The Effect of Insecticide-Treated Mosquito Nets (ITMNs) on Japanese Encephalitis Virus Seroconversion in Pigs and Humans

Prafulla Dutta,* Siraj A. Khan, Abdul M. Khan, Jani Borah, Chandra K. Sarmah, and Jagadish Mahanta

*Regional Medical Research Centre, Indian Council of Medical Research, Division of Entomology and Filariasis,
North East Region, Dibrugarh, Assam, India*

Abstract. The effect of insecticide-treated mosquito nets (ITMNs) on Japanese Encephalitis (JE) virus seroconversion in pigs and humans was studied in Assam, Northeast India. A sharp reduction of seroconversion rate in human and pig was found in treated localities after intervention. A marked reduction was achieved in humans (risk ratio [RR] = 0.28, 95% confidence interval [CI] = 0.16–0.49) and pigs (RR = 0.21, CI = 0.11–0.40) in the Kollolua locality where ITMNs were used on both humans and pigs compared with the other two areas, Athabari and Rajmai, where ITMNs were covering only either humans or pigs. Monitoring of the mosquito population in and around cattle sheds during dusk revealed no significant decline ($P > 0.05$) of vector density during the post-intervention period in study localities. In spite of the high preponderance of potential JE vector outdoors during the post-intervention period, an encouraging line of defense against circulation of JE virus through the use of ITMNs can be achieved in endemic areas.

INTRODUCTION

Japanese encephalitis (JE) is a viral zoonosis transmitted through vector mosquitoes. Pigs serve as the amplifying host and main source of JE virus (JEV) for the mosquitoes, which, in turn, spills over the infection to man.^{1–6} It is a dreaded disease causing high mortality, particularly in children. JE outbreaks occur largely in rural areas. However, outbreaks have occurred in peri-urban and urban populations in several Asian cities. It occurs when the virus from migratory (ardeid) birds is brought into the peri-domestic environment by mosquito bridge vectors to infect pigs. JE has occurred in most of the Asian countries, such as China, Malaysia, Taiwan, and others; this is attributed to their pork-exporting business, because most of the people are still practicing traditional ways of rearing pigs.

Since the first record of JE case in India in 1956 in the state of Tamil Nadu followed by the isolation of the JE virus from wild-caught mosquitoes in 1956, epidemics of JE have engulfed several states of the country. The northeastern region of India (NE region), particularly the upper part of the state of Assam, has been experiencing recurrent episodes of JE with different magnitudes from July to October every year. An epidemiological analysis of JE cases in Assam during the period from 1980 to 1993 showed an annual case load of 295.5 ± 364.17 and a case fatality ratio of 40.9 ± 10.95 .⁷ Cases occur every year, with progression of the disease to newer areas in recent years.

Insecticide materials, particularly pyrethroids, are gaining importance in mosquito control because of their low mammalian toxicity and appreciable insecticidal and excite-repellent impact on mosquitoes.^{8,9} A high percentage of coverage of malaria-endemic communities with insecticide-treated mosquito nets (ITMNs) is considered to be the most effective way of providing protection for highly malaria-vulnerable children and pregnant women.¹⁰ However, it is not clear if ITMNs would have some impact on reducing transmission of JEV. Many mosquito vectors of JE are dusk biters.^{11–13} Hence, use of mosquito nets by humans alone may not show adequate protection. A study was conducted from 2003 to 2006 that kept pig

and human populations under ITMNs to evaluate the efficacy of ITMNs in reducing the JEV transmission in some highly JE endemic areas of the state of Assam, India, where a considerably high JE virus activity has been reported in earlier studies.^{14,15} In the present study, the findings of seroconversion in humans and pigs in study areas during pre- and post-intervention periods have been analyzed and discussed.

MATERIALS AND METHODS

Study area. Four study localities, Athabari, Rajmai, Kollolua, and Madhupur (each consists of two to three villages), having similar population structure, people with similar lifestyles/habit of rearing of pigs, and a similar type of ecological set up were selected for study in the Dibrugarh District of the State of Assam, India. The selected localities experienced previous occurrence of JE. Interlocality distance was about 20–25 km. In Athabari, only the human population was kept under ITMNs. In Rajmai, only the pig population was kept under ITMNs. Both human and pig populations were kept under ITMNs in Kollolua. In Madhupur, no intervention measures were taken. All the localities selected were surveyed to enumerate the human as well as pig population/number of pigsties, etc. The houses and pigsties were marked. The number and different sizes of mosquito nets required (for human and pigs) were ascertained to distribute in the earmarked localities. The owners of the pigs were advised to keep the pigs strictly under impregnated bed nets at night, and the same areas were monitored throughout the study period (Figure 1A–C). Buying and selling of pigs by the owners or any death of pigs during the study period was monitored.

