Detection of *Trichinella* Antibodies in Slaughtered Pigs and Risk Factors Associated with Trichinellosis in Pig Farms in Kaduna Metropolis, Nigeria

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Abstract-An experiment was conducted to detect antibodies against *Trichinella* in slaughtered pigs and to identify farm management practices and risk factors associated with trichinellosis in pigs and pig farms in Kaduna Metropolis, Nigeria. A total of 286 pigs, 186 from slaughterhouses and 100 from pig farms, were tested in this study. Serum samples collected from the slaughtered pigs were tested serologically for antibodies for *Trichinella* by ELISA using excretory-secretory (E/S) antigen. Out of the 186 pig serum samples collected from slaughtered pigs at the main pig abattoir in Kaduna Metropolis, 56 were from juvenile pigs (10.7%) and 130 from adult pigs (14.6%). Male pigs had a prevalence rate of 12.6%, which was slightly lower than that of the females (14.1%). An overall prevalence rate of 13.4% was observed in this study. The age and sex of the animals were not associated with the infection. Also, a survey of 100 slaughtered pigs from 100 pig farms was carried out. From the structured questionnaire administered, 90% of the pig farmers had scavenging pigs, 98% had seen rodents around and within their piggeries, 85% had no rodents control program, 70% feed their pigs with food leftovers, 4% had seen their pigs interact with stray pigs, 16% feed their pigs with dead animals, and 10% had seen snakes around their piggeries. Only 2% were aware of trichinellosis while 30% knew of pork-borne diseases. Significant association existed between all the risk factors studied in the backyard pig farms visited with trichinellosis except for factors such as rodents around and within pig pens. The result also classified the distribution of pig owners based on farm management practices into categories of extensive (55%), semi-intensive (40%), and intensive (5%) with prevalence rates of 96.4%, 70.0% and 0.0% respectively. The survey established serological evidence of trichinellosis in slaughtered pigs in Kaduna Metropolis, Nigeria.

Keywords- Antibodies; ELISA; Farm Management; Kaduna Metropolis; Nigeria; Risk Factors; Trichinella; Trichinellosis

I. INTRODUCTION

Trichinellosis or trichinosis is a parasitic zoonotic disease caused by tissue-dwelling nematodes of the genus *Trichinella*. It occurs worldwide, affecting most mammals, some birds, and some reptiles [1].

The causative organism is acquired by eating *Trichinella*-infected meat and meat products, raw or undercooked pork or wild game infected with the larvae of *Trichinella spiralis*, commonly called the trichina worm. This nematode worm of the genus *Trichinella* and family *Trichinidae* is one of the most widespread zoonotic pathogens in the world. Infection has been detected in domestic and wild animals on all continents except Antarctica, where there is no record of the parasite [2].

Trichinellosis has been reported lately in birds and crocodile in Africa [3]. The sudden death of a captive European brown bear was said to be associated with the consumption of a rat infected with *Trichinella* [4].

More pork is consumed than any other meat in the world, accounting for over 36% of the world’s animal protein intake. It is closely followed by poultry and beef with about 33% and 24% respectively [5]. Research on trichinellosis in humans and animals in Nigeria is limited and has led to a lack of reliable epidemiological data on the disease [6].

Throughout much of the world, nematodes of the genus *Trichinella* have been found to be the causative agents of trichinellosis in humans. It affects a wide variety of mammals, birds, and reptiles, and it occurs worldwide. It is not only a public health hazard but also constitutes an economic problem in porcine animal production and food safety [7].

Due to the predominantly zoonotic importance of the disease, the efforts in many nations have focused on eliminating *Trichinella* from the food chain. The most important source of human infection worldwide is the domestic pig, and *Trichinella spiralis* is the species most adapted to domestic and wild swine [2, 7].

The re-emergence of this parasite is due to certain underlying casual factors, including the important role played by the sylvatic *Trichinella* species, therefore leading to large public health concerns and research intensification [8].

Farm management practices play an important role in the outbreak of trichinellosis in pig farms [9]. The outdoor rearing of swine is a major risk factor because of increased exposure to both sylvatic and synanthropic hosts, and the fact that rodents are primarily responsible for the endemicity of *Trichinella* infection [10].

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A number of risk factors are important in the spread of this disease in the United States, such as the feeding of animals with contaminated waste products, exposure to infected living or dead wildlife, or cannibalism among pigs [11].

