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Occurrence of Leptospirae Antibodies in Abattoir Workers in Parts of North Central Nigeria

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ABSTRACT

Leptospirosis, a zoonotic disease of livestock with worldwide occurrence, can have debilitating and life threatening consequences on humans. Thus to determine the sero-epidemiology of *Leptospira* antibodies in Nigeria, prevalent circulating serovars in the population and provide data for health policy makers, in the control and treatment of leptospirosis, this study was undertaken. Two hundred and sixty-three blood samples from male and female abattoir workers from four locations in north central Nigeria; Benue State, Plateau State, Nassarawa State and the Federal Capital Territory were screened for the antibodies to leptospirae by Microscopic Agglutination Test (MAT) and IgG ELISA techniques, where 87.7 and 81.0% sero-positive rates were obtained, respectively. Nassarawa State had the highest sero-positive rate of 94.3%, while the least rates were found in Plateau State with 82.8%. Across all study locations, more males were seropositive than females. *Leptospira hardjo* was the highest circulating serovar; 66 (28.6%), while, the least was *L. tarassovi* 8 (3.5%). Symptoms of *Leptospira* infection closely mimic those of many febrile illnesses in Nigeria and accurate diagnosis may be missed. Education of susceptible individuals and close collaboration between research and medical workers is advocated to help combat the infection.

Key words: Abattoir workers, leptospirae, Nigeria, sero-prevalence

INTRODUCTION

Leptospirosis, discovered in 1886 by Weil, is a worldwide, contagious, zoonotic disease caused by *Leptospira interrogans*. These are thin, highly motile bacteria, slow-growing, obligately aerobic spirochaetes of the family Leptospiraceae with optimal growth temperature of 30°C. They can be distinguished from other spirochaetes by their unique hook or question mark-shaped ends and are 6-20 µm long and 0.1-0.15 µm in diameter (Li *et al.*, 2010).

Leptospirosis has been identified as a re-emerging, infectious disease and this has been demonstrated by the outbreaks in some countries such as Brazil, India, Southern Asia, Malaysia, Japan and California in the United States (WHO., 2000; Meites *et al.*, 2004).

Leptospirosis has a significant health impact in many parts of the world, particularly the Americas and Asia. It can present life-threatening forms, such as Weil's disease and severe pulmonary hemorrhagic syndrome. Estimates indicate that there are more than 500,000 cases of leptospirosis worldwide each year (Wuthiekanun *et al.*, 2007). The majority of reported cases have severe manifestations for which mortality is greater than 10%. Furthermore, studies in Thailand have shown that leptospirosis may represent up to 20% of febrile illness of unknown origin (Ko *et al.*, 1999).

Human leptospirosis manifests as a wide spectrum of clinical illnesses, ranging from subclinical or mild infections to severe multi-organ failure associated with high mortality and morbidity in different countries (Ahmed *et al.*, 2006; El Jalii, 2008). While, symptoms typically appear after 4-14 days incubation period, incubation period in animals is from 2-20 days (Ramakrishna *et al.*, 2008; Patil *et al.*, 2011; DebMandal *et al.*, 2011). Leptospirosis has been reported in Spain with a prevalence of 67.2% among the high-risk groups. A 52.7% prevalence was also recorded among the high-risk groups in Andaman Islands (Sharma *et al.*, 2006), a prevalence of 22% in Chile and a prevalence of 23.3% in Colombia.

Leptospirosis is transmitted to humans via direct or indirect contact with water, food or soil containing blood, urine and tissue from an infected animal by entering the body through mucous membranes of the eyes, nose and mouth or abraded skin by bathing or accidental immersion in fresh water lakes or rivers or canals contaminated with the urine of the infected livestock.

In Nigeria, Leptospirosis has been reported in Northern Nigeria, Enugu Ibadan, Bauchi and Plateau States from cattle, sheep, abattoir workers and in volunteer blood donors (Ezeh *et al.*, 1990; Agunloye, 2002; Abiayi *et al.*, 2011; Ngbede *et al.*, 2012).

