

## Seroprevalence of Toxoplasmosis, Leptospirosis and Brucellosis in Sugarcane Field-workers in Trinidad and Tobago

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### ABSTRACT

*Sugarcane field-workers, like rice field-workers, livestock farmers and abattoir workers are known to be occupationally exposed to zoonotic agents. The study determined the seroprevalence of immunoglobulins to Toxoplasma gondii (IgM), Leptospira spp (IgM) and Brucella abortus (IgG) in sugarcane field-workers across weighing stations in the island of Trinidad. In addition, the association of risk factors to infections by the three zoonoses was investigated. Blood samples were collected from consenting apparently healthy sugarcane field-workers across the island of Trinidad. Current/acute infection in individuals was determined in the sera of individuals using the enzyme immunoassay (EIA) for T gondii IgM antibodies, enzyme-linked immunosorbent assay (ELISA) for Leptospira spp IgM immunoglobulins and both buffered plate agglutination test (BPAT) and competitive ELISA for B abortus IgG antibodies. The seroprevalence of IgM immunoglobulins to T gondii was 15.7% (64 of 407) and to Leptospira spp was 0.7% (5 of 704) and the difference was statistically significant ( $p < 0.05$ ;  $\chi^2$ ). All 704 samples tested for B abortus IgG immunoglobulins were negative. All risk factors (age, gender, race and type of work done) were not statistically significantly ( $p > 0.05$ ;  $\chi^2$ ) associated with infections by T gondii and Leptospira spp. It was concluded that sugarcane field-workers in Trinidad were at high risk of acute toxoplasmosis and, to a lesser extent, to leptospirosis. The fact that the four risk factors studied were not significantly associated with T gondii and Leptospira spp infections suggests that they may not be important in the epidemiology of both diseases in the population studied.*

**Keywords:** Toxoplasmosis, Leptospirosis, Brucellosis, sugarcane workers, Trinidad

## Seroprevalencia de la Toxoplasmosis, la Leptospirosis y la Brucelosis en los Trabajadores de las Plantaciones de Caña de Azúcar en Trinidad y Tobago

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### RESUMEN

*Se sabe que los trabajadores de las plantaciones de caña de azúcar – al igual que los trabajadores de los campos de arroz, la ganadería y los mataderos – se hallan expuestos a agentes zoonóticos debido a su ocupación. El estudio determinó la seroprevalencia de las inmunoglobulinas en relación con Toxoplasma gondii (IgM), Leptospira spp (IgM) y Brucella abortus (IgG) en los trabajadores cañeros a lo largo de las estaciones de pesaje en la isla de Trinidad. Además, se investigó la asociación de factores de riesgo de infecciones por las tres formas de zoonosis. Se obtuvieron muestras de sangre a lo largo de la isla de Trinidad, tomadas de trabajadores cañeros de apariencia saludable, que dieron su consentimiento. La infección aguda presente en los individuos, fue determinada en sus sueros mediante el inmunoensayo enzimático (IEE) para anticuerpos de T gondii IgM, el ensayo inmunosorbente vinculado a enzimas (ELISA) para inmunoglobulinas frente a Leptospira spp IgM, y la prueba de aglutinación tamponada en placa (BPAT) así como el ELISA competitivo para anticuerpos de B abortus IgG.*

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*Palabras claves: toxoplasmosis, leptospirosis, brucelosis, trabajadores cañeros, Trinidad. La seroprevalencia de inmunoglobulinas IgM para T gondii fue de 15,7% (64 de 407) en tanto que para la leptospira spp fue 0,7% (5 de 704). La diferencia fue estadísticamente significativa ( $p < 0.05$ ;  $\chi^2$ ). Las 704 muestras sometidas a la prueba de inmunoglobulinas para B abortus IgG, fueron negativas. Los factores de riesgo (edad, género, raza y tipo de trabajo realizado) no estuvieron significativamente asociados ( $p > 0.05$ ;  $\chi^2$ ) de manera estadística con las infecciones por T gondii y Leptospira spp. Se llegó a la conclusión de que los trabajadores cañeros de Trinidad presentaban un alto riesgo de toxoplasmosis aguda y, en menor medida, de leptospirosis. El hecho de que los cuatro factores de riesgo estudiados no estaban significativamente asociadas con T gondii y a infecciones de Leptospira spp, sugiere que puede que no sean importantes en la epidemiología de ambas enfermedades en la población estudiada.*

West Indian Med J 2010; 59 (1): 15

**Palabras claves:** Toxoplasmosis, leptospirosis, brucelosis, trabajadores azucareros, Trinidad

## INTRODUCTION

Human infections by *Toxoplasma gondii* is worldwide and has been associated with several risk factors (1, 2). Although chronic or subclinical infections by *T gondii* are most prevalent in occupationally exposed individuals as detected by IgG immunoglobulins (3, 4), acute infections (IgM), particularly those acquired for the first time early in pregnancy, have been documented to cause abortions, miscarriages and other clinical signs and symptoms (5, 6). In Trinidad and Tobago, studies have reported the frequency of *T gondii* IgM immunoglobulins in cord blood of newborns and in pregnant women at health centres (7, 8) but no report exists for the general population or for high risk groups in the country.