Estimation of the likely impact of trichinellosis in developing countries like Nigeria is very difficult because little information is available [12, 13]. Hence, this study seeks to determine the prevalence of *Trichinella* in slaughtered pigs in Kaduna Metropolis, Nigeria, using Enzyme Linked Immunosorbent Assay (ELISA) and to identify farm management practices and risk factors associated with the transmission of trichinellosis in pig farms.

II. MATERIALS AND METHOD

A. Study Area

The study was carried out from March 2013 to July 2013. The samples for this research were obtained from Angwa Television pig slaughterhouse. It is the main pig abattoir located in the Kaduna south local government area of Kaduna State, Nigeria. Pigs to be slaughtered were brought from different parts of Kaduna Metropolis and from 100 backyard pig farms in the area.

B. Sampling Plan

The pig slaughterhouse was visited for a consecutive twenty-four days and blood samples were collected from eight slaughtered pigs on each day of the visit. The pigs were selected by consecutively skipping one slaughtered pig and collecting blood samples from the next pig on queue. Also 100 pigs from 100 backyard pig farms were selected randomly within Kaduna Metropolis and tested for antibodies for *Trichinella*. Close-ended structured questionnaires were administered to the pig farmers/owners. The studied farms were managed under extensive, semi-intensive, and intensive systems.

C. Sample Collection and Processing

Blood samples were obtained from the jugular veins of the sampled pigs and were placed into labeled sample bottles free of anticoagulant. Data on the sex and age (juvenile or adult) of the sampled pigs were recorded. The samples were transported to the laboratory in an ice-packed box. Centrifugation was performed at 3000rpm for 10 minutes to obtain the serum. The sera were dispensed into clean, labeled serum bottles and stored at -20°C until the test was conducted.

D. Enzyme-linked Immunosorbent Assay

Serum samples were tested serologically using an ELISA kit from Innovative Diagnostics (ID.vet) Montpellier-France. Exactly 190μl of Dilution Buffer 2 and 10μl of the Negative Control were added to wells A₁, A₂ after wells A₁, A₂ and were left blank. Also 190μl of Dilution Buffer 2 and 10μl of the Positive Control were added to wells A₃, A₄ and A₅, A₆. Then 190μl of Dilution Buffer 2 and 10μl of each sample were added to the remaining wells. The microplates were then incubated for 45 minutes at room temperature (29°C). The contents of the wells were emptied and each well was washed three times with about 300μl of the Wash Solution. Drying of the wells between washings was avoided. Conjugate 1x was prepared by diluting the Concentrated Conjugate 10x to 1:10 in Dilution Buffer 3. Exactly 100μl of the Conjugate 1x was added to each well and the plates were again incubated at room temperature for 30 minutes. The wells were emptied again and each was washed 3 times about 300μl of the Wash Solution. Drying of the wells in between washings was prevented. Next, 100μl of the Substrate Solution (tetramethylbenzidine:TMB) was added to each well. Incubation of the plates was carried out for 15 minutes at room temperature, but this time in the dark. Exactly 100μl of the Stop Solution was added to each well to stop the reaction. Visual reading was done and an ELISA plate reader was used to read and record the Optical Density (O.D) at 450 nm. Visual reading indicated that in positive samples, a blue solution appeared in the wells which later became yellow after addition of the stop solution. Negative samples showed no coloration as specified by the kit manufacturers.

E. Interpretation of ELISA Tests Results

The ELISA test results were calculated based on the manufacturers’ protocols and recommendations as follows:

For each sample, the sample positive percentage value (S/p) was calculated as

\[
S/P = \frac{OD_{sample} - OD_{negative\ control}}{OD_{positive\ control} - OD_{negative\ control}} \times 100
\]

where OD= Optical Density.

The test results were interpreted as Negative, if the S/P ratio ≤ 50%, and as positive if the S/P ratio > 50%.

We considered samples with positive results in the multi-species ELISA (IgG antibodies) to be truly positive and samples negative to be truly negative.

F. Determination of Prevalence (%) of Trichinella Antibodies in Pigs

Percentage prevalence was calculated using the formula:

\[
\text{Percentage prevalence} = \frac{\text{Number of positive samples}}{\text{Total number of samples}} \times 100\%
\]
Prevalence (%) = \frac{\text{Number of serum positive samples for trichinellosis}}{\text{Total number of samples}} \times 100

overall prevalence for pig trichinellosis (%) = \frac{25}{186} \times 100 \approx 13.4% 

G. Statistical Analysis

The computer statistical software, Statistical Programme for Social Science version 19.0 (SPSS v 19.0) for Windows® (SPSS Inc., Chicago, IL., USA), was used for the statistical analysis of the data. Chi-square was used to test for an association between trichinellosis and farm management practices, trichinellosis and other risk factors in pig farms.

