

# Zoonoses and Food Hygiene News

Vol. 20 No. 2 April to June 2014

ISSN: 2091-0932 (Print), 2091-0940 (Online)

Government of Nepal, Registration Number: 148/049/050

***This Issue has been Supported by International Development Research Centre (IDRC), Ottawa, Canada***

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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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## **SERO-PREVALENCE OF *Toxoplasma gondii* IN PREGNANT WOMEN OF BHAKTAPUR DISTRICT**

*Alina Prajapati, Ranjana Gupta & Durga Datt Joshi*

### **ABSTRACT**

Toxoplasmosis is one of the diseases of veterinary and medical importance caused by an obligate intracellular protozoan parasite *Toxoplasma gondii*. There is scarce information about the epidemiology of *T. gondii* infection in pregnant women in different regions of Nepal. Therefore, this study aimed at determining the sero-prevalence of *T. gondii* among pregnant women in Bhaktapur district. A total of 50 blood serum samples of pregnant women attending prenatal check up in Bhaktapur Hospital were collected and structured questionnaire were administered. All the collected serum specimens were tested for IgG anti-*T. gondii* antibodies by enzyme-linked immunosorbent assay (ELISA). The overall sero-prevalence of toxoplasmosis in the study area was 22% among pregnant women. No significant association were observed between anti-*T. gondii* IgG antibodies and any of the possible risk factors viz, cat ownership, playing habit with cats, working in garden, raw and pork meat consumption, drinking untreated water, age group, level of education and occupation during study period.

**Key words:** ELISA, Epidemiology, IgG antibodies, Risk factors, Toxoplasmosis

### **INTRODUCTION**

Toxoplasmosis is a zoonotic disease commonly transmitted to human and other mammals by ingestion of food or water contaminated with

oocysts shed by cats, by eating undercooked or raw meat containing infective tissue cysts, and via transplacental transmission (Dumetre 2003 and Dubey 1988). It has a wide geographical distribution infecting up to one third of global population and a wide range of other warm blooded animals including pigs and avian species (Sukthana 2006 and Miller et al. 1972). Nearly half of the Nepalese people are estimated to be *Toxoplasma* seropositive (Rai et al. 2011). Among meat animals, pigs showed the highest prevalence i.e. 79.6% (Rai et al. 1996) indicating pork as one of the potential source of infection.

*T. gondii* infection in immunocompetent individuals is usually asymptomatic but can cause severe effect in immuno-compromised persons such as AIDS patients and congenitally infected individuals. It usually leads to a wide variety of manifestations in the fetuses and infants including mental retardation, seizures, blindness, spontaneous abortion, and still birth (Jones et al. 2001). Studies on epidemiology of *T. gondii* in pregnant women from Bhaktapur district are lacking. Therefore, present study sought to determine the sero-prevalence and assess some risk factors associated with *Toxoplasma gondii* amongst pregnant women of the district during the study period.

### **MATERIALS AND METHODS**

A cross-sectional study was conducted to determine the sero-prevalence of toxoplasmosis and associated risk factors in pregnant women of Bhaktapur district. A total of 50 blood samples were collected from pregnant women in this study from June to July, 2012 and brought to the laboratory of National Zoonoses and Food Hygiene Research Centre, Kathmandu for ELISA testing. The serum was separated from the whole blood by centrifugation at 3000 rpm for 30 minutes. The serum samples were tested for IgG anti-*T. gondii* antibodies using commercially available "DS-EIA-ANTI-TOXO-G-FAST" ELISA kit (DSI S.r.l, Saronno, Italy) following the manufacturer's instructions. Data obtained were then analyzed using SPSS software (version 16.0). Structured questionnaire was prepared and administered among all the pregnant women along with the blood sample collection.

### **RESULTS AND DISCUSSION**

#### **Sero-prevalence of *T. gondii***

The overall sero-prevalence of anti-*T. gondii* IgG antibodies among the pregnant women in Bhaktapur district was 22% (See table no. 1). Similar type of study was conducted in Nepalese pregnant women and overall prevalence was 55.4% (Rai et al. 1998).

**Table no. 1: Overall sero-prevalence of toxoplasmosis**

Species	No. of samples tested	No. of positive samples	Prevalence %
Pregnant women	50	11	22

#### **Socio-demographic description of the study population**

For age wise distribution, 68% pregnant women were in the range of 15-25 years. Majority of them (78%) were house wives and 20% were illiterate. Forty eight percent of the pregnant women were

within the second trimester. Majority of the pig farmers and pregnant women did not know about toxoplasmosis.

