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The Use of Participatory Disease Surveillance, Tuberculin Test and Polymerase Chain Reaction to Determine the Presence and Prevalence of Avian Tuberculosis in Layers Within Gwagwalada and Kuje Area Councils, Abuja, Nigeria: A Cross-Sectional Study

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Abstract

Introduction: Avian tuberculosis (ATB) is a zoonotic chronic wasting disease of birds with serious economic implications to poultry farmers as well as to public health, particularly in immunocompromised patients. We therefore aimed to determine the presence, prevalence and specie (*Mycobacterium avium*) of ATB affecting layers in Abuja, Nigeria.

Methods: We conducted a cross-sectional study. We adapted a checklist and conducted participatory disease surveillance (PDS) in 12 farms and a live bird market (LBM). We used systematic random sampling method to select 395 layers that were screened for ATB using avian PPD. Antemortem and postmortem were conducted on those birds that tested positive. Organs and tissues with lesions were obtained, DNA was extracted, and polymerase chain reaction (PCR) was conducted for confirmation of *Mycobacterium avium* infection. Data was analyzed using PDS method of analysis (ranking and scoring) and descriptive statistics.

Results: The study established the presence of ATB in commercial laying birds in Abuja. Using PDS, 3/13 (23.1%) locations identified the presence of ATB. Avian tuberculosis, the disease of interest, ranked eleventh. On biosecurity measures, 3/12 (25%) farms had strict movement restriction, 50% of the farms had other animals on the farm aside the layer and / broilers under intensive management, foot dips were absent in 25% of the farms, 25% had foot dips that were not in use, only a farm (8%) disinfected vehicles before entering. Only 12/23 (52.2%) farms agreed to the Tuberculin test with a 47.8% rejection rate. Out of the 12 farms sampled, 5 (41.7%) were positive for ATB. The overall prevalence of ATB was 2.8% (11/395). Layers 105 weeks old and above had the highest prevalence (4.8%) and cachexia (14.3%). Four out of the eleven (36.4%) layers that turned out positive were cachexic. All the layers that were positive for avian PPD turned out positive as *M. avium* using PCR analysis.

Conclusion: We have demonstrated the existence of ATB within Gwagwalada and Kuje Area Councils (ACs) which is a potential threat to the poultry industry and public health. The farm prevalence of 41.7% signifies the need for tuberculin test in all the layer and breeder farms in Abuja to confirm the status of ATB on farms and to curtail the spread of ATB. We have also revealed the lack of knowledge with regards to the availability and relevance of tuberculin test among poultry farmers and the need for sensitization of poultry farmers on ATB and improvement on their biosecurity measures on farms.

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Background

Avian Tuberculosis (ATB) is a zoonotic, insidious, chronic wasting disease with global distribution affecting all species of birds (1). Various species of Mycobacteria are responsible for the disease; the common causative agent being Mycobacterium avium spp. avium (MAA) (2). Mycobacterium avium complex (MAC) comprises a group of closely related non-tuberculous mycobacteria (NTM) species and subspecies which are from veterinary and opportunistic human pathogens. These include M. avium subsp. avium, M. avium subsp. paratuberculosis, M. avium subsp. silvaticum previously called "wood pigeon Mycobacterium", M. avium subsp. Hominissuis, M. intracellulare (2,3), M. scrofulaceum and M. fortuitum (4). Avian tuberculosis is a serious global threat to a majority of domestic and wild birds as well as immunocompromised individuals; the disease has global importance in the public health arena (5). It is classified as a List B disease by the World Organization for Animal Health (6). Globally, there is increased incidence and prevalence of these disease due to pulmonary Mycobacterium avium complex (MAC) infection. Non-tuberculous mycobacteria consist of more than 150 species (5) and are ubiquitous in natural and man-made environments(7). Birds are important agents to spread MAC to humans (8) through environmental contamination with fecal droppings which increases public health concerns (9). Immunocompromised patients may contract the pathogen during handling of infected birds or consumption of improperly cooked meat from infected birds(10). Mycobacterium avium causes a serious disseminated bacterial infection in up to 40% of patients with advanced HIV infection (11). Identification of mycobacterial species and subspecies is relevant in determining their significance, pathogenicity, diagnosis, epidemiology, infectious sources and transmission routes for developing effective control program (1,12). The successful control of mycobacterial diseases is contingent on early diagnosis of infection and the successful treatment of contagious cases (13).

The epidemiology of avian tuberculosis is relevant in the poultry sector especially in birds that stay long on farms such as layers, breeders and local chickens considering the fact that the poultry industry in Nigeria is the most advanced component of the livestock subsector (17). Also, in 2013, Nigeria was rated first in Africa by producing over 26 billion eggs from 57 million commercial layers and ranked 5th in poultry meat production in Africa (14). Livestock production accounts for about 6-8% of the Gross Domestic Product (GDP) and 20-25% to the value added to agriculture, and it contributes about 36.5% to the aggregate protein intake of Nigerians (15). The poultry industry contributes over 25% of the Agricultural Gross Domestic Product (AGDP) of the Nigerian economy and the industry is worth over 10 trillion USD, creating over 20 million direct and indirect employments to Nigerians (14). Poultry is one of the main agricultural industries in the country and the most commercialized of the agricultural sectors, with a net worth of USD 1.7 billion per year (16). The industry comprises about 180 million birds (15) – the second largest chicken population in Africa after South Africa – producing 650 000 tons of eggs and 300 000 tons of meat in 2013 (14,17).

