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Research Article

PIG SERO-SURVEY AND FARM LEVEL RISK FACTOR ASSESSMENT FOR JAPANESE ENCEPHALITIS IN NEPAL

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Abstract

The objectives of this cross-sectional study was to determine the seropositivity status of Japanese encephalitis virus (JEV) in pig and ascertain the farm level risk factors in Nepal. Serum samples were collected from 181 pigs in 91 farms of Rupandehi and Kapilbastu districts and tested for antibodies by IgG ELISA. Survey of farmers in each farm was carried out to determine the farm level risk factors. A total of 20 out of 181 (11%) serum samples tested positive for JEV antibodies. There was no statistically significant difference in seropositivity with age ($p>0.05$), sex ($p>0.05$) and breed ($p>0.05$) of pigs. Farm level infection was significantly associated with closeness to rice fields ($p<0.05$), closeness to standing water sources ($p<0.05$), wild bird exposure ($p<0.05$) and mosquito bite to pigs ($p<0.05$). These factors need to be addressed while formulating prevention, control and management strategies of JEV in pig and hence in human.

Keywords: Japanese encephalitis; Japanese encephalitis virus; Pig; Risk factor

Introduction

Japanese encephalitis (JE) is a mosquito borne zoonotic disease caused by an arbovirus of flaviviridae family. It is the single largest cause of viral encephalitis in the world, with annual case reports ranging from 30,000-50,000 (Solomon, 2006). In Nepal, JE has been endemic in southern region since late 1980s. With appearance of JE cases in Kathmandu valley in 1997, cases have been reported from other hill districts and in recent years JE cases are reported even from mountain districts (Partridge *et. al.*, 2007); Impoinvil *et. al.*, 2011). Ardeid wading birds are considered as the primary enzootic hosts of Japanese encephalitis virus (JEV) and *Culex tritaeniorhynchus* is the predominant mosquito vector (Buescher and Scherer, 1959; Philip-Samuel *et. al.*, 2000). Pigs are the major amplifying host and owing to this fact, pig husbandry may increase the risk of human JEV transmission as pigs are often kept in close proximity to households (van den Hurk *et. al.*, 2009). Human and other domestic animals are considered dead-end or incidental hosts as they can acquire JEV but have no role in virus transmission (Buescher and Scherer, 1959). Though JEV is persistent in Nepal since long, the complex transmission dynamics and risk factors of JE are not well understood. So, this cross sectional study was carried out in two JE endemic districts, namely Rupandehi and Kapilbastu, of Nepal in order to estimate the sero-positivity of JEV infection in pigs and to identify the farm level risk factors to pigs.

Materials and methods

Study area

This cross sectional study was conducted from March to September 2013 in two JE endemic districts of Nepal, Rupandehi and Kapilbastu. Two to four VDC's, which were the active pig farming sites, were selected in each district. Within the selected communities pig farms were counted and then 48 pig farms in Rupandehi and 43 pig farms in Kapilbastu districts were selected randomly by lottery after tabulation of all farms.

Serological study

For serological analysis, inclusion criteria was made. If total pig number in farm was equal or less than 3 then only one pig was sampled. If more than 3 pigs were there then, 1 per 3 pigs was sampled. However, no more than 4 pigs were sampled from each farm. Serum samples were collected in month of July. Pigs less than 3 months were excluded. 3-5 ml blood was collected aseptically from ear vein with standard procedure and centrifuged at 3000 rpm for 5 minute to collect serum. Serum samples were transported to National Zoonoses and Food Hygiene Research Center's

laboratory maintaining cold chain for test of JEV antibodies. The IgG ELISA, an enzyme immuno-assay designed to detect the IgG antibody to JEV in the swine serum, was performed according to the standard protocol of manufacturer Wuhan Unibiotest Co. Ltd., China. As per the manufacturer the sensitivity and specificity of the kit are 92 % and 90 % respectively.

Questionnaire survey at farm

A set of questionnaire was formulated to gather information regarding (i) age, sex and breed (exotic or local) of pig and (ii) exposure of pigs in that farm to probable risk factors including mosquito bites, closeness to rice field and stagnant water sources (which are potential mosquito breeding sites) and wild birds exposure that can be source of JEV transmission to farm. Questionnaire was delivered to one representative from each of 48 farms in Rupandehi and 43 farms in Kapilbastu districts.

