Trop Anim Health Prod
DOI 10.1007/s11250-015-0986-1

SHORT COMMUNICATIONS

Serological evidence of type 2 (North American genotype) porcine reproductive and respiratory syndrome virus in Nepal

Barun Kumar Sharma[1,2] · **Salina Manandhar**[2] · **Brecht Devleesschauwer**[3,4]

Received: 18 August 2015 / Accepted: 15 December 2015

Abstract Porcine reproductive and respiratory syndrome virus (PRRSV) has spread throughout Asia, causing significant losses to commercial farmers and smallholders. However, little is known about PRRS in Nepal, a South Asian country with a gradually increasing pig industry. In 2011, a pilot project was initiated to identify the status of PRRSV in pigs of the Kathmandu Valley of Nepal. Out of 98 serum samples, 31 (32 %; 95 % CI 23–42 %) were found positive by ELISA. All positive samples belonged to the type 2 (North American) genotype. Molecular evaluation by real-time PCR however did not yield positive results. At the herd level, seropositivity was associated with a history of abortion and premature birth. Veterinarians, farmers and government should be aware of this threat to the Nepalese pig industry and initiate an appropriate response.

Keywords Diagnosis · Nepal · Pigs · PRRS · Risk factors

Introduction

Nepal is an agrarian country where more than 65 % of the people depend on agricultural activities for their livelihood (MOAD 2014). Livestock is an integral part of the agrarian economy, and different animal species are being raised, each with their own religious, cultural, nutritional and agricultural value. Traditionally, pigs have been associated with underprivileged social groups and have not been given much consideration in improvement programmes. As a result, pig husbandry and pork production is at an early stage of development compared to other livestock systems. Pig husbandry is however growing in Nepal, due to reduced cultural biases against pigs and government programmes supporting pig husbandry for poverty alleviation and food security (Devleesschauwer et al. 2013; Dhakal et al. 2014)

Even though the import of pork and other pig-derived products is still limited (Deka et al. 2014), threats of introduction, spread and establishment of transboundary animal diseases have increased in Nepal (Postel et al. 2013). Porcine reproductive and respiratory syndrome (PRRS) is a disease of swine that was first recognized in the USA in 1987 (Keffaber 1989) and is associated with late-term reproductive failure and severe pneumonia in neonatal pigs (Olanratmanee et al. 2014). Since its appearance, it has been identified (clinically, serologically or both) throughout all of the major swine production areas of North America, Europe and Southeast Asia (Neumann et al. 2005). PRRS has already been recorded and reported from neighbouring countries including India, China and Bhutan (World Organisation for Animal Health 2014). The highly virulent variant of the PRRS virus (PRRSV) first emerged in China and Vietnam in 2006 (Tian et al. 2007) and rapidly spread in pigs in Southeast Asia (Nguyen et al. 2015). The epidemic affected not only large commercial farms but also backyard pig farms, creating serious problems for the global swine industry. The rapid spread of PRRSV in different parts of the world in a short span after its first recognition suggests that it may soon have a worldwide distribution.

✉ Brecht Devleesschauwer
brechtdv@gmail.com

[1] Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

[2] Department of Livestock Services, Ministry of Agricultural Development, Kathmandu, Nepal

[3] Emerging Pathogens Institute and Department of Animal Sciences, University of Florida, PO Box 110910, Gainesville, FL 32611, USA

[4] Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium

Published online: 28 December 2015

PRRS is a relatively unknown disease in Nepal. In 2011, the Food and Agriculture Organization of the United Nations supported a pilot project to identify the status of PRRSV in pigs of Nepal. This paper reports on the findings of this study.

Materials and methods

Study setting

The study was performed during June–July 2011 in the Kathmandu Valley of Nepal, which comprises the Kathmandu, Lalitpur and Bhaktapur districts. The pig population in the study area was estimated to be 9480 (Kathmandu), 7200 (Lalitpur) and 2811 (Bhaktapur) (MOAC 2010). Pigs in these areas are mostly raised along the riverbanks of the valley with some commercial farming in clusters outside the ring road of Kathmandu Valley.

Sampling strategy

All pig-raising clusters located within the selected districts and that were willing to participate in the survey were included in this study. Within the clusters, herds were randomly selected, and within the selected herds, pigs were selected by systematic random sampling. As this was a pilot study aimed at observing the presence of PRRSV, we aimed to collect a total of 100 serum samples. This would have been sufficient to have a 99 % probability of detecting a disease with a design prevalence of 5 %.