The involvement of PDS / participatory epidemiology (PE) in the study was to demonstrate the presence of ATB in the study site, to serve as a pointer to the possibility of obtaining the disease in the study area since literature reports that it is a rare disease in commercial poultry farms due to the high level of biosecurity in such farms before engaging the use of avian PPD in determining the presence of ATB. Participatory epidemiology advanced from veterinary epidemiology and has been used for early warning of infectious diseases and in disease control within resource-limited settings. The skill was developed by combining participatory methods and expert communication skills to expedite the involvement of animal caretakers and owners considering their knowledge, experience, and motivations to identify and assess animal disease problems (18). The relevance of social perceptions and drivers has received increased recognition by epidemiologists resulting in increased use of PE tools in epidemiological studies (19).

Participatory studies involves the use of a wide range of methods in data collection; focus group discussions, observation, free listing, ranking (simple ranking, pair-wise ranking), causal flow analysis, open-ended stories, genograms, role playing, use of none verbal cues (body mapping) and photo voice (20–22). Participatory disease surveillance is conducted on the disease of interest for generating Information to develop a case definition, ascertain the existence of or estimate of prevalence, incidence, morbidity and/or mortality of disease of interest (19). In Nigeria, reports have suggested the prevalence of ATB in birds, animals and humans (23,24). In humans, co-infections of Mycobacterium tuberculosis complex (MTC) and nontuberculous mycobacteria (NTM) have been identified by Cadmus et al., (25). Studies have speculated on the prevalence of ATB in some parts of the country (24,26) which has indicated the need for such a study in Abuja. The prevalence and effect of ATB is largely unknown and grossly underestimated in Nigeria due to the paucity of data since few studies have so far been conducted in the country on ATB in poultry. The determination of the prevalence of ATB will provide a picture of the likelihood

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of the threat of this disease to the poultry industry. The economic losses due to ATB is not known because backyard farmers, commercial farmers and breeders do not test for the disease in their flocks. Our research questions for the study were; what is the possibility of occurrence of ATB in layers within Gwagwalada and Kuje Area Councils? What is the prevalence of ATB in layers within Gwagwalada and Kuje ACs? Which organs will indicate gross lesions for those birds that turn out positive for the Tuberculin test? Which specie of Mycobacterium causes avian tuberculosis in layers within Gwagwalada and Kuje ACs? We therefore determined the presence of ATB, assessed biosecurity practices on farms that could allow its spread, determined the prevalence ATB and specie of Mycobacteria causing ATB in Abuja to provide insight into the epidemiology of the disease in order to inspire control efforts of the disease.

Methods

The aim, design and setting of the study

Abuja is the capital of Nigeria, located in the central region of the country, north of the confluence of rivers Niger and Benue. It has a projected population of 3,564,126 in 2016 (27). Abuja consists of six local councils, comprising the city of Abuja; Abuja Municipal Area Council (AMAC), Abaji, Gwagwalada, Kuje, Bwari and Kwali. The projected population of 2016 for Gwagwalada was 402,000 while that of Kuje was 246, 400(28). In 2018, the national data on the estimated population of poultry was 193, 578,483 while that of the Federal Capital Territory (FCT), Abuja was 2,358,753(29). We conducted a cross-sectional study using qualitative (PDS) and quantitative study methods. The entire study period was between from 1st of December 2020 to 30th January, 2022. The general objective of the study was to determine the presence of ATB, the knowledge, attitude, and practices (biosecurity measures) observed by poultry farmers that could enable transmission of ATB within Gwagwalada and Kuje ACs. Specific objectives were to detect the presence of ATB in layers within Gwagwalada and Kuje ACs using participatory disease surveillance (PDS), detect the presence of M. avium infection using avian purified protein derivative (PPD) in layers within Gwagwalada and Kuje ACs, to determine the presence of gross pathological lesions in layers that tested positive to avian PPD, confirm the presence of M. avium in avian PPD positive layers using polymerase chain reaction within Gwagwalada and Kuje ACs.

We defined backyard poultry as a semi-intensive management system managed by the family and consists of average range flock size of 50 to 2000 birds characterized by subsistence market-oriented production, indigenous and improved breed, basic production and husbandry practices, basic application of biosecurity measures and medium to low productivity levels and located within the owners' house premises" whereas "commercial or integrated poultry farm is an intensive / commercial poultry management sector that includes great-grandparent and grandparent hatcheries as well as layer and broiler farms; categorized into commercial peri-urban entreprises raising between 2000 to 5000 birds, clustered commercial entreprises raising more than 5000 birds and commercial poultry integrators with over 100,000 and above number of flocks". Layers are matured female chickens kept for egg production. Live Bird Market is a facility at which live poultry or hatching eggs are congregated for sale or to be slaughtered and dressed for sale to the public or restaurants or to be sold live for any purpose.

