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## SERO-PREVALENCE OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) IN PIGS OF DIFFERENT DEVELOPMENTAL REGIONS OF NEPAL

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### Abstract

Porcine reproductive and respiratory syndrome (PRRS) is a highly infectious viral disease of swine. This disease is becoming threat to pig industry of every corner of the world. As the status of this disease in Nepal is not properly understood so far, a cross-sectional sero-prevalence study was carried out in four development regions. A total of 200 blood samples were collected from major pig producing areas of eight districts of four development regions of Nepal and the serum samples were tested for antibodies against PRRS virus by rapid antibody detection kit (Immunochromatographic test using colloidal gold immunoassay: Schenzhen Lvshiyuan biotechnology Co. Ltd, China). Among the tested serum samples, 37 were found to be positive for PRRS indicating 18.5% prevalence of the disease. Statistically the prevalence difference was significant between different development regions and between topographical regions ( $P < 0.05$ ) of the country, but no significant difference was observed in age, sex and housing systems of pigs.

Presence of antibodies in serum against PRRSV in the unvaccinated pig population of the country clearly indicates that pigs of the surveyed regions of Nepal have already been exposed to PRRS virus and the disease should be given adequate attention during differential diagnosis of porcine respiratory diseases in Nepal. Further studies are needed for molecular characterization of the virus and to develop control strategies against this disease in the country.

**Keywords:** PRRS virus; Pig; Sero-prevalence; Nepal

### Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a highly infectious viral disease of swine. Globally, PRRS is an economically important disease of pigs and it causes over \$664 million loss in USA pork Industry alone (Batista *et al.* 2002; Lunney *et al.* 2010). It causes economically great loss in the swine industry worldwide through causing respiratory disease in neonatal pigs and reproductive failure in sows (Albina, 1997; Hopper, 1992; Neuman *et al.*, 2005). This disease was first reported in North America in 1987, and now prevalent in almost all pig production areas of the World (Keffaber, 1989). A positive-stranded RNA virus belonging to Arteriviridae family, PRRS virus (PRRSV), is responsible to cause PRRS (Balasuriya *et al.*, 2008; Zimmerman *et al.*, 2006). This Virus has been manifested as constantly changing virus, representing very big challenge for controlling this disease (Kapur *et al.*, 1996; Nelsen *et al.*, 1999). There are broadly two genotypes of this virus, the European (type I) and the North American (type II) (Ellingson, 2010; Wensvoort *et al.*, 1992). However, the symptoms of these two genotypes are similar and the genomes are diverging by only 40% (Cavenagh, 1997).

Each genotype contains genetically diverse several subtypes and strains which differ in the virulence and pathogenicity in the herds (Kim *et al.*, 2007). This disease has been named differently as porcine endemic abortion reproductive syndrome (PEARS), blue ears disease, mystery swine disease, swine infertility and respiratory syndrome, swine reproductive failure syndrome (Cesar *et al.*, 2010). Pigs of any age are known to be the only known host of the PRRS (Albina, 1997). This syndrome can manifest in two forms, respiratory and reproductive form (OIE general information sheet). In respiratory disease the signs include, pneumonia, reduced food consumption, debilitation, chronic recurring illness and often high mortality. In reproductive form, poor sow performance will be evident with signs like return to estrous, premature farrowing, abortion, still birth and high pre-weaning mortality of piglets (Wensvoort, 1993). PRRSV is transmitted through direct and indirect contacts from infected pigs or even vertically to fetuses (Jenny *et al.*, 2006). Virus is excreted in various body secretions including saliva, nasal secretion, urine, semen, milk, and colostrums (Wills *et al.*, 1997; Yoon *et al.*, 1993).

PRRSV can spread by transport vehicles, farm supplies, personal attires, insects, fomites etc. to long distance as well (Otake, 2002; Report adhoc, 2008 ). Infected pig sheds PRRSV in feces for 7 days and it can persist in herds for years (Stevenson *et al.*, 1994; Terpstra, 1991). The virus is persistent in infected pigs and remains in low levels within the animals but eventually decreases as the time passes on. As, PRRS is listed in the OIE "Terrestrial Animal Health Code" so all the OIE Members are obliged to report outbreaks to the OIE according to the OIE Terrestrial Animal Health Code (OIE general inform. sheet). Vaccination against this disease has been done with the use of live and dead vaccines in order to provide immunity to pigs against the PRRSV. Similarly, other management factors such as pig herd management, introduction of properly developed gilt and proper implementation of bio-security measures in the pig farm have been implied in order to protect pigs from the disease (Dee *et al.*, 1996; Fano *et al.*, 2005). In Nepal, previously PRRS was relatively unknown disease but in last few years several cases of PRRS has been suspected (Prajapati, 2013). Among its two neighboring countries, China pig industry has already been badly affected with this disease (Li *et al.*, 2007; Zhang *et al.*, 2007) while there are some reports of PRRS outbreak in India as well ([www.pigsite.com](http://www.pigsite.com)). One study conducted in 2011, Sharma has shown 29% seropositivity in samples collected from Kathmandu (Sharma, 2011). However, the geographical distribution of this disease and its economic impact in swine industry of Nepal is unknown. So, this study was conducted with the aim of determining the status of PRRSV infection in pigs raised at different geographical regions of Nepal.

