

## Risk Factors Assessment and Detection of Japanese Encephalitis Circulating Antibodies in Pig, Duck, and Human of Chitwan District, Nepal

Dhurba DC<sup>1\*</sup>, Ranjana Ranabhat<sup>2</sup> and Swyam Prakash Shrestha<sup>3</sup>

<sup>1</sup>Institute of Agriculture and Animal Science, Rampur Campus, Nepal

<sup>2</sup>Agriculture and Forestry University, Chitwan, Nepal

<sup>3</sup>Animal Health Research Division, Nepal Agriculture Research Council, Nepal

\*Corresponding Author: Dhurba DC, Institute of Agriculture and Animal Science, Rampur Campus, Chitwan, Nepal.

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### Abstract

A cross-sectional study was carried out from November 2015 to April 2016 in Chitwan district of Nepal. A set of questionnaire was developed, pre-tested in Chitwan district and then survey was carried out. A total of 99 pig blood samples, 102 duck blood sample and 100 human blood samples were collected. JE circulating antibodies IgG in pig and duck and IgM in human were detected from JEV ELISA kit. The data were collected, coded, computed and analyzed by Epi Info 7 and MS- EXCEL 2013. Chi-square and Fisher exact test was used to find out the association of risk factors. The study showed that 8.08% (8/99) of pig serum samples and 5.88% (6/102) of duck serum samples were positive to JE circulating IgG antibodies. In case of human, out of 100 serum samples 100% (100/100) were negative for JE circulating IgM antibodies. There was no significant difference ( $p > 0.05$ ) in the sero-positivity of JE circulating antibodies in pig and duck according to age, sex, breed, proximity of pig/duck farm to rice/paddy field, water sources and exposure to wild birds. Mosquito avoiding practices were significantly associated with occurrence of JE ( $\chi^2 = 9.95$ ;  $p < 0.05$ ).

**Keywords:** Japanese Encephalitis; Antibodies; Risk Factors; Chitwan

### Abbreviations

AHRD: Animal Health Research Division; JE: Japanese Encephalitis; NARC: Nepal Agriculture Research Council; NHRC: Health Research Council

### Introduction

Japanese Encephalitis (JE) is a mosquito borne zoonotic disease caused by an arbovirus of Flaviviridae family [1]. JE virus is an enveloped RNA virus and is antigenically related to St. Louis encephalitis (SLE) virus, Rocio virus, West Nile virus and several other flavivirus [2]. The virus is an enzootic cycle between mosquito and pigs or mosquito and ardeid birds [2]. Pigs are known as important amplifier of the virus [3]. *Culex tritaeniorhynchus* is found in abundance in the rice field ecosystem of the endemic areas during the transmission season. During dawn and dusk *Culex tritaeniorhynchus* become active [4,5] and the average flight range of 1.5 km [6]. *Culex tritaeniorhynchus* prefer rice field [7-10] though it breeds in open sunlit temporary and permanent habitats with vegetation.



tributes such as number and breed of pigs and/or ducks raised, management, proximity to rice fields, standing water etc. (2) farmers exposure to known risk factors such as proximity to rice fields, to pig barns, to duck, standing water etc. (3) specific practices used by the farmer for themselves, their pigs, and their family including vaccination and mosquito avoidance.

**Blood sample collection**

After questionnaire was filled, the number of pigs, duck and human sample were collected from Jugular vein, wing vein and cubital vein respectively. If the total number of pigs was less than or equal to 3 then only one pig was sampled and if the number of pig was more than that 1 pig per 3 pigs were sampled. However, it was made sure that the highest number of pig in one farm was not more than that. It means, if the farm had more than 12 pigs then only 4 were sampled irrespective for the farm size [19]. In case of duck, if the total number of ducks was less than or equal to 3 then only one duck was sampled and if the number of duck was more than that 1 duck per 3 ducks were sampled. However, it was made sure that the highest number of duck in one farm/household was not more than that. It means if the farm had more than 12 ducks then only 4 were sampled irrespective for the farm size.

After the research proposal has been approved by Ethical Clearance Board of Nepal Health Research Council (NHRC), questionnaire was filled and human blood sample were collected from those peoples who reared pig and/or duck and blood sample was taken for this research.

Immediately after collection of blood 3 ml form each pig, duck and human, samples were taken into cool box and allowed to clot follow by centrifugation at 3000 rpm for 5 minutes and the separated serum samples were stored at -20°C. Then the samples were transported by maintaining cold chain to the virology laboratory of AHRD, NARC, Khumaltar, Lalitpur for further test.

