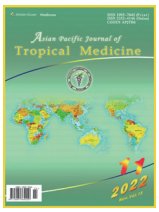

Original Article

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/1995-7645.359788

Impact Factor: 3.041

Prevalence and risk factors associated with Japanese encephalitis virus infection in swine population of Assam, India

Mir Hussain[1], Himani Dhanze[1]✉, Deepa Mehta[1], M. Suman Kumar[1], Ravi Kumar Gandham[2], Megha Gupta[1], AG Barua[3], K.P Suresh[4], Balbir B Singh[5]

[1]*Division of Veterinary Public Health, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, India*

[2]*Division of Animal Biotechnology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, India*

[3]*Department of Veterinary Public Health, College of Veterinary Science, Assam Agricultural University, Guwahati, Assam, India*

[4]*ICAR-National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru, India*

[5]*Centre for One Health, Guru Angad Dev Veterinary & Animal Sciences University, Punjab, India*

ABSTRACT

Objective: To assess the prevalence of Japanese encephalitis virus (JEV) and associated risk factors in the swine population of Assam.

Methods: A total of 432 swine serum and blood samples were collected from Barpeta and Sonitpur districts of Assam and were screened for the presence of JEV antibodies. Information related to risk factors was collected using a self-designed questionnaire from 120 swine-rearing farmers. Linear-mixed models were used for prevalence estimation. Univariate and multivariate regression models were constructed to evaluate the association of demography, season and management practices with JEV positive status.

Results: Overall, the JEV infection prevalence was 51.6% at farm and 47.1% at slaughter premises. Phylogenetic analysis of partial sequence of envelope gene of two positive field samples revealed that both isolates belonged to genotype Ⅲ JEV. Isolate 1 shared a common clade with human isolates while isolate 2 belonged to the same clade as that of other JEV swine strain isolated from India. The final multivariate model showed that two factors including monsoon season (Adjusted *OR* 5.6; 95% *CI* 2.1-14.9; *P*<0.001) and water logging in the area near the pig shelter (Adjusted *OR* 16.9; 95% *CI* 6.1-47.3; *P*<0.001) were associated with greater odds of swine being infected with JEV.

Conclusions: High prevalence of JEV in swine population of Assam state indicates a significant risk of virus transmission to humans while risk factor study underlines the urgent need for awareness campaigns in the Assam.

KEYWORDS: Japanese encephalitis virus; Genotypes; Prevalence; Risk factors; Swine

1. Introduction

Japanese encephalitis (JE) is a re-emerging mosquito-borne flaviviral zoonotic disease prevalent in large parts of Asia. JE is one of the leading causes of childhood mortality and morbidity in

Significance

Assam state has reported the highest number of human JEV cases in India during the last decade. The present study showed high prevalence of JEV in swine population of Assam. The genotype Ⅲ JEV was identified in swine population with one of the isolates sharing a common clade with human isolates, indicating spillover of infection. Risk factors associated with JEV infection can be utilized by health authorities to implement sentinel surveillance and awareness programs in endemic areas to prevent transmission of virus to humans.

✉To whom correspondence may be addressed. E-mail: hdhanze@yahoo.co.in

How to cite this article: Hussain M, Dhanze H, Mehta D, Kumar MS, Gandham RK, Gupta M, et al. Prevalence and risk factors associated with Japanese encephalitis virus infection in swine population of Assam, India. Asian Pac J Trop Med 2022; 15(11): 503-510.

Article history: Received 29 June 2022 Revision 16 October 2022
Accepted 21 October 2022 Available online 30 November 2022

countries of Southeast Asia and Western Pacific region[1,2]. It is estimated that 67 900 human JE cases occur every year worldwide with 30% case fatality[3] and permanent psychiatric or neurological disorders may occur in 30% of encephalitis cases[4].

Swine play an important role in JEV transmission cycle as they aid in pre-epizootic amplification of the virus owing to the multiple factors including high natural infection rate (98%-100%), high viraemia that remains optimal enough to infect mosquitoes for up to 4 days, vector mosquito propensity to feed on swine and high birth rates[5]. The JE virus (JEV) transmission from swine to humans takes place through the bite of *Culicine* mosquitoes, mainly the members of *Culex vishnui* group, which breed in water pools and flooded rice fields, and bite primarily during the twilight hours[6]. Swine is a good sentinel for forecasting of JE outbreaks as it seroconverts 2-3 weeks before infection occurs in humans[5,7]. The coincidence of JE sero-positivity in swine population with the number of human JE cases reported in a particular region is well known[8].

