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Polymerase Chain Reaction (PCR) a Molecular Technique for Diagnosing Newcastle Disease in Local Chickens

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ABSTRACT
This study aimed at detecting Newcastle Disease virus (NDV) in local chickens sourced from local markets using Polymerase Chain Reaction (PCR) technique. Thirty six local birds obtained from three local markets in Uyo, Uruan and Itu, in Akwa Ibom State, Nigeria was used in the experiment. The general clinical signs noticed in the diseased birds when observed by a veterinarian during post-mortem examination included, depression, severe prostration, somnolescence, reduction in normal vocalizations, decrease in food and water intake, huddling behavior, ruffled feathers and greenish diarrhea among others. Three organ samples-proventriculus, trachea and spleen were collected from some of the birds after postmortem investigation, processed and the targeted viral RNA was extracted using the RNA extraction kit. Genome amplification was performed with PCR using specific primers to target the gene. Amplification results produced an amplicon product of 121 base pairs (bp). The PCR product samples were then visualized using agar gel electrophoresis and viewed using the unified gel documentation system. Amplification results showed the three samples to be positive for the DNA bands corresponding to the targeted band of the NDV gene fragment. The results of this study confirm that the PCR method is capable and therefore applicable for diagnosing Newcastle Disease in local birds.

INTRODUCTION

Poultry, the largest livestock group, account for more than 30% of all animal protein. It represents an important sector in animal production that has the backyard flocks accounting for a huge majority, especially in the developing countries, where it is raised to meet household food demands and as additional sources of incomes. This method implies low bio-security measures and high risk of infectious diseases, such as Newcastle disease or zoonosis such as highly pathogenic avian influenza[1].

Newcastle disease is a contagious bird disease affecting many domestic and wild avian species and transmissible to humans.It is an important infectious disease of poultry that is caused by virulent strains of Avian Paramyxovirus-1, which is a single strand non-segmented negative sense RNA virus[2]. The epizootics of Newcastle Disease in poultry continue to occur in Asia, Africa, Central and South America while in Europe, sporadic epizootics occur[3].

Newcastle disease is an economically important disease and also a major threat to poultry industry[4]. According to variation in strains of NDV, the rate of mortality and morbidity in a flock is variable[5]. Isolation of virus and serological diagnostics, such as hemagglutination inhibition test, enzyme-linked immunosorbent assay and molecular diagnostic tests like real time PCR confirmed the presence of velogenic Newcastle disease virus[6].

Globally, Newcastle disease is economically one of the most important diseases causing substantial loss in poultry industry. Sudden high mortality in a flock in the absence of premonitory clinical signs occurs when susceptible species are exposed to highly virulent strain of Newcastle disease. Diagnosis based on clinical sign is not accurate because it resembles highly pathogenic avian influenza[7]. The PCR provides a greater understanding of disease processes as well as a foundation for their diagnosis-molecular diagnosis based on molecular findings than on physiological symptoms. Thus using it to diagnose Newcastle disease in poultry gives high accuracy of the specific strain of the virus. This will make it possible to contain the disease, reduce its severity and thus increases the economic efficiency in poultry farming[8]. More so its application in molecular genetics has led to improvement in animal production through the candidate gene technique[9-11].

This study was therefore carried out to assess the diagnostic capacity of PCR as a molecular technique for Newcastle disease in local chickens.

