Molecular Detection of Avian Infectious Bronchitis Virus from Chickens in Sokoto State, Nigeria

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Abstract: **This research aimed at detection of infectious bronchitis virus (IBV) from chickens in Sokoto State, Nigeria using molecular technique. Three hundred (300) tracheal and cloacal swab samples from 150 chickens were taken in some selected areas of each of the four Agricultural zones of Sokoto State, namely; Sokoto, Tambuwal, Isah and Gwadabawa, pooled together to make 100 samples. The chickens manifested some clinical signs of infectious bronchitis (IB) and the samples were used for reverse transcription polymerase chain reaction (RT-PCR). Out of the 100 samples, 84 had RNA with the required purity and concentration. The overall result of the PCR showed 11(13.09%) positives. From all the tested exotic chickens, 12.50% were positive and out of all the tested indigenous ones, 13.50% were positive. Adults had 8.83% and young 16.00% positives. From the tested males, 17.65% were positive and from the tested females, 10.00 % were positive. The results for the zones showed that out of the tested chickens in Sokoto zone, 14.00% were positive, Tambuwal had 0.00% positives, Gwadabawa 8.33% and Isa 18.75%. In conclusion, the study has provided information on status of IBV in the study area which showed low prevalence of the virus at the period of sample collection. Continuous adoption of strategies to the field situation in Sokoto State was recommended. Indigenous poultry keepers should be enlightened and be encouraged by veterinarians to consider vaccination as a mean of prevention of poultry diseases like IB in the study area.**

Key words: **Infectious bronchitis virus (IBV), chickens, Sokoto State, polymerase chain reaction (PCR).**

I. INTRODUCTION

Infectious bronchitis virus (IBV), a single stranded enveloped virus of the genus *gamma Corona virus* and family *Coronaviridae* isan economically important pathogen of chickens and also the causative agent of infectious bronchitis (IB). IB is an acute, highly contagious disease of mostly chickens, characterized by upper respiratory tract symptoms and urinary and reproductive systems failures (Cavanagh and Gelb, 2008). IBV was first reported in 1931, since then the virus has spread to all parts of the world, the incidence of the disease being reported more often during the cooler months (Gary *et al.,* 2009). Reports suggest a shift in tissue tropism, and an extended host range into bird species such as the guinea fowl (*Numida meleagris*) and the partridge (*Alectoris* species) (Liu *et al.*, 2006). Diagnosis of IB is achieved by serological and molecular techniques (Gary *et al.,* 2009; Bande *et al*., 2016).As conventional virus isolation technique has been too cumbersome, RT-PCR, a molecular technique on reverse transcribed RNA has replaced it being it rapid and highly sensitive for the detection of IBV viral RNA (Zwaagstra *et al*., 1992). Samples frequently used include swab materials from the trachea, lungs and kidneys of infected chickens (Sylvester *et al*., 2006). Tracheal swabs are preferred and are obtained as soon as clinical signs of the disease are evident (Swayne *et al*. 1998). Results of RT-PCR to detect IBV showed moderate to low prevalence in different countries (Dergham *et al*., 2009; Mohammad *et al.,* 2013). Ducatez et al., (2009) reported prevalence of the virus in some states in Nigeria (Sokoto State inclusive) to be18 % using RT-PCR. Since then, there has not been survey of the virus using molecular technique in the study area which necessitates this study in order to establish its current status.

II. MATERIALS AND METHODS

2.1 Sampling frame

In this study, 150 each of tracheal and cloacal swab samples of chickens from Sokoto, Isa, Gwadabawa and Tambuwal agricultural zones of Sokoto state were conveniently collected between 2016 and2017making 300 samples. The samples wereobtained from exotic (commercial) broilers, pullets & layers from commercial poultry houses and indigenous (local) chickens from back yard poultry and live bird markets. Types

of chickens, age, sex and location (zone) were used as sampling frame in the study (table 2.1).The number of different variables was taken considering availability and owners' cooperation. The study was carried out from November 2016 to February 2017. Table 2.2 showed areas where samples were collected.