Statistical analysis

Statistical analysis was done by using SPSS software version 16. The serological status of JEV infection was determined as positive percentage as a whole and the relationship of age, sex, and breed with sero-positivity was determined by using Chi-squared test. Based on test result, each farm was considered as infected/positive if at least one pig sampled is found to be positive on ELISA test and clean if none of the pigs sampled were positive. The farmer's response regarding various probable risk factors was then compared with farm infection status and farm level risk factors were determined by using Chi-squared test or Fisher exact test where appropriate. The statistical significance level used was $P<0.05$.

Results and discussion

A total of 181 sera samples, 103 from Rupandehi and 78 from Kapilbastu districts, were collected from pigs for detection of IgG antibody against JEV infection. Out of 181 serum samples 11% (20/181) were positive to JEV antibodies by ELISA test. The sero-positivity was 9.7% and 12.8% in Rupandehi and Kapilbastu districts respectively. In one previous study, sero-prevalence of JE in pigs was 48.1% in various 16 districts of Nepal which is greater than our study result (Pant, 2006). However, prevalence in pigs of other four districts Sindhupalchowk, Dolakha, Solukhumbu and Kavrepalanchowk was 16.7%, 4%, 6.6% and 44.6% respectively (Thakur *et. al.*, 2012). The variation in results may be due to variation in geographical locations as well as test procedures used and season of blood collection. We collected blood particularly in July considering the fact that human cases generally peak in August (Bist and Shrestha, 2005).

Possible association of factors including age, sex and breed of pigs were also considered in this study for JEV infection. Pigs were grouped into two categories, one from 3 to 6 month and another more than 6 month. On average 10.9% (14/129) of pigs having age group less or equal to 6 months were positive while 11.5% (6/52) pigs of age group more than 6 months were found to be positive. Regarding sex of pigs, 10% (8/80) of males and 11.9% (12/101) females were found positive and considering the breed 12.1% (7/58) of locals and 10.6% (13/123) exotic breed pigs tested positive. However, there was no significant association of seropositivity with district (0.5), breed ($p=0.76$), age ($p=0.89$) and sex ($p=0.68$) (Table 1). Thus, this study suggests that all pigs despite their district, breed, age and sex, are equally exposed and at risk of JEV infection. Thakur *et. al.* (2012) also reported no association of JE virus infection with gender of pigs. The reason for higher infection rate in females and older pigs may be due to the fact that female and older pigs were kept for longer duration in farm and got more exposure time to infected mosquito bite.

Out of 91 farms, 48 in Rupandehi and 43 in Kapilvastu, 22% (20/91) were positive for JEV infection. Regarding farm and closeness to rice field 26.4% (19/72) farms within 1 kilometer were infected with JEV which was higher than farms located at more than 1 kilometer distance from rice field (5.3%, 1/19). Rice fields are the preferred development sites for *Culex tritaeniorhynchus* as well as the main foraging sites for the water birds; which provide an important transmission site for infectious and susceptible birds and mosquitoes to meet (Impoinvil *et. al.*, 2011). In Gorakhpur district and Mandya district of Karnataka of India where irrigated rice agriculture system is extensively developed, occurrence of JE was closely associated with high vector densities either breeding in the rice fields or canal system (Mishra *et. al.*, 1983; Kanojia *et. al.*, 2003). In this study we found significant association of closeness of rice field with farm level infection ($p=0.048$). The infection rate was higher in farms close to the rice field (26.4%) than in farms far from rice fields (5.1%).

