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# ISOLATION AND PREVALENCE OF PATHOGENIC *LEPTOSPIRA INTERROGANS* IN SLAUGHTERED CATTLE IN TWO ABATTOIRS IN SOUTHWESTERN NIGERIA

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## SUMMARY

Leptospirosis is a waterborne bacterial disease, transmitted to humans through contaminated water, usually by urine of rodents that are chronically infected by the pathogenic strains. The prevalence and incidence of leptospirosis in slaughtered cattle in relation to the risk of exposure of abattoir workers and the public consumers was determined in this study. The objective of this study was to evaluate the prevalence of bovine leptospirosis in slaughtered cattle in southwestern Nigeria. Kidney samples from 108 cattle were examined. Samples were collected from Bodija abattoir in Ibadan, capital city of Oyo State, and Lafenwa abattoir in Abeokuta, capital city of Ogun State. *Leptospira* was isolated in Ellinghausen-McCullough-Johnson-Harris broth medium (EMJH). Pathogenicity test was carried out in Guinea pigs. Gross and histopathological lesions were observed in their

kidneys. *Leptospira* species were isolated from 89 (82.4%) out of 108 kidneys from the slaughtered cattle. Twenty (95.2%) out of 21 kidneys and 69 (79.3%) out of 87 kidneys collected from Ibadan and Abeokuta respectively were positive. Only 31 (28.7%) (9 kidneys from Ibadan and 22 from Abeokuta) kidneys showed visible macroscopic changes, while histomorphological changes such as interstitial nephritis, tubular nephrosis and tubular protein cast were observed. Death of guinea pigs that were inoculated occurred within 24 hours to 168 hrs. The isolation of *leptospira interrogans* and the pathology associated with kidneys obtained in this study indicates that cattle slaughter in public abattoir in South-western Nigeria may be sources of exposure and infection to abattoir workers and the public to leptospirosis.

## INTRODUCTION

Leptospirosis is a zoonosis of ubiquitous distribution, caused by infection with pathogenic *Leptospira* species. The spectrum of disease caused by leptospires is extremely wide, ranging from subclinical infection to a severe syndrome of multiorgan infection with high mortality. The syndrome, icteric leptospirosis with renal failure, was first reported over 100 years ago by Adolf Weil in Heidelberg (Levett, 2001).

Documented information on the role of cattle in the epidemiology of leptospirosis in Nigeria is scanty. At

present, there is no specific control strategy against leptospirosis in Nigeria as little is known about the epidemiology of the infection. Cattle, sheep and goats are known in Nigeria to be kept in smallholder units in close proximity with their owners, thus infection with *leptospira* may pose human health hazard. Hence, the present work was designed to determine the prevalence of leptospirosis in cattle, by leptospire isolation in EMJH medium and histopathological changes associated with infected cattle in south-western Nigeria.

## MATERIALS AND METHODS

This project was carried out in Ibadan and Abeokuta the capital cities of Oyo and Ogun States respectively, in the south western Nigeria. The animals were slaughtered in the central metropolitan abattoirs in Ibadan and Abeokuta where more than 500 and 200 heads of cattle respectively, are slaughtered daily. One-hundred and eight kidney samples from 108 different cattle with unknown leptospirosis history, slaughtered at the abattoirs were selected for the study. Approximately 10-15g of kidney sections each was taken for bacteriological and pathological evaluation.

(Difco®-USA) with the addition of 10 % of Rabbit serum and 5-fluorouracil (400 mg/L; Sigma®-USA and chloranphenicol (5 mg/L; Sigma®-USA), nalidixic acid (50 mg/L; Inlab®-BR), neomycin (10 mg/L; Sigma®-USA) and vancomycin (10 mg/L; Acros®-USA). Each sample was inoculated into EMJH medium tubes, incubated at room temperature (28-30°C) in the dark and examined under dark field illumination at intervals of 10 days to check for the growth of leptospires for at least three months. The bacteria load was manually counted with a Petroff Hausser counting chamber for experimental infection. Ten guinea pigs of either sex each weighing 150 to 200 grams were inoculated intraperitoneally with 1 ml of randomly selected isolates of the culture leptospiral ( $1 \times 10^6$ ). Two normal

The isolation of *Leptospira* was made from direct inoculation of two drops of blood in 5 mL of Ellinghausen-McCullough-Johnson-Harris broth medium (EMJH)



guinea pigs were inoculated with EMJH medium as the negative controls.

