Research Article

# Occurrence of *Leptospira* Species from Pigs in Selected Local Government Areas of Kaduna State, Nigeria

**Bridget Adah[1]*, Clara Kwanashie[2], Haruna Kazeem[2] and Samuel Mailafia[1]**

*[1]University of Abuja, Nigeria; [2]Ahmadu bello university, Zaria, Nigeria.*

**Abstract** | The paucity of information on the occurrence of porcine leptospirosis in Kaduna State, Nigeria predicated this study. The research was conducted to isolate *Leptospira* species from pigs. The organisms were isolated using Ellinghausen-McCullough-Johnson-Harris (EMJH) enrichment and basal medium, and identified using dark field microscopy. A total of two hundred (200) blood samples and two hundred (200) urine samples each were collected from pigs in Kaduna state, Nigeria. A total of 9 (4.5%) of the cultured samples were positive for the *Leptospira* organisms and % isolation from blood sample was 7 (3.5%), while 2 (1%) came from urine samples. Our results demonstrated that the total prevalence of *Leptospira* organisms in pigs in these areas was 4.5%. Considering the economic and zoonotic significance of this organism, we recommend that proper handling of pork and associated by products, as well as occupationally predisposed personnel should be of great importance especially pig farmers and veterinarians. The findings of this study indicates the need for further research to illuminate predisposing factors, patterns of distribution and clinical manifestation of leptospirosis in porcine inhabitants in different regions in Nigeria.

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***Correspondence** | Bridget Adah, University of Abuja, Nigeria; **Email:** bridget.adah@uniabuja.edu.ng
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## Introduction

Leptospirosis is an infectious and highly contagious commercially important disease of livestock which is widely distributed in the world (Bharti *et al.*, 2003). It is caused by *Leptospira* organism within the phylum: Spirochaetes, order: *Spirochaetales,* family: *Leptospiraceae,* and Genus *Leptospira* (Scolamacchia *et al.*, 2010). The genus comprises of saprophytic and pathogenic species with members also assembled into Serovars based on their antigenic relatedness with over 250 recognized serovars and each serovar acts differently within its maintenance host (Ngbede *et al.*, 2013).

It is a disease that affects humans, mammals, birds, amphibians and reptiles (Langston and Hueter, 2003). Leptospirosis has been reported to be an indigenous disease in Nigeria with prevalence between 1.7% - 23% in cattle and 26% - 29% in human (Boey *et al.*, 2019).

The organism can survive in cold water environment; infections are most likely to occur in wet seasons than in dry seasons (Thiermann *et al*., 1985). The disease is an occupational hazard to swimmers through contact with rivers, streams or lakes which has been tainted with urine of rodents and wild animals (Bharti *et al*., 2003; Adah *et al*., 2018).

Definitive diagnosis of leptospirosis is usually provided by bacteriologic culture of the infecting organism (Toyokawa *et al*., 2011). In the selection of proper tissues or samples for isolation purposes, it is necessary to determine the stage of the disease. In cases of acute disease, isolation should focus on blood samples (Sukl *et al*., 2017). In the chronic form after seroconversion, the isolation should be ventured from the urine (Boey *et al*., 2019). Where there is a clinical disease or presence of an aborted foetus, isolation should be ventured from the kidney, liver and the aqueous humor (Faine, 1994). Due to production and reproductive losses which includes manifestations like agalactiae, decreased productivity and infertility, stillbirth, abortion, and low productivity due to the disease, leptospirosis is reported to be a major cause of economic/industrial losses in the livestock production (Agunloye *et al*., 2002). As a result of the public health and zoonotic importance attached to leptospirosis, there is need for creating awareness about the diseases worldwide (Alonghom *et al*., 2017; Burriel, 2010). The under reporting and paucity of information about the clinical confirmation and availability of required diagnostic tools has lead to the misdiagnosis of Leptospirosis for other interconnected diseases. Leptospirosis is often complicated in Nigeria due to negligence and unavailability of diagnostic tools and data, which is also seen in many other under developing countries worldwide (LERG, 2010).