2.2. Sampling Method

Purposive sampling method was adopted in this study as described by Paul (2004), targeting chickens manifesting some clinical signs of IB without history of vaccination against the disease (indigenous chickens in the study area are not usually vaccinated against poultry diseases). Chickens less than six months of age were considered as young while those above six months were considered as adults.

2.3. Sample Collection and Preservation

The swab samples were collected for viral RNA isolation and RT-PCR. Each swab was placed in a sterile tube containing 1mL of Phosphate buffered saline (PBS) as described by Martin *et al.,* (2006) and transported to laboratory in cold conditions. The swabs were scrapped on the side of the tubes to remove the contents from the swabs heads. Three swabs from the same zone, sex, age group and route were pooled together in order to reduce the number of samples and increase viral RNA concentration(100 pooled tracheal and cloacal swab samples) and stored at -20^{0}C until analysis as described by Jonathan (2015).

Table 2.1: Sampling frame

Variables	Number of pooled samples
Type	
Exotic	40
Indigenous	60
Age	
Adults	42
Young	58
Sex	
Male	42
Female	58
Zone	
Sokoto	54
Tambuwal	10
Gwadabawa	16
Isah	20

Table 2.2: Areas where samples were collected

Local Chickens	Commercial Chickens
Sokoto meat and vegetables market	Tabanki farms Sokoto
Unguwar Rogo poultry market	Maska farms Sokoto
Achida Market	Dan Uda Farms Sokoto
Illela Market Tambuwal market	Ambarura farms Guiwa Lowcost Sokoto A backyard Poultry Mabera Sokoto
Two households in Achida	Two backyard Poultry in Lowcost Sokoto
Two households in Illela	Three backyard Poultry in Tamaje
	Sokoto
Two households in Tambuwal	Three backyard poultry in Achida
	Three backyard poultry in Illela
	Three backyard poultry in Tambuwal

2.3 Viral RNA Extraction

A QIAamp® (QIAGEN®, Germany) viral RNA extraction mini kits were used for the extraction as described by the manufacturer. The kits were stored at room temperature before use. RNA extraction was done for the 100 pooled samples. Purity and concentration of the 100 extracted viral RNA were determined using a J.P Selecta series 2005® UV spectrophotometer. Both RNA extraction and determination of purity and concentration of the RNA were carried out in Central Veterinary Research Laboratory, of the Usmanu Danfodiyo University Sokoto.

2.4 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

i. Reverse transcription (cDNA synthesis)

The extracted RNAs were reverse transcribed to form cDNA. This was performed with **5**µL of denatured RNA sample. Two micro liters (2 µL) of gDNA remover and 7uL of RNAse free water (Qiagen). The mixture was incubated at 42^{0}C for 2 minutes. This was then added to another mixture containing 1µL of reverse primer UTR 41, 4µL of quantiscript® RT-buffer and 1µL of quantiscript® reverse transcriptase and incubated at 42^{0}C for 30 minutes and inactivated at 95^{0}C for 3 minutes. The cDNA was stored at -20^{0}C.

ii. Polymerase Chain Reaction (PCR)

Integrated DNA Technologies IDT® primers (Coral vial, IOWA, USA) were used for the PCR. The cDNA was screened for IBV using PCR targeted to the highly conserved un-translated region 3'UTR of the virus genome with primers designed by Cavanagh *et al.* (2002).Forward **(**Sense specific) primer UTR11 was first used in combination with reverse (anti-sense specific) primer UTR 41to amplify a 266 base pair DNA fragment. Sequences of the primers are shown in table 2.3.

IBV vaccine-strain H120 was used as positive control for RT-PCR optimization. Five (5) µL of cDNA sample was used with 12.5µL QIAGEN® master mix (MgCl2, dNTP) 2X (Coral vial, IOWA, USA) 1µL each of forward and reverse primers and 5.5µl of RNAse-free water to a final volume of 25µL. The reaction mixture was transferred into AB® thermocycler (Applied biosystem). The thermal profile for the PCR was 94˚C for 3 minutes, followed by 35 cycles of 94 ˚C for 30 seconds, 48˚C for 30 seconds and 72˚C for 1 minute, followed by 72^0 C for 10 minutes.

