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Full Length Research Paper

Prevalence of respiratory viruses in ducks, chickens and turkey flocks in Benue state

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The prevalence of Avian influenza, Newcastle disease, Infectious bronchitis and Infectious Laryngotracheitis viruses as well as Avian pneumoviruses among ducks, chickens and turkey flocks was studied in Benue State in March 2009. One hundred and ninety- four cloacal/pharyngeal samples comprising of one hundred and sixty-four cloacal and thirty pharyngeal swabs were taken from apparently healthy ducks and turkeys, as well as sick local and exotic chickens from16 of the 23 local Government areas of the state. Sample sites were markets, suburbs, commercialized intensive poultry farms and village settlements. Sample swabs of ducks turkeys and chickens species were taken into in eppendorf tubes containing viral transport media and transported on ice in a cooler box. These were delivered to the laboratory and kept at -20℃ until tested. Polymerase chain reaction technique was used for analysis of samples. Out of the 194 samples collected 94 (48.5%) tested positive for NDV and 65 (34.0%) were positive for infectious bronchitis while one (0.01%) sample was positive for ILTV. The results obtained showed that there were no avian influenza viruses in the samples collected from farms markets and suburbs in Benue State, but Newcastle disease virus, Infectious bronchitis virus and ILTV were present. One hundred and twenty nine chickens were tested and 61 (47.0%) were positive for NDV while 41 (32.0%) were positive for IBV. Thirty one ducks were tested and 7 (23.0%) of them were positive for NDV while 9 (29.0%) for IBV. Thirty four turkeys were tested, 25 (74.0%) were positive for NDV while 15 (49.0%) for IBV. This study has shown that Newcastle disease, infectious bronchitis and infectious laryngotracheitis are prevalent in Benue State among chickens, ducks and turkeys, and infectious laryngotracheitis is of extremely low prevalence.

Key words: Prevalence, respiratory viruses, duck, chickens, turkey, Benue State, Nigeria

INTRODUCTION

Respiratory viral diseases such as New-Castle disease, Infectious bronchitis, Infectious laryngotracheitis, Pneumoviral infections and Avian influenza are among diseases that could jeopardize the health status of the bird and could nearly wipe out the industry of a country if not adequately researched into and controlled (Beard, 1998). Considerable research work has to be done with regards to studying the prevalence of the diseases in some localized areas of Nigeria with a view to working out modalities for prevention and control. Avian flu and New Castle disease, apart from their economic impact on the industry, also share zoo-anthroponotic credentials (Hayden and Croisier, 2005). Newcastle disease for instance causes clinical infections characterized by reddening, excessive lacrimation, edema of the eyelids, conjunctivitis, and subconjunctival haemorrhage in humans as cited by Alexander (2003).

Oluyemi and Robert,(2000) maintained that the control of these diseases in free range, intensive and semi-intensive poultry farms has remained one of the greatest challenges to producers of poultry products. In Benue state, the poultry or avian stock share also in the

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worldwide disease epidemic pool and are seriously at risk of exposure to viral diseases because of the very hot climate that predisposes the animals to stress which precipitates the proliferation of the organisms as well as the generally prevalent sub-standard prophylactic and husbandry inputs. With regards to the highly pathogenic avian flu whose outbreak could occur with devastating fatalities, it is conceivable that the low pathogenic Avian flu virus (LPAI) might be resident in some parts of the State at the time of conducting this study. Serological findings elsewhere in Nigeria have reported infections of type A influenza viruses in poultry and pigs (Adeniji *et al*., 1993). It is not impossible therefore to rule out low grades flu virus activities among avian species in other parts of the country which could have predicated previous H5N1 pandemic.

Studies have shown that some of our local chickens do carry and possibly excrete the causative agents for diseases like ND and IB. Owoade *et al.* (2006) reported high seroprevalence for 1B, AI, AP and Avian pneumoviruses. Lack of adequate diagnostic facilities has always hindered research work on these diseases in the state. This has resulted to epileptic outbreaks due to wrong diagnosis and incorrect application of control measures especially application of good control programmes (Adu *et al*., 1985).