Participatory disease surveillance

Participatory disease surveillance was conducted in Gwagwalada live bird market (LBM) and twelve farms that met the inclusion criteria for sampling and gave their consent to be sampled within Gwagwalada and Kuje ACs. A pre-advocacy visit was conducted at Gwagwalada LBM and all the poultry farms in various settlements that were sampled. Meeting was arranged with the poultry butchers at the LBM and poultry management staff and workers at the various farms according to the suitability of time, place, and convenience. Cardboards, counters (beans), permanent markers and android phone were used for the study. The geographical coordinates of each meeting point were obtained using an android mobile phone. Each team member was assigned a role (note taker, observer, tool applicator and the facilitator) before moving out. To avoid bias, the PDS Team did not mention ATB during the discussion process. Pictures on ATB lesions on the android phone was presented to the farmers by the facilitator to aid in the identification of the disease. We adapted a check list based on previous PDS studies (30–32) with some modifications to suite the objectives of our study. The following tools were used during the PDS (33): (a) check list consisting of mutual introduction, identification of respondents, sources of chickens and feed, poultry diseases, source of water, biosecurity on farm (foot dip, disinfection of vehicles, presence of other animals on farm and movement on farm), questions and advice. (b) Methods used in data collection were, scoring and ranking, simple ranking, and proportional pilling (33). (c) Visualization includes transect walk/ walk survey around the farms (33).

Study population

Our inclusion criteria were all backyard and commercial farms that had layers above 24 weeks old within Gwagwalada and Kuje ACs while the exclusion criteria were layers 24 weeks old or less during the period of the study.

Sampling

As part of the biosecurity measures, we ensured that not more than two farms were visited in a day and observed all

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biosafety measures. We calculated our sample size using the method of Thrusfield for cross-sectional surveys (34), a total of 395 layers were sampled. Administration of avian PPD was done using systematic sampling method to select the bird to be tested from twelve farms (10 commercial and 2 backyard poultry) within Gwagwalada and Kuje ACs. Administration of avian PPD, was conducted by 2 trained research assistants; one research assistant was responsible for restraining the layers properly for the PPD administration while the other assistant was responsible for marking the birds with permanent markers to indicate those layers that were administered avian PPD for easy identification between 48–72 hours after administration.

Tuberculin Test

Onderstepoort Biological Products; Avian PPD 2500 with Reg. N0. G4085 (Act 36/1947) from Onderstepoort Veterinary Institute (OVI), South Africa was used for the test. Exactly 0.1ml (containing 2500 International Units (IU) of standard avian purified protein derivative (PPD) was administered using needle of approximately 10 x 0.5mm2 (insulin syringe). The avian PPD was administered intradermal into one of the wattles of the layers, the other wattle was used as control. The test was read between 48 hours to 72 hours. A positive reaction was detected by swelling or induration at the site of inoculation ranging from a small firm nodule approximately 5mm in diameter to gross edema extending into the other wattle and down the neck (35).

Ante-mortem and Post-mortem Inspection

Layers that tested positive after avian PPD administration were bought from the owners and transported to the laboratory for post-mortem inspection. For the post-mortem inspection, visual examination, palpation of tissues and organs and incisions were conducted for nodular and granulomatous lesions. The entire procedures was carried out based on the standard methods of postmortem examination in poultry (36,37). Factors such as sources of birds, breed, body conformation, predilection site/sites of lesions (specified organ) and body condition score to determine the impact of these factors on the disease picture were obtained.

Tissue Sample collection and Transportation

Infected tissues and organs with gross pathological lesions were collected, packaged using standard procedures and stored (refrigerated at-4OC) in the laboratory at the University of Abuja Veterinary Teaching Hospital throughout the 3 months' period of sample collection (December 2020 to February 2021). Thereafter, in June 2021, stored samples were packaged using ice packs and cold boxes and were transported to the National Veterinary Research Institute (NVRI), Vom, Plateau State for PCR analysis. Processing of Tissue Samples for PCR

DNA extraction

Stored tissue samples were removed from the freezer and thawed before the commencement of the DNA extraction process. ZYMO RESEARCH Quick-DNA™ Tissue/Insect Miniprep Kit; was used for extraction of DNA from the lesions that were collected. The DNA extraction was done according to the manufacturers' protocol.

Polymerase Chain Reaction (PCR)

Two primers (P) were used in the PCR; M. avium F and R; P1 (5´-GCCGCCGAAACGATCTAC) and P2 (5´--AGGTGGCGTCGAGGAAGAC) (38) to define the host range of IS1245 and to generate a 427 bp fragment. PCR amplifications were performed in a Gene Amp PCR system 9700 (PE Applied Biosystems, Foster City, Calif.), according to the method described by Telenti et al. (39): 30 cycles of 1 minute at 94OC, 65 OC and 72OC, with an extension cycle of 10 minutes at72OC.

Quality Assurance

An advocacy visit was made to each farm and LBM (poultry butchers) for sensitization and fixing of suitable time to ensure full participation of the key informants/focus group discussion where applicable on the subject matter. Team members were trained on application of PDS tools on the field and the various functions of the PDS. Direct observation such as transect walks or walking surveys were used in assessing available infrastructure, farm environment, activities on farm such as slaughter and dressing of chickens, working conditions of farm workers and biosecurity measures; potential drivers of disease (dumping of poultry manure on farm premises, presence of water bodies, animal movements and interactions). Distance examination of animals for signs of disease was conducted (19).