The isolation of the organism is the preliminary bases by which all other antigen detection methods are evaluated (OIE, 2010). Cultural isolation of the organism is not only depending on the number of infectious organisms but also depends on the pathogenicity of the organisms (Kartrin *et al*., 2018). One vital superiority of bacteriologic culture and identification of *Leptospira* is the fact that, any serovar can be cultured, detected and subsequently identified (Collins *et al*., 2021). It can also aid in typing for various strains, required for epidemiological studies involving many serovars within particular species in different geographical locality (Vanina *et al*., 2018). This study is remarkable as it will supply information required for diagnostic and control strategies of the disease in Nigeria.

The aim of this study is to determine the occurrence of *Leptospira* organism from urine and blood of porcine species in Kaduna state, Nigeria and to establish the epidemiological implications and distribution of the disease in the study area.

## Materials and Methods

### *Study area*

The study was carried out in parts of Kaduna State (Zaria, Kaduna metropolis and Kafanchan), Nigeria. Zaria is located between latitude 11°04′N and longitude 7°42′E, covering an area of 300km$^2$ and with a population of about 408,198 (Nimyel and Namadi, 2019). The vegetation is Northern Guinea Savannah zone, with rainfall ranging from 0.0 to 816.0 mm/month and temperature of 17°C to 33°C. Kafanchan is a town in Southern Kaduna located between latitude 9°34′N and longitude 8°18′E, with an estimated population of 83,092 (Nimyel and Namadi, 2019). Kaduna metropolis is located between latitude 10°31′N and longitude 7°26′E, covering an area of 46.053 km$^2$ and with a population of about 736,000 (Nimyel and Namadi, 2019).

### *Study design*

Cross-sectional study was designed using convenience sampling based on availability of pigs and willingness of pig farmers and abattoir owners to participate in this research. The sampling was carried out daily from June to August, 2012. Sampling covered areas of Zaria, Kaduna metropolis and Kafanchan which are the three senatorial zones and also constitute the nuclei for pig rearing in Kaduna state. In each farm, abattoir, household and market visited, samples were collected from 50% of the animals available to come about the sample size which was determined as recommended by Thrusfield (2007). The age, sex, breed, management practice, source of animals and location were recorded.

### *Sample collection and processing*

Blood and urine samples were taken from 200 pigs in Kafanchan, Kaduna metropolis, and Zaria. About 10ml of blood was collected aseptically using 20ml syringe and 18 gauge needles, after proper restraining of animals, which was collected directly

from the anterior vena cava of the heart of each pig using the hypodermic needle and syringe. About 5 ml each from the 10mls of blood collected was transferred into 2 clean test tubes with one containing heparin (anticoagulant) for culture and the one without heparin for serology, and both were labeled appropriately. The screw capped blood samples were transported immediately to the Ahmadu Bello University, Veterinary Microbiology Laboratory in a leak proof container with ice packs for processing (Miller *et al*., 1990).

Midstream urine samples were collected into sterile sample bottles after cleaning of the vulva in females and prepucial area of males using the clean catch urine sampling method. The pigs were encouraged to urinate using the massage method. Five mililiters of the collected urine collected directly unto a sterile screw cap bottle containing 0.5ml of filtered (0.4µm) 40% formaldehyde and transported to the laboratory placed in an ice packed container (4°C) as soon as possible for processing (Sakhaee *et al*., 2007).

### *Media preparation*

Ellinghausen McCulough modified Johnson Harris (EMJH) semi solid medium (Difco™ USA) was used for culture and isolation. The media was prepared following the manufacturers specification for each sample (urine and blood). 2.3 g of the EMJH basal media (Difco™ USA), was weighed electronically and dissolved in 900ml of distilled water. It was then heated over a Bunsen burner flame to enable complete dissolution of the medium. The dissolved solution was sterilized via autoclave for 15mins. It was allowed to cool down to 40°C, and 100ml of the EMJH enrichment medium (Difco™ USA) was added. 200µg/ml of 5 fluorouracil antibiotics was added to prevent growth of contaminants. The media was then dispensed into culture tubes at 5ml per culture tube (Sakhaee *et al*., 2007).