MATERIALS AND METHODS

Thirty-six local birds were sourced from reputable markets in Akwa Ibom State, Nigeria where they are abundant, consisting of twelve local birds obtained from each of the three local markets (Uyo, Itu and Uruan). The birds were not vaccinated against any endemic diseases before they were purchased for the experiment as attested to by the traders. The experiment was carried out under proper management practices, with water and feed provided *ad libitum.* After some weeks (6-7 weeks), the birds came down with a disease. The clinical signs consisted of depression, severe prostration, somnolescent, reduction in normal vocalizations and decrease in food and water consumption, huddling behavior, ruffled feathers and greenish diarrhea. A wide range of consistent and progressive neurological signs including tremors of head and neck, inability to stand, torticollis, paresis, paralysis, convulsions, rolling or circling movements, in coordination, loss of balance and recumbence with pedaling movement, flapping movements of the wings and unusual positions of head and appendages of the diseases are common to Newcastle disease virus (NDV). Post-mortem examination was carried out by a veterinary doctor and Newcastle Disease was suspected. Organ samples (proventriculus, trachea and spleen removed from the dead birds after dissection by a veterinarian)were collected for confirmation and taken to the laboratory for PCR analysis using an ordinary physiological buffer solution (water) and a known Newcastle isolate as negative and positive control, respectively.

RESULTS AND DISCUSSIONS

The mortality rate and organs used for PCR analysis is shown in Table 1. Samples collected were spleen, proventriculus and trachea. The recorded mortality rate from the three markets in the local government areas were 66.67% (Uruan), 50% (Uyo) and 33% (Itu) out of the 12 birds that were sourced from each of them respectively. Highest mortality at Uruan could mean that the NDV in the area is more virulent when compared to Itu and Uyo local governments.

The spread of the disease was rapid and the mortality rate was high. The general clinical signs consisted of depression, severe prostration, somnolescent, reduction in normal vocalizations, and decrease in food and water consumption, huddling behavior, ruffled feathers and greenish diarrhea. A wide range of consistent and progressive neurological signs including tremors of head and neck, inability to stand, torticollis, paresis, paralysis, convulsions, rolling

Table 1: Mortality rate and organs used for PCR

Location	Number	Mortality (%)	Samples collected
Uruan	12	8 (66.67)	Spleen, proventriculus and trachea
Uyo	12	6 (50)	Spleen, proventriculus and trachea
Itu	12	4 (33.33)	Spleen, proventriculus and trachea

or circling movements, in coordination, loss of balance and recumbence with pedaling movement, flapping movements of the wings and unusual positions of head and appendages could be noticed in the affected flock were in agreement with findings of Gowthaman *et al*.[12].

The birds exhibited general depression, dullness, loss of appetite, greenish diarrhea, somnolescence and respiratory distress such as labored breathing, increased rales, wheezing, and open-mouthed breathing and enteric signs such as watery/tenacious mucus discharge from the nostrils. Dergham *et al*.[13] and Roussan *et al*.[14] also had similar findings in broiler flocks.

The hemorrhage in the proventriculus is similar to the findings of Adi *et al*.[15], Alexander *et al*.[16] and Terregino and Capua[17]. Okoroafor and Onyema also reported hemorrhage on the proventriculus. The spleen was severely atrophied, this is in agreement with the findings of Okoye *et al*.[18] which reported swollen and hemorrhagic kidneys and congestion of the liver and heart. These were however in disagreement with our own findings.

The greenish diarrhea observed in this study (Fig. 1) was similar to the findings of Victor. Gowthaman *et al*.[12] also reported that there was strip with numerous red streaks of congestion/hemorrhage on the mucosal surface of the trachea and catarrhal exudates in the lumen, similar to findings of this study. There was no hemorrhage in the cecal tonsils in our studies compared to the work carryout by Onyema.

The lesions in the gastrointestinal tract of the birds were similar to the findings of Okoroafor and Onyema *et al*. (2019). There were lesions on the trachea. This finding contradicts the finding of Okoye *et al.*[18] who reported that the trachea and the lungs showed no lesion. Okoye *et al*.[18] reported swollen and hemorrhagic kidneys and congestion of the liver and heart, which disagrees with the findings in this study.

The PCR result in Fig. 2 showed that sample 1 (lane 1) had bands similar in width to the positive control but thinner compared to other samples. Sample 2 (lane 2) with the thickest band formation has the highest amounts of the gene fragments as such a higher amount of gene fragments of NDV compared to samples 1 and 3 with sample 3 having a higher gene fragment than sample 1. Sample 3 (lane 3) had bands that are thicker in size when compared to sample 1.