All procedures (administration of avian PPD, antemortem and postmortem inspection) were conducted based on standard procedures. Research assistants were trained before the commencement of sampling. Systematic random sampling was used. Mycobacterium avium positive control obtained from OVI was optimized and used for the PCR analysis.

Data analysis

Data from PDS was analyzed based on the analytical procedure of PDS data (33); simple ranking and proportional pilling were used in obtaining the poultry diseases occurring on farms based on their importance (predominance of occurrence on farm) followed by ranking and scoring. A table was created where all diseases scored on each of the farms were imputed and computed (33). Computation indicated in S1 Table. Microsoft Excel was used in the descriptive analysis of the results.

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Results

Participatory Disease Surveillance

Detection of the presence of ATB using PDS

Participatory disease surveillance was conducted in 12 farms and 1 LBM; 3/13 (23.1%) identified the presence of ATB (Table 1). They reported that they have seen such a disease in slaughtered chickens based on the pictorial description of ATB that was shown to them.

Relative importance of ATB for PDS

The most common disease in poultry houses (mostly layers) based on the PDS conducted is Newcastle Disease followed by salmonellosis, coccidiosis, Chronic Respiratory Disease and fowl cholera while the list was avian Influenza (Table 1). Avian Tuberculosis, the disease of interest ranked eleventh (Table 2).

Biosecurity on the farms

Nine (75%) out of the 12 farms had boreholes as a means of their water supply, 2 (17%) of the farms were supplied by the municipal water system while a farm (8%) had a well that uses a machine to pump water in the farm. Among the twelve farms sampled, only a farm disinfects vehicles before entering the farm and the farm also has disinfectant dip at the gate when vehicles pass through. Another farm also had disinfectant dip for vehicles before entering the farm (8%) the rest of the farms do not disinfect vehicles. Twenty-five percent of farms had movement restriction where as 75% had restriction only at the poultry house level. Three (25%) out of the twelve farms had strict movement restriction on the farm while the rest (75%) of the farms allow entry into their farm but not into the poultry houses where the birds are kept. Six (50%) of the farms had other animals on them aside the layer and/ broilers under intensive management while the rest didn't. Animals present on the farms are dogs, local chickens, ducks, turkeys, geese, goats, guinea fowls and calves. Foot dips were absent in three (25%) of the farms while three other farms had foot dips that were not in use while six farms had foot dips in front of all their pens that were being used. Poultry manure kept in 5/12 (41.7%) of farms.

Table 1: Diseases, total score and ranking from PDS in Gwagwalada and Kuje area councils in Abuja, Nigeria

Diseases	Total Score	Ranking
Newcastle Disease	1070	1
Salmonellosis	1010	2
Coccidiosis	730	3
Chronic Respiratory Disease	650	4
Fowl Cholera	590	5
Prolapse	330	6
Gumboro	300	7
Fowl pox	300	7
Ectoparasites	280	8
Marek	220	9
Egg binding	180	10
Avian tuberculosis	130	11
Worms	120	12
Hypocalcaemia	110	13
Cannibalism	80	14
Bumble feet	70	15
Infectious Laryngotrachitis	60	16
Avian influenza	50	17

Prevalence and organs affected

Out of 23 poultry farms (2 backyards and 21 commercial farms) approached within Gwagwalada and Kuje ACs for the Tuberculin test, only 12 (52.2%) agreed to the test; there was 47.8% rejection rate. A total of 12 farms were sampled out of which 5 (41.7%) farms were positive for ATB. The farm prevalence of ATB was 1/12 (8.3%) in Gwagwalada area council and 4/12 (33.3%) in Kuje ACs. The overall prevalence within Gwagwalada and Kuje ACs for ATB was 2.8%. Gwagwalada area council had an ATB prevalence of 1% (2/191) while Kuje had 4.4% (9/204). Layers from 105 weeks old and above had the highest prevalence of ATB (4.8%), followed by layers between the ages 25 to 52 weeks with 4.2% prevalence while those with the least prevalence of ATB (0.6%) were between 53 to 104 weeks old (Table 2).

Four out of the eleven (4/11) chickens accounting for 36.4% that turned out positive were emaciated (cachexia). Overall emaciation rate for the sampled layers as 3.8% (15/395); 3.6% emaciation for layers between 25-52 weeks, 0% for weeks 53-104 and 14.3% for layers above 105 weeks old (Table 2). Fig 1, indicates picture of a layer positive for the Tuberculin test with marked induration on the wattle while Figs 2 and 3 shows grows pathological lesions (heart, intestine, liver and kidney) extracted from layers that turned out positive for the Tuberculin test. A total of 23 gross lesions from organs and tissues were harvested; heart 11 (48%), liver 5(22%), intestine 3(9%) and uterus 3 (13%) and egg 1(4%) (Fig 4). Results of the PCR for infected tissues and organs is shown in Table 3 and Fig 5.