The products were screened for the presence of a specific fragment in comparison with a Gene Ruler 100 bp DNA

Ladder (Promega) after electrophoresis was done using 1.5% agarose gel pre-stained with ethidium bromide DNA Gel stain (Sigma Aldrich® Missouri USA) and 1X Tris borate EDTA (TBE) buffer according to standard (85V, 3.00A,300W) for 45 minutes. Positive bands were checked on the gel using the UV trans illuminator of the BioRad® gel documentation device.

Table 2.3 Primers used in the study

Name	Sequence (5′ to 3′)	Target Region
UTR11	GCTCTAACTCTATACTAGCCTA	UTR
UTR41	ATGTCTATCGCCAGGGAAATGTC	UTR

Cavanagh *et al.* (2002)

2.5 Data Analysis

Simple percentages were used to analyze the obtained data.

III. RESULTS

3.1 Viral RNA extraction

Out of the 100 extracted RNAs, 84 passed purity and concentration test and were used for RT-PCR.

Over all prevalence

Out of the 84 samples tested for PCR, 11 were positive (13.09%). They are as follows: TR/B/SK/05, TR/B/SK/06, TR/B/SK/23, TR/AF/SK/07, CL/AM/SK/06, TR/YM/IS/15, CL/YM/IS/15, CL/P/SK/05, CL/YM/GW/08, TR/AF/IS/04, TR/YF/SK/16.

The Samples were designated as route/type/location/sample ID number. TR=Trachea; CL=Cloaca; B=Broiler; P= pullet; (L= layer but none was positive); AF=Adult female (Local); AM=Adult male (Local); YM=Young male (Local); YF=Young female (Local); SK=Sokoto; GW=Gwadabawa; and IS=Isa.

From the results, there were 7 positives from tracheal swabs and 4 positives from cloacal swabs. Also, the results showed that out of the 11 positive samples obtained, 36.36% were from exotic chickens while 63.64% were from indigenous ones. Out of the 84 tested, 4.76% of the exotic were positive and 8.33% of the local chickens were positives. Out of all exotics tested, 12.5% were positive and out of all local ones tested, 13.5% were positive. Adults had 27.27% positives and young chickens had 72.73% positives. Out of the total number (84) tested, adults had 3.57% positives and young chickens had 9.53% positives. Out of the adults tested, 8.83% were positive and out of the young ones tested, 16% were positive. Out of the 11 that were positive, 54.55% were cocks and 45.45% were hens. Out of the 84 tested, cocks had 7.15% positives and female chickens had 5.95% positives. Out of all the males tested, 17.65% were positive and out of all the females tested, 10% were positive.

From chickens tested in Sokoto zone for PCR, 63.64% were positive, no positive was obtained from those tested in Tambuwal zone (0%), Gwadabawa zone had 9.09%positive and Isa zone had 27.27%positives. Out of the 84 tested, Sokoto zone had 8.34% positives, Tambuwal zone had 0% positive, Gwadabawa had 2.38% positives and Isa zone had 7.14% positives. Out of all the chickens that were tested from Sokoto zone, 14% were positive, 0% was positive from Tambuwal zone, out of those tested from Gwadabawa zone 8.33% were positive and out of all those tested from Isa zone, 18.75% were positive (table 4.7).

The gel image of the PCR where 266bp was amplified using UTR primers and Biolabs® 100bp ladder, showed appearance of bands corresponding to the targeted position on DNA ladder, indicating the amplicons that were positive (figure. 3.1).