Impregnation of mosquito nets. Nylon mosquito nets with 156 holes/in² (12 holes horizontally and 13 holes vertically per square inch) were used for insecticide impregnation with a synthetic pyrethroid compound Deltamethrin 2.5% (K-othrine, Bayer Cropscience India Limited, Gujrat, India) emulsifiable concentrate (EC) at a dose of 25 mg/m² according to the technique recommended by World Health Organization (WHO).¹⁶

Distribution of impregnated mosquito nets. Mosquito nets were distributed among the human and pig populations of the specified localities in the month of February 2004 after surveying the population (Table 1). It was made sure that all the

*Address correspondence to Prafulla Dutta, Regional Medical Research Centre, Indian Council of Medical Research, Division of Entomology and Filariasis, North East Region, Post Box No. 105, Pin 786001, Dibrugarh, Assam, India. E-mail: duttaprafulla@yahoo.com

FIGURE 1. (A–C) Different types of pigsties covered with insecticide-treated mosquito nets (ITMNs) in study sites.

family members of the villages as well as the individual pigsties got the ITMNs as per their requirement. Reimpregnation of the nets was done in every 6 months, because earlier studies have shown that the effect of pyrethroid can successfully reduce vector density for up to 6 months.^{17,18} Efficacy is reduced if retreatment with pyrethroid is delayed beyond 6 months.¹⁹ The proper use of ITMNs during the time of sleeping and the advantages of its use were explained to the residents. The proper use of ITMNs by the study population (covering beds as well as pigs) was monitored through unannounced sniff checks during evening hours.

Blood sample collection for serological tests. Blood samples were collected from human and pigs at monthly intervals in all of the four study localities. For pre-intervention study, the collection of samples was done from March 2003 to January 2004. In the post-intervention period, the collection was started in March 2004 and stopped in March 2006. About 5 mL of sample from 20 school-going children (8–15 years old) and the same volume of sample from 8 to 10 pigs (above 6 months old) were collected from each site per survey. School-going children and pigs were screened for the presence of anti-JEV antibody before use as a subject for the study. A cohort of 250 children

and 120 pigs showing negative results was selected in the month of February in each year in four earmarked localities as subjects for subsequent monitoring of JE seroconversion pattern. The individuals to be bled in each month were selected randomly and marked accordingly (in case of pigs, ear clipping was done as a mark of identification) from the selected cohort from respective localities. Necessary ethical clearance was obtained during formulation of the study. Permission from the Government Health Department as well as the school authority was obtained, and information regarding project methodology and objectives was explained in advance. The names of children, their guardians, and the animal owners' names were recorded for further identification. Informed consent was taken from guardians of children and owners of the pigs. The collected blood samples were brought to the laboratory, and the serum of each sample was separated and stored in 2-mL centrifuge vials at 4°C for detection of the JE antibody. Detection of JEV antibody in pig sera was done by hemagglutination inhibition (HAI) test using JE antigen (raised from JE P20778) obtained from the National Institute of Virology (ICMR), Pune, India, using the method of Clarke and Casals.²⁰ Human sera were tested for detection of immunoglobulin M (IgM) antibodies by monoclonal antibody capture (MAC) enzyme-linked immunosorbent assay (ELISA) kits obtained from National Institute of Virology (NIV), Pune, India.

Adult mosquito collection. Adult mosquito collections were conducted at fortnightly intervals from each of the four study localities. One cattle shed housing six to eight cattle in each village was pre-identified for the adult mosquito collection, and collection commenced 30 minutes after sunset and continued for 1 hour. Three entomologically trained technicians were engaged in the collection by suction tubes so as to efficiently collect the maximum number of mosquitoes resting in and around the cattle sheds as well as on the animals in the sheds. The collected mosquitoes were brought to the laboratory in 12 × 12 × 12-cm Barraud cages wrapped in moistened muslin cloth. Mosquitoes were identified to species using taxonomic keys,^{21–23} and the numbers of monthly collected mosquitoes of each species from different localities were entered into SPSS-13. The mean ± standard deviation (SD) of each species was calculated by using SPSS-13.

Statistical analysis. Statistical analysis was done to compare the effects of ITMNs in differently treated localities: (1) seroconversion rate of humans in intervention versus non-intervention localities, (2) seroconversion rate of pigs in intervention versus non-intervention localities, and (3) seroconversion rate of humans and pigs among intervention localities. Rates in 1, 2, and 3 were analyzed using the EPI INFO version 6.1 statistical package to test the statistical significance among the variables under study.

