Research Journal of Veterinary Sciences

ISSN 1819-1908

Academic Journals Inc.

www.academicjournals.com

Research Journal of Veterinary Sciences 8 (2): 21-28, 2015
ISSN 1819-1908 / DOI: 10.3923/rjvs.2015.21.28

Seroprevalence of *Leptospira* Antibodies in Cattle Slaughtered for Sale in Some North Central States of Nigeria

[1]E.A. Abiayi, [2]H.I. Inabo, [2]E.D. Jatau, [1]A.A. Makinde and [3]T.T. Sar
[1]Central Diagnostic Laboratory, National Veterinary Research Institute, Vom, Plateau State, Nigeria
[2]Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
[3]Department of Biological Sciences, University of Agriculture, Makurdi, Benue State, Nigeria

Corresponding Author: E.A. Abiayi, Central Diagnostic Laboratory, National Veterinary Research Institute, Vom, Plateau State, Nigeria Tel: +2348034612525

ABSTRACT

Leptospirosis, a disease of livestock, has great economic impact on the agricultural industry because it causes abortions, stillbirths, infertility, reduced milk production and death in livestock. Moreover, when successfully transmitted to man, it may result in multisystem febrile illness, with hepatic, renal and pulmonary involvement and high mortality rates. This research determined the sero-epidemiology of *Leptospira* antibodies using, Microscopic Agglutination Test (MAT) in slaughtered cattle in selected states of North Central Nigeria. A prevalence rate of 57.9% for *Leptospira* antibodies were recorded for the slaughtered cattle. The most prevalent circulating serovars in the study locations were Hardjo 65 (27.8%), followed by Grippotyphosa 60 (25.6%). Older cattle were more likely to be seropositive, p = 0.000002. However, no significance was observed in seropositive rates between bulls and cows. The data generated by this study will help provide baseline information, as well as give latest indications about the extent of the problem in the study areas, assist in control and prevention of the condition in cattle and ultimately disease in the susceptible population.

Key words: Seroprevalence, *Leptospira*, serovars, MAT, ELISA, cattle, Nigeria

INTRODUCTION

Leptospirosis is a contagious, zoonotic disease of worldwide occurrence, caused by *Leptospira interrogans*. These are bacterial Spirochetes of the genus *Leptospira*. They belong to the family Leptospiracea, are thin, highly motile, slow-growing and obligately aerobic. They have optimal growth temperature of 30°C and measure 6-20 μm in length by 0.1-0.15 μm in diameter and are distinguishable from other spirochetes by the unique hooked ends they posses (Li *et al*., 2000).

Leptospirosis, a zoonotic disease, is an important threat to public health worldwide as it and other infectious diseases makeup about 70% re-emerging infectious diseases in recent times (Meites *et al*., 2004; Cutler *et al*., 2010).

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

Leptospirosis is transmitted to humans via direct or indirect contact with water, food or soil containing blood, urine and tissues from an infected animal. The spirochetes enter the body through mucous membranes of the eyes, nose and mouth or abraded skin, during bathing or

accidental immersion in fresh water, lakes, rivers or contaminated canals. Infection in Livestock is via grazing in fields or grasses contaminated by the urine of infected rats and other mammals, by drinking contaminated water from puddles, ditches and gullies where there is regular traffic of either wild or farm mammals and by direct or indirect contact with the semen of infected animals (Vijayachari *et al*., 2008).

The carrier cattle, goats and sheep secrete *Leptospira* in their urine without clinical signs of the disease, because of the tendency of the bacteria to accumulate in the kidneys. The leptospires are maintained in nature by chronic renal carriage. Therefore these livestock play an important role in the epidemiology of the disease (Fang *et al*., 2015; Vallee *et al*., 2015; WHO., 2015).

This study was carried out to help generate data on epidemiological burden, which is largely unknown of leptospirosis in livestock in Nigeria, especially in the North Central States, where a large percentage of the population depend on livestock for sustenance, often in very close proximity, risking disease transmission to and spread among humans.

It is also hoped that findings of the study will help concerned government agencies achieve prioritization, dedication and significantly improve allocation of resources to the prevention and control of the disease.

To achieve the mentioned objectives, the seroprevalence of leptospirosis in cattle slaughtered for sale in North Central Nigeria, was undertaken (Abiayi *et al*., 2011).