III. RESULTS

An overall prevalence rate of 13.4% was obtained in this study (25/186 samples collected) for slaughtered pigs. Table 1 shows the prevalence of trichinellosis in pigs by age and by sex.

| Age group          | Number of serum samples examined | Number of positive samples | Prevalence rate (%) |
|--------------------|----------------------------------|---------------------------|---------------------|
| Juveniles (1-2 years) | 56                              | 6                         | 10.70               |
| Adult (>2 years)    | 130                             | 19                        | 14.72               |
| Total              | 186                             | 25                        | 13.44               |

A prevalence of 10.7% was recorded among the juvenile pigs (1-2 years) while 14.6% was observed among the adult pigs (>2 years), resulting in the overall prevalence of 13.4%.

Out of the 87 male pig sera examined, a prevalence of 12.6% was recorded and one of 14.1% for the 99 female pigs examined was observed.

From the close-ended structured questionnaire administered to the 100 pig owners/farmers, the data obtained showed the distribution of pig owners based on farm management practices. Fifty-five per cent (55%) of them practiced extensive system while 40% and 5% practiced semi-intensive and intensive systems, respectively. Pigs from extensive farming systems had trichinellosis with prevalence of 96.4%, while those reared under semi-intensive conditions showed a prevalence of 70.0%, and those under strict intensive care had no detectable case of trichinellosis. When the data were tested statistically, a significant association at p ≤ 0.001 existed between farm management practices and Trichinellosis, as seen in Table 2.

| Farm management type | Number of sample | Number positive (%) | \( \chi^2 \) | p-value |
|----------------------|------------------|---------------------|-------------|---------|
| Extensive            | 55               | 53 (96.4)           | 32.896      | < 0.001** |
| Semi-intensive       | 40               | 28 (70.0)           |             |         |
| Intensive            | 5                | 0 (0.0)             |             |         |

** = significant association exists at p ≤ 0.001

Table 3 presents the relationship between the various risk factors and trichinellosis in the study population. Ninety per-cent had scavenging (search through waste/refuse for food) pigs, 98% had seen rodents around and within their pig pens, 85% had no rodent control programme, 70% feed their pigs with food leftovers, 4% had seen their pigs interact with stray pigs and 16% allowed their pigs to feed on dead animals. Ten per-cents of the respondents had seen snakes around their piggeries. Only 2% of the respondents were aware of trichinellosis while 30% had knowledge of pork-borne diseases. A statistically significant association (\( \chi^2 = 12.037, p = 0.001 \)) exists between scavenging pigs and trichinellosis. Other factors that exhibited significant association (p ≤ 0.05) with trichinellosis included the absence of rodent control practices on the farms, the feeding of pigs with leftovers, the feeding of pigs with dead animals, the interaction of stray pigs with farmers’ pigs, snakes around piggeries, and the farmers’ awareness of trichinellosis and other pork-borne diseases. The presence of rodents around and within piggeries showed no association (\( \chi^2 = 1.738, p=0.087 \)) with Trichinella infection.
The prevalence rate of 13.4% positive pigs for *Trichinella* antibodies slaughtered in Kaduna metropolis as recorded in this study is slightly lower and similar to the prevalence rate of 15.0% recorded by [6] in Oyo State, Nigeria [14], recorded a prevalence of 26.6% from a survey in Markurdi, Benue State, Nigeria. This variation could be based on cultural preferences, religious beliefs or tradition of the people inhabiting the various locations. This view point is upheld by the Food and Agricultural Organization of the United Nations, which holds that the utilization and consumption of animal species vary as cultural preferences and religious beliefs are observed [5].

Reference [15] reported a prevalence of trichinellosis 5.2% in Oyo State using Pepsin Digestion technique. The Pepsin Digestion Technique has the limitations of being unable to detect all larvae present in the carcasses of the animals, and is thus poor at detecting low-level infections [16]. Powdered pepsin has also been observed to be carcinogenic and the commercial type is not readily available [17].

Reference [18] recorded a prevalence of Trichinelllosis of 16.0% among African giant rats in northern Nigeria. The giant rats that dwell in pig-rearing areas were those said to harbor the parasite. Reference [19] observed sero-prevalences varying from 0.09% to 29.6% between 1914 and 1999 in China. Reference [20] in Argentina, [21] in Ecuador, [22] in Mexico, and [23] in the United States reported sero-prevalences of 19.9%, 5.7%, 2.5%, and 0.013% in swine, respectively.