For each locality, the risk ratio (RR) less than 1 indicates a reduction of seroconversion relative to reference locality, and differences are statistically significant when the confidence intervals do not overlap 1. In parentheses are the 95% confidence intervals. The *P* value indicates the significance level for the interaction term. A significant interaction term indicates that the RRs are significantly different between the localities.

The difference in vector density between the pre- and post-intervention period and year-wise difference (between pre-intervention year and first year of intervention as well as between the first and second years of intervention) in human and pig seroconversion rate was tested by using a Student *t* test.

TABLE 1

Pre-intervention baseline data and details of intervention measures from March 2004 to March 2006 in four study localities

Locality	Size of the locality (km ²)	Total no.		Population		No. of ITMNs	
		Houses	Pigsty	Human	Pig	Human	Pig
Only humans covered under ITMNs (Athabari)	1.7	260	48	1,200	286	560	–
Only pigs covered under ITMNs (Rajmai)	1.5	200	42	960	264	–	42
Both humans and pigs covered under ITMNs (Kollolua)	2	300	100	1,300	349	670	100
Non-intervention (Madhupur)	1.8	260	95	1,265	311	–	–

FIGURE 2. Status of monthly sero-positivity percentages in human populations in treated and untreated localities during the pre- and post-intervention periods.

RESULTS

Pre-intervention findings. In 2003, before the initiation of intervention measures, the monthly seroconversion rate was found to be very pronounced in humans and pigs in four study localities. Monthly seroconversion rates in humans in all four localities were in the range of 12.5–80% in humans (Figure 2) and 11.11–60% in pigs (Figure 3). Analysis revealed that, during pre-intervention period, there were no significant differences ($P > 0.05$) in seroconversion in humans (Table 2) and pigs (Table 3) among the localities to be earmarked as intervention and non-intervention.

Similarly, with regards to the entomological observations conducted during the pre-intervention year, presence of both vector and non-vector species of JE virus was documented in all four localities (Table 4). The potential eight JE vector species are *Culex vishnui*, *Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, *Cx. fuscocephala*, *Cx. gelidus*, *Cx. whitmorei*, *Mansonia*

FIGURE 3. Status of monthly sero-positivity percentages in pig populations in treated and untreated localities during the pre- and post-intervention periods.

uniformis, and *Ma. annulifera*, and they were encountered in all the study localities. Of the three members (*Cx. vishnui*, *Cx. tritaeniorhynchus*, and *Cx. pseudovishnui*) of the vishnui subgroup, which is regarded as the main potential JE vector, *Cx. tritaeniorhynchus* (29.33 ± 43.92 to 36.92 ± 22.46) was found to be dominant species in all four localities, and it was followed by *Cx. vishnui* (22.33 ± 24.90 to 29.91 ± 18.98) and *Cx. pseudovishnui* (7.92 ± 7.52 to 13.33 ± 16.50). Among the Mansonioidea, *Ma. uniformis* was dominant (32.16 ± 43.58 to 68.08 ± 38.48) over *Ma. annulifera* (5.17 ± 6.49 to 10.59 ± 5.58). The presence of other vector species *Cx. fuscocephala*, *Cx. gelidus*, and *Cx. whitmorei* was found in similar pattern in all four localities. The preponderance of *Cx. fuscocephala* was more than *Cx. whitmorei* and *Cx. gelidus* (Table 4).

Post-intervention findings. The reduction of seroconversion in treated localities during the post-intervention period was prominent in both humans (Figure 2) and pigs (Figure 3) compared with the pre-intervention period. The results revealed that monthly seroconversion rates in humans were

TABLE 2

Seroconversion of humans during the pre- (2003) and post-intervention periods (2004–2006) against JEV infection in intervention and non-intervention localities

Locality	Pre-intervention		Post-intervention	
	Risk ratio	Significance	Risk ratio	Significance
Only humans covered under ITMNs (Athabari)	0.97 (0.67–1.39)	0.971	0.33 (0.20–0.56)	< 0.001
Only pigs covered under ITMNs (Rajmai)	1.01 (0.70–1.45)	0.922	0.44 (0.28–0.71)	< 0.001
Both humans and pigs under ITMNs (Kollolua)	0.99 (0.69–1.42)	0.957	0.28 (0.16–0.49)	< 0.001
Non-intervention (Madhupur)	1		1	

TABLE 3
JE seroconversion in pigs during the pre- (2003) and post-intervention (2004–2006) periods in intervention and non-intervention localities