MATERIALS AND METHODS

Study area: Nigeria has an area of 923,768 sq km inhabited by a population of more than 140, 003, 542 (NPC., 2006). The country is divided into 6 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 (FCT) Abuja (Fig. 1).

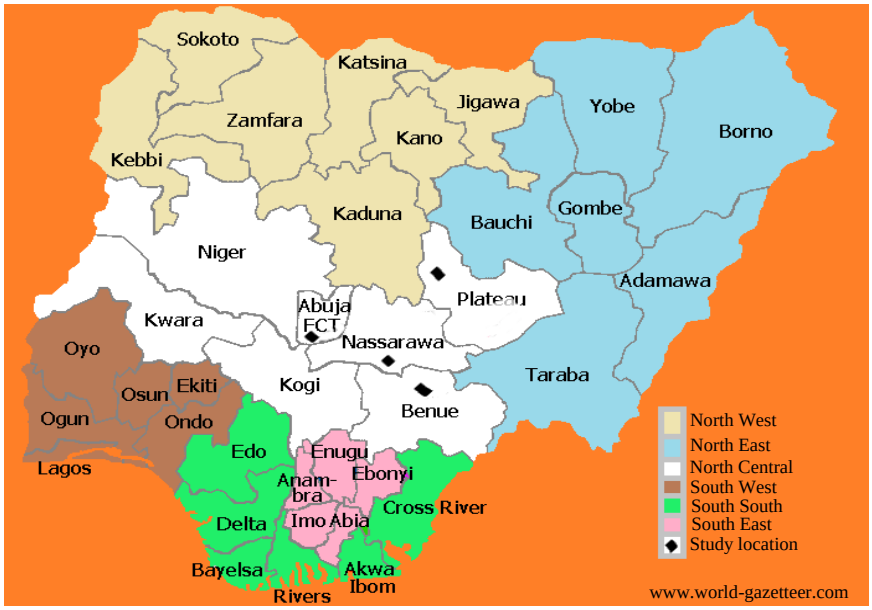

Fig. 1: Map of Nigeria showing the six geo-political zones and study locations

The North-Central zone is the home of 18,971,965 Nigerians, making up 13.46% of the total national population. The study areas are important agrarian states, where high numbers of livestock are raised for food or commerce. They are also routes of migration of nomadic cattle herdsmen for decades and are centrally located within Nigeria.

These states are well known high density cattle production areas (Damina *et al*., 2011). The Guinea Savannah vegetation is very conducive for livestock, poultry and crop production. The major livestock reared include cattle (including exotic breeds), sheep, goats and pigs. Consumption of meat, eggs, milk and their products forms a large part of the diet the inhabitants.

Ethical approval: Permissions were obtained from the veterinary officers in charge of the abattoirs in the study areas.

Abattoirs in the selected areas were visited twice weekly, having pre-information that approximately 40, 45, 52 and 60 cattle were slaughtered daily at major abattoirs in Benue, Plateau, Nasarawa and FCT, respectively. At each visit, blood samples were collected from all cattle slaughtered daily until the desired sample size was attained. Other animals like pigs, sheep and goats slaughtered at the same abattoirs were excluded from the study.

Study design: A descriptive cross sectional study design was employed.

Sample size calculation: The cattle sample size was calculated using the equation at prevalence rates of 53.7% based on convenience as below:

$$n = \frac{z^2pq}{L^2}$$

Where:
n = Desired sample size (minimum statistically analyzable)
z = Standard normal distribution at 95% confidence interval = 1.96
p = Known prevalence of the condition (53.7%)
q = 1.0-p
L = Allowable error (taken as 5% = 0.05)

Thus,

$$n = \frac{1.96^2 \times 0.537 \times 0.463}{0.05^2} = 382$$

5% non response rate (or attrition rate) = 19

Therefore, sample size, n = 382+19 = 401 cattle.

Preparation of hyper immune serum in rabbit: Young healthy rabbits weighing between 3-5 kg and confirmed *Leptospira* antibody free by MAT were used.

The following antigens; *L. canicola*, *L. australis*, *L. icterohaemorrhage*, *L. pomona*, Tarassovi, *L. grippotyphosa* and *L. hardjo* were obtained from the bacteriology unit of Onderstepoort veterinary Institute, Pretoria, South Africa. Serovar antigens, cultured for 14 days in EMJH media

Table 1: Protocol for rabbit hyper-immune serum preparation

Programs	No. of days	Volume of antigen (mL)
Days of week		
Friday	1	1
Wednesday	6	2
Monday	11	4
Monday	18	6

without 5-fluorouracil, to give an approximate count of 2×10^8 leptospires per mL were inoculated intravenously in the marginal vein of the ear as using the protocol in Table 1.