Blood was collected from the ear vein of the pigs. The blood sample was labelled and placed vertically in a stand and allowed to clot. The sample was then centrifuged at 2000 rpm for 15 min and the serum was separated. The separated serum was stored in a cryovial, labelled and placed in a cool box and brought to the Central Veterinary Laboratory (CVL), Tripureshwor, Kathmandu, where it was stored at −20 °C until further testing.

Serological and molecular analyses

The serum samples were first analysed for the presence of antibodies to PRRSV by using the AniGen PRRS Ab ELISA 4.0 kit (Bionote Inc., Korea). This test detects anti-PRRSV antibodies, without making a distinction between the European and North American genotypes. According to the manufacturer's instructions, a sample to positive ratio cutoff of 0.4 was applied.

A subset of the samples was submitted for confirmation testing to the World Organisation for Animal Health (OIE) reference lab for PRRSV, i.e., the Department of Swine Diseases at the Polish National Veterinary Research Institute. Samples were tested by a commercial ELISA (HerdChek X3, IDEXX) and an in-house indirect ELISA that allowed discriminating antibodies to type 1 (European genotype) and type 2 (North American genotype) PRRSV (Stadejek et al. 2007). If sufficient volume remained, samples were subsequently tested by real-time PCR for PRRSV (PRRS NextGen, Tetracore).

Risk factor analysis

At the moment of sampling, herd-level information was collected, i.e., number of animals raised, type of breed, rearing practices (mixed vs. separate housing), type of feed provided, use of Classical Swine Fever vaccine, and any history of piglet mortality at birth, abortion, and premature birth. Univariable logistic regression, performed in R 3.2.0 (R Core Team 2015), was used to assess the association between these variables and the herd-level prevalences. For the prevalence estimates, 95 % exact binomial confidence intervals (CI) were calculated using the prevalence package in R (Devleesschauwer et al. 2014).

Results

In total, 98 serum samples were collected of pigs originating from 24 herds (with a median of 3 pigs per herd [range 1–13]). Out of these, 31 (32 %; 95 % CI 23–42 %) were found positive. The herd-level prevalence was 50 % (95 % CI 29–71 %). A history of abortion ($P=0.013$) and a history of premature birth ($P=0.001$) were the only two factors significantly associated with PRRSV seroprevalence (Table 1). The effect of breed could not be assessed because of a poor definition of breeds during the survey.

Out of 98 serum samples, 39 (30 ELISA positive and 9 ELISA negative) were tested at the OIE reference lab. The IDEXX ELISA test results corresponded to those obtained at the CVL. Using the in-house indirect ELISA, all positive samples were found to react with the North American PRRSV genotype (type 2). Thirty-four samples were also tested with real-time PCR, but were found negative.

Discussion

PRRS has been known to occur in East and Southeast Asia since several decades (Neumann et al. 2005) but received renewed attention due to the emergence of highly pathogenic PRRSV strains in China and Vietnam in 2006 (Tian et al. 2007), which rapidly spread to other countries in Southeast Asia (Nguyen et al. 2015). However, little is known about the PRRSV epidemiology in South Asia, Recently, Rajkhowa et al. (2015) reported the first outbreak of

Table 1 Univariable associations with herd-level prevalence for anti-PRRSV antibodies

Variable	Odds ratio	95 % confidence interval	*P* value
District (Bhaktapur)	—	—	—
District (Kathmandu)	0.48	0.08–2.75	0.390
District (Lalitpur)	0.13	0.00–1.41	0.120
Number of animals	0.98	0.93–1.02	0.230
Mixed pig rearing	0.81	0.33–1.95	0.649
Hotel/restaurant waste provided	1.05	0.90–1.21	0.513
Self-made feed provided	0.81	0.34–1.92	0.637
CSF vaccination applied	1.36	0.52–3.88	0.548
Piglet mortality observed	0.91	0.33–2.65	0.850
Abortion observed	3.20	1.32–8.31	0.013
Premature birth observed	4.68	1.91–12.3	0.001

PRRS in India, which occurred in 2013 in the eastern state of Mizoram, bordering Bangladesh and Myanmar. We report the findings of what was, to our knowledge, the first study on PRRSV in Nepal. In the Kathmandu Valley, nearly one in three pigs was seropositive, and seropositive pigs were found in half of the herds. In 2014, a screening of 200 pigs in and outside of the Kathmandu Valley showed a seroprevalence of 19 % (K.C. et al. 2015). As vaccination against PRRSV has not yet started in Nepal, these results show that PRRSV has been present in Nepal since at least 2011. We identified the presence of type 2 (North American genotype) PRRSV in Nepal, mimicking the situation in India (Rajkhowa et al. 2015) and most Southeast Asian countries (Jantafong et al. 2015). As attenuated PRRSV vaccines are genotype-specific, knowledge on the circulating strains is crucial for planning control efforts (Jantafong et al. 2015). Unfortunately, we could not obtain molecular confirmation. As the samples were collected irrespective of clinical signs, the animals may have been immune and no longer infected at the time of sampling.