In this study, adult pigs were observed to be more infected than the juvenile pigs, (14.7%) compared with 10.7%. This matches the findings of [15], [20], and [6]. The reason for this may be the fact that adult swine naturally scavenge more than the younger ones, and are thus more likely to be exposed to predisposing factors such as scavenging, rodents and reptiles, interactions with wild animals and cannibalism.

The prevalence in female pigs was higher (14.1%) than that of males (12.6%), a result which differed slightly from that obtained by [6], but agreed with that of [14]. This could be because both Kaduna Metropolis and Makurdi are part of the northern region of Nigeria, and is probably connected with the fact that female pigs scavenge for themselves and their piglets. This scavenging could potentially cover large areas, which could bring them in contact with wild pigs [24] and other predisposing factors.

The relatively high prevalence in pigs observed in this study could be due to the predominantly extensive farm management practices which constitute a major predisposing factor in the transmission of trichinellosis among domestic pigs in Kaduna Metropolis (96.4%). This is closely followed by the semi-intensive system (70.0%) and the intensive system (0.0%). This trend was also observed by [6] and also [25]. The extensive and semi-intensive systems of farming and backyard rearing exposed the animals to risk factors like free-ranging/scavenging, interactions with stray and wild pigs, rodents and reptiles. Also the awareness of pig owners and/or farmers of the disease and its transmission among pigs were very low. Thus, this deficiency in knowledge did not encourage pig farmers and producers to design management systems that would prevent or drastically reduce the risk of exposure to the parasite [26, 27].

The study also revealed that, there was significant statistical association between the following risk factors and trichinellosis: scavenging pigs, lack of rodent control in farms, feeding pigs with food leftovers, pigs feeding on dead animals, interactions of farmers’ pigs with stray pigs, snakes around piggeries, and the farmer’s level of awareness of the disease. This is attributed to the extensive and semi-intensive mode of farming swine in Kaduna Metropolis.

Reference [25] identified some risk factors associated with trichinellosis in backyard pig farms in Zaria, Kaduna State, Nigeria, to include the presence of rodents, rodent control and access to dead pigs. Zaria and Kaduna metropolis are major urban cities in Kaduna state [28], also established that the access of pigs to live wildlife and wild carcasses on the farm were

| Risk factors from pig farms          | Number of respondents (N=100) | Number of pigs tested positive (%) | \( \chi^2 \) | p-value |
|--------------------------------------|-------------------------------|-----------------------------------|--------------|---------|
| Scavenging pigs                      | 90                            | 52 (57.8)                         | 12.037       | 0.001** |
| Rodents around/within pigsty         | 98                            | 46 (46.9)                         | 1.738        | 0.187   |
| No rodent control                    | 85                            | 47 (55.3)                         | 15.649       | < 0.001** |
| Feed their pigs with leftovers       | 70                            | 27 (38.6)                         | 15.851       | < 0.001** |
| Pigs to feed on dead animals         | 16                            | 16 (100.0)                        | 100.00       | < 0.001** |
| Stray pigs interact with their pigs  | 4                             | 2 (50.0)                          | 48.980       | < 0.001** |
| Seen snakes around their             | 10                            | 3 (30.0)                          | 27.835       | < 0.001** |
| Awareness on Trichinellosis          | 2                             | 1 (50.0)                          | 49.495       | < 0.001** |
| Aware of pork-borne diseases         | 30                            | 18 (60.0)                         | 51.220       | < 0.001** |

** = significant association exists at \( p \leq 0.001 \)

IV. DISCUSSION

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significantly associated with trichinellosis, while waste feeding was not an important risk factor in the northeastern United States.

V. CONCLUSION

This survey concludes that, pigs in Kaduna Metropolis, Nigeria harbor antibodies for Trichinella with a high prevalence. Extensive and semi-intensive farm management systems practiced by pig farmers and pig producers aid the transmission of trichinellosis in Kaduna Metropolis. Allowing pigs to scavenge, the lack of proper rodent control, the feeding of pigs with food leftovers, the access of pigs to dead animals, the interaction of domestic pigs with stray pigs, the presence of reptiles like snakes and the level of awareness of pig farmers were important risk factors in the spread of Trichinellois in pigs. The result of this study, therefore, is of public health importance because pork and pork products are considered very important sources of daily animal protein and sources for delicacies in Kaduna Metropolis. Trichinellosis is not only a public health hazard, but represents an economic problem for pig production and food safety.

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