Leptospirosis is considered to be one of the most widespread zoonoses globally (9) but an inapparent form of the infection is known to be most prevalent (10, 11). Reports exist on seroprevalence studies of *Leptospira* spp infections in the general population as well as in individuals who are occupationally exposed (11–14). Cases of clinical leptospirosis in humans have also been documented in several countries, including risk factors associated with infections (15–19). Both clinical and subclinical leptospirosis have been reported in the human population in Trinidad and Tobago (20–23).

Brucellosis is an important zoonosis of both economic and public health significance (24, 25). In areas where the disease is prevalent in livestock, human brucellosis cases have been recorded, particularly, in individuals occupationally exposed (26–28). Until 1998, Trinidad and Tobago was recognized as a brucellosis-free country by the Office of International Epizootics (OIE) but with the epidemic of brucellosis in cattle and water buffalo (*Bubalus bubalis*) as reported by Fosgate *et al* (29, 30), that status was withdrawn.

The country adapted a test and slaughter policy to successfully eradicate the disease in animals.

This study was conducted to determine the frequency of immunoglobulins to *T gondii*, *Leptospira* spp and *B abortus* in apparently healthy sugarcane field-workers and to investigate any relationship between selected factors and infection

by the three zoonoses.

## SUBJECTS AND METHODS

The Veterinary Public Health Unit (VPHU) of the Ministry of Health and the Sugarcane Farmers Association, assisted in contacting the sugarcane farmers across the country to elicit their participation in the study. The location of all sugarcane weighing stations was also identified during the 2006 sugarcane harvesting season. The study design was to collect blood samples from consenting individuals (cane-cutters, harvesters, loaders, drivers, office workers) associated with the industry. Overall, a total of 704 samples (Table 1) were collected from individuals/farmers at 30 weighing stations across the country.

On scheduling visits to the sampling venues, all workers were informed of the voluntary nature of the project and during the visits each participant completed a consent form. In addition, each consenting participant completed a questionnaire which elicited demographic data (ethnic group, age, marital status and gender) and other risk factors for toxoplasmosis (type of work done).

For blood sample collection, qualified phlebotomists drew 5 mL of blood from the median cubital veins of each individual into vacutainer tubes without anticoagulant. Blood samples were transported to the laboratory ice-cooled and stored overnight at 4°C after which serum was harvested following centrifugation.

The enzyme immunoassay (EIA) test kits (Diamedix, Maimi, USA) with appropriate positive and negative controls, supplied by the manufacturer, were used to detect IgM immunoglobulins to *T gondii*. A total of 407 samples, based on the limited number of test kits available for the study, were randomly selected but representative of the various weighing stations and the workers performing different duties at these stations.

To detect *Leptospira* IgM immunoglobulins, an enzyme-linked immunosorbent assay (ELISA) as described was used (31). For this study, titres of 1:160 or greater were

Table 1: Sources of human samples studied

Identification of sugarcane weighing stations	Number of sugarcane workers tested
Caroni	16
Calcutta	7
Milton	11
Todds Road	22
Forres Park	25
Dubfries	27
Devil's Hole	12
Penal	14
Hindustan	20
Brother's Road	27
Malgretoute	14
Gobin Village	20
Williamsville	19
Reform/Massey Lands	20
Cocoa	11
Cedar Hill #1	9
Cedar Hill #2	20
Cemetery Road	28
Cola Reinzi	39
Norma Giwar	27
Oropouche # 1	18
Oropouche # 2	24
Oropouche # 3	24
Oropouche # 4	30
Oropouche # 5	51
Oropouche # 6	32
Oropouche # 7	39
El-Hueto	33
Exchange Pen Yard	7
USM Delivery	38
<b>Total</b>	<b>704</b>

considered positive.

*Brucella abortus* immunoglobulin was detected in serum samples using the buffered plate agglutination test (BPAT) as earlier described (32) followed by the competitive ELISA (33) on all BPAT-suspect positive samples.

All participants were assured that the results would be made available to them through the Veterinary Public Health unit of the Ministry of Health and the data treated with strictest confidence.

The frequency of detection of immunoglobulins to *T gondii*, *Leptospira* spp and *Brucella abortus* in sugarcane workers were compared with risk factors for infections and analysed using the Statistical Package for Social Sciences (SPSS), version 10. All statistical tests were two-sided and interpreted at the 5% level of significance using the chi-square ( $\chi^2$ ) test.