Assam is a Northeastern state of India and is home to 34.5 million human population, more than 80% of the population is inhabitant of rural areas and 36% falls below poverty line[9]. People are economically dependent on agriculture and rice is grown in two-third of the total cultivated land. Assam has the largest swine population (15.86%) in the country[10,11]. Swine and cattle egrets, found in abundance in rural areas near paddy field ecosystems in Assam play an important role in JE virus transmission[12]. JE is endemic in several parts of India including Assam. The state has reported approximately 46.95% of total JE positive cases in the country during the year 2019[13]. The cases of JE in humans have been appearing in sporadic and epidemic forms in Assam, since 1976. The maximum number of 4 573 human JE cases were reported from Assam state of India, leading to 1 121 deaths during the last decade[13].

The human cases of JE have also been reported from Sonitpur and Barpeta districts of Assam[12,14] albeit the status of JE in swine populations of these districts has not been previously explored. Limited studies have been conducted in other parts of the state to understand the prevalence of JE in the swine population[15,16]. Taking this into account, the current study was envisaged to understand the prevalence and the risk factors associated with JE in domestic pigs (Sus scrofa domesticus) in the Assam state.

2. Materials and methods

2.1. Ethical approval and consent from the of the farm/animal owners

The study protocol was assessed and approved by the Deemed University, ICAR-Indian Veterinary Research Institute (approval No. F.4-6/5925/18-Acad). The field samples were collected by veterinarians adhering to the regulations and guidelines on animal husbandry and welfare, Government of India. The samples were collected after taking consent of the farm/animal owners.

2.2. Study area

The current study was conducted in Barpeta (26°32′N 91°00′E) and Sonitpur (26°63′N 92°8′E) districts of Assam, India during July 2019 to June 2020. The Assam state shares the international borders with Bangladesh and the Kingdom of Bhutan. The climate of state is characterized by heavy monsoon downpours and has four distinct seasons including summer/pre-monsoon (March to May), monsoon (June to September), post monsoon/autumn (October to November), and winter (December to February). The two predominantly rural districts were selected due to the typical epidemiological setting of JEV in these regions which include backyard pig farming and waterlogged paddy fields. Human cases of JE have been reported from these districts[12], but the status of JE infection in swine population(s) remains unknown. As per the Ministry of Health and Family Welfare, Government of India, Barpeta and Sonitpur are part of the JE endemic areas of Assam[14].

2.3. Sample size estimation and collection

The sample size (n=432) was calculated using Epi InfoTM software assuming 17% prevalence[15,16], swine population of 11 blocks of the two districts Barpeta and Sonitpur (n=53 486)[11], 95% confidence level, 5% absolute precision and a design effect of 2. Based on the convenience in terms of willingness of farmers to allow sample collection from their animals, a total of 120 samples were collected from 60 households of 5 blocks of Barpeta and 60 households of 6 blocks of Sonitpur districts. In addition, 312 samples were collected from the block-level (above-mentioned district blocks) backyard slaughter premises. A total of 108 blood samples each were collected during four distinct seasons, as previously described. Overall, 215 samples from Barpeta and 217 samples from Sonitpur were collected. Apprcoximately 2-3 mL blood was collected in serum and blood collection tubes (BD Vacutainer®) from the ear vein of pigs.

2.4. Questionnaire design

A detailed questionnaire was designed to understand the risk factors associated with Japanese encephalitis. The questionnaire

consisted of information relating to farmers including vaccination of children against JEV, history of JE in the family, knowledge about JE; information relating to swine husbandry practices such as total number of pigs owned by the household, distance between the pig barn and other animals in the shed, distance of pig shelter from owner's home, total number of shelters available for the pigs, hygienic condition of the pig barn, water-logging in the area, presence of mosquitoes in the shed, birds seen near the pig barn, and questions related to swine age, sex and the nutritional status. However, the requisite information could only be collected from 120 pig farmers of the selected districts as the information from 312 slaughtered pigs was not available.

2.5. IgG and IgM ELISA

The previously standardized recombinant NS1 protein-based indirect IgG ELISA[17] and IgM ELISA[8] were used to screen the swine serum samples for antibodies against JEV. The recombinant protein (NS1) was purified using available JEV *NS1* gene clones, maintained in the Division of Veterinary Public Health, Indian Veterinary Research Institute. The diagnostic sensitivity and specificity of the ELISA kits was 91% and 97% for IgG ELISA and 95.3% and 98.6% for IgM ELISA, respectively.