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Table 2: Indicating the total number of layers (n=395) that were sampled, number sampled per age group, number of positive layers and number emaciated in Kuje and Gwagwalada Area Councils, Abuja, Nigeria

Age groups (AG) in weeks	No. of layers by AG	% by AG	No. of layers Positive by AG	% Positive by AG	No. of layers Negative by AG	% Negative by AG	No. emaciated layers/AG	% emaciated/ AG
25-52	165	41.8	7	4.2	158	95.8	6	3.6
53-104	167	42.3	1	0.6	166	99.4	0	0
105>	63	15.9	3	4.8	60	95.2	9	14.3
Total	395	100	11	2.8	384	97.2	15	3.8

Table 3: Results of samples tested for *M. avium* using PCR

Layer ID	Heart (H1-H11)		Liver (L1-L5)		Uterus (U1-U3)		Intestine (I1-I2)		Egg (E1)	
	lesion	PCR	Lesion	PCR	Lesion	PCR	Lesion	PCR	Lesion	PCR
L1	+	+	+	+	+	+	+	+	NL	NT
L118	+	+	+	+	+	+	+	+	NL	NT
L129	+	+	+	-	+	+	NL	NT	NL	NT
L154	+	+	+	+	NL	NT	NL	NT	NL	NT
L320	+	+	+	+	NL	NT	NL	NT	+	+
*L162, L165, L192, L200, L230 and L323	+	+	NL	NT	NL	NT	NL	NT	NL	NT

+ (positive); - (Negative); NL-No lesion; NT-Not tested;* L162, L165, L192, L200, L230, L323 are positive layers that had lesions in the heart only. All the layers that were positive for avian PPD turned out positive for *M. avium*. Also, all samples were positive except for one of the liver samples (L3) (Table 3 and Figure 5).

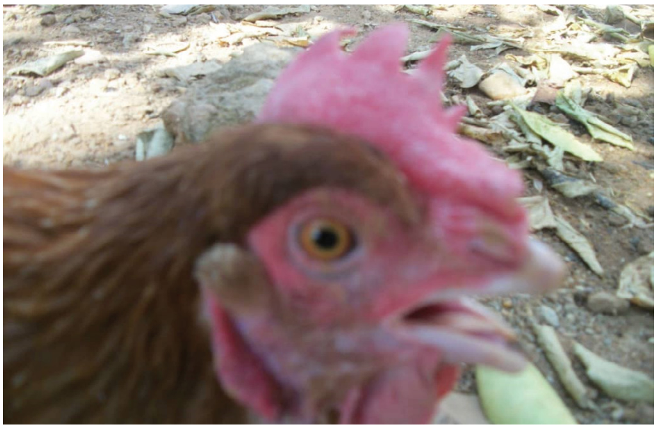

Figure 1: Showing a positive reactive layer with swollen wattles after 48 hours of Avian PPD administration

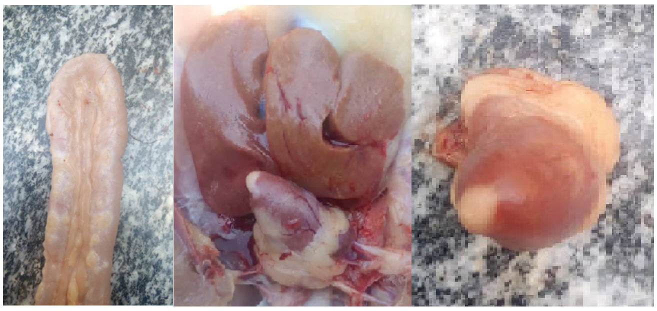

Figure 2: Arrows showing Lesions on the intestine (a) and heart (b & c)

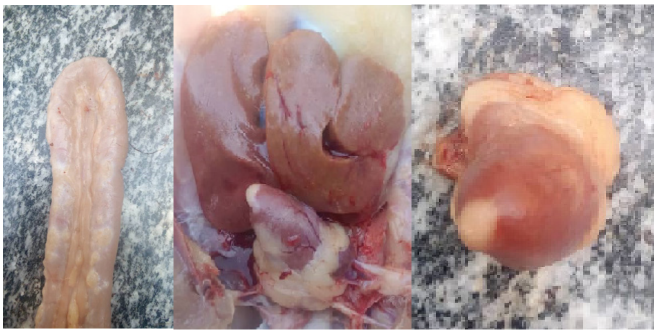

Figure 3: Arrows showing lesions (a) and caseous necrotic tissue (b & c) found in the uterus of an infected layer (150 weeks old) that tested positive to avian PPD

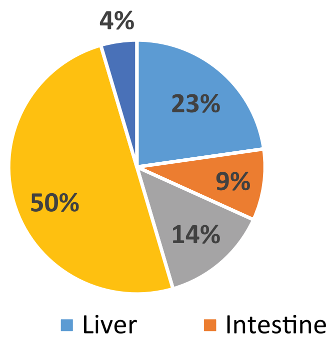

Figure 4: Distribution of harvested tissues and organs with gross lesions from positive birds

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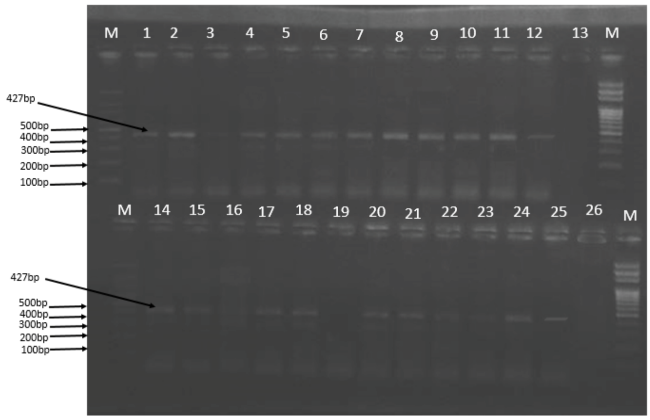