Table 1: District, age, sex and breed wise seropositivity of JE in pigs in study areas

Parameters		**ELISA result (%)**		**P value**
		Positive	**Negative**	
District	Rupandehi	10 (9.7)	93 (90.3)	0.5
	Kapilvastu	10 (12.8)	68 (87.2)	
Age	≤ 6 month	14 (10.9)	115 (89.1)	0.89
	> 6 month	6 (11.5)	46 (88.5)	
Sex	Male	8 (10)	72 (90)	0.68
	Female	12 (11.9)	89 (88.1)	
Breed	Local	7 (12.1)	51 (87.9)	0.76
	Exotic	13 (10.6)	110 (89.4)	

Table 2: Farm level JE infection and association with various contextual risk factors

Parameters		Farm JE infection (%)		P value
		Positive	Negative	
Closeness of farm to rice field	≤ 1 Km	19 (26.4)	53 (73.6)	0.048
	> 1Km	1 (5.3)	18 (94.7)	
Closeness of farm to standing water sources	≤ 1 Km	18 (37.5)	48 (62.5)	0.048
	> 1Km	2 (8)	23 (92)	
Mosquito exposure at farm/bite to pigs	Yes	17 (28.3)	43 (71.7)	0.04
	No	3 (9.7)	28 (90.3)	
Wild bird exposure to farm	Yes	15 (31.9)	32 (68.1)	0.018
	No	5 (12.8)	39 (87.2)	

Around 37.5% (18/66) farms located within 1 kilometer and 8% (2/25) farms located more than 1 kilometer distance from stagnant water sources were found to be positive for JEV infection. Besides rice fields other stagnant water sources provide breeding sites for *Culex* mosquitoes which commonly exploit stagnant water with high organic content for development (Buescher and Scherer, 1959; Solomon, 2006). In this study farm level infection was significantly associated with closeness to the stagnant water sources (p=0.048).

Forty-seven farms encountered wild birds and among them 31.9% (15/47) farms were positive for JEV infection while only 12.8% (5/44) of farms where farmers said they don't encounter wild birds were positive. The birds encountered included crow, sparrow, crane, water fowls, wading birds and other wild birds. Viremia and/or sero-conversion to JEV has been observed in over 90 wild and domestic bird species belonging to a number of different avian species. Viremic migratory birds and even bats, especially fruit bats (Megachiroptera), may be involved in distant transport. In considering the expansion of genotype 1 in Asia, the migratory birds were found to have important role in the importation of JEV into new territories [Banerjee *et. al.*, 1984; Nga *et. al.*, 2004). This study also showed farm level infection having statistically significant relationship with wild bird exposure in farm (p=0.018).

Around 28.3% (17/60) of farms where pig farmers had reported they had seen mosquitoes biting pigs were found to be infected compared to 9.7% (3/31) of farms where farmers had not seen mosquitoes biting pigs. Regarding farm infection the closeness of farm to rice fields (p=0.048), closeness to stagnant water sources (p=0.048), wild bird exposure on farm (p=0.018) and mosquito exposure to farm (p=0.04) all were found to be significantly associated (Table 2). Japanese encephalitis virus has been isolated from over 30 species of mosquitoes. However, paddy breeding mosquitoes of the *Culex vishnui* subgroup, particularly *Culex tritaeniorhynchus*, are the major vectors of JEV (van den Hurk *et. al.*, 2009). Wind-blown infected mosquitoes have been suggested for the dispersal of JEV in China and into Australia (Mifune, 1965; Ritchie and Rochester, 2001) . This study showed that mosquito bite exposure to pigs is significantly associated with farm level infection status (p= 0.04). Phukan *et. al.* reported that the practice of paddy cultivation, proximity of houses to water bodies and suitable climatic factors were the most important environmental factors associated with several outbreaks in Northeast India as that provides mosquito breeding sites (Phukan *et. al.*, 2004). This study showed closeness to rice field, proximity to water bodies, exposure to wild birds and exposure to mosquito bites as important environmental risk factors for JEV infection at farm level.

This study showed that pigs were infected with JEV irrespective of study districts, sex, age and breed. We found closeness to rice field, closeness to stagnant water sources, wild bird exposure and mosquito exposures being the risk factors for farm level JEV infection in Nepal. Vaccination of the pigs is found to be effective for controlling this disease in other countries, however, feasibility of vaccination in pigs needs further exploration in resource poor country like Nepal. Since the JEV transmission cycle is very complex and involves multiple aspects of land use pattern, pig husbandry, climate change, human-animal bonding, access and use of health practices, poverty, awareness and preventive measures used etc. further studies and explorations are required for designing efficient prevention and control strategies.

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Conflict of interest: None

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