mizoram (54.02%) and Arunachal Pradesh (33.33%) were showing increasing trend compared to previous reports (Pegu *et al*. 2017), who reported 17.91% in Mizoram and 12.5% in Arunachal Pradesh. Rajkhowa *et al*. (2016) had reported that illegal trade of pigs through the porous international border with Myanmar continues to be the main route of entry for porcine reproductive and respiratory syndrome virus (PRRSV) in Mizoram. This illegal trade may be a reason for spread of viral diseases of pig including PCV2. Garam *et al*. (2016) in their study on rotavirus infection in Arunachal Pradesh had reported that high density of pig population in some districts and unorganized type of pig farming leads to seroprevalence of viral infection.

Size of drift or sounder: PCV2 prevalence was highest in group where 5–10 animals were kept (60.47%) and lowest in group a single animal was kept (38.64%) (Table 2). Similar observations were noticed by Fraile *et al*. (2009) who opined that size and location of the farm has significant effects on the risk of PCV2 infection in the piglets and the bigger the farm, the easier the transmission of PCV2.

Sex wise: The seroprevalence of PCV2 was more in female animals compared to male animals (Table 3). Soria and Segales (2012) opined that, in a farm, the breeding animals are the ones that maintain the infection and transmit to their offspring. But, Correge (2001) reported that higher susceptibility of castrated males to PCV2 infection.

Symptoms wise: Samples from animals having multi-systemic involvement showed a higher prevalence (88.24%) whereas samples from animals with no clinical symptoms showed prevalence of 34.33% (Table 4). PCV2 is considered to be the key agent in the development of a number of diseases like enteric form, respiratory form, systemic form, post weaning multi-systemic wasting syndrome (PMWS)

Table 1. State wise PCV2 seroprevalence

State	Samples	IgM	IgG	IgM+IgG	Total	%
Tripura	14	0	4	1	5	35.71
Mizoram	87	1	39	7	47	54.02
Manipur	103	0	45	0	45	43.69
Nagaland	25	0	0	1	1	4
Meghalaya	21	0	2	2	4	19.05
Arunachal Pradesh	6	0	2	0	2	33.33
Sikkim	50	0	47	0	47	94
Total	306	1	139	11	151	49.35

Table 2. Drift/Sounder size PCV2 seroprevalence

Size of Drift or Sounder	Samples	IgM	IgG	IgM+IgG	Total	%
Single	54	1	15	6	22	40.74
2 to 5	101	0	35	5	40	39.6
5 to 10	43	0	26	0	26	60.47
Above 10	108	0	63	0	63	58.33
Total	306	1	139	11	151	49.35

χ^2_{23}, 5.2868; χ^2_{14}, 4.4677; χ^2_{24}, 7.3248. Significant at 5% level ($P<0.05$).

Table 3. Sex wise PCV2 seroprevalence

Sex	Samples	IgM	IgG	IgM+IgG	Total	%
Male	109	0	38	7	45	41.28
Female	197	1	101	4	106	53.3
Total	306	1	139	11	151	49.35

Table 4. Symptom-wise PCV2 seroprevalence

System-wise	Samples	IgM	IgG	IgM+IgG	Total	%
General systemic state	32	0	16	1	17	53.13
Alimentary system	63	1	31	2	34	53.97
Reproductive system	37	0	19	4	23	62.16
Respiratory system	69	0	32	2	34	49.28
Nervous system	1	0	1	0	1	100
Skin	20	0	4	0	4	20
Multi-systemic	17	0	13	2	15	88.24
No symptoms	67	0	23	0	23	34.33
Total	306	1	139	11	151	49.35

χ^2_{38}, 7.4863. Significant at 5% level (P<0.05)

Table 5. Age-wise PCV2 seroprevalence

Age	Samples	IgM	IgG	IgM+IgG	Total	%
Below 3 months	67	0	24	1	25	37.31
3–5 months	78	0	32	2	34	43.59
6–12 months	59	1	42	4	47	79.66
Above 1 year	102	0	41	4	45	44.12
Total	306	1	139	11	151	49.35

χ^2_{23}, 18.0849; χ^2_{34}, 19.2823. Significant at 5% level (P<0.05).

Table 6. Management system

Management system	Samples	IgM	IgG	IgM+IgG	Total	%
Intensive system	166	0	68	8	76	45.78
Semi intensive system	124	1	60	2	63	50.81
Extensive system	16	0	11	1	12	75
Total	306	1	139	11	151	49.35

and porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC), proliferative and necrotizing pneumonia (PNP) with clinical signs of weight loss/emaciation, tachypnoea, dyspnoea, icterus and pallor in pigs of weaning and post-weaning age (Segales 2012). Lymphadenomegaly may also be present (Afolabi *et al*. 2017). Association of PCV2 with reproductive disorders like mummified foetus, abortion, repeat breeding, still birth and necrotizing myocarditis of the foetus had been reported (Salogni *et al*. 2016). Baro *et al*. (2015) narrated about the intestinal disorders in growing and finishing pigs associated with PCV2 referred as PCV2-enteric disease (PCV2-ED). Anoopraj *et al*. (2012) described about PCV2 etiology in pneumonia and wasting disease of pigs. Cantile and Youssef (2016) described that PCV2 produces nonsuppurative or granulomatous encephalitis with gliosis. PCV2 is also responsible for skin lesions of red to purple colour in affected pigs and can cause PDNS (Segales 2012). Coming to the asymptomatic animals, the prevalence was 34.33%. Segales (2009) reported PCV2 positive animals in an apparently normal farm with no symptoms of the disease and concluded that the dynamics of the PCV2 infection can be very similar on farms with or without disease. Similarly, Shen *et al*. (2010) could detect PCV2 viremia in healthy sows and their pre-suckle piglets.

Agewise: The highest seroprevalence (79.66%) was recorded in 6–12 months age group and lowest was in 3–5 months age group (43.59%) (Table 5). The seroprevalence was more in growers and adult animals. This was in agreement with the earlier reports (Csank *et al*. 2011).

Management system: Extensive system of management showed 75% and intensive system of management showed 45.78% of PCV2 seroprevalence (Table 6). There is a strong link between the occurrence of diseases and pig production systems and farm scales. Smallholder pig production systems are usually linked to poor hygiene and low bio-security with few barriers to potential contacts between the pigs, humans and wildlife. This facilitates disease transmission from wildlife to pig, pig to pig and pig to human. The reasons for more prevalence in extensive system may be due to lack of observation leading to delayed diagnosis of serious diseases, lack of investment in pig health by their owners, uncontrolled movement of pigs, difficulty in applying preventive or control measures, contact with wild pigs, pigs from other herds and carcasses of pigs (Thomas *et al*. 2013).

This comprehensive study on PCV2 infection revealed an overall seroprevalence of 49.35% in NEH states of India. The present study warrants in depth studies in this region involving larger pig population as well as devising control measures to prevent the occurrence of this economically important viral disease of pigs.

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