2.6. RNA extraction, RT–PCR and Real time RT–PCR

The RNA was extracted from JEV infected Vero cells (positive control) and from the field swine blood samples using the Trizol LS (Invitrogen) reagent as per manufacturer's instructions. A two step RT-PCR assay was used for screening of samples using published primers specific for *NS1* gene[18]. The samples that were positive for *NS1* gene of JEV were further screened using self-designed degenerate primers (Supplementary Table S1) targeting envelope gene of JEV for the purpose of nucleotide sequencing. The reaction mixture and amplification conditions for the degenerate primers targeting envelope gene of JEV were same as that of *NS1* gene. The TaqMan probe-based two-step real time RT-PCR was also used to screen field swine blood samples for JEV and for quantification of viral copy numbers in field samples[19]. The standard curve was prepared using cDNA of JEV and the viral copy number in each positive sample was calculated.

The positive control consisted of GP78 strain of JEV maintained in Vero cell lines in Division of Veterinary Public Health, Indian Veterinary Research Institute, India.

2.7. Sequencing and phylogenetic analysis

RT-PCR products (n=2) of the envelope gene of JEV were sequenced using Sanger sequencing method from Eurofins Genomics, India. The resulting base pair sequences were aligned using BioEdit Sequence Alignment Editor with reference sequences of different JEV genotypes derived from NCBI GenBank database. Phylogenetic analysis of JEV was performed for the envelope gene by comparing with published sequences of JEV isolates to know the genotype of JEV circulating in swine population of Assam, India (Supplementary Table S2). A multiple and pairwise alignment was generated by ClustalW program in MEGA X software and best evolutionary model for estimating genetic distances between sequences was estimated using the 'Models' function. Phylogenetic tree was constructed by neighbor-joining method based on Kimura-2 parameter model.

2.8. Outcome and explanatory variable(s)

The JE positive status of swine was considered as an outcome variable using combination of tests in parallel *viz*., considered positive if positive in either of IgM ELISA, IgG ELISA, RT-PCR or real time RT-PCR. A total of 16 explanatory variables were considered in the present study. These included place and month of sample collection, number of pigs per household, distance of pig shelter(s) from the farmer's home, number of pig shelters in a household, number of pigs in each shelter, number of pig farms in the vicinity, hygienic condition of the farm, water logging in the area, presence of mosquitoes in the shed, presence of ardeid birds near the farm, age and sex of animals, type of pig shelter, presence of cattle, sheep, goat and/or other animals near pig shelter(s).

2.9. Statistical analyses

All statistical analysis was conducted in R statistical program (R statistical package version 3.4.0, R Development Core Team (2015), http://www.r-project.org), except when mentioned otherwise.

To account for clustering, the disease prevalence was estimated using linear mixed models with the variable sampling site (Farm or Slaughter premises) as a fixed effect and district as a random effect. Sampling weights were also applied in the prevalence estimates to account for disproportionate sampling at the district level. The R packages 'Survey', 'Remotes', and 'Syvlme' were used in the analysis. The overall prevalence was calculated after using combination of tests in parallel *viz*., considered positive if positive in

either of IgM ELISA, IgG ELISA, RT-PCR or real time RT-PCR.

The Cohen's Kappa and its 95% confidence intervals were calculated using Epitools epidemiological calculators (http://epitools.ausvet.com.au) to determine the diagnostic agreement between serological and molecular tests.

For risk factor investigation(s), univariate binomial logistic regression models were constructed to understand the association of explanatory variables with the outcome variable and *Chi*-square test was applied. The Fisher's exact test was applied to two explanatory variables (presence of mosquitoes in the shed and type of pig shelter) as conditions of the *Chi*-square test were violated. However, the explanatory variables where Fisher's exact test was applied were also tested during the multivariate logistic regression analysis. Initially, the explanatory variables with a univariate *P* value (likelihood-ratio *Chi*-square or Fisher's exact) of <0.25 and with <10% missing values were included. The model was constructed using forward selection (likelihood ratio), stepwise approach. Variables with a *P* value >0.25 in the univariate analysis were also tested in the final model. The final model included the explanatory variables with a *P* value of <0.05. The explanatory variables in the final model were also tested for biologically important two-way interactions. The variance inflation factors were used to test for multi-collinearity in the final model. Likelihood ratio *Chi*-squared goodness-of-fit statistic and residuals were used to determine adequacy of the final model.