Pathological analysis showed that there was hemorrhage in the proventriculus (Fig. 3), the spleen was severely atrophied and that there were lesions on the trachea.

In Table 2, the PCR results were recorded as positive with a '+' sign and negative with a '-' sign. The Lasota live ND vaccine used as the positive control contains the targeted 121 bp NDV genome and Gene Ruler 100 bp DNA Ladder helps estimate the size of the nucleotides separated and detect whether the desired gene sequence was amplified.

Band formations along lanes 1-3 in Fig. 2 indicate that all the three samples are NDV positive. Formation of sharp bands indicates optimal PCR program and materials were applied in this study to produce good quality amplification products. Results show that samples bands formed within the 100-200 bp range, which suggests the presence of the targeted 121 bp NDV gene fragment in all three samples. Pelt-Verkuil *et al*.[20] stated that comparing band intensities of sample bands to a control of known quantity, such as the DNA ladder, helps yield a measure of the amount of transcript in the samples. Hence, difference in band intensities corresponds to the amount of virus present in samples. Difference in sample band thickness may be used to estimate the amount of product relative to the DNA ladder. This has implications for and will be of benefit to breeders as well since it will allow for the breeding value and genetic worth of an individual to be assessed even at an early age with a view to engineering animals that will express desired traits[9].

Fig. 1: Greenish feaces showing a clinical sign of Newcastle disease virus

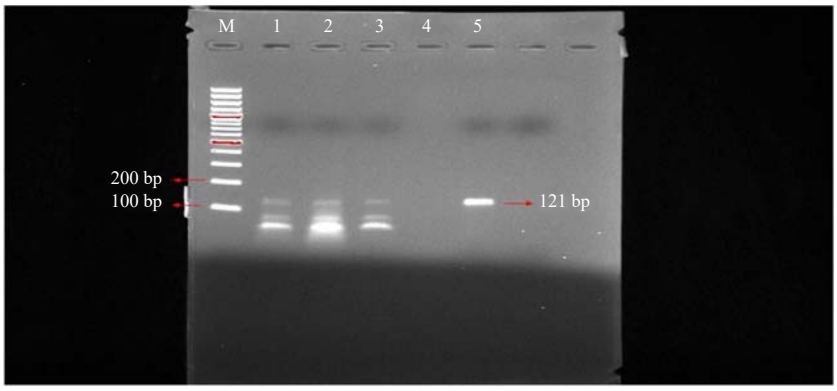

Fig. 2: NDV-specific amplicons visualized by agar gel electrophoresis
Lane M: Molecular weight marker, Lane 4: Negative control, ordinary physiological buffer solution (water), Lane 5: Known Newcastle isolate, Lanes 1-3: Samples, Lane 1, 2, 3 shows the positive samples of NDV with BP (base pair) of 121 bp

Fig. 3: Haemorrhage at the proventriculus

Table 2: Polymerase chain reaction results

No.	Organ sample	Lane	Results
1	Trachea	1	+
2	Proventiculus	2	+
3	Spleen	3	+
4	Positive control	4	+
5	Negative control	5	-

+: Positive, -: Negative

The presence of the gene fragments of NDV is a pointer to the fact that the causative agent with the full compliments of its genome is intact in the affected birds, thus causing the disease in the birds and subsequently their death. Clinical diagnosis according to Abdisa and Tagesu[7] gives conflicting result as it could lead to diagnosing a different disease in error whereas PCR has the propensity, as a molecular technique, for an accurate diagnosis. Therefore, the use of molecular technique in diagnosis will give high accuracy, as was the case in this study, in identifying the virus causing the disease with a view to containing it with respect to treatment and its spread in the flock[8].

CONCLUSION

Clinical diagnosis is prone to error as it could lead to conflicting results, whereas molecular technique (PCR) gave a more accurate diagnosis. It is obvious therefore that its usage will definitely revolutionize disease diagnosis and treatment, it is thus recommended.

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