This research work is conducted to evaluate the presence of Avian influenza, Infectious bronchitis, Newcastle disease, Infectious laryngotracheitis viruses and Avian pneumoviruses among chickens, turkeys and ducks in Benue state with a view to working out a programme that would prevent their outbreaks.

MATERIALS AND METHODS

Sample selection

Samples were collected from apparently healthy ducks, turkeys and local chickens. Samples from exotic breeds were from apparently sick birds looking birds, some of which had the following clinical signs like coughing, nasal discharges emaciation, dehydration, anorexia and torticollis. Sensitization of the people was done by the veterinary staff of the Ministry of Animal and Forest Resources of each Local Government area before embarking on sample collection and this made the collection process and accessibility to farmers and markets easier. Hand gloves, nose or mouth masks and boots were used during collection period.

Sample collection

A total of 194 samples were collected, 32 samples from ducks; 32 from turkeys and 130 from chickens. Pharyngeal and Cloacal swabs were performed on each of the birds. The trachea of a live bird was swabbed by inserting a dry sterile swab into the pharynx and gently swabbing the wall, and the swab was placed in VTM, while the cloacal swab was done by inserting a swab deeply into the vent and gently swabbing the wall. The swab with fecal material and the laryngeal swabs were placed in the eppendorf tube containing the transport medium (VTM). The other ends of the swab stick were cut with a pair of scissors so that the cover of the eppendorf tube would be properly closed. These were kept on ice in a cooler. The period of collection lasted for 13 days. All the samples were kept in the freezer at -20°C in the Laboratory before testing.

RNA/DNA isolation and purification from samples

Isolation of viral RNA (Avian influenza virus, NDV, IBV and APV) and ILT DNA Virus from samples and its purification was done using Qiagen DNA/RNA minikit. Five hundred and sixty µ1 (560µ1) of Buffer AVL containing carrier RNA was pipetted into a 1.5ml microcentrifuge (eppendorf) tube. Viral transport medium containing swabs was mixed by pulse-vortexing, and then 140µ1 of medium was added to each eppendorf tube containing Buffer AVL. This was mixed by pulse-vortexing for 15 seconds. The solution was then incubated at room temperature (25-30°C) for 10minutes**.** After incubation, the 1.5ml microcentifuge tube was briefly centrifuged to remove drops from the inside of the lid and 560µ1 of ethanol (96-100%) was added to the sample, mixed by pulse-vortexing for 1 seconds and tube briefly centrifuged to removed drops from inside the lid. Then 630µ1 of the solution in step three was carefully applied to the QIAmp spin column, closed and centrifuged at 8000rpm for 1 minute. The QIAmp spin column was then placed into a clean 2ml collection tube and the tube containing the filtrate was discarded.

Another 630µ1 of the solution was again applied into the QIAmp spin column without wetting the rim, the cap closed and again centrifuged at 8000rpm for 1 minute. The QIAmp spin column was then placed in a 1.5ml eppendoff tube, and the tube containing the filtrate discarded. The QIAmp spin column was carefully opened and 60µ1of Buffer AE (Elution buffer) added, incubated at room temperature for 1 minute and then centrifuged at 8000rpm for 1 minute, thus yielding viral RNA from the sample and viral DNA in case of laryngotracheitis. The vaccines for various viral diseases were similarly treated and used as positive control

Reverse transcription (RT)

For RNA viruses (NDV, IBV, APV, AIV) reverse transcription was done to convert the RNA to cDNA in order to be used for PCR. The procedure is as follows: Five microlitres (5µ1) of extracted RNA was diluted with

5µ1 RNAase free water (ratio 1:1). This was then incubated at 65°C for 5 minutes in the PCR machine. Thereafter, it was brought out and chilled on ice.

