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Research Article

Swine Sero-Status of Japanese Encephalitis among Kathmandu and Morang Districts of Nepal

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Abstract

Japanese Encephalitis (JE) a vector borne zoonoitc disease caused by arbovirus of Flavivirus and transmitted by Culex tritaeniorhynchus additionally pig acts as amplifying host for the virus. A total 115 swine serum samples 100 pig farmers from each district were tested against Japanese encephalitis virus (JEV) infection. The collected samples were analyzed by using "Porcine Encephalitis B Virus Antibody Rapid Test Kit". Among them 17% samples were positive for JE while 83% samples were negative for JE. Total 18% were positive for JE in Morang district while 115.4% were positive for Kathmandu district. There was no significant difference in prevalence of JE in these two districts ($p>0.05$). In Kathmandu, the prevalence was 15.4% while in Morang it was slightly higher, 18%. 9.5% younger pigs of 3-9 months of age were positive for JE and 20.6% of age above 9 months were positive of JE but were no significant ($p>0.05$). Similarly, 17.9% female pigs and 14.6% male pigs were positive for JE however difference was not significantly different. In case of breed, no association of pigs with seropositivity ($p>0.05$), 7 (13.5%) out of 52 local breed pigs and 12 (19.1%) out of 63 were JE positive. The prevalence rate of JEV was higher in Morang district among study area which might be due to several factors like lack of education in pig farmers, non vaccinated pig population and lack of awareness regarding risk factor of JEV.

Keywords: Japanese encephalitis; japanese encephalitis virus; pigs; kilometre; serum

Introduction

Japanese encephalitis (JE) is a mosquito borne zoonotic disease. It is caused by an arbovirus of Flaviviridae family (Lindenbach and Rice, 2001). First clinical identification was made in 1871 in Japan and previously this disease was known as "summer encephalitis" (Mackenzie *et al.*, 2007). The virus responsible for Japanese encephalitis B (JEB) was re-isolated in 1933 and ultimately characterized in 1934, when it was experimentally inoculated into monkey brain and successfully reproduced the disease (Jani, 2009). The ability of this virus to infect pigs, bovines, dogs and sheep was found in 1954 (Pond *et al.*, 1954). It is now well established that this virus exists in enzootic cycle between mosquitoes and pigs or mosquitoes and ardeid bird's (Gubler, 2007). Culex tritaeniorhynchus is the predominant mosquito vector of JE (Samuel et al., 2000) that becomes active during dawn and dusk (Baik and Joo, 1991) and has average flight range of 1.5 Km (Henrich et al., 2003).

The first epidemic of JEV in Nepal was reported in 1978 from a southern Rupandehi district (Joshi, 1983). In Nepal,

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JE has been endemic in southern region since 1980s. Morang district has long history of JE since the late 1980's. Later JE cases reported from Kathmandu valley in 1997 and in subsequent years became endemic (Patridge et al., 2007; Pant, 2009 and Impoinvil et al., 2011). Pig farming is also increasing not only in these two districts but all over the country due to of its lucrative profit, requiring comparatively small investment, quick return providing nature and being a highly prioritized sector by government as means of poverty alleviation through cooperative approach. Total pig population was 723613 in 1996/97 which is increased by 83% in 20 years and reached to 1328036 by 2017/18. According to the record of 2017/18, the number of pigs in Morang district was 70715 and in Kathmandu valley it was 8665 (MoAC, 2017). It is now well known that JE is firmly established in Morang and Kathmandu districts both with several cases being admitted to different area hospitals each year.

Farming pattern and the closeness or nearness to the risk factors like rice field, stagnant water sources, mosquitoes, wild birds, pig farms etc. play vital role in transmission and existence of Japanese encephalitis. The lacks of prevention practices like vaccination to pigs and human as well as mosquito avoiding practices compounds the risk of JE (Khanal, 2012).

This study is aimed to find out the level of prevalence of JEV in different topographical region (Morang considered as terai district, lies in tropical and Kathmandu as hilly district, lies in temperate region) for further extensive research for JE virus and its genotype which will help for future extension education opportunities in these communities.

Material and Methods

Based on the previous JE outbreak and history of JE, study was carried out in Kathmandu and Morang district of Nepal

Within Kathmandu district four communities were included under this study, viz: Gothatar, Gokarna, Balaju and Jadibuti-Manahara which were the active pig farming sites. In Morang district three pig farming communities were selected for this study, viz: Urlabari, Biratnagar sub-metropolitan city and Madhumalla. These areas were selected based on the discussion with the local district governmental officials and field level veterinarians.

Sampling Procedure

Farm Count in The Study Area

Total pig farms in the four study sites were counted by visiting those areas under the guidance of community leaders and local para-veterinarians. Sample size was then calculated compromised to 100 pig farm families in each district by random proportional sampling method.