**JE ELISA test for pig, duck, and, human sera**

Pig and Duck IgG ELISA was performed according to standard protocol of manufacturer - Shenzhen Lvshiyuan Biotechnology Co., Ltd, China. Human IgM ELISA was performed according to standard protocol of manufacturer 'Standard Diagnostic Inc'.

**Data entry and analysis**

The open ended questions were classified and converted to close ended form, answers coded and entered in to Epi Info 7 and Microsoft excel-2013. Descriptive statistics was used for analysis and frequencies, sum, range, mean etc. were determined, Chi- square and Fisher extract test was used as a test of association with p < 0.05 selected as the level of statistical significance.

**Result**

A total of 100 human serum samples were collected from those who were reared pig and/or duck were tested for JEV IgM captured ELISA and the result was 100% (100/100) negative to JE circulating IgM antibodies.

Demographic profile		Sero-positivity (%)	OR (95% CI)	χ <sup>2</sup> value	P value
Age group	3 - 6 months	1 (2.13%)	1	5.13	0.07
	6 months - 1 yr	3 (10.34%)	5.31 (0.53-53.66)		
	>1 yr	4 (17.39%)	9.68 (1.015-92.38)		
Sex	Female	3 (7.14%)	1	Fisher's exact test	1.00
	Male	5 (8.77%)	0.8 (0.18-3.55)		
Breed	Cross	6 (7.89%)	1	0.02	0.90
	Local	2 (8.70%)	0.9 (0.17-4.79)		
Proximity of farm to rice field	Less than 1 km	6 (23.08%)	1	Fisher's exact test	1.00
	More than 1 km	1 (25.00%)	0.9 (0.08 - 27.45)		
Proximity of farm to water sources	Less than 1 km	6 (23.08%)	1	Fisher's exact test	1.00
	More than 1 km	1 (25.00%)	0.9 (0.08 - 27.45)		
Exposure to wild bird	No	1 (11.11%)	1	4.75	0.1
	Not noticed	1 (10.00%)	0.89 (0.05 - 16.66)		
	Yes	5 (45.45%)	6.66 (0.61 - 73.02)		

*Table 1: Sero-positivity of JE circulating antibodies in Pigs to various demographics.*

Demographic profile		Sero-positivity (%)	OR (95% CI)	$\chi^2$ value	P value
Age group	3 - 6 months	0 (0.00%)	1	3.328	0.189
	6 months - 1 yr	4 (7.50%)	2.3E5 (0.0-1.0E12)		
	> 1 yr	2 (11.76%)	3.8E5 (0.0-1.0E12)		
Sex	Female	3 (5.08%)	1	0.161	0.688
	Male	3 (6.98%)	1.40 (0.27-7.30)		
Breed	Cross	2 (5.88%)	1	Fisher exact test	1.00
	Local	4 (5.88%)	1 (0.17-5.75)		
Proximity of farm to rice field	Less than 1 km	6 (19.35%)	Na	Fisher exact test	0.567
	More than 1 km	0 (0.00%)	Na		
Proximity of farm to water sources	Less than 1 km	6 (20.00%)	Na	Fisher exact test	0.571
	More than 1 km	0 (25.00%)	Na		
Exposure to wild bird	No	1 (7.69%)	1	2.074	0.355
	Not noticed	1 (11.11%)	1.4 (0.08-27.6)		
	Yes	4 (26.67%)	4.4 (0.42-45.25)		

**Table 2:** Sero-positivity of JE circulating antibodies in Ducks to various demographics.

Practices	Positive Farms (%)	OR (95% CI)	$\chi^2$ value	P value
Yes	3 (7.32%)	1	9.95	0.002**
No	10 (38.46%)	0.1263 (0.0306 - 0.5207)		

**Table 3:** Mosquito avoiding practices in pig and duck farms.

\*\**: There was significant association in the mosquito avoiding practices JE infection status in the farms ( $\chi^2 = 9.95, p < 0.05$ ).*

**Discussion**

From all the eco-regions of the Nepal, Japanese encephalitis has been reported. The majority of the JE cases occur in the lowland plains or Terai region of Nepal. Generally, the outbreak of JE started in the 1<sup>st</sup> week of August peaked in the 1<sup>st</sup> week of September and ended in the last week of October [14].