Locality	Pre-intervention		Post-intervention	
	Risk ratio	Significance	Risk ratio	Significance
Only humans covered under ITMNs (Athabari)	0.99 (0.62–1.58)	0.971	0.92 (0.64–1.31)	0.635
Only pigs covered under ITMNs (Rajmai)	1.03 (0.62–1.69)	0.922	0.23 (0.12–0.43)	< 0.001
Both humans and pigs under ITMNs (Kollolua)	0.99 (0.62–1.56)	0.957	0.21 (0.11–0.40)	< 0.001
Non-intervention (Madhupur)	1		1	

found to be reduced significantly ($P < 0.05$) in all of the three intervention localities during the first year of the post-intervention period compared with the pre-intervention period. The estimated monthly human seroconversion rate in all of the treated localities decreased significantly more ($P < 0.05$) in the second year than in the first year of intervention. The reduction of pig seroconversion was also significant ($P < 0.05$) in all of the treated localities except in the Athabari locality, where only humans were covered under ITMNs ($P > 0.05$). In Madhupur, the non-intervention locality, no significant difference in monthly seroconversion rates in humans (Figure 2) and pigs (Figure 3) was observed in the post-intervention period compared with the pre-intervention period.

There was a significant reduction in human seroconversion in all of the three intervention localities compared with the non-intervention locality ($P < 0.001$) (Table 2). The RR in the locality (Kollolua) using ITMNs in both human dwellings and pigsties was 0.28 (95% confidence interval [CI] = 0.16–0.49), which is a 72% reduction of seroconversion compared with the non-intervention locality. The Athabari locality, where only humans were covered under ITMNs, and the Rajmai locality, where only pigs were covered under ITMNs, exhibited 67% (RR = 0.33, 95% CI = 0.20–0.56) and 56% (RR = 0.44, 95% CI = 0.28–0.71) reduction, respectively (Table 2).

When the effectiveness of interventions in the reduction of seroconversion in pigs was compared, it was observed that the effectiveness was more prominent in Kollolua (covering both humans and pigs under ITMNs) followed by Rajmai (covering only pigs under ITMNs) and Athabari (covering only humans under ITMNs) (Table 3). JE seroconversion in pigs was 79% (RR = 0.21, CI = 0.11–0.40) lower in Kollolua (covering both humans and pigs under ITMNs) compared with Madhupur, the non-intervention locality. Whereas in Rajmai (only pigs covered under ITMNs) and Athabari (only humans covered under ITMNs), it was 77% (RR = 0.33, CI = 0.12–0.43) and 8% (RR = 0.92, CI = 0.61–1.26), respectively. The differences were statistically significant ($P < 0.001$) in Kollolua, where both humans and pigs were covered under ITMNs, and Rajmai, where only pigs were covered under ITMNs. In Athabari, where only humans were covered with ITMNs, although there was some degree of reduction in seroconversion, it was not significant ($P = 0.635$) during post-intervention period (Table 3).

Comparison of seroconversion among the different intervention measures depicted that the JE seroconversion in humans and pigs was lowest in Kollolua (covering both humans and pigs under ITMNs) than in the other two localities, where only humans (Athabari) or only pigs (Rajmai) were covered under ITMNs (Table 5). JE seroconversion in human was 1.23 times higher in Athabari (covering only humans under ITMNs) and

1.6 times higher in Rajmai (covering only pigs under ITMNs) compared with Kollolua (covering both humans and pigs under ITMNs). The differences were not statistically significant in both Athabari ($P = 0.595$) and Rajmai ($P = 0.141$). The locality (Athabari) where only humans were covered under ITMNs showed significantly higher ($P < 0.001$) seroconversion in pigs (RR = 4.4, CI = 2.28–8.50) compared with the locality (Kollolua) where both humans and pigs were covered under ITMNs. Although the locality (Rajmai) where only pigs were covered under ITMNs showed 1.13 times higher seroconversion in pigs than the locality (Kollolua) where both humans and pigs were covered under ITMNs, the difference was not significant ($P = 0.822$).

Monitoring of entomological data during the post-intervention phase in all four localities indicated the prevalence of eight reported vector species as observed in the pre-intervention period (Table 4). Information in Table 4 was further subjected to a Student *t* test, and the results at the 0.05% level of significance indicated no significant difference ($P > 0.05$) in the mean density of vectors in all of the study localities during the post-intervention period compared with the pre-intervention period.

The benefit in terms of reduction of seroconversion rate in humans (Table 2) and pigs (Table 3) was observed in all of the three intervention localities during the post-intervention period compared with the pre-intervention period.

During surprise checks to verify the proper use of ITMNs over beds at night, it was observed that almost all of the families in the intervention localities accepted the measure of using ITMNs both in human dwellings and pigsties. Initially, in our first visit, some people reported a feeling of suffocation while sleeping under ITMNs, and some of them were found to sleep protruding their head out of the net. However, motivation was effective, and the repetition of the same behavior was not found later.