Sample Size Determination

To determine the sample size prevalence of Japanese encephalitis as reported by other researchers previously in Nepal were used, average was calculated and by using the Cochran's formula the sample size for this study was determined. Based on previous studies JE positivity was fund to be 47.94%. The appropriate sample size was then calculated at 95% level of significance (Thrusfield, 2002).

$$n = [(Z_\alpha)^2 p_{exp}(1-p_{exp})]/d^2$$

Where;

n = number of samples

Z = Z value at α level of significance

P_{exp} = expected prevalence of JE in pigs indifferent tropical and sub tropical region

d = desired absolute precision

At 95% confidence level and 10% of the desired absolute precision, the estimated sera sample size was 96. Total pig sera samples collected for this study was however, 115.

Inclusion Criteria for Pigs

Generally, age was considered to be more than 3 for easiness in blood collection through ear vein and in order to reduce stress to piglet and get farmers' cooperation. One pig was selected in each group of 3 pigs but if the farm had less than 3 pigs then one was selected.

Blood Collection and Serum Separation

About 5 ml blood was taken from ear vein aseptically with standard method. Immediately after collection of blood, samples were taken into cool box and allowed to clot followed by centrifugation at 3000 rpm for 5 minutes and serum was separated. The serum samples were transported to National Zoonoses and Food Hygiene Research Centre's laboratory for further test maintaining cold chain.

Rapid Test Kit

Rapid test kit was used to find out the prevalence of JE in pig. The rapid test kit used was "Porcine Encephalitis B Virus Antibody Rapid Test Kit" developed by All-biotest Co. Ltd, China. This test kit is based on dot immunogold method to detect swine sera samples for the specific antibody against JEV with high sensitivity (98%) and specificity (92.5%) in less than 2 hours.

The test procedure was:

- Two drops of washing solution was dispended into the reaction well and waited until the liquid be absorbed completely.
- Two drops (or 100 micro liter) of sample was added into the reaction well and waited until the liquid be absorbed completely.
- The red color was gently removed, and two drops of washing solution was dispensed into the

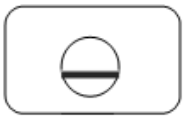
reaction well, and waited until the liquid be absorbed completely.

- Four drops of immunogold solution was dispensed into the reaction well and waited until the liquid was absorbed completely.
- Two drops of washing solution was dispensed into the reaction well and waited until the liquid was absorbed completely. Then the results were studied.

Test Results Interpretation

- ***Negative***

There was wine red color reaction on parallel line, but no color on upright line.



- ***Positive***

Both on parallel line and upright line could be seen wine red color reaction.

- ***Void***

No color reaction on parallel line.

Statistical Analysis

Data collected were entered into SPSS version 16. Chi square test was used to determine the association or non-association of variables and p value less than 0.05 was taken as significant for the study.

Results

Swine Sero-Survey Result

A total of 115 sera samples were collected from pigs for detection of antibody against JEV infection. 50 samples were collected from Morang and 65 from Kathmandu valley. In total 19 samples were found to be positive and remaining 96 were negative. There was no significant difference in prevalence of JE in these two districts ($p>0.05$). In Kathmandu, the prevalence was 15.4% while in Morang it was slightly higher, 18% (Fig.1).

The other animal factors taken under study were age, sex and breed. There was no significant difference in age wise prevalence of JE infection ($p>0.05$). 42 pigs were of age 3 to 9 month. Out of these 42 younger pigs, 9.5% (4) were positive. There were 73 pigs of age more than 9 month and out of them 20.6% (15) were positive (Fig. 2).

No significant relation found between sex of pigs and sero-positivity against JEV infection ($p>0.05$). Out of 67 female 12 (17.9%) were positive and out of 48 male 7 (14.6%) were positive (Fig. 3).

Likewise, Fig.4 shows that there was no association of breed of pig with seropositivity as well ($p>0.05$). There were 52 local breed pigs and among them 7 (13.5%) were positive while out of 63 exotic breed pigs 12 (19.1%) were positive.