Despite its importance, as a public health threat, this zoonotic disease is rarely diagnosed in most health care facilities in Nigeria. Contemporary data on the burden of disease in these high risks groups and in livestock are not available in North Central States (Nasarawa, Benue, Plateau and the Federal Capital Territory). Likewise, prioritization and dedication of resources to the prevention and control of disease by the government has to be improved. Therefore, the need to investigate the Sero-Epidemiology of *Leptospira* antibodies in Abattoir workers in some selected states of North Central Nigeria.

MATERIALS AND METHODS

Ethical approval: Informed consent was obtained from the abattoir workers prior to the study. All abattoir workers, who were 18 years and above and present at the abattoirs at the period of the study from (2010-2013) and all consenting respondents were included in the descriptive cross sectional study.

Study area: Nigeria has an area of 923,768 sq km inhabited by a population of more than 140,003,542 (NPC., 2006) with a population density of 152 persons per sq km, 48% urban population distribution and 52% rural population distribution. The country is divided into six geopolitical zones North-West, North-East, North-Central, South-East, South-West and South-South. The North-Central zone of Nigeria consists of Nasarawa State, Niger State, Benue State, Kwara State, Kogi State and Plateau State. These six states surround the Federal capital territory (FTC), Abuja (Fig. 1). The climate in the North-Central zone of Nigeria is moderate in humidity and with mean temperature of more than 35°C in low areas near the Rivers Benue and Niger with altitude of less than 750 m to the cold mean temperature of less than 25°C in Jos Plateau with altitude of more than 1750 m.

Blood sample collection and human sera preparation: Whole blood was collected from a total of 263 abattoir workers across the four north central states selected using standard procedure as adopted by WHO (2003). Butchers were swabbed with 70% ethanol. The 4 mL of blood was collected with 5 mL syringes and transferred into plain glass tubes in an insulated box. Urine samples were also collected into wide mouth universal bottles and transported in cool boxes to the laboratory for preliminary analysis. Blood samples were left overnight to clot at 4°C before centrifugation. Sera were separated, labeled and stored at -20°C, while the urine samples were cultured within 3-4 h of collection.

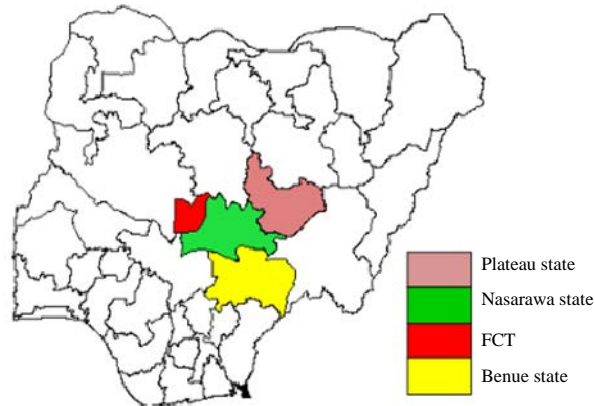


Fig. 1: Map of Nigeria highlighting study locations

Serological identification of leptospire: This was done using standard procedures as outlined by WHO (2003).

Microscopic agglutination screening test: Sorensen's buffer, antigens, test and positive control sera were brought to room temperature before use. The 25 μ L Sorensen's buffer was dropped in all the wells of Microtitre plates of row 3-10. The 5 μ L of the test sera was placed in well of row 3-8. The 5 μ L diluted positive sera were placed in row 9. The 25 μ L antigen was placed in wells of rows 3-10, giving a final dilution of 1/11. The mixture was gently shaken for 30 sec and Incubated in the dark in a moist chamber at 37°C for 2 h. Positive controls were included in every plate, when a new antigen was to be used. End point was that dilution where 50% agglutination was seen or where 50% of the leptospirae disappeared.

