IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 11, Issue 2 Ver. II (February 2018), PP 11-16
www.iosrjournals.org

Prevalence of Leptospira Spp. Serovar Bratislava in Pigs from, kaduna State, nigeria using competitive -Elisa.

Adah BMJ[1],Kwanashie CN[2], Prof. Kazeem HM[2], And Mailafia S[1]
[1]*Department of Veterinary Microbiology, University of Abuja, Nigeria.*
[2]*Department of Veterinary Micrbiology, Ahmadu Bello University Zaria, Nigeria.*
Corresponding Author: Adah BMJ1

Abstract: *The scarcity of information on porcine leptospirosis in Zaria and areas of Kaduna State led to this study, in order to detect antibodies to Leptospira in pigs, and to isolate Leptospira from pigs in Kaduna state Nigeria. The methodologies involved serological survey using competitive Enzyme Linked Immuno-Sorbent Assay (cELISA). Five hundred (500) porcine blood (whole blood) samples were collected for serology. The percentage distribution of antibodies to Leptospira detected using C - ELISA was 75 (15.83. This study showed that antibodies to Leptospira are present in pigs in Kaduna state, Nigeria. Based on the findings of this study, recommendations are; proper hygienic handling of pigs and porcine raw materials in farms, households, markets, teaching hospitals and abattoirs, by personnel like veterinarians, farmers, butchers, sewer workers, pork meat consumers that are at high risk of contracting leptospirosis. There is need for public health awareness and education on prevention /control of leptospirosis, as the disease is of great public health significance.*

Keywords: *Prevalence, Leptospira spp. serovar Brastislava, Pigs, C-ELISA, Kaduna, Nigeria.*

Date of Submission: 03-02-2018 Date of acceptance: 20-01-2018

I. Introduction

Leptospirosis is also known as Weil`s disease, Weil`s syndrome, canicola fever, cane field river, nanukayami fever, 7-day fever, rat catcher`s yellows, Fort bragg fever, and pretibial fever (James et al., 2006). These bacteria are long, motile spirochaetes that can be either free living in the environment or found as parasites in animal hosts (Ricardo et al.; 2008 Gompf, 2006). The incubation period is usually 5–14 days, with a range of 2–30 days (Terpstra et al., 2003a)It has been reported in over 150 mammalian species such as cattle, goat, sheep, pigs, man, dog, bats, marsupials, cat, rat, mice, and bufalo (Christopher et al., 2011; Ngbede et al, 2012). Both saprophytic and pathogenic species presently exist in nature, but saprophytic species, such as Leptospira biflexa, live in water and soil and do not infect animals (Skyes, 2011). Advantage of bacteriologic culture is the possibility of isolating Leptospira species of any serovar. However, bacteriologic culture procedures are too expensive and too slow for routine use, because fresh samples are necessary and 4 to 6 months may be required for conclusive results (Bolin et al., 1989b; Sarah et al., 2015), thereby necessitating the use of rapid screening test like ELISA (Bhatia et al., 2015).ELISA has been shown to be a highly sensitive and suitable diagnostic test for routine screening of leptospirosis due to its high specificity and sensitivity (Thiermann and Garrett, 1983; Yan et al., 1999; OIE, 2008; Ngbede et al., 2012). In addition, the organisms are needed for typing, as specific antigens in serological tests and for determination of pathogenicity/ vaccine production (Thiermann, 1984; Banihashemi, 2013).Leptospirosis occurs worldwide (Hua – wei et al., 2015), wherever there is a risk of direct or indirect contact with the urine of infected animals (Colleen et al., 2015). Theoretically, any mammal is capable of being infected by any serovar of Leptospira interrogans (Christopher et al., 2011). However, optimal conditions for survival are a warm and wet environment, with neutral or slightly alkaline water (Colleen et al., 2015; Senaka et al., 2015).