Similarly, mix 2 of RT protocol was then prepared and added to the PCR tube containing 7µl of mix 1 that was brought out from the PCR machine and incubated in the PCR machine at 37°C for 2 minutes. It was then brought out and 1µ1 of M-MLV was added to the mix and then taken to the PCR machine where it was incubated at 37°C for 50 minutes and then 70°C for 15 minutes. The end product of this was cDNA of the various RNA viruses. It is this cDNA that was then used for PCR protocol.

Polymerase chain reaction protocol

Avian influenza virus(AIV)

This was carried out by dispensing 22.5µ1 of AI PCR mix into each 200µ1 microcentrifuge tube and 2.5µ1 of cDNA added to make a volume of 25µ1 in each tube. Microcentrifuge tubes containing cDNA and PCR mix were then placed in a thermocycles for amplification by first carrying out an initial denaturation of the cDNA at 94°C for 5 minutes and another for 30 seconds, annealing at 60°C for 1 minute, elongation at 72°C for 1 minute, the second to fourth steps were repeated 39 times to make a total of 40 cycles before a final elongation was done at 72°C for 5 minutes. The PCR product was then held at 22°C in the thermocycler before subjecting it to electrophoresis

Newcastle disease virus

This was carried out by dispensing 23µ1 of ND PCR mix into each 200µ1 microcentrifuge tube and 2µ1 of cDNA added to make a volume of 25µ1 in each tube. Microcentrifuge tubes containing cDNA and PCR mix were then placed in a thermocycler for amplification by first carrying out an initial denaturation of the cDNA at 94°C for 3 minutes, and again for 30 seconds, annealing at 58°C for 30 seconds, elongation at 72°C for 1 minute, the second till fourth steps were repeated 34 times to make a total of 35 cycles, before a final elongation was done at 72°C for 5 minutes. The PCR product was then held at 22°C in the thermocycler before subjecting it to electrophoresis.

Infectious bronchitis virus

This was carried out by dispensing 22.5µ1 of infectious bronchitis PCR mix into each 200µ1 microcentrifuge tube and 2.5µ1 of cDNA added to make a volume of 25µ1 in each tube. Microcentrifuge tubes containing cDNA and PCR mix were then placed in a thermocycler for amplification by first carrying out an initial denaturaiton of the cDNA at 94°C for 5 minutes and again for 30 seconds, annealing at 53°C for 30 seconds, elongation at 72°C 1 minutes, the second to fourth steps were repeated 34 times to make a total of 35 cydles before a final elongation was done at 72°C for 7 minutes. The PCR product was then held at 22°C the thermocycler before subjecting it to electrophoresis.

Laryngotracheitis virus

This was done by dispensing 24µ1 of LT PCR mix into each 200µ1 microcentrifuge tube and 1µ1 of DNA added to make a volume at 25µ1 in each tube. Microcentrifuge tube containing PCR mix and DNA were then placed in a thermocycler for amplification by first carrying out an initial denaturation at 94°C for 5 minutes, another denaturation at 94°C for 30 seconds, annealing at 61°C for 30 seconds, elongationa at 72°C for 1 minute, the second to fourth steps were repeated 30 times to make a total of 31 cycles before a final elongation at 72°C for 5 minutes. The amplified DNA was then held at 4°C in the thermocycler subjecting it to electrophoresis.

Avian pneumovirus (APV)

This was carried out by dispensing 22.8µ1 of APV PCR mix into each 200µ1 microcentrifuge tube and 2.2µ1 of cDNA added to make a volume of 25µ1 in each tube. Microcentrifuge tubes containing cDNA and PCR mix were then placed in a thermocycler for amplification by first carrying out an initial denaturation of the cDNA at 94°C for 5 minutes and again for 30 seconds, annealing at 50°C for 30 seconds, elongation at 72°C for 1 minute. The second to fourth steps were repeated 34 times to make a total of 35 cycles before a final elongation was done at 72°C for 7 minutes. The PCR product was then held at 22°C in the thermocycler before subjecting it to electrophoresis.

