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Zoonoses and Food Hygiene News, published four times a year, provides a medium for disseminating technical information on matters related to zoonoses and food hygiene generated in the world, particularly in Nepal. The editors welcome submissions on these topics with appropriate illustrations and references. The views and opinions expressed in the News are those of the authors.

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Seroprevalence of Japanese encephalitis in Pig and Human of Morang District

Yanamani Nepal, Durga Datt Joshi, Anita Ale, Santosh Dhakal, Bol Raj Acharya and Minu Sharma

Abstract

A total of 50 pig blood serum samples were collected from 3 different areas of Morang district during the period of September to November, 2011. The serum samples were tested at NZFHRC laboratory by using "Porcine Encephalitis B Virus Antibody Rapid Test Kit". Result showed 9 out of 50 pigs serum samples positive suggesting 18% prevalence of Japanese encephalitis virus infection in pigs of Morang district. At the same period, a total of 10 serum samples of Japanese encephalitis suspected patients were also collected from 3 major hospitals of Morang district. When tested with 'SD Bioline JEV IgG/IgM Rapid Test Kit' followed by 'SD JEV IgM Capture ELISA Test Procedure', one out of ten (10%) human serum samples were found to be positive for Japanese encephalitis antibody.

Keywords: Japanese encephalitis, Japanese encephalitis virus, Morang, Pig

INTRODUCTION

Japanese encephalitis (JE) is a viral disease caused by an enveloped positive single stranded RNA virus belonging to family *Flaviviridae* (Monath *et al.*, 1983). It is a zoonotic disease, infecting mainly animals and incidentally man (Prompiram *et al.*, 2011). It is the leading cause of viral encephalitis in the world, with 30,000–50,000

cases and 10,000 deaths reported annually (Solomon, 2006). It is transmitted in an enzootic cycle involving water birds, domestic pigs, mosquitoes, humans and other non-avian vertebrates. Pigs act as important amplifier of the virus (Nidaira *et al.*, 2009), and birds can also be involved in its amplification and spread. The infection in pigs might show reproductive disorders such as abortion in sow, infertility in boar and neurologic signs in piglets (Ghoshal *et al.*, 2007). Reproductive losses can reach upto 50 – 70% whereas mortality in non-immune, infected piglets can approach to 100%. Most human JE Virus (JEV) infections are asymptomatic or cause a non-specific febrile illness. Less than one percent of JEV infections results in symptomatic neuroinvasive disease (Halstead, 2008).

Japanese encephalitis was first recorded in Nepal in 1978 from Rupandehi district. Total cases admitted in hospitals that year were 422. The highest cases in Rupandehi district (119 cases) was followed by Morang district (100 cases), Parsa district (38 cases), Kapilvastu district (32 cases) and Banke district (32 cases) (Khatri *et al.*, 1981). Since then, JE infection has been reported in animal reservoirs and in humans throughout the Terai region, which borders India (Pant, 2006; Akaiba *et al.*, 2001; Bista and Shrestha, 2005). Morang district lies on the southern part of eastern Nepal, bordering Bihar of India and is an endemic region for JE. From 2005 to 2010, 121 human cases of Japanese encephalitis were reported from Morang (WHO/IPD, 2012).

Data about the seroprevalence in domestic animals, birds and human will help in understanding about status of JE viral infection and developing prevention and control strategies. Therefore, this study was conducted with the objective of determining the prevalence of Japanese encephalitis in pig and suspected human cases of Morang.

MATERIALS AND METHODS

This study was designed as a descriptive cross-sectional study and carried out from September to November, 2011. For pig blood sample collection three different sites namely, Biratnagar, Uurlabari and Indrapur were selected. Biratnagar included whole Biratnagar sub-metropolitan city, Uurlabari included Uurlabari and Madhumalla VDC's whereas, Indrapur included Indrapur and Banigama VDC's. All these VDC's and municipalities are active pig farming areas and had Japanese encephalitis outbreaks recorded by WHO-IPD in the last 5 years. From Uurlabari, Biratnagar and Indrapur, 16, 16 and 18 pig blood samples were randomly collected respectively. All pigs sampled were of age more than 6 month so as to make certain that they were exposed to the JE season of that year. Breed and sex of each pig being sampled was recorded. During sampling, 5 ml blood was collected from the ear vein. Blood was centrifuged at 5000 rpm for 5 minutes and serum was separated. Serum was stored at 4°C and then transported to National Zoonoses and Food Hygiene Research Centre, Kathmandu by maintaining the cold chain. Antibody against porcine Japanese B encephalitis virus in serum were tested by using "Porcine Encephalitis B Virus Antibody Rapid Test Kit" provided by "All-biotest Co; Ltd. China". This test is an immune-chromatographic test, using Dot-immunogold method. This test can detect specific antibody against JEV in less than two hours.