II. Materials And Methods

Experimental Area, Animals And Sampling

The study was carried out in parts of Kaduna State (Zaria, Kaduna metropolis and Kafanchan), Nigeria. Zaria is located between latitude $11^{o}04$ N and longitude $7^{o}42$ E, covering an area of $300km^2$ and with a population of about 408,198. The vegetation is Northern Guinea Savannah zone, with rainfall ranging from 0.0 to 816.0 mm/month and temperature of 17^{0}C to 33^{0}C (Mortimore, 1970). Kafanchan is a town in Southern Kaduna located between latitude $9^{0}34$N and longitude $8^{0}18$E, with an estimated population of 83,092 (Archibong, 2006). Kaduna metropolis is located between latitude $10^{0}31$N and longitude $7^{0}26$E, covering an area of 46.053 km^2 and with a population of about 6,066,562 (Fletcher *et al*., 1996). The sampling was carried

out from June to August, 2012 following the methods of Levett, (2001). Sampling covered areas of Zaria, Kaduna metropolis and Kafanchan, which are major areas for convergence of pigs in Kaduna state. Demographic data such as age, sex, breed, management practice, source and location of the animals were recorded.

Serological Analysis

Serology was done using, the Linnodee Porcine Leptospira bratislava cELISA (Solid Kit), which was obtained from Linnodee Animal Care Laboratory in Ireland. The solid kit is a competitive ELISA based on a unique monoclonal antibody to L. bratislava specific antigen with high specificity, and high sensitivity. The kit is safe and easy to use, suitable for screening large numbers of sera, and with an assay time of 180 minutes. It detects IgM antibodies in porcine serum (Linnodee, 2010).

Coating of Antigen: Addition of 100µl / well of L. Bratislava antigen diluted 1/100 in coating buffer was done. Then the plates were Sealed and incubated for 30 minutes at 37°C with shaking. This was washed x3.

Blocking of the Plate: Addition of 100µl / well of blocking buffer (x1) was carried out, then the plates were sealed and incubated for 30 minutes at 37°C with shaking. After which the plates were washed x3. For each washing step, the test wells were washed with at least 200µl/well of diluted wash buffer. Following the final wash, removal of residual wash buffer was done by inverting the plate and blotting firmly on absorbent paper.

Sample incubation: Addition of 100µl / well of test and control samples were carried out. Plates were then sealed and incubated for 45 minutes at 37°C with shaking. Then, wash x4. Four wells contained sample diluent only as this was used as the control OD.

Incubation with Monoclonal Antibody: Addition of 100µl / well of the anti-*L.*Bratislava monoclonal antibody diluted 1/800 in blocking buffer. Plates sealed and incubated for 30 minutes at 37°C with shaking. The plates were washed x3.

Conjugate incubation: Addition of 100µl / well of peroxidase conjugate diluted 1/7000 in blocking buffer was carried out. Plates were sealed and incubated for 30 minutes at 37°C with shaking. Were wash x3.

Substrate incubation: 100µl / well of TMB-E substrate was added. Plates were then incubated in the dark at room temperature for 10mins. 50µl / well of stop reagent was added. The plates were read at 450nm.
The monoclonal antibodies compete with anti-LPS antibodies in serum. The specific porcine antibodies if present bind to the L. bratislava antigen and inhibit binding of the monoclonal antibody.
The positivity of a sample was determined using the following calculation:
% Inhibition = [(Control OD – Test Serum OD)/ Control OD] x 100.
Results were interpreted as follows:

1. The result is considered positive if % Inhibition > 40%
2. The result is considered negative if % Inhibition ≤ 40%

Statistical Analysis

Results were subjected to simple descriptive statistical methods e.g. bar chart and tables. The data obtained were analyzed using Chi-square analysis and statistical package for social sciences (SPSS) version 20.0 (SPSS, Chicago, IL, USA). Values of $P < 0.05$ were considered significant.

III. Results

Prevalence of Leptospira antibodies from serum samples of pigs

Percentage inhibition of greater than 40% which was considered as positive had a frequency of 75 (15.83%) and percentage inhibition less than or equal to 40% considered to be negative was 405 (84.37%). A calculated percentage occurrence of 15.83% was obtained. (Table 1)

Table 1: Percentage distribution of IgM antibodies to Leptospira detected by c-ELISA

Serum sample tested	Frequency	Percentage (%)
Positive	75	15.83
Negative	405	84.17
Total	480	100

Table 2: Distribution of Leptospira antibodies according to age in Kaduna State, as determined by cELISA.

Age group	Result of cELISA		Total (%)
	Positive (%)	Negative (%)	
0 – 5 months	19 (23.2)	63 (76.8)	82 (100)
6 – 10 m0nths	17 (13.5)	109 (86.5)	126 (100)
11- 15 months	30 (15.2)	168 (84.8)	198 (100)
16 – 20 months	8 (14.0)	49 (86.0)	57 (100)
21 – 25 months	0 (0.0)	12 (100)	12 (100)
26+ months	1 (20)	4 (80)	5 (100)
Total	75 (15.83)	405 (84.4)	480 (100)

(Chi square = 6.414; p=0.268; p>0.005)

Table 3 : Distribution of antibodies to Leptospira according to sex in Kaduna State, as determined by cELISA.