Although we could identify the presence of PRRSV in Nepal, much is to be learned about its epidemiology and impact. In this study, we could not identify any transmission-related risk factors. This is likely to be related to the overall poor biosecurity status in the Nepalese pig industry. For instance, feeding of animal waste and kitchen leftovers from hotels and restaurants is common practice. For bigger hotels and restaurants, pork is imported from different countries with reported cases of PRRSV. The use of kitchen leftovers may thus imply a certain risk for the introduction of PRRSV (Hall and Neumann 2015).

Information on the extent and impact of PRRSV in Nepal is currently lacking. Our study was limiting in size and geographical scope. K.C. et al. (2015) showed that PRRSV is widespread in Nepal, with a higher seroprevalence found in the Western Development Region versus other Development Regions, and in the Terai versus the Hills. Our study showed a significant association with abortion and premature birth, as can be expected for PRRSV. Further work is however needed to demonstrate the actual clinical and economic impacts of PRRSV in Nepal.

The presence of this transboundary animal disease in Nepal calls for an improved vigilance of all stakeholders. Field veterinarians and technicians should be made aware about the disease, including its clinical signs, post-mortem lesions and other diagnostic features. Awareness programmes for farmers covering biosecurity, pig disease transmission and control, and quarantine measures should be regularly conducted, as most of them have little or no knowledge on these topics. Finally, the relevant government agencies should draft and implement a contingency plan for monitoring and controlling the disease.

Conclusion

We demonstrate the presence of PRRSV in Nepal, and more specifically the type 2 (North American) genotype. However, much remains to be learned about the actual epidemiology and impact of the disease. Veterinarians, farmers and government should be aware of this threat to the pig industry and initiate an appropriate response.

Acknowledgments We are grateful to Dr Tony Williams, Dr Ram Krishna Khatiwada, Dr Kishan Chand Thakuri, Dr Bal Bahadur Chand, Dr Damodar Sedai, Dr Khadak Singh Bisht, Dr Bishnu Bahadur Adhikari, and others who have supported this study. Similarly, the Department of Livestock Services (DLS), Directorate of Animal Health (DAH), and Central Veterinary Laboratory (CVL) are also acknowledged for giving permission to conduct this study.

Compliance with ethical standard

Funding This study was funded by the FAO Technical Assistance to Avian Influenza Control Programme (grant number UTF/NEP/061/NEP).

Conflict of Interest The authors declare that they have no competing interests.

References

Deka, R. P., Grace, D.,, Lapar, M. L.,, Lindahl, J.,, 2014. Sharing lessons of smallholders' pig system in South Asia and Southeast Asia: A review. Presented at the National Conference on Opportunities and Strategies for Sustainable Pig Production, Guwahati, India, 20–21 December 2014. Nairobi, Kenya: ILRI. https://cgspace.cgiar.org/handle/10568/53928. Accessed 24 Nov 2015

Devleesschauwer, B., Pruvot, M.,, Joshi, D. D.,, De Craeye, S.,, Jennes, M.,, Ale, A.,, Welinski, A.,, Lama, S.,, Aryal, A.,, Victor, B.,, Duchateau, L.,, Speybroeck, N.,, Vercruysse, J.,, Dorny, P.,, 2013. Seroprevalence of zoonotic parasites in pigs slaughtered in the Kathmandu Valley of Nepal. *Vector Borne and Zoonotic Diseases* 13, 872–876.

Devleesschauwer, B., Torgerson, P.,, Charlier, J.,, Levecke, B.,, Praet, N.,, Roelandt, S.,, Smit, S.,, Dorny, P.,, Berkvens, D.,, Speybroeck, N.,, 2014. prevalence: Tools for prevalence assessment studies. R package version 0.4.0. http://cran.r-project.org/package=prevalence. Accessed 24 Nov 2015.