Determination of positive samples

A 2% gel mixture was melted by heat in a microwave oven. Ethidium bromide (1.5µ1) was added to the gel in the conical flask after cooling. The Agarose gel was cast by pouring into a mold containing combs (14 slots) to form wells in the gel and allowed to gel. The combs were removed and the gel placed in an electrophoresis tank containing 0.5% TBE.

Five microlitre (5µ1) of 1kb ladder (invitrogen) was loaded in the first and last wells of the gel in the electrophoresis tank. Then, 5µ1 of PCR product mixed with 2µ1 gel loading dye including positive control were

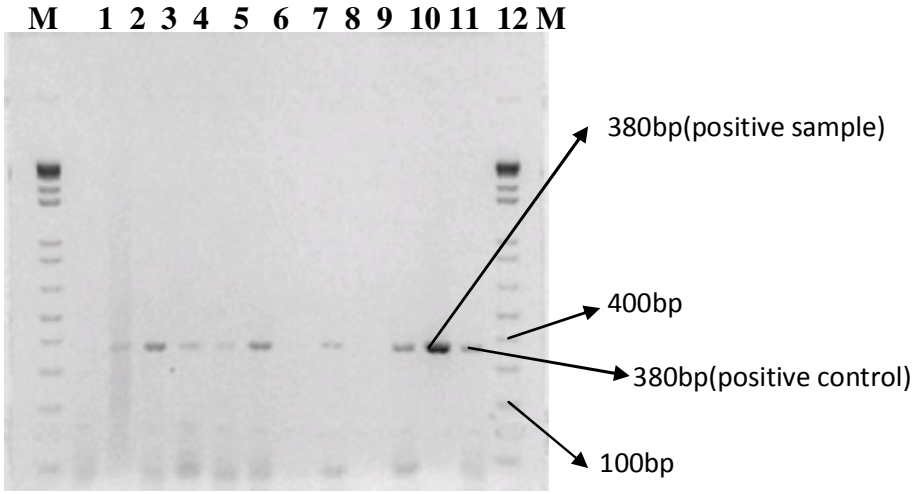

Figure 1. PCR result for IBV detection showing positive for IBV at Lanes 2-6,8,10-12 (380bp).

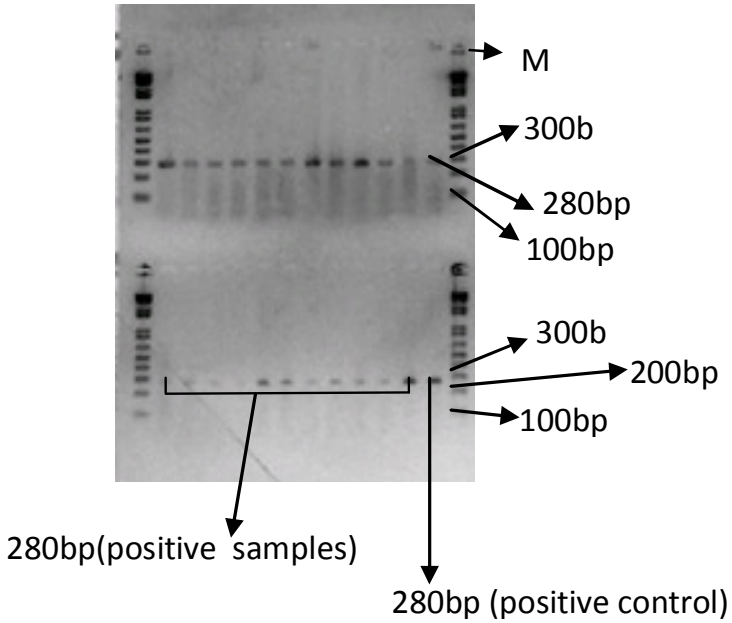

Figure 2. PCR result for NDV detection showing positive for NDV at all lanes (280bp).

carefully loaded in wells after the first market towards the last marker. This was done using a single channel micro-pipette without breaking up the gel and then subjected to electric current. This was subjected to electrophoresis at constant 120 volts for about 25 minutes using consort model E455 microcomputer electrophoresis power pack.