DISCUSSION

After 2 years of intervention (2004–2006), the study shows that the use of ITMNs can appreciably cut down JEV transmission in a community vulnerable to this disease. The people in the study localities customarily rear pigs for their livelihood. Nearly 80% of the population depends on agriculture for livelihood. Livestock plays a vital role in it, and pig rearing is an important aspect of the livestock sector. Because pigs are considered to be the amplifying hosts of primary importance in the natural cycle for JE virus and are important sources of the virus for transmission to humans,^{4,5,24,25} a pig–mosquito cycle, operating throughout the year, seems to be one of the maintenance mechanisms of the virus in a particular area.²⁶

TABLE 4
Relative prevalence of JE vector species in intervention and non-intervention localities during the pre- and post-intervention periods

Mosquito species	Only humans covered under ITMNs (Athabari)			Only pigs covered under ITMNs (Rajmai)			Both humans and pigs covered under ITMNs (Kollolua)			Non-intervention (Madhapur)		
	Pre-intervention	Post-intervention	P value	Pre-intervention	Post-intervention	P value	Pre-intervention	Post-intervention	P value	Pre-intervention	Post-intervention	P value
<i>Cx. vishnui</i> group												
<i>Cx. tritaeniorhynchus</i>	29.92 ± 4.42	34.22 ± 15.36	0.406	29.33 ± 43.92	31.00 ± 17.45	0.891	36.92 ± 22.46	38.16 ± 18.55	0.868	34.67 ± 38.25	35.16 ± 23.53	0.975
<i>Cx. vishnui</i>	27.50 ± 6.65	31.17 ± 20.93	0.567	22.33 ± 24.90	27.08 ± 14.84	0.568	29.91 ± 18.98	28.39 ± 12.81	0.783	25.00 ± 26.41	32.15 ± 19.13	0.420
<i>Cx. pseudovishnui</i>	12.75 ± 4.49	13.27 ± 7.85	0.811	13.33 ± 16.50	10.67 ± 5.92	0.614	7.92 ± 7.52	10.82 ± 6.23	0.278	12.40 ± 10.33	10.77 ± 5.52	0.974
Mansonia group												
<i>Ma. uniformis</i>	40.33 ± 51.05	35.46 ± 31.82	0.466	32.16 ± 43.58	38.54 ± 39.82	0.379	46.58 ± 72.03	55.10 ± 53.34	0.586	68.08 ± 38.48	73.90 ± 41.19	0.213
<i>Ma. annulifera</i>	6.42 ± 7.79	1.08 ± 0.88	0.463	5.17 ± 6.49	6.25 ± 6.03	0.392	7.42 ± 10.84	8.60 ± 8.06	0.596	10.59 ± 5.58	11.55 ± 0.08	0.172
Other vector species												
<i>Cx. fuscoccephala</i>	23.58 ± 16.27	18.51 ± 13.37	0.261	18.08 ± 31.07	21.63 ± 15.09	0.611	27.08 ± 31.08	32.89 ± 27.68	0.577	27.75 ± 28.54	23.73 ± 11.06	0.679
<i>Cx. whitmorei</i>	5.33 ± 3.20	4.16 ± 2.70	0.173	3.83 ± 4.72	4.12 ± 2.39	0.619	19.83 ± 6.08	18.45 ± 5.63	0.595	5.67 ± 5.67	4.61 ± 2.01	0.589
<i>Cx. gelidus</i>	3.17 ± 2.16	2.08 ± 1.92	0.109	2.74 ± 3.13	2.08 ± 1.74	0.718	16.92 ± 4.25	15.82 ± 3.72	0.585	4.00 ± 3.83	3.32 ± 1.55	0.603

Furthermore, the experimental studies have shown that pigs circulate JEV in titers sufficient to infect mosquito vectors.³ In the present entomological study, eight incriminated vectors responsible for transmission of JE in India²⁷ were detected in the study localities. There was not much difference in outdoor vector density between the pre- and post-intervention period in all four localities. The use of pyrethroid-impregnated nets indoors (houses) or in pigsties had no impact on the reduction of outdoor resting mosquitoes in cattle sheds or surroundings from where the mosquitoes were collected during dusk hours. The cattle were kept equal distances from human dwellings and pigsties. Therefore, this might be the reason for having no significant difference of vector population in and around the cattle sheds, where there was no effect of ITMNs to reduce the vector population. This finding is consistent with the view that pyrethroid-impregnated bed nets have only a small effect on outdoor biting densities of mosquitos.²⁸ Immigration of younger mosquitoes from outside the treated area (pigsty and house) might be another reason for the lack of significant decrease in vector population during the pre- and post-intervention periods. This study reveals that, despite the high preponderance of potential vectors in study localities in the post-intervention period, an encouraging line of defense against circulation of JEV in community through the use of ITMN can be achieved.