Figure 5: PCR gel electrophoresis picture; M (Molecular size marker); Lane 1 to 11(tissue samples of the heart, H1 to H11); Lane 12 (Positive Control); Lane 13 (Negative Control); Lane 14 to 16 (uterus samples, U1 to U3); Lane 17-21 (Liver samples, L1 to L5); Lane 22 to 23 (Intestines, I1 to I2), Lane 24 (Egg, E1); Lane 25 (Positive Control); Lane 26 (Negative control)

Discussion

Participatory disease surveillance was conducted on various farms within Gwagwalada and Kuje ACs. The estimation of farm prevalence obtained using PDS was quite lower than what was obtained when active search of the disease was conducted. Results of the PDS indicated Newcastle Disease (ND) as the most common disease on the farms. This result is similar to those obtained from other PDS studies conducted in Plateau, Borno, and Benue states where ND was also top on their ranking scale(30–32,40). Other top diseases from the current study were Salmonellosis, followed by coccidiosis, chronic respiratory disease (CRD) and fowl cholera also similar to what was obtained in these other studies(30,31,40). Avian tuberculosis, the disease of interest, ranked 11th, indicating that the disease is not commonly known by farmers and may not be a common occurrence due to the chronic nature of the disease and can only be detected in birds that stay long on farms such as layers, breeders and local chickens. Some farmers identified the disease based on the picture of the lesions that was shown to them. To the best of our knowledge, this is the first PDS in the country that identified the presence of ATB in poultry in Nigeria. This indicates the need for farmers' sensitization with regards to ATB.

In some of the farms visited, layers in the large poultry houses were not of the same age groups which is also a danger to the younger layers. In a farm that tested positive to ATB, the poultry houses were very dirty coupled with mixing of 3 different age groups of layers, with the older layers being about 3 years old. Literature reveals persistence of infection within flocks associated with keeping older stocks without proper hygiene(41). Furthermore, chickens were slaughtered and processed on the farm premises and part of the raw meat was given to the dogs on farm for consumption. Majority of the farms (83.3%) practiced battery cage system management with about 2 to 3 layers per cage while the remaining ones were on deep litter. Maintaining birds closely confined under stressful conditions provide favorable ways for spread of the disease(41).

Poultry manure is usually kept and processed within the farm premises and close to the poultry houses. The method of processing seen in one of the farms involved spreading of the manure for sun drying and a labourer comes to turn the manure from time to time to ensure that the moisture content is well reduced. This could also be a source of infection to birds on the farm. Unfortunately, some of the birds in these farms tested positive to ATB; invariably the environment is contaminated with *M. avium* since the mycobacterium is usually shed in the feces of chickens and can survive for long period of time in the environment. Moreover, wild birds may be exposed to *M. avium* when the organism is present in the manure that is spread on fields or deposited within the poultry vicinity. This serves as a major hazard for domestic and wild birds, and other domestic animals that roam within the farm premises. Manure merchants come to the farms to purchase manure and transport it to other locations either within Abuja or to other states of the federation and the manure is reportedly used in vegetable gardens and farms that indicates a further spread of the disease through this means of transporting the manure from one location to the other and selling to farmers. Also, these manure merchants can serve as a means of spread of the disease from one farm to another by visiting several farms on the same day unaware of the disease and liable not to take the necessary precautions in limiting or curtailing the spread of the disease. Another factor that could contribute to the spread of this disease is the fact that the poultry farms are usually clustered; located close to each other; most times within a kilometer of each other, with the possibility of spread to other environments via the wind leading to environmental contamination. (42). Litter, pens, equipment, and pasture contaminated with excreta of infected domestic birds and the hands, feet, and clothing of attendants play an important role in disease transmission (41,43).

In all the farms visited, no special clothing was used by the workers except for the use of boots by workers in some of the farms. The biosecurity measures on the farms sampled needs to be improved upon. Total movement restriction was observed in only 25% of the farms while the remaining farms allow customers and visitors entry on the farm, even though entry into the pens in all the farms is not allowed. There is a need for more movement control on the farms to limit disease transmission. The lack of foot dips (25%) in some farms and lack of use of foot dips (25%) even when available is a serious threat to biosecurity on farms and may be a possible factor in

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the spread of this disease giving the high farm prevalence that was obtained in the study. Two of the farms had disinfectant dips for vehicles entering the farm and only a farm disinfects the vehicle with disinfectant spray before entering the farm. Other animals such as local chickens, ducks, geese, goats and dogs moved around in 50% of the farms which is also a signal for easy transmission of diseases from the animals to the layers on the farm. There was high rejection rate (47.8%) of the poultry farms that were approached to conduct the Tuberculin test. None of the farms where tuberculin test was administered or approached with the request were aware of the tuberculin test for chickens. This made the acceptance of the Tuberculin test on the various farms a tedious process. Sensitization on the test had to be conducted along with documented information on avian PPD attached with the letter of introduction and several visits to the farms in most cases (up to four or five visits per farm) before the acceptance of the test by eligible farms. Some of the big commercial farms (more than 80,000 birds) did not agree to the tuberculin test due to limited knowledge on the value and availability of the avian PPD for the Tuberculin test; others gave excuse on biosecurity measures while others said that their birds will not be used for experimental purpose even after several attempts of debriefing on the essence of the test.

