Seroprevalence of Peste des petits ruminants in small ruminants in the North Eastern Region of India

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Summary
A seroprevalence study of the peste des petits ruminants (PPR) in small ruminants was carried out in the different states (Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura) in the North Eastern Region (NER) of India using serum samples collected from April 2017 to March 2018. A total number of 4,163 sera [sheep (n = 508) and goats (n = 3,655)] collected from 345 epidemiological units/villages covering 176 tehsils/blocks in NER were screened by competitive ELISA kit for the detection of PPR virus antibodies. The results revealed that the seroprevalence of PPR in small ruminants in Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura was 34.3%, 10.3%, 4.7%, 15.7%, 14.7%, and 5.5%, respectively with an overall 14.5% prevalence.Association between the presence of antibodies and goats has been showed to be significant ($p < 0.01$) at the NER level and within every single state. This manuscript highlights the need for continuous monitoring of this important disease as for the severe economic impact PPR may have in the affected countries.

Peste des petits ruminants (PPR), otherwise known as 'Plague of small ruminants' or 'Kata' is a world organization for animal health (OIE) notifiable and economically important transboundary animal disease (TAD) of sheep and goats caused by the Small ruminants morbillivirus (SRMV), formerly known as PPR virus, a member of the genus *Morbillivirus* of the family *Paramyxoviridae* (http://ictvonline.org/virusTaxonomy.asp). It is an acute and highly contagious viral diseases of primarily sheep and goats and is currently emerging to cause infection in camels (Balamurugan *et al.* 2014b) and clinically manifested by high fever, discharges from eyes and nasal orifices, oral necrotizing and erosive ulcers, stomatitis, gastroenteritis, diarrhea and bronchopneumonia (Balamurugan *et al.* 2014b). This transboundary nature of the disease is one of the foremost constraints in enhancing the productivity of the small ruminants in enzootic countries in Africa, the Arabian Peninsula, the Middle East, and Central and South-East Asia. PPR has a massive potential to cause huge economic losses and it significantly impacts the livestock sector economy and food security of the enzootic countries (Govindaraj *et al.* 2016). After the eradication of rinderpest, a global agreement was stretched on the requirement to eradicate PPR, with the adoption of a PPR Global Control and Eradication Strategy (GCES) to make the world free from PPR by 2030 (OIE and FAO 2015a) due to huge socio-economic impacts of PPR. The Food and Agriculture Organization and OIE conjointly initiated an international strategic design for control and eradication with a goal of gathering all stakeholders behind the PPR- Global Eradication Programme (PPR-GEP) and mobilizing the supplementary support required for the eradication (OIE and FAO 2015a) and launched PPR-GEP for the period 2017-2021, into action with the adoption of a PPR GCES.

In India, sheep and goats play a pivotal role in the socio-economic development of rural households and are generally referred for 'Any Time Money (ATM)' to rural farmers. These animals are constituting an important productive asset of settlers, landless, marginal, and small landholders and it generates a flow of income and employment

throughout the year for their livelihood. The disease is enzootic and a number of outbreaks are occurring regularly, in different parts of India throughout the year (Balamurugan *et al.* 2014b). PPR is a major constraint in small ruminant production causing huge economic losses in terms of morbidity, mortality, productivity losses with trade restriction (Balamurugan *et al.* 2014b, Singh *et al.* 2004a). Further, the molecular characterization of PPR virus (PPRV) and its phylogenetic analyses revealed that the Asian lienage IV as the sole circulating in India (Shaila *et al.* 1996, Dhar *et al.* 2002, Balamurugan *et al.* 2010, Muthuchelvan *et al.* 2014).

For the control of disease, India adopted focused vaccination campaigns (within a radius of 3-10 km to contain the disease in outbreak situation) in some of the states since 2002 (Singh *et al.* 2009) and in strategic vaccination mode through implementation of a national control program on PPR (PPR-CP) in some of the states even before the global framework was planned (Balamurugan *et al.* 2016, Govindaraj *et al.* 2019). Nevertheless, neither a surveillance plan nor a systematic post vaccination monitoring or evaluation was initiated to assess the effectiveness of the vaccination program. In India, several outbreaks go unrecorded due to under-reporting or non-reporting (Balamurugan *et al.* 2011) and further, in spite of vaccination, disease outbreaks are being reported regularly in some of the states or sporadically in regularly vaccinated or geographically restricted regions of India.