**Table no. 2: Distribution of *T. gondii* in pregnant women on basis of demographic characteristics**

Demographic characteristics	Sero-prevalence		Total no. (%)
	Positive no. (%)	Negative no. %	
<b>Age group</b>			
• 15-25 year	10 (29.41)	24 (70.59)	34 (68)
• 26-35 year	1 (6.25)	15 (93.75)	16 (32)
<b>Religion</b>			
• Hindu	9 (20.45)	35 (79.55)	44 (86.27)
• Buddhist	1 (20)	4 (80)	5 (9.81)
• Christian	1 (100)	0	1 (1.96)
<b>Occupation</b>			
• Housewife	8 (20.51)	31 (79.49)	39 (78)
• Office	2 (40)	3 (60)	5 (10)
• Student	0	1 (100)	1 (2)
• Business	1 (20)	4 (80)	5 (10)
<b>Level of education</b>			
• Illiterate	2 (20)	8 (80)	10 (20)
• Primary Level	1 (33.33)	2 (66.67)	3 (6)
• Secondary level	6 (21.43)	22 (78.57)	28 (58)
• More	0	9 (100)	9 (18)
<b>Gestational age</b>			
• First trimester	1 (8.33)	11 (91.67)	12 (24)
• Second trimester	5 (20.83)	19 (79.17)	24 (48)
• Third trimester	5 (35.71)	9 (64.29)	14 (28)
<b>No. of pregnancy (Parity)</b>			
• One	7 (25.9)	20 (74.1)	27 (54)
• Two	3 (15.8)	16 (84.2)	19 (38)
• > than two	1 (25)	3 (75)	4 (8)

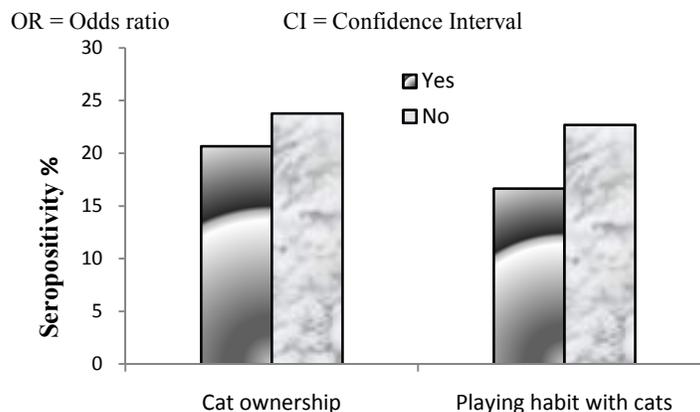
### Factors associated with seropositivity

In the bivariate analysis, various possible risk factors were analyzed with *T. gondii* infection viz, cat ownership, playing habit with cats, working in garden, raw and pork meat consumption, drinking untreated water, age group, level of education and occupation. Among 50 pregnant women who participated in the study, 12% had habit of playing with cat, 20% used to eat pork, 26% were found to have a habit of eating raw/undercooked meat, 90% found to drink untreated water and 54% had reported to work in garden. But, in present study, no significant relations were observed between anti-*T. gondii* IgG antibodies and any of these possible risk factors.

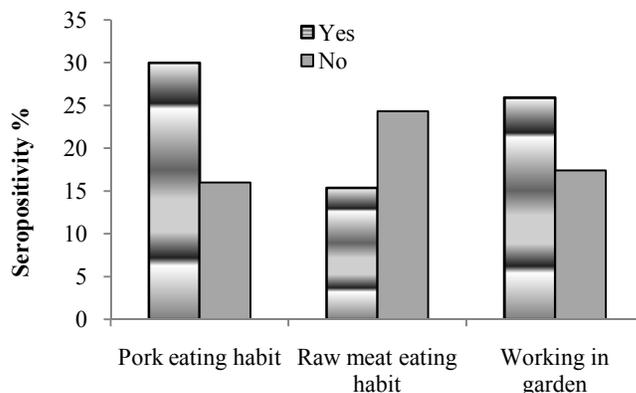
**Table no. 3: Factors associated with *Toxoplasma gondii* infection among the pregnant women (n=50) in Bhaktapur district.**

Characters	Sero-prevalence		P value
	Positive no. (%)	Negative no. (%)	
<b>Cat ownership</b>			
Yes	6 (20.69)	23 (79.31)	0.7927
No	5 (23.81)	16 (76.19)	
<b>Playing habit with cats</b>			
Yes	1 (16.67)	5 (83.33)	0.604
No	10 (22.73)	34 (77.27)	
<b>Working in garden</b>			
Yes	7 (25.93)	20 (70.07)	0.353
No	4 (17.39)	19 (82.61)	
<b>Raw meat eating habit</b>			
Yes	2 (15.38)	11 (84.62)	0.404
No	9 (24.32)	28 (75.68)	
<b>Consumption of pork</b>			