The present study has depicted that the risk for transmission of JEV in pig-farming areas can be minimized by implementing the use of deltamethrin (K-Othrine)-treated ITMNs to cover the pigsties properly. This was found to be effective in deterring mosquitoes from pigs, thereby breaking the bridge of contacts with the reservoir, the amplifying host of the JE virus. Rao and Gajanana²⁹ reported that Deltamethrin-treated curtains can reduce 89–100% of indoor light trap collections of the *Cx. vishnui* subgroup, the vectors of JE. Pigs newly introduced into the areas may carry JEV infection, but with proper housing in ITMNs, the incidence of spillover infection to humans will be minimal, and the uninfected pigs will also be protected from getting infected. The current study shows that the lowest risk of JE incidence in humans and pigs was recorded in Kollolua, where both humans and pigs were covered under ITMNs, compared Athabari, where only humans were covered under ITMNs, and Rajmai, where only pigs were covered under ITMNs. This significant reduction of incidence of JEV seroconversion is attributable to the effect of the coverage of ITMNs on both humans and pigs. Although in Rajmai, where the human population was not covered under ITMNs, there was a significant decline of JE seropositivity in humans because of coverage of surrounding pigsties with ITMNs. This probably blocked the spillover of JE infection to humans through mosquito bites. The locality (Kollolua) covering both humans and pigs showed markedly more (72%) protection from JE infection in humans than the locality (Athabari) covering only humans with ITMNs (67% protection) and the locality (Rajmai) covering only pigs under ITMNs (56% protection). Thus, the coverage of pigsties and humans with ITMNs can confer enhanced protection to the communities vulnerable to JEV infection. A previous study in China suggested that the use of pyrethroid-impregnated bed nets significantly reduced the JE incidence in humans in the intervention group compared with the non-intervention group.²⁸ Bed nets impregnated with pyrethroid insecticide greatly decreased the risk of JEV infection among children under 10 years old, as noted in another study carried out in China.³⁰

TABLE 5

Comparison of JE seroconversion in humans and pigs during the post-intervention (2004–2006) period within the intervention localities

Locality	Human		Pig	
	Risk ratio	Significance	Risk ratio	Significance
Only humans covered under ITMNs (Athabari)	1.23 (0.61–2.38)	0.595	4.40 (2.28–8.50)	0.001
Only pigs covered under ITMNs (Rajmai)	1.60 (0.85–3.01)	0.141	1.13 (0.48–2.56)	0.822
Both humans and pigs under ITMNs (Kollolua)	1		1	

The people of JE-prone areas can be motivated through information, education and communication (IEC) activities/awareness campaigns regarding this dreaded disease so that they participate in this program. By using ITMNs for their self-protection as well as the protection of their pigs from mosquito bites, the people of JE-prone areas can efficiently check the JEV transmission in their community.

ITMNs are easy to use devices for preventing mosquito-borne diseases such as JE, malaria, etc. According to the WHO, the use of ITMNs is one of the most cost-effective interventions against malaria.³¹ The absence of reliable surveillance data, difficulties of vaccine production, and shortages and high costs of the commercial vaccine are thought to restrict the implementation of vaccine programs, especially in low-income countries.^{32,33} A number of reports on systemic and neurological adverse effects have also raised concerns over the safety of the commercially available vaccine.^{34–39}

The interventional strategy using ITMNs will have an important epidemiological implication in bringing down the JEV activity in the pig-rearing communities without disturbing their social custom of rearing pigs, which they have been carrying on for ages. As well, this may provide a cost-effective way to reduce JEV transmission and supplement the relatively high cost of vaccines.

Received May 13, 2010. Accepted for publication November 21, 2010.

Acknowledgments: The financial assistance received from the Indian Council of Medical Research (ICMR), New Delhi, India, under the Northeast Initiative Fund for conducting this study is gratefully acknowledged. The cooperation and help received from the State and District Health Authority is also acknowledged with thanks. The authors thank Mr. Niranjana K. Baruah, Mr. Pabitra K. Doloi, Dr. Binanda Saikia, and Mr. Mrinal Bora for their excellent technical assistance received during this study.

Financial support: The project was funded by the Indian Council of Medical Research, New Delhi, India.