The DNA band was viewed by placing the gel on an uv trans-illuminator (UVP TS-20) beneath a Kodak ID 3.5 camera connected to a computer. The image displayed was captured with the Kodak Id 3.5 camera and viewed on the display unit of the connected computer.

Determination of positive band

The primers F_1P_1 and F_1P_2, N791 and N1129, Chen F and Chen R, g-dig F and g-dig R and APV $G1^+$ and APV G6 were designed specifically for NDV, IBV, AIV, LTV and APV respectively (Figures 1 and 2). The presence of band in any sample screened for ND, IB, AI, LT and APV

Table 1. Positive results for each disease from each local Government.

LGRs	Total of spls	AIV (%)	NDV (%)	IBV (%)	ITV (%)	APV (%)
Makurdi	55	0	10(0.05)	22(0.11)	1(0.01)	0(0.0)
Gboko	9	0	5(0.03)	1(0.01)	0	0
Tarkaa	7	0	4(0.02)	1(0.01)	0	0
Guma	2	0	2(0.02)	0	0	0
Konshisha	4	0	1(0.01)	0	0	0
Orbi	9	0	2(0.02)	1(0.01)	0	0
Logo	10	0	10(0.05)	6(0.03)	0	0
Vandeikya	4	0	4(0.02)	0	0	0
Ukum	8	0	8(0.04)	6(0.03)	0	0
Katsina-Ala	20	0	16(0.08)	9(0.05)	0	0
Okpokwo	13	0	0	0	0	0
Gwer	11	0	10(0.05)	8(0.04)	0	0
Gwer West	15	0	0	0	0	0
Orju	8	0	3(0.02)	1(0.01)	0	0
Otukpo	10	0	10(0.05)	10(0.05)	0	0
Kwande	9	0	9(0.05)	0	0	0
Total	**194**	**0**	**94(48.5)**	**65(34.0)**	**1(0.01)**	**0**

confirmed the presence of NDV, IBV, AIV, LTV and APV in such sample

RESULTS

All local government councils

A total number of 194 samples comprising of thirty (30) Pharyngeal swabs and one hundred and sixty-four(164) cloacal swabs were collected(Table 1). Ninety-five samples(95) from fourteen(14) local government areas out of the 16 visited were positive for Newcastle disease virus (Figure 3) representing 49.0%(fig4.0). Sixty-five samples from eleven local government areas were positive for infectious bronchitis virus representing 34.0% prevalence rate (Table 2). Only one local government area was positive for ILTV and that was Makurdi local government area.

Out of the 194 samples collected for this study, 94 were positive for NDV (49.0%), 65 were positive for IBV(34.0%) and only one was positive for ILT(0.01%) as shown in Table 1.

Positive result for NDV, IBV, AND ILTV in different types of birds

130 chickens were tested and 61(47.0%) were positive for NDV, 41(32.0%) for IBV and 1(0.01%) for ILTV. 32 ducks were tested and 7(22.0%) were positive for NDV, while 9(28.0%) for IBV. And 32 turkeys were tested and 25(78.0%) were positive for NDV and 15(47.0%) for IBV (Table 2).

The PCR results for the IBV detection showed a positive band on lanes 2-6, 8, 10-12 (380bp). While for the NDV detection, the positive bands were observed on all the lanes (280bp) and for the case of ILTV it was seen on lane 133 (296) (Figures 1, 2 and 3).