Even though PPR is enzootic in India, some states in the North Eastern Region (NER) are either free from disease or have a few sporadic outbreaks reports. PPR is of increasing importance and likely to extend its geographic distribution especially in NE states, as PPR outbreaks have been reported in Assam (Balamurugan *et al.* 2014a, Chaudhary *et al.* 2013, Majumder 1997) and in Tripura since 2010 (Muthuchelvan *et al.* 2014). Nevertheless, systematic epidemiological surveys have not been conducted except for a few isolated studies (Balamurugan *et al.* 2019). Earlier studies on the seroprevalence, generating the baseline data on PPR seroprevalence in goats of NER (Balamurugan *et al.* 2014a, Begum *et al.* 2016, De *et al.* 2016, Devi *et al.* 2016, Karam *et al.* 2018), have generally indicated that transboundary migration of animals is related to PPR outbreaks in naïve areas. Moreover, studying the prevalence of antibodies would help in the implementation of proper disease control strategies such as vaccination (Balamurugan *et al.* 2014c). Therefore, a cross-sectional seroprevalence survey is being employed in this study to determine the prevalence of PPRV antibodies in NER of India.

North Eastern Region (NER) is the easternmost region of India representing both a geographic and political-administrative section of the country and it comprises Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura states of India. This region was purposively selected, as the PPR has been reported from this region since 2010 (Balamurugan *et al.* 2014a, Muthuchelvan *et al.* 2014). Further, some of the NER states did not implement the national PPR control program. However, Arunachal Pradesh and Sikkim states were excluded in the study due to the ecological niche of hilly terrain and valley, geographically isolated nature of states, the topology of the region. The states under analysis in this study, in NER, have 7.44 and 0.56 million sheep and goat individuals, respectively, as per Basic Animal Husbandry Statistics (BAHS), 2012 (http://www.dahd.nic.in/).

A cross-sectional prevalence study was conducted between April 2017 and March 2018 to ascertain the prevalence status of PPR virus antibodies in small ruminants population in the epidemiological units (epi-units) of different states in NER. The initial hypothesis is that PPR antibodies are homogeneous or independent in the populations of the epi-units in the study region. The rural societies live in villages consisting of clusters of households that follow similar animal husbandry and socio-economic activities. Hence, the village is considered as a distinct epi-unit in this study as described earlier (Balamurugan *et al.* 2009). Accordingly, a list of villages in various blocks/tehsils in different districts in the state and their sheep and goat populations in each of the state was prepared and in order to have a sizeable population, the villages (epi-units) having more than 200 sheep and goats (with inclusion and exclusion criteria) were shortlisted for the sampling frame.

The sample size for the present study was determined as per Cochran, (Cochran 1963) formula $N = Z^2 [p (1 - p/e^2]$ by using epitools, where N = sample size, Z = 95% confidence level, p = 30% proportion [animal unit-level prevalence of 30% was considered as per GCES (OIE and FAO 2015b) as well as based on the prevalence of PPR before the implementation of vaccination in India (Singh *et al.* 2004a)], e is the precision level (5%). Based on these inputs a total sample size of 323, were determined (http://epitools.ausvet.com.au/content.php?page=1Proportion). However, after considering the attrition rate of 10%, the total sample size was 356. The multistage stratified random sampling procedure was adopted for collecting serum samples.

In the first stage, the states were stratified and in each stratum (state), 60 sampling primary epi-units (villages) were equally allocated randomly using R software (R_Core_Team 2014). In the next stage in each of the selected villages, the number of secondary units (animals) were