3. Results

3.1. Prevalence of JE infection

The frequency distributions of 432 samples screened in the analysis have been presented in Supplementary Table S3. The JE prevalence estimates are presented in Table 1. Overall, the JE infection prevalence was found to be 51.6% (95% *CI* 50.5-52.6) at farm and 47.1% (95% *CI* 45.4-48.8) at slaughter premises (Table 1). Highest prevalence was recorded using real time RT-PCR (farm premises=38.3%; slaughter premises=39.4%) followed by IgM ELISA (farm premises=19.9%; slaughter premises=12.7%), conventional RT-PCR (farm premises=12.5%; slaughter premises=6.7%) and IgG ELISA (farm premises=5.1%; slaughter premises=5.8%) (Table 1). The diagnostic agreement between serological (IgG and IgM ELISA) and molecular assays (RT-PCR and Real time RT-PCR) was 11.9% (95% *CI* 3.4%-20.4%).

Table 1. Prevalence of Japanese encephalitis virus infection in swine estimated using linear-mixed models after accounting for clustering at the district level and adjusting for sampling (applying sampling weights) in Assam (India), 2019-2020.

Sampling location	Beta	Prevalence	95% *CI*	*P* value
IgG ELISA				
Farm	5.1	5.1	4.0-6.2	Reference
Slaughter premises	0.7	5.8	3.9-7.5	0.07
IgM ELISA				
Farm	19.9	19.9	19.3-20.6	Reference
Slaughter premises	7.2	12.7	11.8-13.6	<0.001*
Conventional RT-PCR^				
Farm	12.5	12.5	11.2-13.8	Reference
Slaughter premises	5.8	6.7	3.7-9.8	<0.001*
Real time RT-PCR				
Farm	38.3	38.3	38.1-38.5	Reference
Slaughter premises	1.1	39.4	38.8-40.1	<0.001*
Overall positive (tests in parallel)				
Farm	51.6	51.6	50.5-52.6	Reference
Slaughter premises	4.5	47.1	45.4-48.8	<0.001*

Sampling weights (district Barpeta=0.097; Sonitpur=0.903). ^Sampling weights were not assigned due to model non-convergence (minimal differences). *P<0.05.

3.2. Infection level and genotypic characterization

The viral copy number ranged from 10 copies to 10^6 copies/reaction, with maximum positive samples (50%) having copy number less than 100/reaction (Table 2) and this might be the reason for less detection rate in conventional RT-PCR. Comparatively high viral copy number was found in samples collected during monsoon and post monsoon seasons as compared to winter and summer seasons (Table 2).

Table 2. Description of season-wise Japanese encephalitis virus positive swine samples with different viral copy numbers.

Viral copy No.	Monsoon	Post monsoon	Winter	Summer	Total positive samples
10^6	3	3	0	0	6
10^5	3	1	0	0	4
10^4	5	3	2	2	12
10^3	6	4	3	1	14
10^2	15	11	8	10	44
10	8	5	41	26	80
Total	40	27	54	39	160

The sequences of the two positive samples were submitted to NCBI GenBank and accession numbers were obtained (MW201807 and MW201808). Phylogenetic analysis of partial sequence of envelope gene of the samples AJE2 and AJE3 revealed that both the sequences belonged to genotype Ⅲ of JEV. The isolates from this study were found to fall into two different clades. The isolate AJE2 (MW201807) shared a common clade with human isolates GP78 (isolated in 1978 in India), GP05 (Isolate from 2005 JE epidemic in Uttar Pradesh, India), and 89P131 (Japanese isolate), while isolate AJE3 (MW201808) belonged to the same clade as that of the strain JEV/SW/IVRI/395A/2014 which has been previously isolated from aborted pig foetus in U.P, India (Figure 1).