Yes	3 (30)	7 (70)	0.382
No	8 (16)	32 (64)	
<b>Drinking water</b>			
Treated	1 (20)	4 (80)	0.699
Untreated	10 (22.2)	35 (77.8)	
<b>Age group</b>			
15-25 year	10 (29.41)	24 (70.59)	0.064
26-35 year	1 (6.25)	15 (93.75)	
<b>Level of education</b>			
Illiterate	2 (20)	8 (80)	0.618
Literate	9 (22.5)	31 (77.5)	
<b>Occupation</b>			
Housewives	9 (22.5)	31 (77.5)	0.618
Others	2 (20)	8 (80)	



**Fig1. Toxoplasmosis based on cat ownership and playing habit with cats**



**Fig2. Distribution of *T. gondii* infection in pregnant women with different characters**

### CONCLUSION

The prevalence among pregnant women was lower compared to those reported in other regions of Nepal. Prevalence was observed higher in the age group 15-25 year indicating that most of the infections occur during the early life.

### ACKNOWLEDGMENTS

Authors are heartily indebted to National Zoonoses and Food Hygiene Research Centre (NZFHRC) for providing guidance and lab facilities. Thanks to Dr. Bimal Prasad Dhakal, Medical Superintendent, Bhaktapur Hospital for granting permission to do the research work.

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### Serological diagnosis of Human serum samples for Japanese Encephalitis in Nepal By Rapid Detection Test: A short communication

National Zoonoses and Food Hygiene Research Centre (NZFHRC)

Japanese Encephalitis (JE) is a mosquito borne flavivirus infection of humans as well as swine, horse and other domestic animals. It is a leading cause of childhood encephalitis in Southeast Asia and annually a remarkable number of deaths due to the same are recorded worldwide (WHO 1998). JE virus is the most important cause of endemic encephalitis worldwide, with an estimated 35000 to 50000 cases and 10000 to 15000 deaths annually (Solomon, 2003). The number of cases have been continuously increasing in South East Asia and in Indian Sub continent among people living in poor socio-economic condition and sanitation. Due to the high case fatality rate and frequent residual neuropsychiatric sequelae in the survivors, JE is a significant public health problem in Southeast Asia (Solomon, 1997).

JE cases are not homogenously distributed across Nepal. The disease was first confirmed in 1978 in western Terai of Nepal near the borders of India (Joshi, 1983). It has regularly been reported since then with epidemic peaks every 2-5 years (Bista and Shrestha, 2005). Due to the complexity of interactions between landscape, vectors, reservoirs, and hosts, the epidemiology of JE remains poorly understood in Nepal as well as in many other parts of Asia. Limitations and variability in surveillance systems, health care systems and diagnostic capacity in endemic areas may be leading to misclassification of JE cases and non-JE cases as well as creating errors in estimating the acute encephalitis syndrome (AES) distribution, incidence and impacts. Many estimates of JE incidence depend on hospital based studies which may fail to capture cases with mild symptoms and the population outside the reach of the healthcare system. JE has now been documented in 54 of the 75 districts of Nepal, including 24 hill and mountain districts, and is considered to be endemic in the Kathmandu Valley as well as in 24 other districts (Pant 2009).

The World Health Organization Regional Office for South-East Asia (SEARO/WHO) has pointed out four major strategies for JE

prevention and control; health education, vector control, immunization of people and pigs, and epidemic preparedness and response (SEARO/WHO, 2002). Other countries like Japan, South Korea and Taiwan have successfully controlled JE by human and pig vaccination, improved pig farms, change in agricultural practices and improved living standards (Erlanger et al, 2009 and Igarashi, 2002). The investments required to achieve these changes is beyond the current economic capacity of Nepal. Even regular human vaccination is not affordable or sustainable at the present time. Low-cost alternatives need to be found to address the SEARO/WHO recommendations.

Every year during the rainy and post -rainy seasons in Southern Nepal, JE and other viral disease suspected patients become prevalent. The currently available methods for the laboratory diagnosis of JE (e.g., serology and virus isolation) are often tedious as well as time consuming to carry out. So, there is a need for rapid screening to identify the infection of JE.