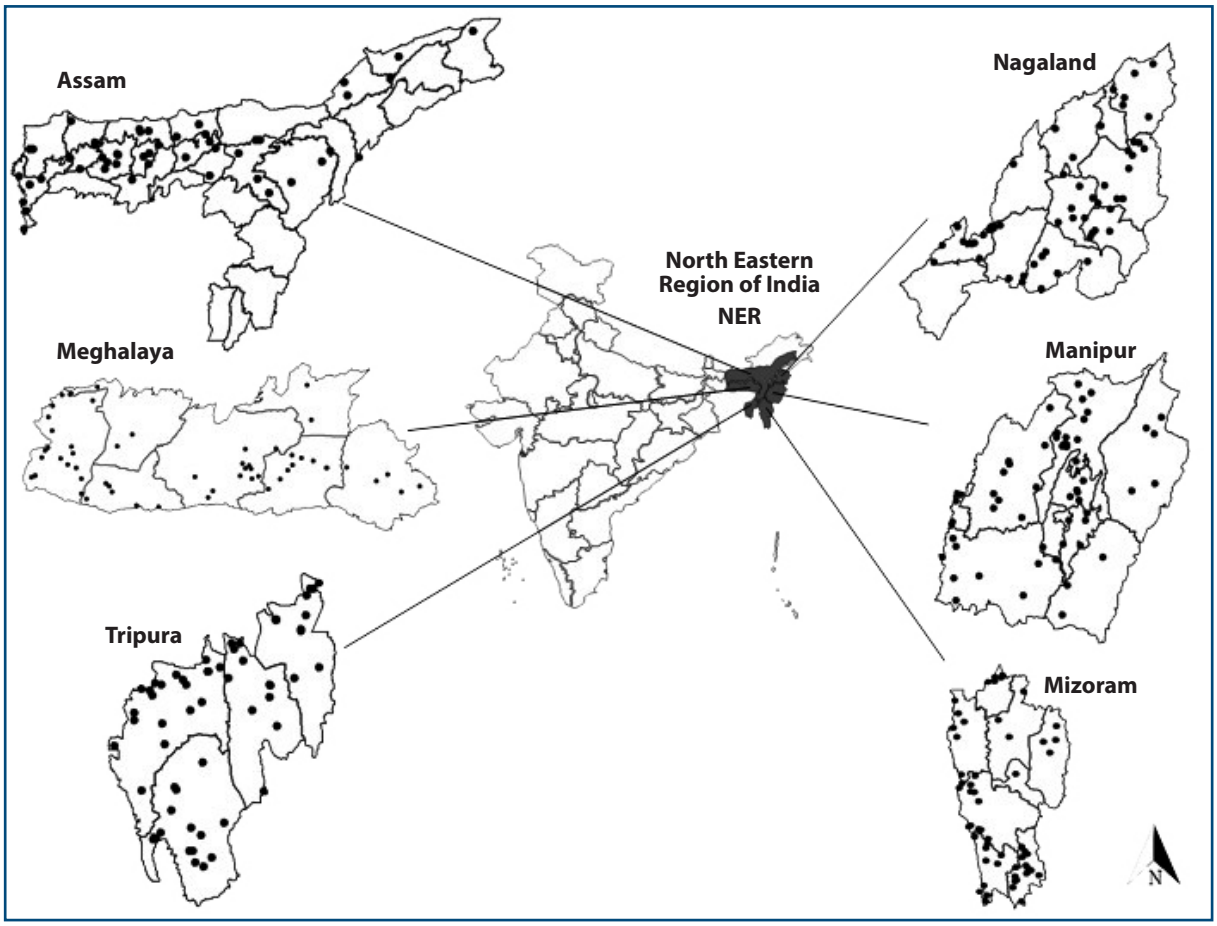

Figure 1. *The epi-units (villages) location are depicted (as • a dot) in the GIS Map of the studied states in the North-Eastern Region (NER) of India.*

calculated by the hypergeometric distribution as per GCES guidelines by considering an animal unit-level prevalence of 30% (OIE FAO 2015b) in small ruminants. The maximum level of 11 samples to be collected was determined based on the sheep and goat populations in each epi-units. Thus, a maximum of 1,320 secondary animal units [660 for each target (sheep or goats) species] was established using epi-calculator https://www.nivedi.res.in/Nadres_v2/Epical/stratified/random_sampling.php.) for this study.

In each epi-unit/village, sera were collected randomly through All India Coordinated Research Project on Animal Disease Monitoring and Surveillance (AICRP on ADMAS), a collaborating center of ICAR-NIVEDI, in the respective states. In the epi-unit, where only either sheep or goats where present, a maximum of 11 samples of either species was collected. The sample epi-units under examination are in GIS Map (Figure 1) using QGIS Software 2.18.6 version. The collected sera were labeled and transported in an ice-cool shipment container to the laboratory. Samples were stored at - 20 °C until further use.

All the sera were tested by using competitive ELISA kit (Singh *et al.* 2004b).

The seroprevalence was estimated by the number of positive animals versus numbers of tested animals as per the method described by Thrushfield (2005). The chi-squared test (χ^2) was carried out in MS-Office Excel 2016 as per the method described by Snedecor and Cochran (Snedecor and Cochran 1967) to understand the association of PPRV antibodies in sheep and goats across states and districts within every single state in the NER of India.