Enzyme-linked immunosorbent assay (ELISA): All samples and reagents were brought to temperatures of between 15-25°C before use. The 10 μ L of subject sera were added to 390 μ L of dilution buffer to give a 1: 40 in dilution. The 100 μ L negative control and 100 μ L positive control were added to wells #1 and 2, while 100 μ L test sera were placed in remaining wells at room temperature for 10 min, after which it was washed and excess wash buffer removed. Two drops of enzyme conjugate were added to each well, including the reagent blank, positive and negative control well. The plates were incubated at room temperature for 10 min, after which they were washed again. Two drops of chromogen were added to each well and incubated at room temperature for 5 min. A blue color formed in some wells was due to the presence of a specific antibody to the antigen. Finally, 2 drops of the stop solution was added to each well, which developed yellow coloration of varying intensities, proportional to the concentration of the IgG antibody. An ELISA plate reader was set to a wavelength of 450 and 620-650 nm used for the reading of the plate.

Statistical analysis: All data was fed into SPSS 16 (full version) and Epi Info (ver 3.5.3) and analyzed using Chi square at 95% level of confidence, to determine levels of association between variables.

RESULTS

Two hundred and thirty one (231) (87.8%) sera were positive for *Leptospira* antibodies by MAT with agglutination reaction at titer 160-2560, while 213 (81%) of the total samples screened by IgG ELISA were positive for *Leptospira* antibodies, respectively (Table 1).

From the 263 serum samples collected from Benue, Nasarawa, Plateau and FCT, the highest sero-prevalence of (94.3%) was found in Nasarawa, followed by FCT with (88.1%). Plateau had the least prevalence of 82.8%. Chi-square analysis showed no significant difference ($\chi^2 = 3.64, p>0.05$) in the rate of occurrence of leptospirosis among Abattoir workers by location (Table 2).

Of the 263 serum samples collected from Abattoir workers by gender, the highest prevalence of 94.3% was again found in Nasarawa, followed by FCT (88.1%). Plateau had the least prevalence of 82.8%. Chi-square analysis indicated no significant association ($\chi^2 = 3.64, p>0.05$) in the rate of occurrence of leptospirosis among Abattoir workers by sex (Table 3).

The prevalent serovars circulating per state in the study area were as follows; FCT had *grippotyphosa* 20 (8.7%), *australis* 16 (6.9%), *hardjo* 12 (5.2%) serovar *tarassovi* 3 (1.3%) had the least prevalence, respectively. Benue showed the following rates; icterohaemorrhage 18 (18.8%), *grippotyphosa* 13 (5.6%), while the least prevalent was *Tarassovi* 1 (0.4%). Nasarawa serovars were *hardjo* 22 (9.5%) and *icterohaemorrhage* 10 (4.3%) (Table 4).

Table 1: Prevalence of leptospirosis by MAT and IgG ELISA among Abattoir workers

Parameters	Frequency			
	MAT		IgG ELISA	
	No.	(%)	No.	(%)
Positive	231	87.7	213	81.0
Negative	32	12.2	50	19.0
Total	263	100.0	263	100.0

Table 2: Seroprevalence of leptospirosis by MAT among Abattoir workers

Location	No. screened	No. positive	No. negative	Prevalence (%)
Benue	62	54	8	87.1
FCT	84	74	10	88.1
Nasarawa	53	50	3	94.3
Plateau	64	53	11	82.8

χ^2 : 3.64, df: 3, p: 0.302 (p>0.05)

Table 3: Seroprevalence of leptospirosis in Abattoir workers by gender

Location and sex	No. screened	No. positive	Percentage
Benue			
Male	58	50	86.2
Female	4	4	100.0
Total	62	54	87.1
FCT			
Male	57	51	89.5
Female	27	23	85.2
Total	84	74	88.1
Nasarawa			
Male	53	0	94.3
Female	0	50	0.0
Total	53	50	94.3
Plateau			
Male	45	37	82.2
Female	19	16	84.2
Total	64	53	82.8

χ^2 : 3.64, df: 3, p: 0.302 (p>0.05)