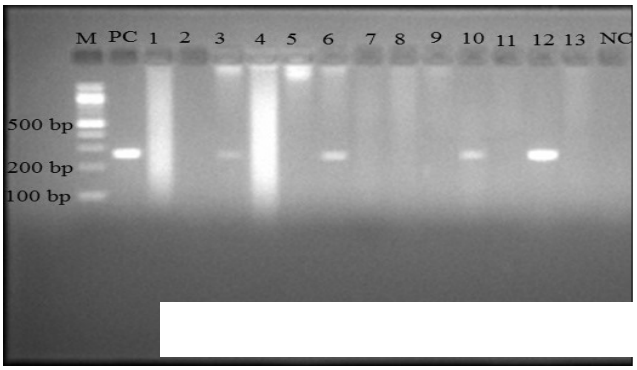

Figure 3.1Representative of elecrophoresis gel images of the PCR

Table 3.1: Results of the PCR in Chickens of various Categories

Variables	Positive in each category (%)	Positive out of total number (84) tested(%)	Positive out of all positives (%)
Exotic	12.50	4.76	36.36
Indigenous	13.50	8.33	63.64
Adults	8.83	3.57	27.27
Young	16.00	9.53	72.73
Male	17.65	7.15	54.55
Female	10.00	5.95	45.45
Sokoto	14.00	8.34	63.64
Tambuwal	0.00	0.00	0.00
Gwadabawa	8.33	2.38	9.09
Isa	18.75	7.14	27.27

IV. DISCUSSION

This is the first work in which tracheal swabs were used for investigating IBV using RT-PCR technique in Sokoto State. The overall result after amplification of UTR region from tracheal and cloacal swabs of 150 sampled chickens in Sokoto State showed 13.1% positives and 86.9% negatives. This is close to 18% reported by Ducatez *et al*. (2009) in similar study conducted between 2002 and 2007 in Nigeria (Sokoto state inclusive) and Niger Republic using cloacal swabs and

lung samples. They reported that in northern Nigerianlive bird markets and in Niger backyard poultry, IBV infections seemed to be less common. The detection of IBV from non-vaccinated birds by RT-PCR indicated that these flocks had experienced field exposure to IBV. The percentage positives (13.1%) recorded in this work was less than the 58.8% PCR positives reported by Dergham *et al.* (2009) in chickens tested in Jordan as well as the 63.4% in Egypt reported by Selim *et al*. (2013) after testing 205 tracheal swabs from commercial chickens using PCR in 2012. Similar research conducted in Iran by Mohammed *et al,* (2013)reported higher percentage than what was recorded in this study of 36.6%.

The result showed higher positives in indigenous (local) breeds than in exotic (commercial) breeds tested (63.64% and 36.36% respectively). All the exotic chickens that appeared to be positive belonged to the young category of age group; these included 3 broilers and 1 pullet, all the tested layers were negative. Selim *et al.* (2013) reported higher PCR percentage positives in broilers tested (65.4%) than in layers (50%) in Egypt also Dergham *et al.*, (2009) reported 64% positives in broiler chickens and a lesser 54.4% positives in layers using PCR in Jordan. It is a common knowledge that biosecurity measures are more imposed in commercial egg producing farms than in broiler farms.

Male chickens tested had 54.55% positives with no much difference compared to the 45.45% positives obtained from the tested female chickens. This could be as a result of same method of management practiced in both sexes of different types of chickens sampled.

The result in the young chickens tested in this study (72.73%) was higher than in the adults with 27.27% positives; young chickens do not have well developed immunity compared to adults.

In this study more positives were obtained from tracheal swabs (63.64%) than from cloacal swabs (36.36%) which support Bande *et al*. (2017) that the upper respiratory tract is the primary replication site for IBV and initial infection starts at the epithelium of Harderian gland down to lower respiratory tract before reaching urogenital and gastro intestinal tracts.

The prevalence of IBV infection in the four agricultural zones in this study indicated widespread distribution of the virus in most parts of Sokoto State except for Tambuwal zone where no positive result was obtained. The percentage positive in Sokoto zone showed highest prevalence (63.64%), Isa and Gwadabawa zones had lower prevalence of 27.27% and 9.1% respectively. The highest prevalence obtained in Sokoto zone might be associated with more poultry commercial activities in the zone resulting to chickens having more contact with each other which further results to contracting various diseases.

V. CONCLUSION

The PCR result showed low prevalence of the virus in Sokoto State.

VI. RECOMMENDATION

1. Clinicians should always investigate IB in cases of sick chickens presented with clinical signs of the diseases.
2. Proper disinfection of veterinary clinics/hospitals and poultry farms should be ensured to minimize spread of IBV.
3. Strategies need to be adapted continuously to the field situation in Sokoto State. Indigenous (local) poultry keepers should be enlightened and encouraged by veterinarians to consider vaccination as a mean of prevention of poultry diseases like infectious bronchitis.
4. Improvement in shelter and management in local chicken production should be encouraged for easy prevention and control of diseases.

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