Human serum samples were collected from suspected cases admitted in 3 major hospitals of Morang district namely, Koshi Zonal Hospital, Biratnagar, Nobel Medical College Teaching Hospital and Research Centre, Biratnagar and Neuro Hospital Pvt. Ltd., Biratnagar. Past

history showed that the case flow rate of JE patients were high in these hospitals. A case was defined under the guidance of WHO (2007) as “having febrile illness of variable severity associated with neurological symptoms ranging from headache to aseptic meningitis or encephalitis”. A total of 10 serum samples were collected within the study period. Patient’s personal details including age, sex, address etc. were also recorded. The serum samples were taken to NZFHRC laboratory maintaining cold chain and analyzed by using rapid test kit ‘SD Bioline JEV IgG/IgM Rapid Test Kit’ followed by ‘SD JEV IgM Capture ELISA Test Procedure’ both developed by Standard Diagnostics Inc., Korea.

RESULT AND DISCUSSION

In Pig

Out of 50 serum samples tested 9 were found to be positive for JE antibody suggesting the seroprevalence of JE in pig of Morang district to be 18% (95%CI: 9.15 to 30.47). Regarding the 3 places in Morang district, Biratnagar sub-metropolitan city had higher prevalence of 25% (4 positive out of 16) followed by Urlabari and Indrapur (Table 1).

Table 1: Percentage of pig serum positive for Japanese Encephalitis antibody in Morang district

Place	No. of samples		Prevalence (%)	
	Total	Positive	Point estimate	95%Confidence Interval
Biratnagar	16	4	25.00	8.50 to 49.89
Urlabari	16	3	18.75	5.00 to 43.01
Indrapur	18	3	16.67	4.42 to 38.97
Overall	50	10	20.00	10.63 to 32.76

In those 50 samples, 27 were of female and 23 of male pigs. The prevalence of Japanese encephalitis infection was higher in female (6 positive, 22.22%) but not significantly different ($P = 0.43$) than in male (3 positive, 13.04%).

For breed wise prevalence analysis the breeds were classified into local breeds and exotic breeds. Pakhribas black, Dharane black, Hurra were included within local breeds and Landrace, Yorkshire, Hampshire and their cross were included under exotic breeds. There were 33 samples of local breeds and 17 samples of exotic breeds. This test result showed slightly higher prevalence in local breeds (18.18%, 6 positive) but not significantly different ($P = 0.98$) compared to exotic breeds (17.65%, 3 positive).

We found a higher prevalence in female pigs and in local breeds but more precise estimates to identify overall burden of JEV infection in different pig groups is only possible with a more comprehensive study. The local breed pigs are mainly raised by free range system whereas the exotic breeds are kept in pig pens. The possibility of more exposure to mosquito bite due to free range system might be the reason for high prevalence in local breeds. Pant (2006) had reported 48.11% prevalence in pigs in a study of 212 serum samples obtained from 10 different districts of Nepal including Morang by performing competitive ELISA procedure. The difference may be because of differences in season, study site, sample size and test procedures.

In human

Overall, 10 serum samples of suspected human cases were collected in Morang district during the study period. Five samples were collected from Nobel Medical College, 4 from Koshi Zonal Hospital and 1 from Neuro hospital. Out of 10 serum samples, 4 were of female and 6 were of male. All these samples were first tested by rapid kit, and one sample tested positive. All these samples were then subjected to ELISA testing which also showed positivity for the same

sample that was positive in the rapid test. The positive case was from Biratnagar, male of 20 year age.

CONCLUSION

This study shows persistence of JE infection in swine of different areas of Morang district. The JE confirmation in one human case even within the limited study period is also indicative of the risk associated with JE virus circulation in the district. In spite of limitations with study size, our finding suggests that JEV is circulating in these study are posing a threat to animals and human populations. For developing better JE control strategies in Morang district this kind of study should be carried out in large scale considering other multiple risk factors of Japanese encephalitis as well. Effective awareness generation program should be launched in the district so that people can adopt preventive measures against JE virus infection.