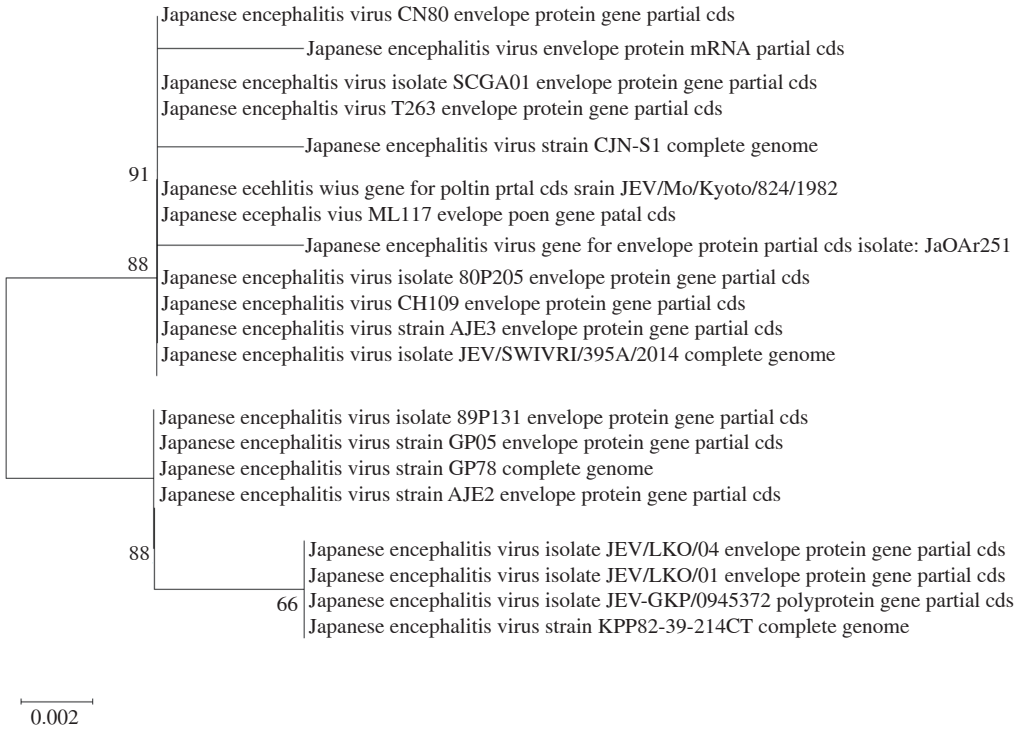

Figure 1. Phylogenetic tree representing nucleotide sequence homology of partial *Envelope* gene sequence of Japanese encephalitis virus isolates.

3.3. Risk factor investigation

The information relating to risk factor investigation was collected for 120 pigs from their respective owners using structured questionnaire when sampled at farm premises. Out of 120 pig owners, only 29% were aware of JE infection whereas vaccination of children against JE was not carried out in either districts of Assam under study. The frequency distribution of 16 explanatory variables has been presented in Supplementary Table S4. The univariate results of the individual animals for the explanatory variables are presented in Supplementary Table S5. The variables including month (monsoon versus post monsoon: *OR* 4.3; 95% *CI* 2.0-9.3; *P*<0.001), hygienic status of the farm (clean versus very dirty; *OR* 119.0; 95% *CI* 6.5-2180.5; *P*<0.001) and water logging in the area (*OR* 14.2; 95% *CI* 5.7-35.6; *P*<0.001) were strongly associated with the JE positive status of a pig (Supplementary Table S5). The final multivariable model at the individual animal level is presented in the Table 3. After adjusting for the other variables, the final multivariable model showed that monsoon season (month of September) was associated with larger odds (Adjusted *OR* 5.6; 95% *CI* 2.1-14.9; *P*<0.001) of pigs being infected with JE virus as compared to the post- monsoon season (month of November). The water logging in the area near the pig shelter was also associated with larger odds (Adjusted *OR* 16.9; 95% *CI* 6.1-47.3; *P*<0.001) of pigs being infected with JE virus as compared to the dry area.

Table 3. Final multivariate model for Japanese encephalitis virus infection status outcome conducted in swine population of Assam (India), 2019-2020.

Parameters	b	SE (b)	Odds ratios	95% *CI*	*P* value
Constant	-2.7	0.5	1		
Month					
November	Reference				<0.001*
September	1.7	0.5	5.7	2.1-14.9	
Water logging in the area					
Absent	Reference				<0.001*
Present	2.8	0.5	16.9	6.1-47.3	

*P<0.05.

4. Discussion

The present study report a high prevalence of JEV infection in swine population of Assam, India. The results can be correlated with the highest number of JE positive human cases (n=962) reported from Assam state of India during the study period of year 2019-2020[13]. The high prevalence of JEV infection in Assam might be attributed to several factors including ecology of the state. The high temperature and humidity during most parts of the year, presence of paddy fields and the prolonged rainy season support the breeding of *Culex* mosquitoes which are involved in the transmission of JEV[20]. The high prevalence of JEV in swine population has been recorded in other Asian countries also. For example, a prevalence of 74.5% (95% *CI* 73.7-86.4) in swine population of ten provinces has been reported from Vietnam[21] whereas 60% prevalence has

been reported in Bali, Indonesia[22]. Sero-surveillance of swine population is an essential component of JE surveillance as the increase or decrease in the level of sero-prevalence in swine helps to indirectly evaluate the likelihood of zoonotic transmission to humans[23,24].