This study indicates high farm prevalence of ATB which reflects the need for commercial poultry farms (layers) to conduct tuberculin test on their farms for early detection and control of ATB on farms. The results indicate that ATB is more prevalent in Kuje AC. This might be due to the terrain of Kuje (an area that has a number of streams across the farm areas) and the abundance of poultry farms in the area which are in clusters. The 0.5% prevalence of ATB in Gwagwalada AC is similar to the results obtained by Madaki(26) in Giwa LGA Kaduna state where the prevalence was 0.52%. The overall prevalence of ATB in this study (2.8%) is higher than what was obtained in a study by Madaki(26) in Kaduna State where the prevalence of ATB among layers was 0.8%(3/384). Similar study was conducted in domestic chickens in Ethiopia(44) with a higher prevalence of 4.26%(12/282). The oldest layers (105 weeks and above) had the highest prevalence (4.8%). Several publications have indicated that older birds are more likely to be infected with *M. avium* due to the chronic nature of the disease in birds. This group of layers also had the highest emaciation rate of 14.3% due to the long duration of infection as a result of the long duration on the farm. This finding is similar to that of a study conducted in Argentina where cachexia and muscular atrophy were frequently associated with older birds infected with *M. avium* infection(45). Massive cachexia was witnessed in four out of the eleven (36.4%) layers that tested positive to ATB (>150weeks old) and when postmortem was conducted, there was atrophy of the breast muscle "knife edge keel", disappearance of body fat and the layer appeared quite smaller than the usual size. The owner complained of reduced egg production which was attributed to the age (spent layers) as well as ATB that was detected on the farm. Layers of 25 weeks' old were found positive for ATB in this study which is not a usual finding. Layers between 25 and 52 weeks' old had a prevalence rate of 4.2% which was much higher than older birds that were between 53 to 104 weeks' old having a prevalence of 0.6%. Literature suggests birds above a year old are more likely to be infected by ATB due to the chronic nature of the disease. Outbreaks of ATB in domestic chickens and ducks less than a year have been reported by several studies in other countries(46,47).

Our finding that there was no pulmonary lesion in all the layers that tested positive to ATB confirms what is obtained in literature—pulmonary ATB is only seen occasionally in pigeons and water fowl (41,48). The Intestinal lesions obtained from this study suggests oral route of infection. Infection acquired through oral ingestion of food and water contaminated with feces is the most common source of infection and spreads throughout the bird's body, the bacilli are oozed from ulcerated lesions of the intestine and voided via the faeces (41,49). Contrary to literature (41) indicating that majority of the lesions are from the intestine, this study revealed lesion on the heart (100%) of layers that tested positive compared to other organs and tissues. The heart indicated the accumulation of caseous lesions on the apex of the heart and some raised patches of whitish lesions on the surface of some of the hearts. Worthy of note also, is that there were more lesions in the liver, and uterus than in the intestine. There were tiny patches of whitish lesions on the liver. On the intestines, there were appearance of massive patches of yellowish tumour-like lesions extensively spread on surface of the gut wall of the intestine. Similar to our study the presence of *M. avium* have previously been confirmed in liver and intestinal tissues of birds(23,50).

Contrary to the opinions that *M. avium* is rarely found in commercial layer farms (46,47), all the presumptively positive samples turned out positive for *M. avium* except for one of the liver samples. All the farms that were positive to avian PPD turned out positive for *M. avium,* indicating that avian PPD is highly effective in the detection of ATB. Studies have confirmed the presence of *M. avium* outbreaks in commercial layer farms(46) and backyard poultry farms in Tunisia(51). Reports have confirmed the outbreak of ATB in commercial domestic Pekin ducks (*Anas platyrhynchos domestica*)(47) and in common pheasants (*Phasianus colchicus*) living in captivity and in other birds, vertebrates, non-vertebrates and the environment(10). A confirmed case of ATB can create a complicated situation in terms of disease control(52). A farm that has confirmed outbreak of ATB needs to stay away from keeping poultry for about two years after taking the necessary

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precautions(52). Unfortunately, this may not be feasible in Nigeria because there is no regulatory policy with regards to the disease; hence, compensation is not available for farmers which could make them adhere to standard procedures when their farm is confirmed ATB positive. Part of the limitations of the study was that quite a number of farms did not allow the administration of avian PPD on their farms (47.8% rejection rate); majority due to lack of information stating that "they will not allow their birds to serve as experimental birds" while the rest due to the outbreak of highly pathogenic avian influenza (HPAI H5N1) during the study period as part of the biosecurity measures that they were observing.