Sex	Result of cELISA		Total (%)
	Positive (%)	Negative (%)	
Male	51 (17.2)	245 (82.8)	296 (100)
Female	24 (13.0)	160 (87.0)	184 (100)
Total	75 (15.83)	405 (84.4)	480 (100)

(Chi square = 1.508; p=0.219; p>0.005)

Table 3: Distribution of Leptospira antibodies according to age in Kaduna State, as determined by cELISA.

Age group	Result of cELISA		Total (%)
	Positive (%)	Negative (%)	
0 – 5 months	19 (23.2)	63 (76.8)	82 (100)
6 – 10 m0nths	17 (13.5)	109 (86.5)	126 (100)
11- 15 months	30 (15.2)	168 (84.8)	198 (100)
16 – 20 months	8 (14.0)	49 (86.0)	57 (100)
21 – 25 months	0 (0.0)	12 (100)	12 (100)
26+ months	1 (20)	4 (80)	5 (100)
Total	75 (15.83)	405 (84.4)	480 (100)

(Chi square = 6.414; p=0.268; p>0.005)

Table 4.4: Distribution of Leptospira antibodies according to location in Kaduna State, as determined by cELISA.

Location	Result of cELISA		Total (%)
	Positive (%)	Negative (%)	
Kafanchan	29 (14.5)	171 (85.5)	200 (100)
Kaduna	8 (16.0)	42 (84.0)	50 (100)
Zaria	38 (16.5)	192 (83.5)	230 (100)
Total	75 (15.83)	405 (84.4)	480 (100)

(Chi square = 0.338; p=0.845; p>0.005)

Table 4.5: Distribution of positive animals according to breed in Kaduna State, as determined by cELISA

Breed	Result of cELISA		Total (%)
	Positive (%)	Negative (%)	
Local	68 (16.4)	346 (83.6)	414 (100)
Exotic	7 (10.6)	59 (89.4)	66 (100)
Total	75 (15.83)	405 (84.4)	480 (100)

(Chi square = 1.462; p=0.227; p>0.005)

Table 4.6: distribution of positive samples based on source of animal

Souce of animal	Result of cELISA		Total (%)
	Positive (%)	Negative (%)	
Household	40 (15.2)	224 (84.8)	264 (100)
Farms	23 (29.9)	54 (70.1)	77 (100)
Abattoir	6 (16.2)	31(83.8)	37 (100)
Market	6 (5.9)	96 (94.1)	102 (100)
Total	75 (15.83)	405 (84.4)	480 (100)

(Chi square = 19.250; p=0.000; p<0.005)

Table 4.7: Distribution of positive samples based on management practice, as determined by cELISA.

Management practice	Result of cELISA		Total (%)
	Positive (%)	Negative (%)	
Intensive	6 (7.7)	72 (92.3)	78 (100)
Semi – intensive	46 (26.9)	125 (73.1)	171 (100)
Extensive	21(9.4)	202 (90.6)	223 (100)
Unknown	2 (25.0)	6 (75.0)	8 (100)
Total	75 (15.83)	405 (84.4)	480 (100)

(Chi square = 27.266; p=0.000; p<0.005)