Dhakal, S., Joshi, D. D.,, Ale, A.,, Sharma, M.,, Dahal, M.,, Shah, Y.,, Pant, D. K.,, Stephen, C.,, 2014. Regional variation in pig farmer awareness and actions regarding Japanese encephalitis in Nepal: implications for public health education. *PLOS ONE* 9, e85399.

Hall, W., Neumann, E.,, 2015. Fresh pork and porcine reproductive and respiratory syndrome virus: Factors related to the risk of disease transmission. *Transboundary and Emerging Diseases* 62, 350–366.

Jantafong, T., Sangtong, P., Saenglub, W., Mungkundar, C., Romlamduan, N., Lekchareonsuk, C., Lekcharoensuk P., 2015. Genetic diversity of porcine reproductive and respiratory syndrome virus in Thailand and Southeast Asia from 2008 to 2013. *Veterinary Microbiology* 176, 229–238.

K. C., M., Joshi, B. R.,, Shrestha, S. P.,, Prajapati, M.,, Kathayat, D.,, Dhakal, S.,, 2015. Sero-prevalence of porcine reproductive and respiratory syndrome (PRRS) in pigs of different developmental regions of Nepal. *International Journal of Applied Sciences and Biotechnology* 3, 218–222

Keffaber, K. K., 1989. Reproductive failure of unknown etiology. *American Association of Swine Practitioners Newsletter* 1, 1–9.

MOAC, 2010. Statistical Information on Nepalese Agriculture 2009/10. Ministry of Agriculture and Cooperatives, Government of Nepal, Singha Durbar, Kathmandu, Nepal.

MOAD, 2014. Statistical Information on Nepalese Agriculture 2013/14. Ministry of Agricultural Development, Government of Nepal, Singha Durbar, Kathmandu, Nepal. http://www.moad.gov.np/uploads/files/Year%20book%202014.pdf. Accessed 24 Nov 2015.

Neumann, E. J., Kliebenstein, J. B.,, Johnson, C. D.,, Mabry, J. W.,, Bush, E. J.,, Seitzinger, A. H.,, Green, A. L.,, Zimmerman, J. J.,, 2005. Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. *Journal of the American Veterinary Medical Association* 227, 385–392.

Nguyen, V. G., Kim, H. K.,, Moon, H. J.,, Park, S. J.,, Chung, H. C.,, Choi, M. K.,, Park, B. K.,, 2015. Evolutionary dynamics of a highly pathogenic type 2 porcine reproductive and respiratory syndrome virus: Analyses of envelope protein-coding genes. *Transboundary and Emerging Diseases* 62, 411–420.

Olanratmanee, E.O., Thanawongnuwech, R.,, Kunavongkrit, A.,, Tummaruk, P.,, 2014. Reproductive performance of sows with and without PRRS modified live virus vaccination in PRRS-virus-seropositive herds. *Tropical Animal Health and Production* 46, 1001–1007.

Postel A, Jha VC, Schmeiser S, Becher P, 2013. First molecular identification and characterization of classical swine fever virus isolates from Nepal. *Archives of Virology*, 158:207–210

R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/. Accessed 24 Nov 2015.

Rajkhowa, T.K., Jagan Mohanarao, G., Gogoi, A., Hauhnar, L., Isaac, L., 2015. Porcine reproductive and respiratory syndrome virus (PRRSV) from the first outbreak of India shows close relationship with the highly pathogenic variant of China. *Veterinary Quarterly* 35, 186–193.

Stadejek, T., Oleksiewicz, M. B.,, Pejsak, Z.,, 2007. Development of ELISA tests for detecting infections of European porcine reproductive and respiratory syndrome virus. *Medycyna Weterynaryjna* 63, 1336–1341.

Tian, K., Yu, X.,, Zhao, T.,, Feng, Y.,, Cao, Z.,, Wang, C.,, Hu, Y.,, Chen, X.,, Hu, D.,, Tian, X.,, Liu, D.,, Zhang, S.,, Deng, X.,, Ding, Y.,, Yang, L.,, Zhang, Y.,, Xiao, H.,, Qiao, M.,, Wang, B.,, Hou, L.,, Wang, X.,, Yang, X.,, Kang, L.,, Sun, M.,, Jin, P.,, Wang, S.,, Kitamura, Y.,, Yan, J.,, Gao, G. F.,, 2007. Emergence of fatal PRRSV variants: Unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. *PLOS ONE*, 2, e526.

World Organisation for Animal Health, 2014. World Animal Health Information Database (WAHID) Interface. http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home. Accessed 24 Nov 2015.