**Table no. 1: Age and sex-wise Japanese Encephalitis positivity among suspected patients visited IOM using RDT kit**

Age group (years)	Male (N=40)	Female (N=24)	Total (N=64)
≤ 30	5 (12.5%)	3 (13%)	8 (13%)
31 – 45	4 (10%)	3 (13%)	7 (11%)
≥46	8 (20%)	5 (20%)	13 (20%)

The descriptive cross-sectional study was conducted from May 2013 to June 2014. A total of 64 serum samples were collected from patients clinically diagnosed as AES or viral fever in Tribhuvan University Teaching Hospital (TUTH) in Kathmandu. Patients' personal details including age and sex 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.

Out of 64, 28 samples were positive for JEV. The sero-positivity was found to be higher in the age group above 46 years and infection rate seemed to be analogous between genders. Overall sero-positivity was found to be 43.75%. Similar type of hospital based study was conducted in Nepal and overall sero-prevalence was 46.7% and 37.1% by the PA and IgM ELISA respectively (Pant et al., 2012).

Definitive diagnosis of JE infections is very critical in serological assays and even in virus isolation. Success of virus isolation is very poor due to rapid development of neutralizing antibodies and it could only be isolated from cerebrospinal fluid (Burke and Monath, 2001). ELISA is more promising tool in recent past and various formats of ELISA are developed from time to time (Xingling et al., 2005; Ravi et al., 2006). IgM capture ELISA is gold standard test for diagnosis of JE infections in human (OIE, 2004). Generally, JE is diagnosed on the basis of clinical symptoms in developing countries like Nepal.

IgM capture ELISA has been the most reliable technique for the confirmatory laboratory diagnosis of JE. But, this technique is expensive and relatively sophisticated. Laboratory diagnosis of JE is difficult to perform in Nepal due to insufficient resources and diagnosis facilities in many health center.

National Public health laboratory is only one referral laboratory for JE diagnosis in Nepal. Due to lack of lab facilities in the peripheral level, patients do not get diagnosis report in time. It takes nearly one week to get the test result. This issue can be overcome by rapid screening technique. Although rapid test is not confirmative test for diagnosis of JEV, it can be used in the health post / hospitals where

ELISA facilities are not available. This also gives a clue to the physicians for differential diagnosis.

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**NEWS**

**South Asia One Health Workshop**

Dr. Padam Raj Bista, Ms. Minu Sharma, and Ms. Meena Dahal of NZFHRC attended the meeting of the South Asia One Health Regional Collaboration meeting to strengthen One Health regional collaboration among India, Pakistan, Nepal, Bangladesh and their neighbors was held on May 29-30, 2014 in Hyatt Regency Kathmandu, Nepal.

**Emergence of Japanese Encephalitis in the highlands of Nepal; Club Himalaya, Nagarkot, Nepal; April 9-10<sup>th</sup> 2014**

Dr. Padam Raj Bista, Ms. Minu Sharma, Dr. Mahendra Maharjan, Ms. Meena Dahal and Mr. Dhan Kumar Pant of NZFHRC have attended the meeting of the Emergence of Japanese Encephalitis in the highlands of Nepal; Club Himalaya, Nagarkot, Nepal; April 9-10<sup>th</sup> 2014. In this meeting following papers were presented:

- Work of National Zoonoses and Food Hygiene Research Centre (NZFHRC)
- Comparative spatial dynamics of Japanese Encephalitis and Acute Encephalitis Syndrome in Nepal

**"ECOHEALTH 2014: Connections for Health, Ecosystems and Society"**

Ms. Minu Sharma, Program Coordinator of NZFHRC will participate in the Steering Committee member of the project to be held on August 9, 2014, Montreal, Canada. The goal of the project is to forecast a strategy for emerging and re-emerging disease research in Asia at the human-animal-environment interface.

She will also participate in the "ECOHEALTH 2014: Connections for Health, Ecosystems and Society" conference which is going to be held on August 10-15, 2014, Montreal, Canada. This conference is organized by The Interdisciplinary Center for Interdisciplinary Research on Well-Being, health, Society and Environment (Cinbiose) and Canadian Community of Practice in Ecosystem Approaches to Health (CoPEH-Canada). Ms. Sharma will present a paper titled "Reducing Vulnerability to the Threat of Japanese Encephalitis Transmission in High-risk Districts in Nepal"

**K.D.M.A. Research Award:**

*Please kindly submit your research work paper on allergy award for the year 2014 for the consideration by the end of October 2014 to KDMART office Chagal, G.P.O. Box 1885, Kathmandu, Nepal, Phone: 4270667, 4274928 and Fax 4272694. This award was established by Late Dr. Durga Datt 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.*

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

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