High sero-prevalence was recorded by IgM ELISA (farm premises=19.9%; slaughter premises=12.7%) as compared to IgG ELISA (farm premises=5.1%; slaughter premises=5.8%) indicating the recent infection of JEV in swine population of Assam. A previous study reported 11.3% sero-positivity in swine population of Kamrup, Jorhat, Lakhimpur, and Goalpara districts of Assam[15]. The sero-positivity of 30% was reported in swine population of Bangladesh, which shares the international border with Assam[20].

The highest prevalence was recorded using real time RT-PCR (farm premises=38.3%; slaughter premises=39.4%) as compared to conventional RT-PCR (farm premises=12.5%; slaughter premises=6.7%) which might be due to the low diagnostic sensitivity of the conventional RT-PCR assay. The higher diagnostic sensitivity of real time RT-PCR as compared to conventional RT-PCR for detection of JEV has also been previously reported[18,19]. The maximum positive samples (77.5%) by real time RT-PCR in the present study were having low viral copy number (≤100/reaction) in part explaining for the low detection rate of conventional RT-PCR. Further, low viral copy number might be the reason behind less antibody response as evident from the difference observed in positivity rate of real time RT-PCR and ELISA. Similar observation was recorded by researchers in case of COVID-19 infection; patients who did not sero-convert were having low viral copy numbers[25].

There was a low agreement between serological and molecular assays. This was expected due to the circulation of virus for shorter periods in the blood and antibody kinetics of JEV in swine. The JEV remains in swine blood for 2-4 days followed by appearance of IgM antibodies at day 5 and later switching of the class to IgG antibodies[6,8]. Since swine infected with JEV do not show any clinical signs except abortion and stillbirth in rare cases[26], it becomes imperative to use both serological and molecular tests to assess the true picture of JEV in swine population.

In the present study, high viremia and the associated highest sero-positivity was found during monsoon and post-monsoon seasons although the antibodies against the virus and viral RNA were detected in swine population across all the seasons. Thus, it can be concluded that JE virus transmission from swine to humans in Assam might occur throughout the year with high chances of transmission during monsoon and post-monsoon seasons. A study has reported round the year presence of JE vectors in Assam with peak during monsoon season[16].

The phylogenetic analysis of partial sequence of envelope gene of two positive JEV samples revealed that both belonged to genotype III and were found to fall into two different clades. The positive sample AJE2 (MW201807) shared a common clade with Indian isolates of human origin which reiterates the fact that there is a spillover of virus from swine to humans. Recently, another study in Assam reported three JEV positive samples from swine with all three belonging to genotype III[15].

The current study demonstrated that monsoon season and water logging in the area were associated with large odds of swine being infected with JE virus infection. Several factors such as presence of water logging in paddy fields, close proximity of swine farming to residential areas, high temperature and humidity, and the rainy season support the breeding of *Culex* mosquitoes and are likely to perpetuate the transmission of JE in the state[27]. During monsoon season, flooding of paddy fields provides a suitable environment for proliferation of the mosquito population and an increase in mosquito population is responsible for spread of JE virus infection in human settlements[28]. A study revealed that 55.1% of the reported JE cases in Assam during the years 2000 to 2002 were from families engaged in swine rearing[29]. Surprisingly, only 29% pig owners of Barpeta and Sonitpur districts of Assam were aware of JE infection, while JE vaccination in children was not carried out in these districts though both the districts have reported human cases of JE in the past. The lack of knowledge of JE amongst most of the pig owners underlines the urgent need for awareness campaigns in the Assam. Awareness campaigns such as "Dastak (A knock at the door)" conducted by government in Uttar Pradesh state of India, have been attributed to the steep fall in the number of cases and deaths for both Acute encephalitis syndrome and JE during 2018[30], reinforcing the importance of education and awareness of farming community.

The current study had some limitations. The non-availability of sampling frame did not allow us for random sampling. In addition, proportion to size sampling could not be conducted. However, we constructed linear-mixed models to account for clustering and adjusted for sampling weights.