## RESULTS

The seroprevalence rates of immunoglobulins to *Leptospira* spp, *Toxoplasma gondii* and *Brucella abortus* are shown in Table 2. The seroprevalence of IgM immunoglobulins to *T gondii*, 15.7% (64 of 407), was statistically significantly

Table 2: Seroprevalence of leptospirosis, toxoplasmosis and Brucellosis amongst sugarcane field-workers

Type of infection	Number of workers tested	Number (%) of workers seropositive
<i>Leptospira</i> spp.	704	5 (0.7) <sup>a</sup>
<i>Toxoplasma gondii</i>	407	64 (15.7) <sup>b</sup>
<i>Brucella abortus</i>	704	0 (0.0) <sup>c</sup>

<sup>a</sup>Using a *Leptospira* IgM enzyme-linked immunosorbent assay (ELISA) using titres of 1:160 and greater as positive

<sup>b</sup>Using enzyme immunoassay (EIA) for *T gondii* IgM

<sup>c</sup>Using the buffered plate agglutination test (BPAT) and competitive *B. abortus* IgG ELISA

( $p < 0.05$ ;  $\chi^2$ ) higher than that of *Leptospira* spp, 0.7% (5 of 704).

The gender, ethnic group, age and type of work done by the sugarcane field-workers did not statistically significantly ( $p > 0.001$ ;  $\chi^2$ ) affect infection by *T gondii* (Table 3).

Although the seroprevalence of *Leptospira* spp IgM

Table 3: Seroprevalence of *Toxoplasma gondii* IgM antibodies in sugarcane field-workers

Category of workers	Number of workers tested	Number (%) of workers seropositive <sup>a</sup> for <i>T gondii</i> antibodies
<b>Gender</b>		
Male	357	60 (16.8)
Female	50	4 (8.0)
<b>Race</b>		
Indian	339	54 (15.9)
African	67	9 (13.4)
Others <sup>b</sup>	1	1 (100.0)
<b>Age (years)</b>		
≤ 20	17	3 (17.7)
21–30	53	11 (20.8)
31–40	75	12 (16.0)
41–50	124	19 (15.3)
>50	138	19 (13.8)
<b>Type of job</b>		
Field workers <sup>c</sup>	296	47 (15.9)
Others <sup>d</sup>	111	17 (15.3)

<sup>a</sup>Using enzyme immunoassay (EIA)

<sup>b</sup>Chinese, Syrians, mixed races

<sup>c</sup>Cane cutters, cutter/harvesters, loaders/cutters, harvesters, harvesters/loaders

<sup>d</sup>Delivery personnel, supervisors, security officers, drivers, laboratory technicians, and office workers

immunoglobulin was higher in male than female workers, in Africans than Indians, in workers aged 20 years or less than in those > 20 years old and in workers who cut or load cane than those who did not, the differences were not statistically significant ( $p > 0.001$ ;  $\chi^2$ ) as shown in Table 4.

## DISCUSSION

Table 4: Seroprevalence of *Leptospira* spp IgM antibodies in sugarcane field-workers

Category of workers	Number of workers tested	Number (%) of workers seropositive <sup>a</sup>
<b>Gender</b>		
Male	313	4 (0.7)
Female	61	1 (1.2)
<b>Race</b>		
African	241	3 (0.5)
Indian	124	2 (2.2)
Others <sup>b</sup>	29	0 (0.0)
<b>Age (years)</b>		
≤ 20	24	1 (4.2)
21–30	108	1(0.9)
31–40	140	0 (0.0)
41–50	209	1 (0.5)
>50	223	2 (0.9)
<b>Type of job</b>		
Field workers <sup>c</sup>	491	2 (0.4)
Others <sup>d</sup>	213	3 (1.4)

<sup>a</sup>Using *Leptospira* IgM enzyme-linked immunosorbent assay (ELISA) using titres of 1:160 or greater as positive

<sup>b</sup>Chinese, Syrians, mixed races

<sup>c</sup>Cane-cutters, cutter/harvesters, loaders/cutters, harvesters, harvesters/loaders

<sup>d</sup>Delivery personnel, supervisors, security officers, drivers, laboratory technicians and office workers

It is evident from the data obtained in the current study that acute or new infections, as detected by IgM immunoglobulins, is significantly higher for *T gondii* compared with *Leptospira* spp and *Brucella abortus*. These three zoonotic pathogens have been established as occupational diseases (2, 11, 25). Sugarcane workers are a high risk group for zoonoses just as are rice field, slaughter house and abattoir workers (4, 19, 27, 34).