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Prevalence of Cysticercosis in Pigs and Its Impact on Public Health

Amir Sadaula, Shyam Kishor Shah, Bodh Raj Adhikari, Krishna Raj Pandey, Anita Ale, Dhan Kumar Pant and Durga Datt Joshi

ABSTRACT

Introduction - Cysticercosis is one of the important parasitic zoonoses of worldwide occurrence. It results from infection of larval stage of *Taenia solium* known as *Cysticercus cellulosae*; a tapeworm belonging to class cestode. In many developing countries *Taenia solium* cysticercosis, classified as neglected tropical disease by the WHO, is an important zoonotic disease of man and pigs and most common in countries with poor sanitation and traditional pig-production systems. The life cycle of the pork tapeworm is maintained by poverty, lack of hygiene and insufficient knowledge of the associated risk factors. Due to increased migration and tourism, its importance has again increased in more developed countries (Flisser *et al.*, 2006). This study was conducted to estimate the prevalence of *Taenia solium* cysticercosis in swine and cysticercosis in slaughter house workers in Chitwan and its impact on public health.

Methodology:

A cross-sectional study with serum samples from 100 pigs from Chitwan district and 20 human samples from slaughter house workers were included in this study. Fresh blood samples were collected and centrifuged at 10,000rpm for 15 minutes and stored in -20°C in NAL. The serum samples were then tested for *Taenia solium* cysticercosis antibodies by serological test sandwich Ag-ELISA in National Zoonoses and Food Hygiene Research Centre (NZFHRC).

Results

Out of 100 slaughtered pigs serum samples 30 (30%, 95% Confidence interval: 21.63% to 39.51%) were found positive for *Taenia solium* cysticercosis. Similarly, 14 out of 20 human serum samples (70%, 95% Confidence interval: 47.72% to 86.84%) of slaughter house workers were found seropositive for *Taenia solium* cysticercosis.

Conclusion - This study has shown that the prevalence of porcine cysticercosis is higher and cysticercosis in slaughter house workers is also high. This is due to the fact that more number of pigs were raised in poorly sanitized condition and lack of proper deworming and lack of proper disposal of swine feces. Prevalence of cysticercosis is very high in slaughter house workers because they are working in poor sanitary condition. The consumption of such raw and uncooked pork meat leads to zoonotic threat.

Key words: Cysticercosis, Ag-ELISA, Swine, Zoonoses

Seroprevalence of *Toxoplasma gondii* in Pregnant Women and Slaughtered Pigs of Chitwan District

Krishna Datt Bhatt, Durga Datt Joshi, Anita Ale, Santosh Dhakal, Yogendra Shah and Dinesh Kumar Singh

Abstract

Introduction: Toxoplasmosis in human and food animals is a worldwide problem. It is an important cause of abortion and neonatal deaths in sheep, goats and pigs and produces wide range of clinical disorders in human including abortion. Humans become infected with *T. gondii* usually by ingesting oocysts in food and water contaminated by cat feces or by consuming tissue cysts in undercooked meat or even through placental transmission (Dubey and Beattie, 1988). Pig meat is considered as one of the principal

meat source of human infection (Dubey, 1986). Closeness with pet animals like cat, increasing pig husbandry, lack of proper antihelminthic treatment practices in animals, habit of eating raw or undercooked meat, lack of awareness etc. all increase the risk of *T. gondii* infection in Nepal. This study was conducted with an objective to determine the seroprevalence of *Toxoplasma gondii* infection in pregnant women and slaughtered pigs of Chitwan district and find their association with other risk factors.

Methodology:

Design: Cross-sectional study. **Sample collection and survey:** Serum samples of 47 pregnant women were collected from 5 different health posts and serum samples of 79 slaughtered pigs were collected from different slaughter houses of Chitwan. Serological examination was done for IgG antibodies against *T. gondii* using ELISA kit developed by DSI, Italy at NZFHRC laboratory. Questionnaire survey was also carried out among the pregnant women for exposure to various risk factors.

Results: Result showed 53.19 % serum samples of pregnant women and 1.27% of slaughtered pigs were found positive for *T. gondii* infection. 65.96% of the interviewed people had a history of consumption of raw or undercooked meat and 85.1% had contact with cats. Of various risk factors surveyed for, playing habit with cat was only found statistically significant ($p < 0.05$, crude OR = 4.327).

Conclusion: The probable reason for low seroprevalence (1.27%) in pig may be due to indoor pig farming system practiced in Chitwan. Low prevalence in pig however, does not mean that there is no risk associated with consumption of raw or undercooked meat. The difference between prevalence rates in the pregnant women and pigs suggests for exposure to other sources of infection to be considered. Pregnant women probably acquired the infection from their association with cats. Further studies should be focused in future on determining various risk factors associated with *Toxoplasma gondii* transmission. The high seropositivity (53.19%) in pregnant women calls for awareness generation program to be launched in Chitwan district with special emphasis to pregnant women.

Key words: Sero-prevalence, *Toxoplasma gondii*, Risk factors, Nepal.