IV. Discussion

Immunoglobulin M (IgM) antibodies to Leptospira was detected using IgM competitive ELISA in 75 sera (15.83%), out of the 480 samples tested (Table 4.1). A percentage occurrence of 15.83% was obtained in this study, which is higher than the earliest study reports on seroprevalence of leptospirosis in Nigeria, which indicated a positive rate of 4.5% in rats (Diallo and Dennis, 1982), and 8.44% in cattle (Ngbede et al., 2012) in Zaria, Kaduna – State. The high percentage occurrence in this present study was attributed to the fact that the previous studies used sandwich ELISA while in this present studies a competitive ELISA kits were used and also, the previous study was targeted towards detecting IgG antibodies to Leptospira which indicates latent infection (Ngbede et al., 2013), while this study was targeted towards detecting IgM antibodies to Leptospira which indicates acute infection. In the previous work their results were obtained from rats and cattle while the present studies were from pigs. The high percentage occurrence in this study may also be as a result of the unhygienic state/poor management practice observed in pig housing / mud flooring, which increases the risk of infection (Terpstra et al., 2003b). The IgM cELISA kits used in this study, is highly specific for Leptospira interogans serovar Bratislava and the value (15.83%) obtained, is higher than the prevalence of 13.7%, reported for L. interrogans serovar Bratislava detected in dogs by Agunloye et al. (2002), in Ibadan, Nigeria. This may be as a result of pigs unlike dogs, wallow in dirty/mud water due to possession of poor sweat glands, hence the need for body temperature regulation, and at such are at greater risk of contracting the disease than dogs, because pigs are usually raised on mud floor houses with poor sanitary conditions (Terpstra et al., 2003b; Bharadwaj et al., 2002)), and infected animals become carriers for live (Thiermann, 1981; Leonard et al., 1992; Faine et al., 1999b; Bharti et al., 2003), they also continue to shed the organisms in urine and genital fluid onto the mud floors (Terpstra, 2006, and leptospires have been reported to persist long in contaminated soil/water for long, hence increasing the risk of infection more on pigs (Kuperk et al., 2000) . The percentage occurrence obtained from this study agrees with the work of Bertherat et al. (1999) who reported a prevalence of 15% in cattle in Gabon, Africa. And lower than the 16% positive rate reported in aborting goats in Ibadan by Agunloye et al. (1997), and 16.7% reported in vaccinated dogs in Ibadan by agunloye et al. (2002), this was attributed to the presence of previous antibodies raised due to previous vaccination (Ngbede et al., 2013), as a result of routine DHLPP vaccination in dogs with live Leptospira polyvalent vaccines and hence, maybe the reason for the increase in circulating antibodies in serum due to previous exposure.The absence of a statistically significant association (p=0.219; p>0.05) (Table 4.2) using IgM cELISA between the presence of Leptospira antibodies in sera and sex indicates that both males and females possess equal risk of contracting the infection. Males had a higher percentage occurrence (17.2%), than was observed in females (13.0%) and this might be as a result of sample size collected in this study because more males were sampled than females (Table 4.2).The absence of a statistically significant association (p=0.268; p>0.05) (Table 4.3) using IgM cELISA between the presence of Leptospira antibodies in sera and age indicates that all age groups possess equal risk of contracting the infection. The highest no of positives was seen among the age group 11 – 15 months (30), this may be due to the fact that, at this age the pigs are said to have reach table size and at such taken to the market or slaughtered, and others at this age are released to roam about and fend for themselves (semi-intensive management).There was no statistically significant association between location (p=0.845; p>0.05) (Table 4.4), breed (p=0.227; p>0.05) (Table 4.5) and seropositivity of pigs, which indicates that all location sampled, as well as both local and exotic breeds all have equal risk of contracting Leptospira interrogans serovar Bratislava, as observed by our findings. Which is in agreement with the report of Ngede et al. (2013) and Adama et al. (2011), that indigenous breed (Local breeds) are more common in this area, which was the case in our study with the indigenous breed being more predominant at the time of sampling, with a higher prevalence (n=430), than the exotic breed (n=70).The presence of a statistically significant association between source of animal (p=0.000; p<0.005) (Table 4.6), management Practice (p=0.000; p<0.005) (Table 4.7) and seropositivity of pigs. which indicates that management practice and the source of animal plays a role in predisposing the animals to infection with the disease, as well as increases the risk of transmitting the disease from one animal to another through constant shedding of the organism in urine, especially in places like markets were unsuspecting farmers and pig owners come to purchase more pigs to increase their stock, as seen in our research where pigs were apparently healthy

yet some were seropositive. Which agrees with the report of Bharti et al. (2003), which says when animals become infected, they might not show clinical signs to the disease but yet remain carriers for life.
Porcine leptospirosis is endemic in pigs in Kaduna state, Nigeria with a percentage occurrence of 15.83% and this study has shown the presence of Leptospira antibodies among pigs in Kaduna state.