It is of significance that all the 704 sugarcane field-workers sampled across 30 weighing stations in the country were apparently healthy at the time of the study while as many as 15.7% were seropositive for *T gondii* IgM immunoglobulins suggestive of acute or new infections. This could be a reflection of new exposure that is subclinical or as a result of IgM immunoglobulins remaining for extended periods of time in the blood as suggested earlier (35, 36). The seroprevalence for *T gondii* IgM immunoglobulins detected in the current study compares favourably with 20% and 2.32% reported in Saudi Arabia and China respectively (37, 38). Relatively higher prevalence rates have been documented in high risk groups with reported prevalence of 25% in Finland (39) and 42.6% in Djibouti (40) when *T gondii* IgG immunoglobulins, which reflect past infection, were assayed for. The fact that 12.3% (50 of 407) of the sugarcane workers were women and most of childbearing age coupled with the finding that 8.0% of the women were seropositive for *T gondii* IgM immunoglobulins cannot be ignored. This is because

primary infection with *T gondii* during early pregnancy has the potential to cause abortion, miscarriage and other clinical manifestations in the neonate or later in adulthood (5, 6). In Trinidad and Tobago, Adesiyun *et al* (7) reported, for the first time, serological evidence of congenital toxoplasmosis (0.4%) in neonates using cord blood. In a most recent study, Ramsewak *et al* (8) reported a seroprevalence of 3.4% and 5.5% for *T gondii* IgM immunoglobulins in pregnant women who attended hospitals and health centres in the country respectively.

Risk factors such as age, gender, ethnic group and types of work, along with other factors usually linked to toxoplasmosis in previous studies (38, 41) were not detected to have a significant effect on the prevalence of *T gondii* IgM immunoglobulins in the current study. Reports on studies conducted earlier on newborns and pregnant women in the country agree with findings in the current study (7, 8).

A seroprevalence of 0.7% for *Leptospira* spp IgM immunoglobulins amongst sugarcane field-workers is considerably lower than the rates reported in Chile, 3.3% (42) and 5.4% found in Vietnam (43) reported for other high risk groups where IgM was also tested. Considerably higher seroprevalence ranging from 37% to 45% was reported in Trinidad and Tobago when IgG immunoglobulins were assayed (34). Although clinical manifestations such as headache, vomiting amongst other signs and symptoms usually associated with clinical leptospirosis were not observed or elicited by the questionnaire administered to sugarcane field-workers in the current study, it has been established that a majority of *Leptospira* infections in humans and animals is asymptomatic (44). Rodents are known to be important reservoirs for leptospirosis and sugarcane field-workers are particularly exposed to cuts and water contaminated by rodent urine in their daily chores in the field (34, 45). The finding that all risk factors studied did not affect acute infection rates by *Leptospira* spp is an indication that other factors, not investigated, may have been responsible for exposure to *Leptospira* spp. Clinical human leptospirosis has been reported in the population in Trinidad and Tobago while subclinical infections have also been reported (20–23).

All workers sampled were seronegative for *B abortus* IgG immunoglobulins suggestive of the lack of human exposure to the pathogen in the country. It is pertinent to mention that an epidemic of brucellosis was reported in cattle and water buffalo (29, 30) in 1998 which resulted in a loss of OIE brucellosis-free status by the country. A successful aggressive test and slaughter policy reduced human exposure during the epidemic which might have been responsible for the failure to detect *B abortus* antibodies in all the sugar cane field-workers tested in the current studies. In other countries, it has been demonstrated that human infections occur when infections are prevalent in the animal population (26, 27, 46, 47).

We report, for the first time, the occurrence of acute infections, demonstrated by IgM immunoglobulins to *T gondii* in sugar cane field-workers in Trinidad and Tobago with all previous documented studies in humans limited to pregnant women or neonatal children. Also of epidemiological significance was the fact that for both *T gondii* and *Leptospira* spp infections, the types of work done in the sugarcane field which were expected to pose different risks to infection did not significantly affect exposure to both zoonotic agents. The implication is that other factors may have been important in the exposure of sugarcane field-workers in Trinidad to toxoplasmosis and leptospirosis and need to be further investigated. Finally, it was concluded that acute or new infections by *T gondii* followed by *Leptospira* spp may be prevalent amongst sugarcane field-workers in the country with infected individuals all asymptomatic.

#### ACKNOWLEDGEMENTS

The contributions of the technical staff of the Veterinary Public Health Unit of the Ministry of Health in the completion of questionnaires during the study are appreciated. We are thankful to Mr David London who was responsible for coordinating the sampling of sugarcane workers across weighing stations and sugarcane farms. We acknowledge the input of Gerard Ramirez for taking blood from all participants in the study.

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## Erratum

Correction to Author's name

Re: Letter to the Editor entitled "Failure to Detect *Angiostrongylus cantonensis* in Rats in Barbados" Published January 2004, in Vol. 53, No. 1, page 58. Authors – PN Levett et al. The initials of the second author should be **KO** Douglas, instead of KA Douglas