Overall, the authors reported a high prevalence of JE virus in swine population of Assam state, indicating a significant risk of virus transmission to humans. It is recommended that health authorities of the state must consider implementation of sentinel surveillance of JE virus in swine population along with the routine vaccination of children, generating awareness in the target populations and mosquito control measures to prevent outbreaks of JE in humans.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgments

We are thankful to the Director, ICAR-Indian Veterinary Research Institute, for providing necessary facilities to conduct the study. We wish to thank Dr. Chandana Choudhury Barua, Professor, Department of Pharmacology, College of Veterinary Sciences, AAU, Assam for providing laboratory facilities for preliminary sample processing and storage. The cooperation received from swine owners and butchers during the sample collection is thankfully acknowledged.

Funding

This study was financially supported by the Department of Biotechnology, Government of India, through the research project entitled 'Genomics assisted pathobiology to identify novel targets for diagnosis and therapeutic intervention(s) of Japanese encephalitis and Leptospirosis'.

Authors' contributions

MH: collection of samples and data collection, and laboratory analysis of samples; HD: concept and design of study, manuscript editing, and guarantor; DM: manuscript preparation, and laboratory analysis; MSK: data analysis (bioinformatics part); RKG: designing of primers and acquisition of funds; MG: laboratory analysis of samples; AGB: initial processing of samples and data collection; KPS: statistical analysis; BBS: data analysis to assess the risk factors, and manuscript review.

References

[1] Turtle L, Solomon T. Japanese encephalitis-the prospects for new treatments. *Nat Rev Neurol* 2018; **14**: 298-313.

[2] Kulkarni R, Sapkal GN, Kaushal H, Mourya DT. Japanese encephalitis: A brief review on Indian perspectives. *Open Virol J* 2018; **12**: 121-130.

[3] Campbell GL, Hills SL, Fischer M, Jacobson JA, Hoke CH, Hombach JM, et al. Estimated global incidence of Japanese encephalitis: A systematic review. *Bull World Health Organ* 2011; **89**(10): 766-774.

[4] WHO. Japanese encephalitis vaccines: WHO position paper. *Weekly Epidemiol Record* 2015; **90**: 69-88.

[5]Van den Hurk AF, Ritchie SA, Mackenzie JS. Ecology and geographical expansion of Japanese encephalitis virus. *Annu Rev Entomol* 2009; **54**: 17-35.

[6] Acha PN, Szyfres B. *Japanese encephalitis. Zoonoses and communicable disease common to man and animals.* Vol. III, Chlamydioses, rickettsioses and viroses. 3rd ed. Washington DC: PAHO; 2003, p. 172-176.

[7] Cappelle J, Duong V, Pring L, Kong L, Yakovleff M, Prasetyo DB, et al. Intensive circulation of Japanese encephalitis virus in peri-urban sentinel pigs near Phnom Penh, Cambodia. *PLoS Negl Trop Dis* 2016; **10**(12): e0005149. https://doi.org/10.1371/journal.pntd.0005149.

[8] Dhanze H, Kumar MS, Singh V, Gupta M, Bhilegaonkar KN, Kumar A, et al. Detection of recent infection of Japanese encephalitis virus in swine population using IgM ELISA: A suitable sentinel to predict infection in humans. *J Immunol Methods* 2020; **486**: 112848. doi: https://doi.org/10.1016/j.jim.2020.112848.

[9] India Population. *Assamstat (2017).* [Online]. Available from: www.Indiapopulation2017.in/population-of-assam-2017.html. [Accessed on 30 June 2019].

[10]Borah M, Halim RA. Dynamics of livestock and poultry sector in Assam: A critical analysis. *Econom Affair* 2014; **59**: 773-782.

[11]Department of Animal Husbandry and Dairying, Goverbment of India. *Nineteenth livestock census.* [Online]. Available from: https://epashuhaat.com/?module=live_dashboard&action=surveydashboard. [Accessed on 25 May 2019].

[12]Dutta BS, Bezborah K, Begum S, Pathak M, Biswas S. A retrospective study of acute encephalitis syndrome with special reference to Japanese encephalitis in tertiary care centre, Tezpur, Assam. *J Entomol Zool Stud* 2017; **5**(6): 1231-1235.

[13]NVBDCP (2020). *Japanese encephalitis.* [Online]. Available from: www.nvbdcp.gov.in/je-cd,html. [Accessed on 11 July 2020].

[14]Press Information Bureau (PIB), Govt. of India, 2019. *Japanese encephalitis (JE) situation in Assam.* [Online]. Available from: https://pib.gov.in. [Accessed on 5 July 2019].

[15]Pegu SR, Choudhury M, Rajkhowa S, Kumar D. Molecular characterization and pathological studies of Japanese encephalitis virus in pigs of Assam. *J Entomol Zool Stud* 2019; **7**(1): 874-878.