Sixteen days after the last inoculation, a small 25 μL volume of blood was tested with the corresponding antigen for high titre agglutination higher than 1/6000 after which the rabbits were bled for maximum serum yield. The hyper immune sera were separated and mixed with an equal volume of glycerin and stored at -20°C.

Cattle blood sample collection: Whole blood was collected from the jugular vein of apparently, healthy cattle presented for slaughter. Blood were collected with a vacutainer after Swabbing the area with 70% ethanol. These were carefully stored upright in an insulated box with ice packs. Blood samples were left overnight to clot at 4°C before centrifugation. Serum was separated, labeled and stored at -20°C until required.

Microscopic agglutination screening test: Sorensen's buffer, antigens, Test and positive control sera were brought to room temperature before use. Twenty five microliter Sorensen's buffer was dropped in all the wells of Microtitre plates of row 3-10. 5 μL of the test sera was placed in well of row 3-8. Five micro liter diluted positive sera were placed in row 9. 25 μL 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.

Statistical analysis: Data obtained was fed into SPSS 16 (full version) and Epi Info (version 3.5.3). Statistical tool used for analysis was Chi square at 95% of confidence level to determine significant levels of association between variables.

RESULTS

Four hundred and four serum samples collected from cattle slaughtered in the three North Central States and Federal Capital Territory (FCT) were screened for the presence of *Leptospira* antibodies by MAT, using a reference panel of 7 *Leptospira serovars*: *L. icterohaemorrhagiae*, *L. tarassovi*, *L. pomona, L. grippotyphosa, L. australis, L. canicola* and *L. hardjo*, which yielded 457 serovar occurrences (Table 2). The serovars to which antibodies were detected included; *L. icterohaemorrhagiae,* 69 (17.08%), *L. tarassovi* 68 (16.83%), *L. pomona* 42 (10.40%), *L. grippotyphosa* 112 (27.72%), *L. australis* 50 (12.38%), *L. canicola* 49 (12.13%) and *L. hardjo* 69 (16.58%). This gave an overall seroprevalence of 16.16% in the slaughtered cattle (Table 3).

The most prevalent *Leptospira* serovar among cattle in the North Central States were *hardjo* 65 (27.8%), *grippotyphosa* 60 (25.6%), *icterohaemorrage* 34 (14.5%). The most prevalent *Leptospira* serovars in the FCT were *grippotyphosa* 25 (10.7%), followed by *hardjo* 15 (5.4%). The least prevalent were *canicola* 7 (3.0%) and *pomona* 2 (0.9%), respectively. The prevalent *Leptospira* serovars in Benue State were *icterohaemorrage* 21 (9.0%) and *hardjo* 12 (5.1%). There were no

Table 2: Seroprevalence of various *Leptospira* serovars in slaughtered cattle in States of North Central Nigeria

		Positive		Negative	
Serovars	Samples screened	No.	%	No.	%
L. icterohaemorrage	404	69	17.08	335	82.92
L. tarassovi	404	68	16.83	336	83.17
L. pomona	404	42	10.40	362	89.60
L. grippotyphosa	404	112	27.72	292	72.28
L. australis	404	50	12.38	354	87.62
L. canicola	404	49	12.13	355	87.87
L. hardjo	404	67	16.50	337	83.42
Total	2828	457	16.16	2371	83.84
Actual seropositive		234			

Table 3: Number of positive reactors to MAT by location

Locations	Samples size	LI	LT	LP	LG	LA	LC	LH	No. (+ve)	No. (-ve)
FCT	101	8	10	2	25	7	7	15	74	27
Benue	101	21	7	2	10	0	4	12	56	45
Plateau	101	2	4	3	11	6	8	22	56	45
Nasarawa	101	3	5	3	14	4	3	16	48	53
Total	404	34 (14.5)	26 (11.1)	10 (4.3)	60 (25.6)	17 (7.3)	22 (9.4)	65 (27.8)	234	170

LI: *L. icterohaemorrage*, LT: *L. tarassovi*, LP: *L. pomona*, LG: *L. grippotyphosa*, LC: *L. canicola*; LH: *L. hardjo*, +ve: Positive, -ve: Negative, Parenthesis: Percent serovar occurrence, FCT: Federal capital territory