Serological test of pig and human serum for JEV infection in Kathmandu and Morang district

Yogendra Shah, Kabita Shahi, Indira Mainali & Durga Datt Joshi

From August 2011 to March 2012, a total of 80 pig blood samples were collected from Morang and Kathmandu districts. Pig blood was collected from ear vein puncture method. The blood samples were collected in sterile, clean, dry and labeled test tube and transported to NZFHRC Laboratory. Samples were tested by rapid detection test (RDT) using "Porcine Encephalitis B Virus antibody Rapid test kit (All-biotest Co; Ltd China)". Out of 80 samples processed, 9 were positive for JEV antibody. The prevalence of Japanese encephalitis in pig was thus 11.25 %.

In total 10 and 6 human samples were collected from the Morang district and Kathmandu district respectively. Blood samples (5 ml from adult and 3 ml from children) were collected from vein puncture method from those suspected patients having high fever with clinical symptoms suggestive of JEV infection as per World Health Organization, 1997. The serum were separated and transported to NZFHRC Laboratory for test. Patients' personal details about the symptoms and age, sex etc. were obtained through a questionnaire method by direct interview. Test was done firstly by screening with "SD JEV IgM/IgG Rapid detection test". Then confirmation laboratory test was performed by "JEV IgM capture Elisa Kit

(Standard Diagnostics Inc, Korea)". Out of 16 serum samples tested for JEV infection, one was found to be positive in both rapid test and ELISA test suggesting the sero-positivity of 6.25% samples tested.

JE project activities reported by NZFHRC

- ☞ **Entomological survey completed:** An entomological survey team of NZFHRC completed early monsoon season mosquito sampling from all four districts – Kathmandu, Morang, Rupandehi and Kapilbastu of Nepal where JE project has been launched.
- ☞ **Stakeholders meeting and syringe distribution in Kathmandu:** Considering the impending JE season in Kathmandu district a one day meeting was arranged on 8th June 2012 at NZFHRC office for clinic in-charge of Kathmandu metropolitan city. The clinic heads were made aware of Japanese encephalitis and syringe and test tubes were distributed for sample collection from suspected cases of JE.
- ☞ **Spatial Analysis and Geographical Information System (GIS) Workshop and Organized by NZFHRC:** Prof. Craig Stephen and Robert Collins from Canada visited NZFHRC on June. During their visit discussion on geographical study and future workplan of JE project was carried out. Two days workshop on ‘spatial analysis and GIS’ was also organized by NZFHRC at Hotel Orchid, Tripureshwor, Nepal. Human and veterinary doctors, sociologists, microbiologists, mid and young career researchers and NZFHRC staffs participated in that workshop. Robert Collin from Canada was the resource person of this workshop.

NEWS

ECOHEALTH 2012: Sustaining Ecosystems and Supporting Health in Kunming, China October 15-18, 2012:

Dr. Durga Datt Joshi, Executive Chairman, Ms. Minu Sharma, Programme Officer and Mr. Bipin Bista, Young Research Career of NZFHRC will be participating on the "ECOHEALTH 2012: Sustaining Ecosystems and Supporting Health" which is going to be held in Kunming, China October 15-18, 2012.

Dr. Joshi and Ms. Sharma will present following papers:

- Ecosystem Approach to Japanese Encephalitis (JE) Control in Nepal – Dr. D. D. Joshi
- Knowledge, attitude and risk factors associated with JE among pig farmers in Kathmandu, Nepal – Ms. Minu Sharma

Eco Health Alliance

Dr. Durga Datt Joshi, Executive Chairman will be participating in the meeting on "Eco Health Alliance". The meeting is being co-hosted by the Public Health Foundation of India and Eco Health Alliance. These two-day meeting will be held at the Hotel Suryaa in New Delhi, India, on July 31 and August 1st, 2012 and is funded by the Rockefeller Foundation.

The ultimate goal of this meeting is to identify strategic pathways for a sustainable ‘One Health’ Alliance of South Asia – a functional network of scientists and policy makers in South Asia across all three sectors relevant to health: human, livestock, and wildlife that will facilitate cooperation and serve as a resource for zoonotic disease control and prevention.

K.D.M.A. Research Award:

Please kindly submit your research work paper on allergy award for the year 2011 for the consideration by the end of December 2012 to KDMART office Chagal, G.P.O. Box 1885, Kathmandu, Nepal, Phone: 4270667, 4274928 and Fax 4272694. This award was established by Dr. D.D. Joshi in 2049 B.S. (1992) on the memory of his wife, the late Mrs. Kaushilya Devi Joshi. The award includes a grant of NCRs. 10,001 with certificate.

***From: Zoonoses& Food Hygiene News, NZFHRC
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