This cross-sectional prevalence study assessed the status of the PPRV antibodies in sheep and goats in NER from April 2017 to March 2018.

The observed percentage seroprevalence of PPR in small ruminants in the studied states of the NER of India were, 34.3%, 10.3%, 4.7%, 15.7%, 14.7%, and 5.5%, in Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura, respectively with the overall prevalence of 14.5%. Further, the results of the chi-square analysis revealed that the association (not independent) of PPR virus antibodies in goats ($\chi^2 = 137.3$, $p < 0.01$) and in small ruminants ($\chi^2 = 330.6$, $p < 0.01$) across the different states in the studied NER of India. It is important to point out that most of the states do not adopt vaccination strategies. State-wise seroprevalence of PPRV

Table I. *Serum samples screened for each state and prevalence of PPR antibodies in small ruminants in the NER of India.*

Name of the states	No. of the tehsil/ block in each state	No. of the village / epi-unit in each state	No. of the serum samples screened			No. of the samples positive in ELISA			Prevalence of PPR virus antibodies (CI - Value at 95%)			Seroprevalence % status in epi-units (No.)		
			Total	Sheep	Goats	Total	Sheep	Goats	Total	Sheep	Goats	< 30	30-70	> 70
Assam	43	48	724	260	464	248	138	110	34.25 (31-38)	53.08 (47-59)	23.71 (20-28)	28	12	8
Manipur	24	59	758	132	626	78	13	65	10.29 (8-13)	9.85 (6-16)	10.38 (8-13)	55	3	1
Meghalaya	25	60	655	-	655	31	-	31	4.73 (3-7)	-	4.73 (3-7)	56	3	1
Mizoram	18	60	682	40	642	107	0	107	15.69 (13-19)	0 (0-9)	16.67 (14-20)	47	12	1
Nagaland	41	59	723	76	647	106	7	99	14.66 (12-18)	9.21 (5-18)	15.3 (13-18)	51	7	1
Tripura	25	59	621	-	621	34	-	34	5.48 (6-10)	-	5.48 (6-10)	55	4	0
Total	**176**	**345**	**4163**	**508**	**3655**	**604**	**158**	**446**	**14.51 (13-16)**	**31.10 (27-35)**	**12.2 (11-13)**	**292**	**41**	**12**

Chi square value: Goats ($\chi^2 = 137.3$, $p < 0.01$); States ($\chi^2 = 330.6$, $p < 0.01$)

antibodies is presented in Table I. Results of single states are available upon request.

The results here provided indicate that the disease incidence is sporadic in the states of NER and in some places the animals are naïve for PPRV infection (Balamurugan *et al.* 2014a, De *et al.* 2016, Singh *et al.* 2004a). Generally, variation in seroprevalence could be due to differences in sample size, prevailing management practices, humidity or season as reported earlier (Singh *et al.* 2004a). However, in the present study, as per the sampling plan, a cross-sectional study was conducted to estimate prevalence with a specified level of confidence and desired precision with the maximum statistical sample size for the finite or large population representing the target population from different epidemiological units of the studied region.

Sporadic nature of the disease in the NE region, especially in Assam and Tripura states, will cause an increase in the number of outbreaks, as there is no vaccination of small ruminants is undertaken at present in the NER, which in turn favors the spread of disease to other parts.

Furthermore, most of the selected epi-units of NER had neither seroprevalence of > 30% (n = 292) nor protective population immunity of > 70% (n = 333), which implies that goat population is free from PPRV antibodies, as there were neither PPR outbreaks reported nor vaccination strategies practiced in NER except focused vaccination in selected outbreaks places.

This cross-sectional study is the first of its kind and strongly suggests that the small ruminants in most of the epi-units had < 30% seroprevalence, which necessitates of a comprehensive intensive active surveillance or vaccination programs.

This information would be very useful in the formulation of effective disease management strategies as well as in the implementation of a national PPR vaccination program in the NER states of India. More systematic, intensive and comprehensive serological active surveillance program in small ruminants along with measurement of clinical prevalence or monitoring of the occurrence of sporadic outbreaks in different clinical forms of PPR in the enzootic areas must be undertaken in order to develop effective control measures/strategies for PPR to make disease-free NER of India.

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