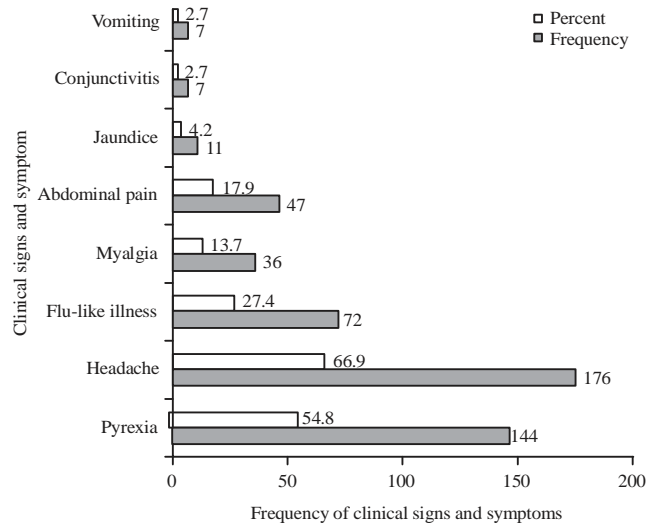


Fig. 2: Abattoir workers with history of leptospirosis symptoms

Table 4: Occurrence of *Leptospira* serovars among Abattoir workers

Location	Sample size	Circulating serovars							No. +ve	No. -ve
		Li	Lt	Lp	Lg	La	Lc	Lh		
FCT	84	5	3	10	20	16	8	12	74	10
Benue	62	18	1	7	13	0	4	11	54	3
Plateau	64	7	2	4	7	9	3	21	53	11
Nasarawa	53	4	2	6	10	2	4	22	50	3
Total	263	34 (14.7)	8 (3.5)	27 (11.7)	50 (21.6)	27 (11.7)	19 (8.2)	66 (28.6)	231	27 (11.7)

Li: *L. icterohaemorrhage*, Lt: *L. tarassovi*, Lp: *L. pomona*, Lg: *L. grippityphosa*, La: *L. australis*, Lc: *L. canicola*, Lh: *L. hardjo*

Table 5: MAT titres of *Leptospira* serovars in seropositive abattoir workers

Serovars	Antibody titres							Total prevalence	
	160	320	640	1280	2560	5120	10,240	No.	%
<i>L. icterohaemorrhage</i>	1	16	12	3	1	0	0	33	12.5
<i>L. tarassovi</i>	0	6	2	0	0	0	0	8	3.0
<i>L. pomona</i>	0	2	15	10	0	0	0	27	10.3
<i>L. grippityphosa</i>	2	25	12	10	1	0	0	50	19.0
<i>L. australis</i>	1	0	23	3	0	0	0	27	10.3
<i>L. canicola</i>	0	0	14	5	0	0	0	19	7.2
<i>L. hardjo</i>	13	20	23	9	2	0	0	67	25.5
Total	17	69	101	40	4	0	0	23	1(87.8)

The highest titre of 1: 2560 recorded in this study occurred with serovar *icterohaemorrhagiae*, *grippityphosa* and *hardjo*. Most titres had values between 160 and 1280. Titre 160 had the highest occurrence of reactors 34.3% (n = 135) followed by titre 320, which occurred 28.9%, titre 640 had 25.6% and titre 1280 had 10.2% (Table 5).

By symptoms of *leptospira* infection among the workers, headache had the highest frequency of 176 (66.9%), followed by pyrexia with 144 (54.8%), vomiting and conjunctivitis had the lowest frequency of 7 (2.7%) (Fig. 2).

DISCUSSION

The occurrence of positive cases of leptospirosis among the abattoir workers is of epidemiological importance. In Nigeria, Abattoir workers constitute a major group at risk of

occupational zoonosis, due to the close contact that exists between abattoir workers and animals/tissue of animals during slaughtering or processing (Ezeh *et al.*, 1991; Ngbede *et al.*, 2012). Leptospirosis presents with protean clinical manifestations such as fever, headache, vomiting, conjunctiva suffusion and myalgia (Patil *et al.*, 2011), which mimic those of other diseases such as malaria, typhoid, hepatitis, lassa fever, dengue, yellow fever, tuberculosis and brucellosis (Yanagihara *et al.*, 2007) which are all endemic in Nigeria.