Authors' addresses: Prafulla Dutta, Siraj A. Khan, Abdul M. Khan, Jani Borah, Chandra K. Sarmah, and Jagadish Mahanta, Regional Medical Research Centre, Indian Council of Medical Research, North East Region, Dibrugarh, Assam, India, E-mails: duttaprafulla@yahoo.com, sirajkhanicmr@gmail.com, abdulmaboodkhan@gmail.com, drjaniborah@gmail.com, icmrredci@ren.nic.in, and icmrredci@hub.nic.in.

Reprint requests: Prafulla Dutta, Regional Medical Research Centre, Indian Council of Medical Research, North East Region, Post Box No. 105, Pin 786001, Dibrugarh, Assam, India, E-mail: duttaprafulla@yahoo.com.

REFERENCES

- Buescher EL, Scherer WF, 1959. Ecologic studies of Japanese encephalitis virus in Japan. IX. Epidemiologic correlations and conclusions. *Am J Trop Med Hyg* 8: 719–722.
- Hurlbut HS, 1964. The pig mosquito cycle of Japanese encephalitis virus in Taiwan. *J Med Entomol* 1: 301–302.
- Carey DE, Reuben R, Meyers RM, 1969. Japanese encephalitis in Vellore, South India. Part V. Experimental infection and transmission. *Indian J Med Res* 57: 282–289.
- Johnsen DO, Edilman R, Grossman RA, Muangman D, Pomsdhit J, Could DJ, 1974. Studies of Japanese encephalitis virus in Chiangmai valley, Thailand. V. Animal infections. *Am J Epidemiol* 100: 57–68.
- Fukumi H, Hayashi K, Mifune K, Schichijo A, Matsuo S, Omori VS, Oda WT, Mogi M, Mori A, 1975. Ecology of Japanese encephalitis virus in Japan. I. Mosquito and pig infection with the virus in relation to human incidences. *Trop Med* 17: 97–110.
- Rodrigues FM, Soman RS, Guttikar SN, Pinto BD, 1981. Antibody response in ardeid birds following experimental infection with Japanese encephalitis virus. *Indian J Med Res* 73: 667–672.
- Baruah HC, Biswas D, Patgiri DK, Mahanta J, 2002. Clinical outcome and neurological sequelae in serologically confirmed cases of Japanese encephalitis patients in Assam, India. *Indian Pediatr* 39: 1143–1148.
- Hougard JM, Zaim SD, Guillet P, 2002. Bifenthrin: a useful pyrethroid insecticide for treatment of mosquito nets. *J Med Entomol* 39: 526–533.
- Ansari MA, Razdan RK, 2000. Relative efficacy of insecticide treated mosquito nets (Diptera: Culicidae) under field conditions. *J Med Entomol* 37: 201–204.
- Curtis CF, Maxwell CA, Magesa SM, Rwegoshora RT, Wilkes TJ, 2006. Insecticide-treated bed nets for malaria mosquito control. *J Am Mosq Control Assoc* 22: 501–506.
- Bhattacharyya DR, Handique R, Dutta LP, Dutta P, Doloi P, Goswami BK, Sharma CK, Mahanta J, 1994. Host feeding patterns of *Culex vishnui* subgroup of mosquitoes in Dibrugarh district of Assam. *J Commun Dis* 26: 133–138.
- Khan SA, Narain K, Dutta P, Handique R, Srivastava VK, Mahanta J, 1997. Biting behaviour and biting rhythm of potential Japanese encephalitis vectors in Assam. *J Commun Dis* 20: 109–120.
- Dutta P, Khan AM, Khan SA, Mahanta J, 2003. A study on mosquitoes feeding on cattle and their relation to zoonotic disease in Assam. *Indian Vet J* 80: 1225–1229.
- Rodrigues FM, Dandawate CN, 1977. Arthropod-borne viruses in north eastern India: a serological survey of Arunachal Pradesh and northern Assam. *Indian J Med Res* 65: 453–465.
- Dutta P, Khan SA, Khan AM, Mahanta J, 2007. Japanese encephalitis B-virus among the porcine population of Assam. *Indian Vet J* 84: 1125–1127.
- Schreck CE, Self LS, 1985. *Treating Mosquito Nets for Better Protection from Bites and Mosquito Borne Disease*. Geneva, Switzerland: World Health Organization. Unpublished Report, WHO/VCB/85.914.
- Curtis CF, Lines JD, Carnevale P, Robert V, Boudin C, Halna JM, Pazar L, Gazin P, Richard A, Mouchet J, Charwood JD, Graves P M, Hossain MI, Kurihara T, Ichimori K, Li Z, Lu B, Majori G, Sabatinelli G, Coluzzi M, Njunwa KJ, Wilkes TJ, Snow RW, Lindsay SW, 1990. Impregnated bed nets and curtains against malaria mosquitoes. Curtis CF, ed. *Appropriate Technology in Vector Control*. Boca Raton, FL: CRC Press, 5–46.
- Salako LA, 2002. National malaria day malaria awareness walks. Text of an address given on the occasion of the malaria awareness work held in Abuja. *J Mal Afr Trop* 1: 6–7.
- Penelope APH, Bernald LN, Margaret SK, Allen WH, Feiko OTK, Jane AA, John EG, John A, John MV, Amos O, Kachur SP, Erik S, Daniel HR, John DS, Aggery JO, Hawley WA, 2003. Efficacy of Permethrin-treated bed nets in the prevention of mortality in young children in an area of high perennial malaria