## Materials and Methods

### Study area

This cross-sectional study was conducted from February to May, 2014 in the eight districts of four development regions of Nepal viz. Dhankuta, Sunsari, Chitwan, Kathmandu valley, Kaski, Rupandehi, Banke and Surkhet. These districts were selected because pig industry is growing rapidly in these areas and there is huge movement of pigs from these areas to rest of Nepal.

### Questionnaire and survey

First of all a set of questionnaire was developed in order to get basic information about i) pig raising ii) housing management iii) health status and iv) vaccination information. Samples were collected in such a manner that it represented the whole district.

### Sample collection

A total of 200 blood samples were collected from major pig producing areas of eight districts of four developmental regions of Nepal. Blood was collected from Radial vein using aseptic technique; centrifuged at 1000 RPM for 3

minutes and serum separated. Separated serum was transported to virology laboratory of Animal Health Research Division, NARC, Khumaltar, Lalitpur in ice cooled box for further laboratory test.

### Tests and testing procedure

The serum samples were tested for the presence of PRRS antibody by using Colloidal Gold Immuno-chromatographic Cards manufactured by Schenzhen Lvshiyuan biotechnology Co. Ltd, China as per manufacturer's instructions. The test was aimed for detection of antibody (European and American strain) against porcine reproductive and respiratory syndrome. Briefly, 100ul of serum sample was added into the well on the test card. The test card was placed on the flat table, incubated for 5-20 minutes, and then result was recorded. Interpretation was carried out as, (i) Positive if control line and test lines were both seen as wine red, the more the antibody thicker the color appeared; (ii) Weak positive if control line and test line were both seen as light wine red; (iii) Negative: only control line was seen wine red and (iv) Invalidation if control line isn't seen wine red.

### Statistical analysis

Data entry and data analysis was done by SPSS version 16.0 and association of different variables were analyzed by chi-square test with significance level defined at the level of  $P < 0.05$ . Graphical representation and tabulation was done in MS excel- 2007.

## Results

Out of 200 serum samples tested, 37 serum samples were found to be positive with PRRS antibody detection kit. It shows 18.5% incidence of the disease in this study. According to the kit statement among positive samples, 21 serum samples were found to be strong positive, and 16 serum samples were found to be weak positive.

There was significant association between different development regions ( $P < 0.05$ ) and PRRS prevalence. Prevalence of PRRS was seen higher (32%) in western development region and least (6%) in eastern development region (table 1). Moreover, among 8 different districts Rupandehi district has higher prevalence (48%) , whereas no samples were positive from Dhankuta district.

The research was carried out in four districts of Terai region- Sunsari, Chitwan, Rupandehi and Banke, and four districts of Hilly region namely, Dhankuta, Kathmandu, Kaski, and Surkhet. Among them 25% (25/100) serum samples were found to be positive in Terai region and 12% (12/100) serum samples were found to be positive from the serum samples of Hill region. There was significant association between topography and PRRS prevalence ( $P < 0.05$ ), Terai region having more prevalence than hills.

Table 1: Total distribution of serum samples and sero-positivity status

Parameters		Sample number	Result		P value
			Positive	Negative	
Development Regions	Eastern	50	3 (6)	47(94)	0.006
	Central	50	11(22)	39 (78)	
	Western	50	16(32)	34(68)	
	Mid-Western	50	7(14)	43(86)	
Districts	Sunsari	25	3(12)	22(88)	ND
	Dhankuta	25	0	25	
	Kathmandu valley	25	6(24)	19(76)	
	Chitwan	25	5(20)	20(80)	
	Kaski	25	4(16)	21(84)	
	Rupandehi	25	12(48)	13(52)	
	Surkhet	25	2(8)	23(92)	
Topography	Terai	100	25(25)	75(75)	0.018
	Hill	100	12(12)	88(88)	
Age	Below 6 month	60	9(15)	51(85)	0.404
	Above 6 month	140	28(20)	112(80)	
Sex	Male	80	14(17.5)	66(82.5)	0.766
	Female	120	23(19.2)	97(80.8)	
Housing System	Intensive	157	32 (20.4)	125(79.6)	0.369
	Semi-intensive	14	1 (7.1)	13(92.9)	
	Free ranging	29	4(13.8)	25(86.2)	

\*ND: Not determined

Similarly, serum samples of two age groups, below 6 month and above 6 month age of pig, were included in the research and it was found that 15% (9/60) serum sample of age below 6 month were found to be sero-positive and 20% (28/140) serum samples of above 6 month were found to be positive in the test. In relation to sex-wise prevalence of PRRS in the result it was found that, 17.5% (14/80) serum samples of male pigs and 19.2% (23/120) serum samples of female pigs were positive(table 1).