For the diagnosis of JE infection various techniques are available but the present study was ELISA technique that was targeted to detect the Anti-JE IgG antibodies in pigs and ducks and Anti-JE IgM in human serum sample.

Out of 99 pig serum sample 8.08% (8/99) were found to be positive in the porcine encephalitis virus Ab ELISA test kit. Ghimire., *et al.* [19] reported a sero-positivity of JE in pigs was 9.70% and 12.82% in Rupandehi and Kapilbastu district respectively. A sero-prevalence of JE in pigs in Kathmandu and Morang was reported to be 15.40% and 18.00% respectively [20]. However, higher level of sero- prevalence of JE in pig of Rupandehi (67.30%) and Kapilbastu (76.30%) was reported earlier [21] but it has used rapid test kit. Thakur., *et al.* (2012) reported a prevalence of JE in pigs of Sindhupalanchowk (16.70%), Dolakha (4.00%), Solukhumbu (6.6%) and Kavrepalanchowk (44.60%). Sero- prevalence of JE in pigs was 48.11% in various 16 districts of Nepal [13]. These differences in the result could probably be due to the geographical variations, sample collection time, season, mosquito avoiding practices in the past and present and test procedures used.

Ghimire., *et al.* [19] recorded a sero-positivity of 12.06% in local, 10.56% in exotic, 10.85% in pigs belonging to age group less or equal to 6 months, 11.54% for pigs belonging to age group more than 6 months, 10.00% of males and 11.88% of females were found positive

to JEV antibodies. There was no significant association of age, sex and breed with the infection of JEV in pigs in Rupandehi and Kapilbastu districts. According to Nepal, *et al.* (2012), 18.18% in local and 17.65% in exotic were positive to JEV antibodies whereas Chapagain [20] found a sero- prevalence of 13.5% and 19.10% in local and exotic pigs respectively. There was no significant association of age and sex with infection of JEV in pigs of Rupandehi and Kapilbastu districts [21].

Pant [13] had conducted a sero survey for antibody to JEV in Nepal from September to August 2004 and found 26.97% positivity in ducks, which is the higher percentage of positivity than this research.

Ghimire., *et al.* [19] was found that 2.54% (4/157) of suspected human sample collected from Rupandehi and Kapilbastu district hospital were positive to JE circulating IgM antibodies. The results of IgM-capture ELISA demonstrated that serologically confirmed cases among JE suspected cases in 1998 were 7/9 (78%), 13/14 (93%) and 21/30 (70%) in Nepalgunj Medical College Hospital, Bardiya District Hospital and Bheri Zonal Hospital, respectively [22].

In 2002, the age specific deaths showed that 51.8% (87 deaths) of the total deaths occurred in the age group 15 years and below, and 48.2% (81 deaths) in the age group above 15 years. However, in 2003, 44.7% (72 deaths) of the total deaths occurred in the age group 15 years and below, and 55.3% (89 deaths) in the age group above 15 years. On cumulative for 2002 and 2003, 52% of the total deaths occurred in the age group 15 years and below, whereas 48% in the age group above 15 years [11]. These differences in the result could probably be due to the geographical variations, sample collection time, season, mosquito avoiding practices in the past and present and test procedures used. Past researches were based on the hospital based JE suspected sample but this research was based on the community based sample i.e. from the owners of pig and duck so that the result may be different than the results of past research also.

According to Ghimire., *et al.* [19] 26.39% of the farms which are close to rice field (< 1 Km) were positive while 5.26% which were far from the rice fields (> 1 Km) were positive to JEV infection and there was significant difference in the JEV infection status according to proximity of the farm to rice/paddy fields ( $p = 0.048$ ). Water bodies can form breeding site so might be responsible for this [23]. There was significant association of exposure of wild bird with JEV infection ( $p = 0.018$ ) i.e. 31.9% [19].

## Conclusion

A cross-sectional study was carried out in Chitwan district to study on the risk factors associated with JE and JE infection status in pig, duck and human. The sero-positivity rate in pigs was 8.08%, in ducks 5.88%, and in human 0%. There was no significant association of age, sex, breed, proximity of rice/paddy field, proximity of water sources and wild bird exposure to pig and duck farms. There was significant association in JEV infection status and mosquito avoiding practices. Out This study showed that risk of JE occurrence can be minimized through mosquito avoiding practices. The awareness program should focus on correct measures to be adopted to decrease the exposure to mosquitoes.

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