Table 4: Frequency of MAT reaction with single or multiple antigen in positive reactors in selected States of North Central Nigeria

	Frequency in cattle		Total	
No. of antigens and locations	No.	(%)	No.	(%)
One				
FCT	41	17.52	120	51.26
Nasarawa	28	11.96		
Plateau	27	11.53		
Benue	24	10.25		
Two				
FCT	11	4.70	38	16.22
Nasarawa	14	5.98		
Plateau	8	3.41		
Benue	5	2.13		
Three				
FCT	13	5.55	41	17.50
Nasarawa	11	4.70		
Plateau	9	3.84		
Benue	8	3.41		
Four				
FCT	9	3.84	35	14.93
Nasarawa	4	1.70		
Plateau	12	5.12		
Benue	10	4.27		
Total	234	99.41	234	100.00

Figure in parenthesis: Total percent of antigen occurrence across study locations, MAT: Microscopic agglutination test, FCT: Federal capital territory, MAT: Microscopic agglutination test, FCT: Federal capital territory

positive reactions to serovar *australis* in the sera collected from cattle in Benue State. The most prevalent *Leptospira* serovars in Plateau State were *hardjo* 22 (9.4%) and *grippotyphosa* 11 (4.7%), while, the least were *icterohaemorrage* 2 (9%) and *tarassovi* 4 (1.7%). In Nasarawa State the most frequent serovars were *hardjo* 16 (6.8%) and *grippotyphosa* 14 (6.0%). The least were canicola 3 (1.3%) and *icterohaemorrage* 3 (1.3%), respectively (Table 3).

Two hundred and thirty-four of the four hundred and four serum samples obtained from cattle showed positive agglutination reaction to *Leptospira interogans* by MAT. One hundred and fourteen (48.7%) showed serological cross reactions to more than one serovar. Thirty 5 (15. 0%) samples were

Table 5: Association between social demographic characteristics of cattle with MAT positivity in some States in North Central Nigeria

Variables	Frequency No. (%)	Seropositive No. (%)	Seronegative No. (%)	Prevalence odds ratio	95% CI	p-value
Age (years)						
≤5	268 (66.3)	176 (65.7)	92 (34.3)	0.38	0.25-0.58	0.000002*
>5	136 (37.7)	57 (47.9)	79 (58.1)			
Sex						
Bull	246 (60.9)	134 (54.5)	112 (45.5)	1.40	0.93-2.11	0.05
Cow	158 (39.1)	99 (62.7)	59 (37.3)			
Breed						
Red bororo	40 (9.9)	34 (85.0)	6 (15.0)	0.21	0.09-0.52	0.00007*
White fulani	364 (90.1)	199 (54.7)	165 (45.3)			
Source						
Indigenous	356 (88.1)	195 (54.8)	161 (45.2)	3.14	1.52-6.49	0.0005*
Neighboring country	48 (11.9)	38 (79.2)	10 (20.8)			
Pregnancy						
No	20 (5.0)	229 (59.6)	155 (40.4)	0.17	0.06-0.52	0.0003*
Yes	384 (95.0)	4 (20.0)	16 (80.0)			
Location						
FCT	101 (25.0)					
Benue	101 (25.0)					
Nasarawa	101 (25.0)					
Plateau	101 (25.0)					

*Significant p-values, MAT: Microscopic agglutination test, FCT: Federal capital territory

positively reactive to four serovars. Plateau State showed the highest frequency of 12 and Nasarawa showed the least frequency of 4. Forty-one (17.5%) of the serum samples were cross reactive to three serovars: FCT with the highest frequency 13 and the least frequency of 8 for Benue State. Likewise, the serum samples that showed positive reaction to 2 serovars were 38 (16.2%) with the highest frequency of 14 for Nasarawa State and the least frequency of 5 for Benue state and 120 (51.3%) samples were positive to just one serovar each, FCT serovars showed highest frequency of 41 (Table 4).

Out of the 404 cattle, the mean age of slaughtered cattle was 4.7±1.2 SD years. Majority of slaughtered cattle were Bulls, 246 (61%), White Fulani breed 364 (90.2%), indigenously sourced 356 (88.1%), while 384 (95%) cattle were non-pregnant (Table 5).