## RESULTS

Culturally, leptospire were isolated from 89 (82.4%) out of 108 kidneys from the two states. This consist of 20 (95.2%) out of 21 kidneys and 69 (79.3%) out of 87 kidneys collected from Ibadan and Abeokuta respectively.

Out of the one hundred and eight (n = 108) kidney samples randomly collected from the two abattoirs, only 31 (28.7%) (9 kidneys from Ibadan and 22 from Abeokuta) kidneys showed visible macroscopic changes. The lesions include multifocal necrotic areas, multifocal petechial ions, icterus and diffuse nephrosis

The specific histopathology lesions observed in samples that tested either positive or negative for leptospire in EMJH expressed in percentages are shown in table 1.

Most of the guinea pigs that were inoculated with the isolates died between 24 and 168hrs (seven guinea pigs) and the remaining three showed signs of infection associated with leptospirosis.

Table 1: Prevalence of specific histopathological lesion in kidney tissue samples collected from Abeokuta and Ibadan that were cultured in EMJH medium.

Histological lesions	Abeokuta abattoir (n=87)		Ibadan abattoir (n=21)	
	Positive (n=69)	Negative (n=18)	Positive (n=20)	Negative (n=1)
Interstitial Oedema	8 (11.6%)	-	1 (5%)	-
Tubular Nephrosis	52 (75.4%)	2 (11.1%)	16(80%)	1 (100%)
Tubular Epithelial Necrosis	47 (68.1%)	4 (22.2)	6 (30%)	-
Interstitial Fibrosis	31 (44.9%)	1 (5.5%)	8 (40%)	-
Interstitial Mononuclear Cells Infiltration	58 (84%)	1 (5.5%)	13(65%)	1 (100%)
Perivascular Mononuclear Cell Infiltration	38 (55.1%)	-	7 (35%)	1 (100%)
Periglomerular Mononuclear Cell Infiltration	32 (46.4%)	-	3 (15%)	-
Glomerular Atrophy	25 (36.2%)	-	4 (20%)	-
Cast	40 (58.0%)	13(72.2%)	11(55%)	1 (100%)
Tubular Dilatation	30 (43.5%)	5 (27.8%)	4 (20%)	1 (100%)
Glomerulonephritis	10 (14.5%)	-	1 (5%)	-
	19 (25.5%)	-	6 (30%)	-
Crystals	31 (44.9%)	4 (22.2%)	6 (30%)	1 (100%)

## DISCUSSION

Leptospire were isolated from 89 out of 108 kidney samples collected from the two abattoirs in the Southwestern Nigeria. The diagnosis was based on either

or both isolation of the leptospira species with EMJH medium at 27°C-30°C and pathogenicity test with guinea pigs. In Nigeria, there is no record of the isolation of

leptospire from animals, as the majority of the data are based on serology (Agunloye *et al* 1997).

The gross renal lesions, such as cortical haemorrhage, multifocal necrosis, diffuse palor and icterus, reported in this study are typical of renal bovine leptospirosis and are consistent with those in previous reported cases in cattle and other animals (Faine *et al.*, 1999). In this study, there is no relationship between gross lesions and isolation of leptospira organism in the kidney samples, since most of the kidney samples without gross lesions were culturally

positive. Histological changes observed in this investigation were in correlation with the reports of other workers (Marinho *et al* 2009). However, Skilbeck *et al* (1988) did not observe significant histopathological lesions in kidneys from which leptospire were isolated. In this study the lesions range from locally extensive cellular infiltrates to diffuse lesions, characterized by tubular nephrosis, glomerular atrophy and renal haemorrhage. Most of the kidneys samples studied presented changes suggestive of leptospirosis in the histopathological lesions of the kidneys in accordance with Faine *et al.*, (1999).

### CONCLUSION

The isolation of *leptospira interrogans* and the pathology associated with the kidneys obtained in this study indicate that cattle slaughtered in the public abattoir in the South-western Nigeria may be sources of the infectious agent to

human population. It is recommended to improve on the sanitation and personal hygiene of abattoir workers and implement a hazard analysis critical control system in the abattoir.

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