These non-specific symptoms exhibited makes diagnosis of leptospirosis difficult (Zhang *et al.*, 2012; Ngbede *et al.*, 2012) reducing the level of awareness of the disease since the diagnosis of febrile illnesses are often limited to malaria and typhoid fever and presentation of jaundice to viral infection with complete neglect to spirochetes infections.

The prevalence of leptospirosis is quite high and alarming. This could be as a result of broken down conveyor machines in the abattoirs or nonexistent facilities, which lead to manual slaughtering of animals and the carcasses carried on the workers heads, with meat containers leaking animal body fluids all over the bodies of workers and cuts and abrasions on the skin facilitate penetration of Leptospire. Indeed, it was observed that minor cuts or injuries sustained during work in the abattoirs did not serve as deterrent from handling animal tissues, which may will have been infected.

The prevalence in this study is higher than the 67.2% among the high-risk groups in Spain and 52.7% also recorded among the high-risk groups in Andaman Islands (Sharma *et al.*, 2006). This may be due to the presence of antibodies against more than one serovar, which may be related to mixed serovar infection or cross-reactivity among serovars at titres 160-2560.

The 24.4% serovar prevalence among abattoir workers corresponds with the study by Ezeh *et al.* (1991), who reported a prevalence rate of 29.5%. Similar results have been reported for other countries; Zamora *et al.* (1990) reported with a prevalence of 22% in Chile and 23.3% prevalence in Colombia.

More males than females work in abattoirs in Nigeria to earn a living, mostly as a result of social and religious considerations. This fact may explain the slightly greater sero-prevalence in males as compared to females. Females mostly run errands such as fetching water and cleaning assistants. This does not place them in direct contact with the fluids of animals. It also lessens their risks of knife cuts and bruises, as they do not butcher animals for sale, considerably reducing chances of leptospire gaining access into them through this route.

However, though not butchers and handlers of the animals in the abattoirs, female workers are also exposed to animal fluids from leaking vessels and containers used to transport and convey butchered meat to point of sales. These fluids may well have contained live leptospirae, which penetrate into the body through the soft membranes of the eyes, mouth and nostrils.

At one of the study locations, Nasarawa State, there were no females Abattoir workers at the time of the study consequently, no samples and data could be taken for analysis. This could be due to the fact that in Nasarawa State, most women and girls traditionally tend to find alternative work at stone quarries, which could explain their absence from the Abattoirs.

Though, most Abattoir workers had at one point or another had shown one or more symptoms consistent and synonymous with *Leptospira* infection, these could not be verified as having been due to clinical leptospirosis, as these symptoms could have been as a result of any number of diseases and other infections common to the study locations, such as typhoid fever, malaria fever and even seasonal outbreaks of conjunctivitis.

CONCLUSION

Occurrence and presence of *Leptospira* antibodies has been established in a susceptible group in North Central Nigeria. What remains to be achieved is a co-ordinated strategy of combating the insidious disease.

Towards this end, community and population based surveillance or prevalence studies should be carried out in other parts of Nigeria, in order to have more data on the burden of leptospirosis in Nigeria, as the burden of the disease ravaging the population is yet unknown and to shed more light on the dynamics of *Leptospira interrogans* infection. Alliances and collaboration should be encouraged between academic, health-care and research institutions for better synergy in *Leptospira* control. While, the academic and research institutions provide data and other information on the disease, the healthcare, policy formulators and implementers use same for effective leptospirosis control strategies.

Further, enlightenment programs on the implication of contact with animal and their tissues without adequate protection and the need for the use of protective clothing for individuals at risk of infection should be embarked upon as a matter of urgency.

There is also the need for leptospirosis to be included by the Integrated Disease Surveillance and Response (IDSR) as one of the notifiable diseases in Nigeria.

These measures will no doubt help in curbing the spread of leptospirosis in Nigeria.

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