Conclusion

This study established the existence of ATB in chickens in Gwagwalada and Kuje Area Councils (ACs). The PDS has established the presence of ATB in 15.4%; 12 farms and LBM. Also, the disease of interest, ATB ranked 11 using the PDS method. Regarding the biosecurity measures, 75% had water supply from their boreholes, 25% movement restriction on farms, 50% of the farms had other animals on the farm aside the layers that were intensively managed, 25% of the farms had no foot dips, 25% had foot dips that were not in use. Possible derivers of infection and spread of ATB based on the PDS conducted were: housing of layers of different age groups, dumping and processing of manure on farm premise, low biosecurity measures on majority of farms, activities of manure merchants and clustering of poultry farms in an area. There was high rejection rate (47.8%) from farms that met the sampling inclusion criteria. There was high farm prevalence, (41.7%) of ATB detected using avian PPD. Out of the 395 layers sampled, the ATB prevalence was 2.8% with 1% (2/191) in Gwagwalada and 4.4% in Kuje ACs. Emaciation was seen in 34.6% of the layers that turned out positive for avian PPD. All layers that were positive for avian PPD turned out 100% positive for *M. avium* using PCR analysis. We recommend strict biosecurity measures in commercial and backyard farms, sensitization of farmers on the relevance of performing tuberculin test on long standing birds on the farm; farmers should raise layers from day old in the farm as we discovered that most of the farms purchase their birds at point of lay which usually come down with diseases like fowl cholera and salmonellosis; and the Federal Ministry of Agriculture and Rural Development (FMARD) needs to develop a policy that regulates the spread of ATB by ensuring that any farm that test positive for ATB is well compensated and the necessary control measures implemented to stop the spread of infection to other farms.

Declarations

Ethics approval and consent to participate

Research protocol was prepared according to ARRIVE guidelines and research was conducted according to the guidelines. All methods conducted are reported in accordance with ARRIVE guidelines (53). Ethical approval was obtained from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with the application number: ABUCAUC/2022/Vet. Pub. Health & Prev. Med/026 and Approval N0: ABUCAUC/2022/026. Furthermore, an introductory letter was issued by the Department of Veterinary Services, Agriculture and Rural Development Secretariate, Federal Capital Territory (FCT) administration after application which was distributed to the farm managers. Also attached to the above-mentioned documents was information on the Avian PPD used for the Tuberculin Test as indicated by Onderstepoort Veterinary Institute (OVI), South Africa where the product was obtained. Some farms gave written informed consent while informed verbal consent was obtained from majority of the sampled farms before commencement of the Tuberculin test after thorough explanation of the research to the farm mangers/owners, followed by sensitization of the farm managers on the test procedure with application letter stating full details of the researcher, organization and associated institutions and other relevant information in order to obtain permission to conduct the tuberculin test on the farms. We informed participants on our intension to publish and assured them that their farms will remain anonymous to the public and will not be linked with ATB in any way since the results for the birds were lumped together and the study purpose was to find out the presence and prevalence of ATB in the study area.

Consent for Publication

Not applicable

Availability of Data and Materials

The data sets supporting the conclusions of this article is (are) included within the article and its additional files.

Competing Interests

The authors declare that they have no competing interests.

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Author's Contributions

AVK; Research concept, development of research protocol, obtaining ethical approval, obtaining letter of introduction and permission from the Federal Capital Territory Authority (FCTA) to conduct research, data collection (PDS, administration of avian PPD, antemortem and postmortem inspection), data analysis and manuscript draft. AVK and BJA; PCR analysis. AVK, BJA, MB, MSB and JK, reviewed, My gratitude to the Nigeria Field Epidemiology

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Supplementary Table 1: Participatory Diseases Surveillance results from various farms and live bird Market within Gwagwalada and Kuje area council, Abuja, Nigeria

Diseases	Gwagwalada Area Council								Kuje Area Council					Total Score	Ranking
	F1	F2	F3	F4	F5	F6	F7	LBM	F8	F9	F10	F11	F12		
NCD	50	70	70	90	20	100	100	90	90	90	100	100	100	1070	1
Salmonellosis	70	0	80	80	100	90	90	100	80	80	90	60	90	1010	2
Coccidiosis	80	90	100	40	90	80	0	0	70	100	0	80	0	730	3
CRD	0	40	90	100	80	0	0	80	100	20	70	0	70	650	4
Fowl Cholera	60	100	60	0	60	70	70	50	0	10	80	0	30	590	5
Prolapse	100	0	0	10	30	0	80	0	0	70	0	0	40	330	6
Gumboro	40	0	50	0	0	60	0	0	0	0	0	90	60	300	7
Fowl pox	0	80	30	20	50	50	0	70	0	0	0	0	0	300	7
Ectoparasites	0	0	0	30	70	0	0	60	0	30	0	70	20	280	8
Marex	30	60	0	0	0	0	0	30	0	50	0	50	0	220	9
Egg binding	90	0	0	0	40	0	0	0	0	0	0	0	50	180	10
ATB	0	50	0	0	0	0	0	0	0	40	0	40	0	130	11
worms	0	0	0	60	0	0	0	0	0	60	0	0	0	120	12
Hypocalcaemia	0	0	40	70	0	0	0	0	0	0	0	0	0	110	13
Cannibalism	0	0	0	0	0	0	0	0	0	0	0	0	80	80	14
Bumble feet	0	0	20	50	0	0	0	0	0	0	0	0	0	70	15
ILT	0	0	0	0	0	0	0	0	0	0	0	60	0	60	16
Avian influenza	0	0	0	0	10	0	0	40	0	0	0	0	0	50	17