References

[1] Abdollahpour, G. R. (1995a). Isolation of Leptospira interrogans serovar Grippotyphosa from a heifer in New South Wale`s. Australian Veterinary Journal, 73: 109 – 110.
[2] Abdollahpour, G. R. (1995b). Bovine leptospirosis with a special reference to Leptospira interrogans serovar Hardjo. A thesis submitted in fulfillment of the requirements for the degree of Doctor of philosophy, Department of Animal Health, University of Sidney. 73 – 84.
[3] Archibong, M. (2006). Kafanchan: Rising from rot wrought by Railways` woes. The Daily Sun, 26.
[4] Adler, B., Faine, S., Christopher, W.L. and Chappel, R.J. (1986). Development of an improved selective medium for isolation of leptospires from clinical material. Veterinary Microbiology, 12: 377-381.
[5] Agunloye, C.A., Oyeyemi, M.O., Akusu, M.O., Ajala, O.O., and Agbede, S.A. (1997). Clinical and serological diagnosis of leptospirosis in aborting West African dwarf goats. Bulletin of Animal Health and Production in Africa, 45: 5-8.
[6] Agunloye, C.A., Alabi, F.O., Odemuyiwa, S.O. and Olaleye, O.D. (2001). Leptospirosis in Nigeria: a seroepidemiological survey. Indian Veterinary Journal, 78(5): 371-375.
[7] Agunloye, C. A., Ajuwape, A. T. P., and Nottidge, H. O., (2002). Comparative study of the prevalence of leptospirosis in vaccinated and unvaccinated dogs in ibadan. Retrieved from Vet.ui.edu.ng on 26th January, 2010. At 8pm.
[8] Anderson, J.F., Miller, D.A., Post, J.E., Johnson, R.C., Magnarelli, L.A. and Andreadis, T.G. (1993). Isolation of Leptospira interrogans serovar Grippotyphosa from the skin of a dog. Journal of American Veterinary Medicine Association, 203: 1550-1551.
[9] Bharti, A. R., Nally, J. E., Ricaldi, J. N., Matthias, M. A., Diaz, M. M., and Lovett, M. A. (2003). Leptospirosis: A zoonotic disease of global importance. Lancet Infectious Disease, 3(12): 757 - 771.
[10] Bolin, C.A., Zuerner, R.L. and Trueba, G. (1989b). Effects of vaccination with a pentavalent leptospiral vaccine containing Leptospira interrogans serovar Hardjo type Hardjobovis infection of cattle. American Journal of Veterinary Research, 50: 2004-2008.
[11] Burriel, A. R. (2010). Leptospirosis: an important zoonotic disease. In Current Research. Technology Education Topics in Applied Microbiology and Microbial Biotechnology, MENDEZ-VILAS A. (ed), Publ. Formatex Research Center, Spain. Volume 1, pp: 687 – 693.
[12] Christopher, A. D., Marvin, J. S., Ryan, C. M., Anne, S., Braden, H., Mark, J., Mary, B., and Joseph, M. V. (2011). Hemoptysis associated with leptospirosis acquired in Hawaii, USA. Emerging Infectious Diseases, 17 (2): 2375 - 2377.
[13] Diallo, A.A. (1978). Public Health Significance of Leptospirosis in Northern Nigeria.
[14] Unpublished PhD. Thesis. Ahmadu Bello University, Zaria, Nigeria. 354pp.
[15] Ellis, W.A., O'Brien, J.J., Neill, S.D., Ferguson, H.W. and Hanna, J. (1982). Bovine leptospirosis: Microbiological and serological findings in aborted fetuses. The Veterinary Record, 110: 147-150.
[16] Ellis, W.A., McParland, P.J., Bryson, D.G. and McNulty, M.S. (1985). Leptospires in pig urogenital tracts and fetuses. Veterinary Record, 117: 66-67.