By Bivariate Analysis the social demographic factors found to be associated with leptospirosis or *Leptospira* infections among slaughtered cattle were: Age group≤5 years (OR = 0.38, 95% CI: 025-0.58), Breed of cattle (OR = 0.21, 95% CI: 0.09-0.52), Source of cattle (OR = 3.14, 95% CI: 1:52-6.49) and Pregnancy (OR = 0.17, 95% CI: 0:06-0.52) (Table 5).

DISCUSSION

The serovar prevalence leptospirosis recorded is not much different from that of the study conducted by Ezeh *et al*. (1991) which reported a prevalence rate of 29.5% in Plateau State, Nigeria. Similar results have been reported for other countries, Zamora *et al*. (1990) reported a prevalence of 22% in Chile, while, Ferro *et al*. (2006) reported a prevalence of 23.3% in Colombia.

There was no significant difference between MAT and IgG ELISA as methods for the diagnosis of leptospirosis. However MAT had a better performance than ELISA. No wonder, MAT is currently the Gold standard for leptospirosis diagnosis. Its demonstrated sensitivity, specificity, positive predictive value of MAT and negative predictive values are in agreement with reports of WHO-ILS (2003).

Almost all the slaughtered cattle were from the nomadic herds located within the north central zone. Sometimes, cattle from neighboring Chad, Cameroun and Niger were brought to the abattoir for slaughter, which was also reported by Damina *et al*. (2011). However, it seemed indigenously sourced cattle, with the observed prevalence and odds ratio were three times more likely to come down with leptospirosis than those sourced from neighboring countries. This may be, as a result of the continuous grazing of the indigenous breeds on so called green pastures around slow moving water, streams etc. Various authors have established that a considerable percentage of "rat-friendly" areas (urban ponds, slow-moving rivers, canals and lakes near farms are contaminated with leptospires due to contamination from rat urine. Since, leptospires cannot replicate outside their hosts, slow-moving water, small pools of water and underground water are at higher risk of contamination by leptospires than larger, faster moving bodies of water, which can dilute the number of leptospires and decrease the risk of cattle infection (Mohan Rao, 2006; Meites *et al*., 2004). In the nomadic pasture system practiced by indigenous nomadic cattle rearers in Nigeria, cattle frequently and commonly drink from small water bodies, which may will have been contaminated, thereby leading to high levels of cattle infection as observed.

More male cattle of age ≤5years were slaughtered during the study period and were observed to be the most seropositive for *Leptospira.* This is hardly surprising; as cattle owners in the study area typically prefer to have more female than male animals in their herds, which they use to propagate the herd. Such females are usually only disposed of or taken to the abattoirs for slaughter when they can no longer reproduce, normally due to old age. The bivariate analysis showed the occurrence of leptospirosis, the following risk factors the age≤5years, the red Fulani breeds and non-pregnant. This observation is supported by the work of Bovet *et al*. (1999) that the older cattle are more likely to test positive to leptospirosis.

The observed non-significance in the prevalence rate of bovine leptospirosis in cattle with respect to sex is most likely, because leptospirosis is not peculiar to any sex, indicating that bulls or cows are at equal risk of contracting the disease, if exposed to sources of infection.

The higher prevalence of *Leptospira* antibodies in FCT could be due to the fact that FCT, being the Federal Capital city of Nigeria attracts more investors in the trade of cattle from farms and villages. This influx of cattle may well have been infected before reaching the FCT abattoirs.

CONCLUSION AND RECOMMENDATIONS

This study showed an overall high prevalence of leptospirosis in cattle slaughtered in north central, Nigeria. It also established the circulating serovars among cattle in the north central states, which demonstrated mix serovar infected or cross-reactivity among serovars. Thus, bovine leptospirosis is endemic in Nigeria and need to be urgently addressed. From the observed trend of increasing prevalence, as, the years go by, if nothing is done to arrest the trend, there may will be an epidemic outbreak of leptospirosis in the nearest future.

Leptospirosis in Nigeria is preventable, since, some of the risk factors are appropriately identified and interventions could be targeted at individual and communities.

REFERENCES

Abiayi, E.A., O.G. Ajani, J.I. Micheal, P.R. Kumbish, M. Odugbo, H.I. Inabo and P. Okewole, 2011. Serological evidence of leptospiral infection in sheep and goats in Benue, Nigeria. Proceedings of the 48th Annual NVMA Congress, November 21-25, 2011, Kwara State, Nigeria.