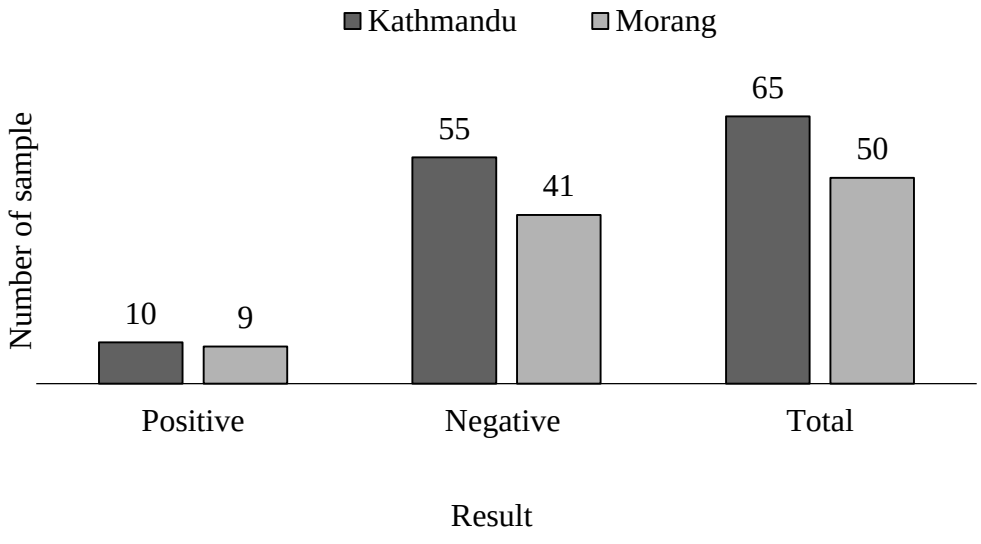


Fig. 1. Swine sero-survey result in Kathmandu and Morang districts

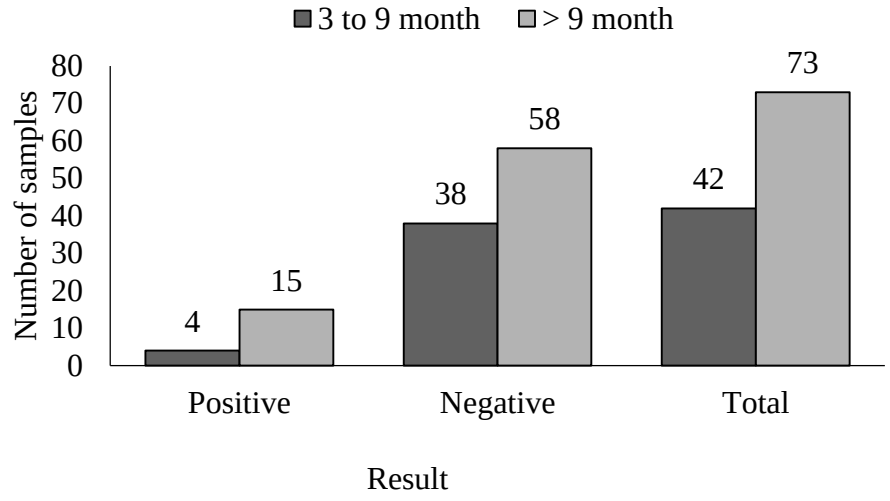


Fig. 2: Age wise JE sero positivity result

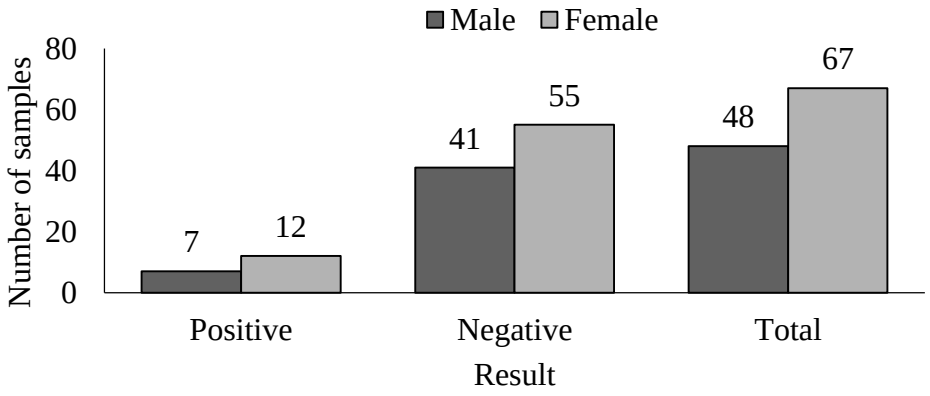


Fig. 3. Sex wise JE sero positivity result

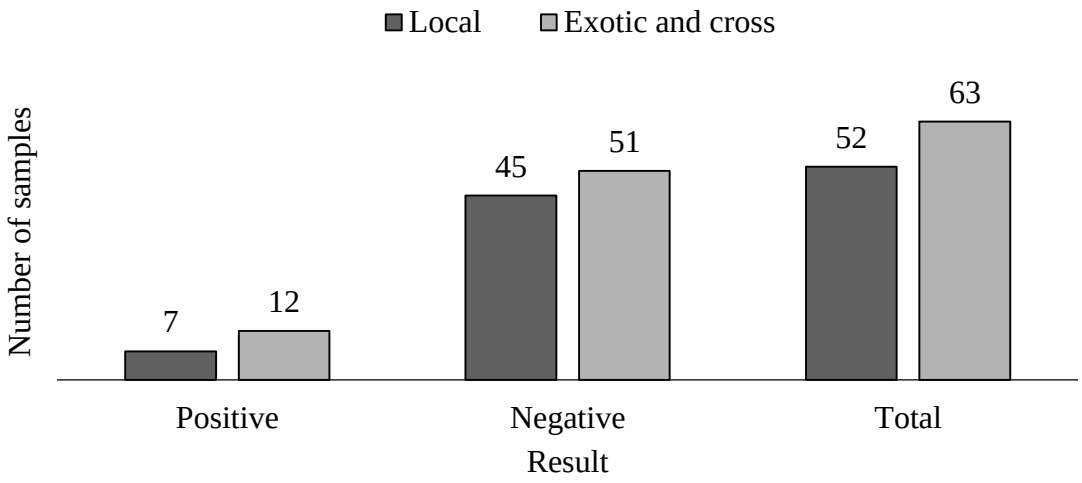


Fig. 4: Breed wise JE sero survey result

Discussion

Comparison of Swine Sero-Status

In total, 19 (16.5%) samples were found to be positive and remaining 96 were negative. There was no significant difference in prevalence of JE in these two districts p>0.05 (figure 1). In Kathmandu, the prevalence was 15.4% while in Morang it was slightly higher, 18%(figure 1). The non-difference indicates how the climate change and other factors are making Kathmandu as well equally vulnerable to mosquito borne diseases like JE.

Seroprevalence of JE in pigs varies considerably across geographic locations. The prevalence rate observed in this study was less than the previous report from various 10

districts of Nepal where 48.11% were positive (Pant, 2006) and another research including 16 districts which showed 55% prevalence (Pant *et al.*, 2006). One cross sectional study conducted from July to August of 2010 in Sindhupalchowk, Dolakha, Solukhumbu and Kavrepalanchowk had shown 16.7% prevalence in Sindhupalchowk, 4% prevalence in Dolakha, 6.6% in Solukhumbu and 44.6% prevalence in Kavrepalanchowk (Thakur *et al.*, 2012). The findings of this study are almost comparable to the findings from other Asian countries like 49% in Bali and 6% in Java of Indonesia (Yamanaka *et al.*, 2010); 33.3% in Tibet (Li *et al.*, 2009) and 4.5% in Ishigaki Island, Japan (Nidaira *et al*., 2009). Study in Rupandehi and Kapilvastu had shown higher prevalence than this study finding. In Rupandehi, out of 55 samples 37 (67.3%) were positive while in Kapilvastu, out of 59 sera 45 (76.3%) were positive (Khanal, 2012). The prevalence of the JE was found considerably different in various locations, and we considered topography and climatic condition could be the factor responsible for this variation. Moreover, the research period and sample collection duration could have made such differences.