DISCUSSION

Benue State, with its large expanse of land has just a sizeable population of poultry farms with the highest concentration in the state capital, Makurdi. Some local government areas have only a few pockets of free range local chickens and ducks living in close association with their owners. Only a few people are into large poultry farming for meat and egg production as a business. The procedure of catching the local chickens and ducks was laborious except when they were brought to the open market. The concentration of chickens, turkeys and ducks in the areas from which samples were not collected was very scanty. Samples collected from among the birds have tested positive for NDV, IBV, and ILTV giving an insight into their prevalence in the state.

Although Owoade *et al.* (2002) had reported serological prevalence of type A influenza viruses in some parts of Nigeria even before its confirmed outbreak in February 2006, there is evidence to prove with this survey that the virus is yet to find its substrate in Benue State as none of the samples tested was positive for influenza virus. Also notwithstanding the confirmed report of its arrival in

Table 2. Prevalence of ND, IB and ILT viruses in chickens, turkeys and ducks in the study area.

S/N	Type of chicken	Total samples collected	NDV (%)	IBV(%)	ILTV(%)
1	Chickens	130	61(47.0)	41(32.0)	1(0.01)
2	Ducks	32	7(22.0)	9(28.0)	0
3	Turkeys	32	25(78.0)	15(47.0)	0
TOTAL		**194**	**93 (147.0)**	**65(107.0)**	**1(0.01)**

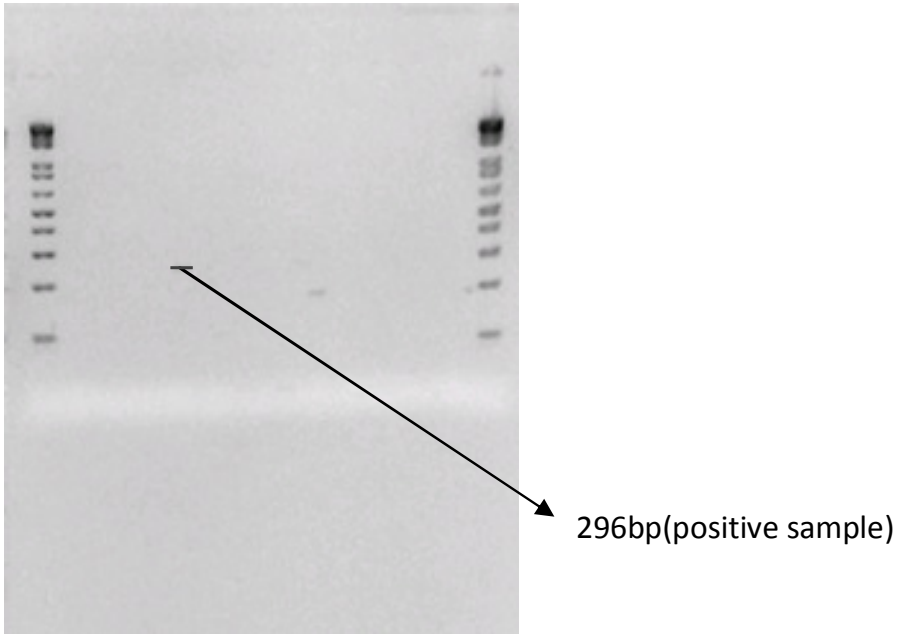

Figure 3. PCR result for ILTV detection showing positive for ILTV at lane 133 (296bp).

Nigeria (WHO, 2006) and the panic it caused scientists and stake holders in the poultry industry as to its epidemic potentiality of spreading to other states of the federation, it is pertinent to emphasize based on this study that the virus had not yet arrived the environmental enclave of the state.

Thirty-two free-range domestic ducks that habituated in close proximity with villagers and looked apparently healthy were sampled across the geographical area of the state and none was found with Avian flu virus. This dispels the belief that as reservoirs they might have been harboring the virus without clinical signs(FAO/OIC/WHO, 2004; Chen *et al*.,2006).This is not to lose sight of a report that some North American wood ducks were found to be susceptible to illness and death from highly Pathogenic avian influenza viruses(Brown, 2006). Turkeys that are known to be involved in natural infection as reported by CIDRAP (2008) also did not have any trace of the virus from the samples collected.