[16]Baruah A, Hazarika RA, Barman NN, Islam S, Gulati BR. Mosquito abundance and pig seropositivity as a correlate of Japanese encephalitis in human population in Assam, India. *J Vector Borne Dis* 2018; **55**: 291-296.

[17]Dhanze H, Bhilegaonkar KN, Rawat S, Kumar HBC, Kumar A, Gulati BR, et al. Development of recombinant nonstructural 1 protein based indirect enzyme linked immunosorbent assay for sero-surveillance of Japanese encephalitis in swine. *J Virol Methods* 2019; **272**: 113705. doi: https://doi.org/10.1016/j.jviromet.2019.113705.

[18]Dhanze H, Bhilegaonkar KN, Kumar GRM, Thomas P, Kumar HBC, Kumar MS et al. Comparative evaluation of nucleic acid-based assays for detection of Japanese encephalitis virus in swine blood samples. *Arch Virol* 2015; **160**: 1259-1266.

[19]Pantawane PB, Dhanze H, Kumar R, Grace MR, Dudhe NC,

Bhilegaonkar KN. TaqMan real-time RT-PCR assay for detecting Japanese encephalitis virus in swine blood samples and mosquitoes. *Anim Biotechnol* 2019; **30**: 267-272.

[20]Khan SU, Salje H, Hannan A, Islam MA, Bhuyan AAM, Islam MA, et al. Dynamics of Japanese encephalitis virus transmission among pigs in Northwest Bangladesh and the potential impact of pig vaccination. *PLoS Negl Trop Dis* 2014; **8**(9): 3166. doi: https://doi.org/10.1371/journal.pntd.0003166.

[21]Lee HS, Thanh TL, Ly NK, Nguyen-Viet H, Thakur KK, Grace D. Seroprevalence of leptospirosis and Japanese encephalitis in swine in ten provinces of Vietnam. *PLoS One* 2019; **14**(8): e0214701. doi: https://doi.org/10.1371/journal.pone.0214701.

[22]Damayanti PAA, Adi AAAM, Astawa INM, Sudarmaja IM, Kardena IM, Swastika IK. Incidence of Japanese encephalitis among children is associated with the presence of pigs in Bali, Indonesia. *Biomed Pharmacol J* 2017; **10**: 1333-1338.

[23]Duong V, Sorn S, Holl D, Rani M, Deubel V, Buchy P. Evidence of Japanese encephalitis virus infections in swine populations in 8 provinces of Cambodia: Implications for national Japanese encephalitis vaccination policy. *Acta Trop* 2011; **120**: 146-150.

[24]Ruget AS, Beck C, Gabassi A, Trévennec K, Lecollinet S, Chevalier V, et al. Japanese encephalitis circulation pattern in swine of northern Vietnam and consequences for swine's vaccination recommendations. *Transbound Emerg Dis* 2018; **65**: 1485-1492.

[25]Masiá M, Telenti G, Fernández M, García JA, Agulló V, Padilla S, et al. SARS-CoV-2 seroconversion and viral clearance in patients hospitalized with covid-19: Viral load predicts antibody response. *Open Forum Infect Dis* 2021; **8**(2): ofab005. doi: https://doi.org/10.1093/ofid/ofab005.

[26]Guerin B and Pozzi N. Viruses in boar semen: Detection and clinical as well as epidemiological consequences regarding disease transmission by artificial insemination. *Theriogenology* 2005; **63**: 556-572. https://doi.org/10.1016/j.theriogenology.2004.09.030.

[27]Khan SA, Narain K, Handigue R, Dutta P, Mahanta J, Satyanarayana K. Role of some environmental factors in modulating seasonal abundance of potential Japanese encephalitis vectors in Assam, India. *Southeast Asian J Trop Med Public Health* 1996; **27**(2): 382-391.

[28]Sharma J, Baruah MK, Pathak. Epidemiology of Japanese encephalitis cases in Dhemaji district of Assam, India. *South Indian J Bio Sci* 2015; **1**(3): 141-144.

[29]Phukan AC, Borah PK, Mahanta J. Japanese encephalitis in Assam, northeast India. *Southeast Asian J Trop Med Public Health* 2004; **35**: 618-622.

[30]Singh AK, Kharya P, Agarwal V, Singh S, Singh NP, Jain PK. Japanese encephalitis in Uttar Pradesh, India: A situational analysis. *J Family Med Primary Care* 2020; **9**(7): 3716.

Publisher's note