- transmission in western Kenya. *Am J Trop Med Hyg* 68: 23–29.
20. Clarke DH, Casals J, 1958. Technique for haemagglutination and haemagglutination inhibition with arthropod-borne viruses. *Am J Trop Med Hyg* 7: 561–573.
 21. Barraud PJ, 1934. *The Fauna of British India Including Ceylon and Burma. Diptera Vol. V. Family Culicidae, Tribes Megarhinini and Culicini*. London, United Kingdom: Taylor and Francis.
 22. Knight KL, Stone A, 1977. *Catalogue of the Mosquitoes of the World (Diptera: Culicidae)*. Maryland: The Thomas Say Foundation, Entomological Society of America.
 23. Reinert JF, 2001. Revised list of abbreviations for genera and subgenera of Culicidae (Diptera) and notes on generic and subgeneric changes. *J Am Mosq Control Assoc* 17: 51–55.
 24. Scherer WF, Moyer JT, Izumi T, Gresser I, McCown J, 1959. Ecologic studies of Japanese encephalitis virus in Japan. VI. Swine infection. *Am J Trop Med Hyg* 8: 698–706.
 25. Rosen L, 1986. The natural history of Japanese encephalitis virus. *Annu Rev Microbiol* 40: 395–414.
 26. Geevarghese G, Shaikh BH, George Jacob P, Bhat HR, 1991. Monitoring Japanese encephalitis virus activity using domestic pigs in Mandya district, Karnataka state (India). *Indian J Med Res* 93: 140–142.
 27. Rodrigues FM, 1984. Epidemiology of Japanese encephalitis in India: a brief overview. Proceeding of National Conference on Japanese Encephalitis, November 3–4, 1982, Indian Council of Medical Research, New Delhi.
 28. Dapeng L, Renguo Y, Jinduo S, Hongru H, Ze W, 1994. The effect of DDT spraying and bed nets impregnated with pyrethroid insecticide on the incidence of Japanese encephalitis virus infection. *Trans R Soc Trop Med Hyg* 88: 629–631.
 29. Rao DR, Gajanana A, 1998. Pyrethroid-impregnated curtains for Japanese encephalitis control. *Curr Sci* 74: 722.
 30. Dapeng L, Konghua Z, Jinduo S, Renguo Y, Hongru H, Baoxiu L, 1994. The protective effect of bed nets impregnated with pyrethroid insecticide and vaccination against Japanese encephalitis. *Trans R Soc Trop Med Hyg* 88: 632–634.
 31. Global Malaria Programme. *Insecticide-Treated Mosquito Nets: A WHO Position Statement*. Available at: <http://www.un.org/millenniumgoals>. Accessed August 26, 2008.
 32. World Health Organization, 1998. Japanese encephalitis vaccine. WHO position paper. *Wkly Epidemiol Rec* 73: 337–344.
 33. Monath TP, 2002. Japanese encephalitis vaccines: current vaccines and future prospects. *Curr Top Microbiol Immunol* 267: 105–138.
 34. Anonymous, 1993. Inactivated Japanese encephalitis virus vaccine. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 42: 1–15.
 35. Nothdurft HD, Jelinek T, Marschang A, Maiwald H, Kapaun A, Loscher T, 1996. Adverse reactions to Japanese encephalitis vaccine in travelers. *J Infect* 32: 119–122.
 36. Plesner AM, Arlien-Soborg P, Herning M, 1996. Neurological complications and Japanese encephalitis vaccination. *Lancet* 348: 202–203.
 37. Takahashi H, Pool V, Tsai TF, Chen RT, 2000. Adverse events after Japanese encephalitis vaccination: review of post-marketing surveillance data from Japan and the United States. The VAERS Working Group. *Vaccine* 18: 2963–2969.
 38. Shlim DR, Solomon T, 2002. Japanese encephalitis vaccine for travelers: exploring the limits of risk. *Clin Infect Dis* 35: 183–188.
 39. Okabe N, 2005. Background of recent JE vaccine issues. *Virus* 55: 303–306.