### *Identification*

The identification of leptospiral organisms was done according to standard methods as described in the World Health Organization (WHO) leptospirosis guidelines (Abdollapour, 1995; Alonghom *et al*., 2017). For blood samples, the sample bottles containing heparin was inoculated with the suspected sample materials, into four 5ml tubes (containing *Leptospira* EMJH medium) with 1–2 drops of blood per tube and was incubated in the dark at 30°C. One drop of each urine sample was inoculated, immediately after sample collection into two 5ml sterile tubes of EMJH medium. Both tubes were incubated in the dark at 30°C based on standard methods (Isenberg, 1992).

All the cultured tubes were observed weekly for growth, which is evident by presence of turbidity, haze or a ring of growth. The culture was also examined microscopically, with dark field illumination at x400 magnification for *Leptospira* organisms, which appear as motile, tightly coiled spirochetes of about 1µm wide and 6–20µm long in size, with hooked end. All incubated culture media was kept for at least 5–8 weeks, after which negative growth tubes were discarded (Sakhaee *et al*., 2007: Ramesh *et al*., 2018).

## Results and Discussion

### *Colonial and microscopic morphology*

A total of 120 (12%) of the culture tubes incubated exhibited clear EMJH medium while two hundred and eighty-eight of the culture tubes showed cloudiness, haze, and turbidity of the EMJH medium. Nine of the culture tubes, which exhibited haze, turbidity and cloudiness showed presence of *Leptospira* species. The nine isolates were identified on dark field illumination at x400 magnification using dark field microscopy to be spiral, motile, and tightly coiled organisms, about 1µm wide and 7–18 µm long. The prevalence rate of *Leptospira* organisms in blood was 288 (3.5%) while the prevalence in urine was 1.0% with an overall prevalence rate of 4.5%.

The variation in prevalence rates obtained in our study was 3.5% from blood and 1.0 %, from urine as shown in Table 1. This shows that the organism can survive in urine, blood and perhaps other bodily fluids.

This research conducted at the bacteriology Laboratory of Ahmadu Bello University, Zaria, documents the occurrence of *Leptospira* organism in the study area as shown in Figure 1. Out of the 400 samples cultured, 9(4.5%) showed presence of *Leptospira*, on dark field illumination from pigs in Kaduna state (Figure 1). Our finding agrees with the previous reports of Agunloye *et al*. (2002) which reported similar prevalence of leptospirosis in Nigeria. This finding also concurs with similar studies from bovine kidneys in Plateau state which yielded the same prevalence rate of 4.5% (Ezeh *et al*., 1989).

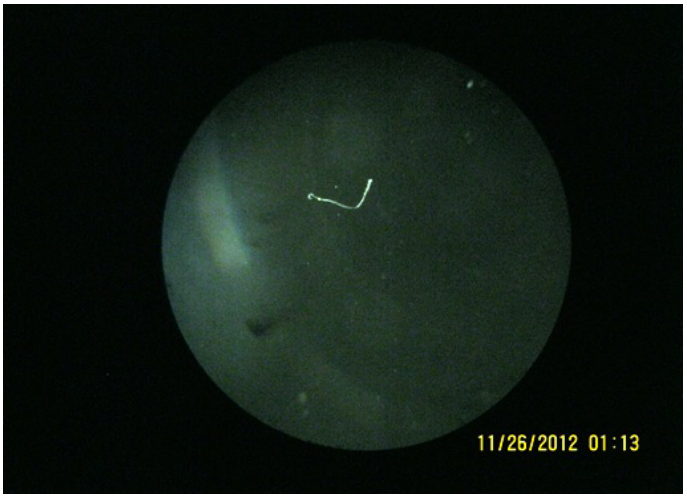


**Figure 1:** *Leptospira specie on dark field microscopy at x400 showing hooked end.*

A higher prevalence rate of 13.5% in humans has been reported in Enugu and its environs of eastern Nigeria by Onyemelukwe (1993). The sporadic nature of leptospirosis has been reported to be a major cause of morbidity and mortality in both animals and man (Bili *et al*., 2017). The differences in prevalence rates may be due to season of the year, with regards to time of sampling and also due to the difference in location, type of samples and sample size (Mohammad *et al*., 2020).

Our prevalence rate is lower than that obtained (4.5%) by Diallo *et al*. (1982) from Brown field rats (*Arvicanthus niloticus*) and this was attributed to the fact that rats serves as reservoir host for *leptospira* species (Diallo *et al*., 1982). The culture negative report obtained by (Diallo *et al*., 1982) from piggery sewage effluents collected at Kano and Kaduna may be as a result of the fastidious nature of the organism to grow (Bili *et al*., 2017).