The serum samples were collected from pigs reared in 3 different housing systems in order to find any significant difference in the prevalence of PRRS. Higher prevalence was seen in intensive system being 20.4% (32/157) followed by in free ranging system 13.8% (4/29) and semi-intensive system 7.1% (1/14).

## Discussion

This study shows there is presence of antibodies against PRRS in the serum samples of pigs of different regions of Nepal. This result signifies that these animals must have been exposed to PRRSV in any time of their life span as vaccination against this disease is not practiced in Nepal and hence possibility of antibodies production through vaccination is completely ruled out.

The sero-prevalence found in this study i.e. 18.5%, is less than the findings of Sharma (2011), where prevalence rate was found to be 29% in Kathmandu district. The geographical variation, sample collection time, test procedures etc. can be responsible for the differences in prevalence. However, in support of Sharma (2011) our study also confirms that PRRS virus is infecting pigs of Nepal and spreading to many regions. Regarding PRRS status in other countries, there was prevalence of 32.6% of PRRS antibody sero-positive samples in pigs of Thailand

which is quite higher than this study (Tummaruk *et al.*, 2013). According to Montagnaro *et al.*, 37.7% (129/342) sero-positive samples were found for PRRS in wild boars in Campania, southern Italy which is also very much higher than our finding (Montagnaro *et al.*, 2010). According to Foti *et al.*, (2008), 31.1% sero-positive samples were found in the ELISA test during a serological survey of detecting PRRSV infection in Italian pigs (Foti *et al.*, 2008).

Results show that there is high prevalence (32%) of the disease in western development region and least (6%) in eastern development region. Statistically significant difference ( $P < 0.05$ ) was observed between the development regions having high prevalence in western development region which might be due to the high movement of pigs and population from India to this region. According to district wise distribution Rupandehi district shows highest (48%) prevalence and but Dhankuta district does not show any positive serum samples of PRRS.

Furthermore, 25% of the positive serum samples were from Terai districts and 12% from the hill districts. The result shows that there is significant difference ( $P < 0.05$ ) between prevalence of disease in Terai and hilly region. This might be due to having maximum pig industry and huge movement of pigs in this area and also the nearby association of the Terai district with the Indian border.

Age wise, there is high prevalence i.e. 20% (28/140) in more than 6 month old pigs than pigs less than 6 month age i.e. 15% (9/60). Similarly, 19.2% (23/120) female serum samples were positive than 17.5% (14/80) of male serum samples. The study shows 20.4 % prevalence of disease in pigs rearing in intensive housing system where as only 13.8% and 7.1 % in free ranging and semi intensive housing system respectively. So, except development region and

topographical difference, there are no significant differences between other variables which signify that pigs of any sex, age, and housing system can be affected with this disease.

## Conclusions

The presence of antibodies in the serum and the fact that there had been no vaccination against this disease in the country clearly indicates that the pigs of different development regions of Nepal had already been exposed to the PRRS virus. As there is no vaccination practices in the country, the positive result refers to natural infection. The result shows that PRRS is circulating in pigs of different developmental regions of Nepal, though in different proportion. Results show that there is high prevalence of disease in Terai than in hills. This might be due to the nearness of these places with the Indian border. Sharing borders with limited restrictions and poor quarantines might be transporting PRRS virus from one direction to other in these countries but research on PRRS is limited on both sides. This result also gives us clue that this is mostly localized in Terai district and pigs of hilly area are not severely affected yet. The high presence of positive case in Kathmandu valley might also be due to supply of pigs from Terai region to the valley.

PRRSV affects pigs irrespective of age, sex and breed. So, all pigs are at risk of PRRSV. It affects pigs irrespective of housing system. Though mountain region is not included in this study, the Terai and Hill districts showing sero-positive pigs for PRRSV indicates the presence of virus in most part of the country. All these circumstances suggest for further detailed study on this disease and its economic impact in pig industry of Nepal to develop suitable control measures against this disease.

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**Conflict of interest:** None

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