Agunloye, C.A., 2002. Leptospiral aggutinating antibodies in sheep and goats in South-West Nigeria. Israel J. Vet. Med., 57: 28-30.

Bovet, P., C. Yersin, F. Merien, C.E. Davis and P. Perolat, 1999. Factors associated with clinical leptospirosis: A population-based case-control study in the Seychelles (Indian Ocean). Int. J. Epidemiol., 28: 583-590.

Cutler, S.J., A.R. Fooks and W.H.M. van der Poel, 2010. Public health threat of new, reemerging and neglected zoonoses in the industrialized world. Emerg. Infect. Dis., 16: 1-7.

Damina, M.S., O.A. Owoludun, S. Chukwukere, J.A. Ameh and M.M. Aliyu, 2011. The use of deletion analysis in the detection of *Mycobacterium bovis*, *Mycobacteium tuberculosis* and *Mycobacterium africanum* among slaughtered cattle in Plateau State, North Central Nigeria. Vet. J., 32: 9-15.

Ezeh, A.O., A.A. Adesiyun, P.B. Addo, W.A. Ellis, A.A. Makinde and C.S. Bello, 1991. Serological and cultural examination for human leptospirosis in Plateau State, Nigeria. Central Afr. J. Med., 37: 11-15.

Ezeh, A.O., P.B. Addo, A.A. Adesiyun, C.S. Bello and A.A. Makinde, 1990. Serological prevalence of bovine leptospirosis in Plateau State, Nigeria. Revue Delevage et de Medecine Veterinaire des Pays Tropicaux, 42: 505-508.

Fang, F., J.M. Collins-Emerson, A. Cullum, C. Heuer, P.R. Wilson and J. Benschop, 2015. Shedding and seroprevalence of pathogenic *Leptospira* spp. in Sheep and Cattle at a New Zealand Abattoir. Zoonoses Public Health, 62: 258-268.

Ferro, B.E., A.L. Rodriguez, M. Perez and B.L. Travi, 2006. Seroprevalence of *Leptospira* infection in habitants of peripheral neighborhoods in Cali, Colombia. Biomedical, 26: 250-257.

Li, C., M.A. Motaleb, M. Sal, S.F. Goldstein and N.W. Charon, 2000. Spirochete periplasmic flagella and motility. J. Mol. Microbiol. Biotecnol., 2: 345-354.

Meites, E., M.T. Jay, S. Deresinski, W.J. Shieh, S.R. Zaki, L. Tompkins and D.S. Smith, 2004. Re-emerging leptospirosis, California. Emerg. Infect. Dis., 10: 406-412.

Mohan Rao, A.M.K., 2006. Preventive measures for leptospirosis: Rodent control. Indian J. Med. Microbiol., 24: 325-328.

NPC., 2006. State population figures. National Population Commission, Nigeria. http://www.population.gov.ng/index.php/state-population.

Ngbede, E.O., M.A. Raji, C.N. Kwanashie, E.C. Okolocha, V.T. Gugong and S.E. Hambolu, 2012. Serological prevalence of leptospirosis in cattle slaughtered in the Zango abattoir in Zaria, Kaduna State, Nigeria. Veterinaria Italiana, 48: 179-184.

Vallee, E., C. Heuer, J.M. Collins-Emerson, J. Benschop and P.R. Wilson, 2015. Serological patterns, antibody half-life and shedding in urine of *Leptospira* spp. in naturally exposed sheep. N. Z. Vet. J., 12: 1-32.

Vijayachari, P., A.P. Sugunan, S. Sharma, S. Roy, K. Natarajaseenivasan and S.C. Sehgal, 2008. Leptospirosis in the Andaman Islands, India. Trans. R.S. Trop. Med. Hyg., 102: 117-122.

WHO-ILS, 2003. Human leptospirosis: Guidance for diagnosis, surveillance and control. http://whqlibdoc.who.int/hq/2003/WHO_CDS_CSR_EPH_2002.23.pdf.

WHO., 2015. Leptospirosis. Fact Sheet, World Health Organization. http://www.searo.who.int/about/administration_structure/cds/CDS_leptospirosis-Fact_Sheet.pdf.

Zamora, J., S. Riedemann, M.I. Montecinos and X. Cabezas, 1990. [Serological survey of human leptospirosis in a high risk population in Chile]. Revista Medica de Chile, 118: 247-252.