[17] Ellis, W.A., O'Brien, J.J., Neill, S.D. and Bryson, D.G. (1986a). Bovine Leptospirosis: Experimental serovar Hardjo infection. Veterinary Microbiology, 11: 293-299.
[18] Ellis, W.A., Songer, J.G., Montgomery, J. and Cassells, J.A. (1986b). Prevalence of Leptospira interrogans serovar Hardjo in the genital and urinary tracts of non-pregnant cattle. Veterinary Record, 118: 11-13.
[19] Ellis, W.A. (1992). Animal leptospirosis: constraints in diagnosis and research. In: Terpstra, J.W. (ed). Proceedings of a CEC/STD3 Research Meeting Zimbabwe, 17- 20 February 1992, pp. 19-30.
[20] Ezeh, A. O., Ellis, W. A., Kmety, E., Adesiyun, A. A. and Addo, P. B. (1989). Bacteriological examination of bovine kidneys for leptospirosis in plateau state, Nigeria. Reviews of science and technology office international epizootic, 8(4): 1005 - 1008.
[21] Ezeh, A. O., Ellis, W. A., and Addo, P. B., Adesiyun, A. A., Makinde and Bello, C. S. (1991).
[22] Serological and cultural examination for human leptospirosis in plateau state, Nigeria. Central African journal of medicine, 37 (1): 11 - 15.
[23] Faine, S. (1982). Guidelines for the control of Leptospirosis Geneva World Health Organisation. Pp. 171
[24] Faine, S. (1994). Leptospira and leptospirosis. CRC press, Boca Raton, Florida. Pp. 353
[25] Faine, S., Adler, B., Bolin, E. and Perolat, P. (1999). Leptospira and Leptospirosis. 2nd edition Medscience. Melbourne. Pp. 296
[26] Gompf, S. G. (2006). Leptospirosis. Electronic Journal of Medicine; 56 - 59.
[27] Isenberg, H. D. (ed) (1992). Clinical Microbiology Procedures Handbook. American Society for Microbiology. Washington D. C. 56 – 89.
[28] James, W. D. and Berger, T. G. (2006). Andrews Diseases of the Skin: Clinical Dermatology, Saunders Elsevier, 216 - 221.
[29] Jimenez-Coello, M., Vado-Solis, I., Cardenas-Marrufo, M.F., Rodriquez-Buenfil, J.C. and Ortega-Pacheco, A. (2008). Serological survey of canine Leptospirosis in the tropic of Yucantan Mexico using two different tests. Acta Tropica, 106: 22-26.
[30] Johnson, R.C., Walby, J., Henry, R.A. and Auran, N.F. (1973). Cultivation of parasitic leptospires: effect of pyruvate. Applied Microbiology, 26: 118-119.
[31] Johnson, R. (1989). Isolation techniques for spirochetes and their sensitivity to antibiotics in vitro and in vivo. Clinical Infectious Diseases, 11(Suppl.6): S1505-S1510.
[32] Langston, C. E. and Heuter, K. J. (2003). Leptospirosis, A re-emerging zoonotic disease. The Veterinary Clinics of North America, Small Animal Practice, 33 (4): 791 - 807.
[33] LERG, (2010). Lepto burden Epidemiology reference group. accessed 23rd March, 2011 at 3:30pm.http://www.who.int/zoonoses/diseases/lerg/en/index5.html.
[34] Miller, D. A., Wilson, M. A., and Beran, G. w. (1990). The effect of storage time on isolation of Leptospira interrogans from bovine kidney. Journal of Veterinary Diagnosis and Investigations. 2: 63 – 65.
[35] Mortimore, M. J. (1970). Zaria. Annals of the Association of American Geographers, 60: 73 – 80.
[36] Ngbede, E. O. (2012). Serological survey for antibodies to Leptospira hardjo in cattle in zaria and environs. Unpublished Msc Thesis, Veterinary Microbiology, Faculty of Veterinary Medicine, ABU, Zaria, Nigeria. Pp. 1 – 120.