In comparison with the isolation rate of *Leptospira* organisms from 3.5% blood and 1% urine, the percentage isolation from blood was higher than that obtained from urine, this might be due to the fact that more animals were sampled at the acute phase of the disease which permits serological responses as indicated in previous studies (Bili *et al*., 2017; Abena *et al*., 2017). Future studies involving leptospirosis should be focused on blood samples, while in chronic diseases; isolation should be from the urine (Udomsak *et al*., 2020).

Leptospirosis is a disease of economic and public health significance (Agunloye *et al*., 2001; Ngede *et al*., 2013), this research showed that the organism occurs more in pigs reared under extensive system than in semi intensive and intensive system as shown in Table 2. The extensive system of pig production in Nigeria allows pigs to roam about and scavenge for food. This may expose those pigs to various surfaces and niches for enzootic and occupational transmission of infection to animals or humans at risk (Ngbede *et al*., 2013: Mailafia *et al*., 2017).

**Table 1:** *Occurrence of Leptospira species from blood and urine sample.*

| Sample type | No positive | Percentage positive (%) | No negative | Percentage negative (%) | Number of samples | Total percentage (%) |
|---|---|---|---|---|---|---|
| Blood | 7 | (3.5) | 193 | (96.5) | 200 | (100) |
| Urine | 2 | (1.0) | 198 | (99.0) | 200 | (100) |
| Total | 9 | (4.5) | 391 | 195.5 | 400 | (200) |

**Table 2:** *Zoographic studies of Leptospira positive cultures.*

| Culture tube no. | Sex | Age (Months) | Location | Breed | Source | Mgt. practice |
|---|---|---|---|---|---|---|
| 7 | Male | 17 | Zaria | Local | Farm | Intensive |
| 9 | Female | 3 | Zaria | Local | Farm | Intensive |
| 10 | Male | 3 | Zaria | Local | Farm | Intensive |
| 15 | Female | 12 | Zaria | Local | Household | Extensive |
| 18 | Female | 2 | Zaria | Local | Household | Semi-intensive |
| 20 | Male | 2 | Zaria | Local | Household | Semi-intensive |
| 27 | Male | 17 | Zaria | Local | Household | Extensive |
| 28 | Male | 15 | Zaria | Local | Household | Extensive |
| 155 | Male | 15 | Zaria | Local | Household | Extensive |

In Nigeria, humans that come in contact with pigs or may be at risk of leptospirosis are the veterinarians, pig handlers, butchers, abattoir workers, coal miners, farmers, laboratory personnel's, rodent control workers and pork meat vendors (Terpstra, 2006; Roman *et al*., 2018). This observation calls for urgent need to conduct more studies on porcine leptospirosis in Nigeria.

## Conclusions and Recommendations

Findings of this research have demonstrated the use of dark field microscopy to determine the occurrence of *Leptospira* organisms in extensive, semi intensive and intensive system of pig production. The total prevalence rate was 4.5% and the prevalence was more (3.5%) in blood than urine (1.0%). This therefore calls for proper hygienic handling of porcine and porcine by products and good management practice. Public health awareness campaign and education, is very necessary as to enlighten the general public and clinicians on the control, economic and public health impact of porcine leptospirosis. This study has shown the occurrence of *Leptospira* organisms in pigs in Kaduna state at a prevalence of 4.5%. Further research on porcine leptospirosis, should be targeted at detecting IgG and the use of Polymerase Chain Reaction (PCR) in the diagnosis of leptospirosis. Veterinarians are encouraged to monitor and report cases of leptospirosis in Nigeria.

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## Novelty Statement

In this study, leptospira specie was isolated from porcine blood and urine samples in Kaduna state Nigeria using cultural method of isolation while prior to this study, there was paucity of information on cultural isolation of Leptospira from porcine specie in Kaduna State, Nigeria using cultural method.

## Author's Contribution

**BMJA:** Contributed to the overall design, laboratory analysis, literature review and writing of the manuscript.

**SM, KCN, and KHM:** Contributed in the review and editing of this manuscript.

### *Conflict of interest*

The authors have declared no conflict of interest.

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