Animal factors like age, sex and breed were taken under study. There was no significant difference in age wise prevalence of JE infection ($p>0.05$). 42 pigs were of age 3 to 9 month. Out of these 42 younger pigs, 9.5% (4) were positive. There were 73 pigs of age more than 9 month and out of them 20.6% (15) were positive. Infection rate was higher in older pigs than younger but not statistically significant. In sex also no significant difference was found ($p>0.05$). Out of 67 female 12 (17.9%) were positive and out of 48 male 7 (14.6%) were positive. The infection rate was higher in female. Similar study in Rupandehi and Kapilvastu districts also found no association of age and sex with infection of JEV (Khanal, 2012). Khanal, 2012 found higher prevalence rate (75.5%) in female than in male (67.3%) and similarly the infection rate was higher in older pigs of age more than 1 year (77.4%) than younger pigs (69.9%). Female pigs are kept for longer duration on farm and males are sold earlier. That means females get more exposure time compared to males which might be the reason for higher sero positivity in females. Similarly, older age pigs are exposed for longer duration to mosquito bite hence more likely to get JEV infection. This may be the reason for higher prevalence in older age groups. But the statistical non association suggests that all pigs irrespective of age and sex are vulnerable to JEV infection.

Likewise, there was no association of breed of pig with seropositivity as well ($p>0.05$). There were 52 local breed pigs and among them 7 (13.5%) were positive while out of 63 exotic breed pigs 12 (19.1%) were positive. Thus this study has shown all pigs despite their breed type, age and sex are equally exposed and at risk of JEV infection.

Conclusion

The prevalence rate of JEV was higher in Morang district might be due to due to several factors such as; lack of vaccination knowledge among pig and farmers, unvaccinated pig population, low level of education level the pig farmer in Morang district. Continuous and repeated vaccination practices in pig population might reduce the incidence of JEV in Morang district. Awareness regarding risk factors and vaccination could be best methods in reducing the JEV in both man and pig in both districts.

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