[37] Ngbede, E. O., Raji, M. A., Kwanashie, C. N. and Okolocha, E. C. (2013). Serosurvey of Leptospira spp serovar Hardjo in cattle from Zaria, Nigeria. Revue Medicine Veterinary, 164(2) 85 – 89.
[38] Nervig, R.M., Cheville, N.F. and Baetz, A.L. (1978). Experimental infection of calves with Leptospira interrogans serotype Szwajizak. American Journal of Veterinary Research, 39(3): 523-525.
[39] OIE (2000) -Office International de epizooties. Manual of Standards for Diagnostic Tests and List A and B diseases Vaccines, pp 165-175.
[40] OIE, (2008). Office des epizootics. Leptospirosis. Manual of Diagnostic Test and Vaccines for Terrestrial Animals. pp. 251- 264
[41] Oie, S., koshiro, A., Konishi, H. and Yoshii, Z. (1986). In vitro evaluation of combined usage of fosfomycin and 5-Fluorouracil for selective isolation of Leptospira species. Journal of Clinical Microbiology, 23: 1084-1087.
[42] Onyemelukwe, N.F. (1993). A serological survey of leptospirosis in Enugu area of eastern Nigeria among people at occupational risk. Journal of Tropical Medicine and Hygiene, 96(5): 301-304.
[43] Plank, R. and Dean, D. (2000). Overview of the epidemiology, microbiology and pathogenesis of leptospira species in humans. Microbes and Infections, 2(10): 1265-1276.
[44] Radostits, O.M., Blood, D.C. and Gay, C.C. (1994) Diseases caused by Leptospira spp. In: Veterinary Medicine. A Textbook of the Diseases of Cattle, Pigs and Horses. Bailliere Tindall, London, 8th Edition. Pp. 758-769.
[45] Ricardo, I., Sagar, G., and Angela, C. (2008). Leptospirosis: The "mysterious" Mimic. Journal of Emergencies, Trauma and Shock, 1(1): 21–33.
[46] Ris, D.R. and Hamel, K.L. (1978). The detection of leptospirae in cattle urine. New Zealand Veterinary Journal, 26: 246,255-6.
[47] Sakhaee, E., Abdollahpour, G. H. R., Bolourchi, M., Hasani, T. A. M., and Sattari, T. S.
[48] (2007).Serologic and Baceteriologic Diagnosis of Bovine Leptospirosis in Tehran Suburd diary farms. Iranian Journal of Veterinary research, 8 (4): 325 – 332.
[49] Sessions, J.K. and Greene, C.E. (2004). Canine Leptospirosis: epidemiology, pathogenesis and diagnosis. Compendium of Continuing Education and Veterinary Practice, 26: 607–622.
[50] Scolamacchia, F., Handel, I.G., Fèvre, E.M., Morgan, K.L., Tanya, V.N., and Bronsvort, B.M.D.
[51] (2010). Serological Patterns of Brucellosis, Leptospirosis and Q - Fever in Bos indicus Cattle in Cameroon. PLoS ONE, 5(1): 8623.
[52] Skyes. (2011). Canine leptospirosis. ACVIM Small Animal Consensus Statement on Leptospirosis: Diagnosis, Epidemiology, Treatment, and Prevention. Journal of Veterinary Internal Medicine, 25: 1–13.
[53] Terpstra, W. J. (2006). Historical perspectives in leptospirosis. Indian Journal of Medical Microbiology, 24(4) 316 – 320.
[54] Thiermann, A.B. and Garret, L.A. (1983). Enzyme-linked immunosorbent assay for the detection of antibodies to Leptospira interrogans serovars Hardjo and Pomona in cattle. American Journal of Veterinary Research, 44: 884-887.
[55] Thierman, A.B. (1983). Bovine Leptospirosis: Bacteriological versus serological diagnosis of cows at slaughter. American Journal of Veterinary Research, 44:2244-2245.
[56] Thiermann, A.B. (1984). Leptospirosis: Current development and trends. Journal of the American Veterinary Medical Association, 184: 722-725.
[57] Thiermann, A.B., Handsaker, A.L., Mooseley, S.L. and Kingscote, B. (1985). New method for classification of leptospiral isolates belonging to serogroup Pomona by restriction endonuclease analysis: serovar Kennewicki. Journal of Clinical Microbiology, 21: 585-587.
[58] Vijayachari, P., Sugunan, A, P., and Shriram, A.N. (2008). Leptospirosis: an emerging global public health problem. Journal of Bioscience, 33: 557 – 569.
[59] Weyant, R.S., Braggi, S.I. and Kaufmann, A.F. (1999). Leptospira and Leptonema. In: Murray, P.R., Baron, E.J., Pfaller, M.A., Trenover, F.C. and Yolken, R.H. (ed). Manual of Clinical Microbiology. 7th edition, Boston, Kluwer Academic Publishers (American Society for Microbiology). Pp. 739-745.
[60] Yan, K.T., Ellis, W.A., Mackie, D.P., Taylor, M.J., McDowell, S.W.J. and Montgomery, J.M. (1999). Development of an ELISA to detect antibody to a protective lipopolysaccharide fraction of Leptospira borgpetersenii serovar Hardjo in cattle. Veterinary Microbiology, 69: 173-187.
[61] Zavitsanou, A., and Babatsikou, F. (2008) Leptospirosis: epidemiology and preventive measures. Health Science journal, 22: 75 – 82.

Adah BMJ "Prevalence of Leptospira Spp. Serovar Bratislava in Pigs from, kaduna State, nigeria using competitive -Elisa.". IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 11.2 (2018): 11-16.