Newcastle disease virus had the highest prevalence rate of occurrence among the five studied viruses and it was seen in ducks, turkeys, local and exotic chicken samples having a percentage of 48.5% of the total number of samples tested, attesting to the ubiquitous nature of the virus among avian species (Nwosu, 1979). Most of the sick exotic birds tested positive for the virus and this agrees with the documentation that Newcastle disease is the most important viral disease of poultry in the world and is found in nearly every species (Oladele *et al*., 2004). A total number of 130 local and exotic chickens were sampled and 61 of these tested positive. The large numbers of positive samples from chickens could be as a result of the fact that they are the natural host of the virus. Reports from farmers were that the birds were off feed and reduction in production was a common feature. This is being reported by Oladele *et al*. (2004) in their works elsewhere about the clinical signs of Newcastle disease. It is no gain saying that an outbreak of Newcastle disease in a locality could have grave economic implications as reported by Oladele *et al*. (2004). Chickens that tested positive for the virus could act as sources of transmission (Alexander, 1988). Seven (7) duck samples tested positive out of 32 and twenty five (25) turkey samples tested positive out of 32. The

presence of the virus in these species can always be a reference point for infection to other birds. Newcastle disease has no reservoir hosts and its availability in any avian species predisposes it to classical infection depending on viral pathotype, viral load, immunogenetic status of the host, stress and other impending bacterial infections (Calnek *et al*., 1997).

Infectious bronchitis virus was also prevalent among samples tested, with a percentage prevalent rate of 34%. Only 9 duck samples collected from only one local Government area were positive for IBV. The number of turkeys sampled was 32 and out of this 25 samples were positive. Infection can be transferred from these species to chickens which are the only species that can come down with natural infection (Hofstad, 1984). Out of the 130 of both local and exotic chickens sampled 61 tested positive for IBV. Low production report from farmers here could also be due to infection with the virus as being reported by Cook and Huggins (1986). Emaciation which is a common clinical manifestation in Infectious bronchitis was also a common feature among the sampled chickens (Gough *et al*., 1992).

The occurrence of Newcastle disease virus and infectious bronchitis virus in ducks and turkeys is evidence that these species are habitats of the viruses as well as the chicken. There is a possibility that ND and IB viruses could persist in this region and be disseminated over other neighboring states through interstate trading with poultry products. Newcastle disease and infectious bronchitis are by this study the most prevalent diseased entities in the state. Hence, it is reasonable to suggest that their successful control could have implied 50% or at least a substantial improvement in the livability of commercial poultry stock. Infectious bronchitis, which no farmer would have envisaged about its existence in farms in this area, is now known to abound. It has with its sister Newcastle disease virus continued to be associated with egg production problems and also caused other losses through the extra cost of remedial treatments and mortality (Oladele *et al*., 2004).

Farmers are expected to begin to shift their attention from diseases that predominantly cause mortalities like cocccidiosis and infectious bursal disease to those like ND and IB which are more important for their impact on egg production. Because of the insidious nature of infectious bronchitis in poultry farms it has not attracted enough attention even though tentatively it had always been diagnosed by veterinary scientists without going a step further towards prevention.

The positive sample for ILTV(0.01%) from among all the samples collected is considered a statistically negligible prevalence rate. This may be one of the reasons why it is usually its sero-prevalence that is reported in Nigeria (Owoade *et al*., 2006).

An efficient surveillance system is central to any preparedness program aimed to detect AIV, NDV, IBV,ILTV, and APV in Benue State. Our data indicate that local and exotic chickens and turkeys would be sensitive indicators of the presence of NDV and IBV circulating in avian species.

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