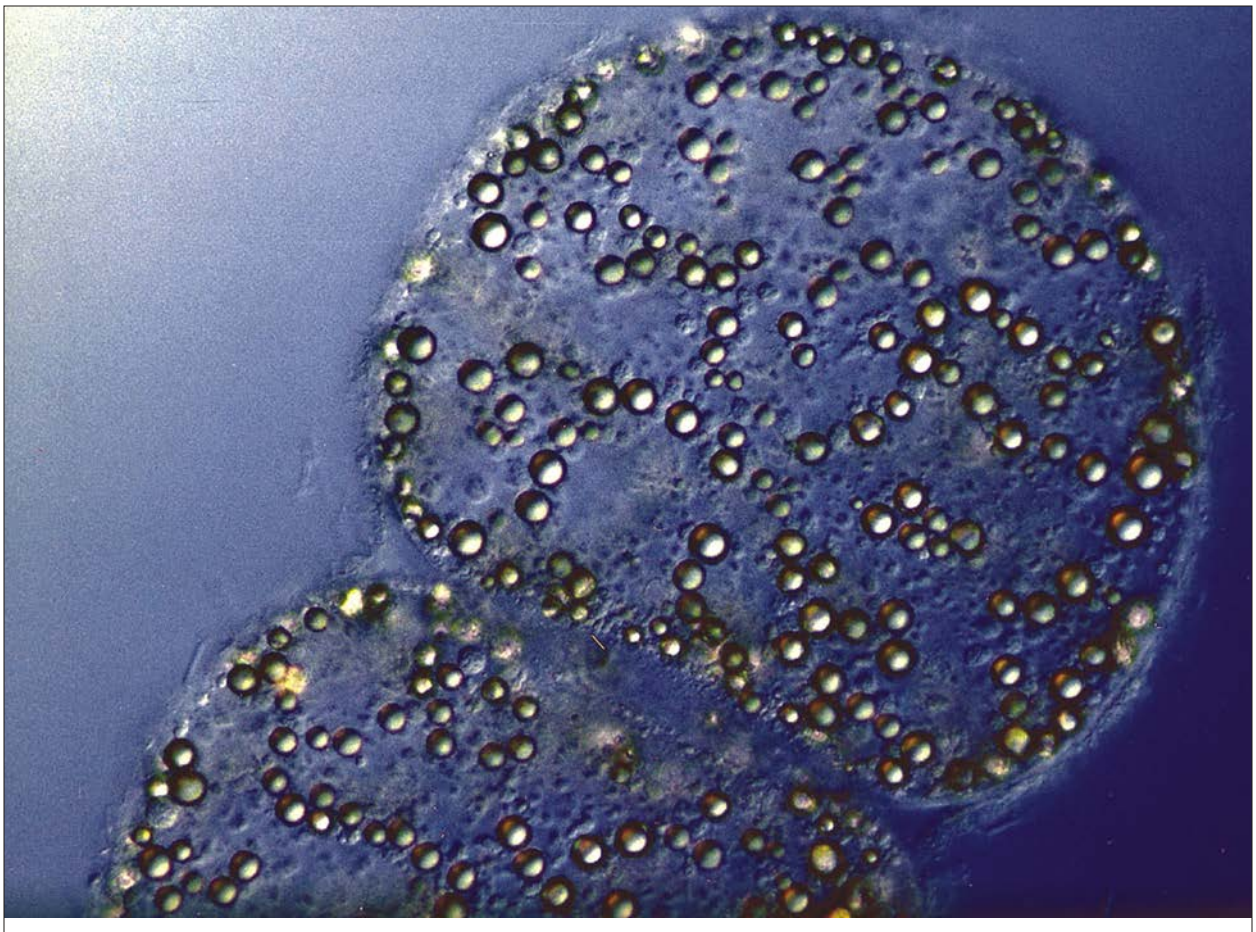

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Research Article
Molecular Detection of Chicken Anemia Virus from Chickens in Yobe South, Nigeria

[1]Ahmed Idriss Jajere and [2]Ahmed Ibrahim Jajere

[1]Department of Biological Sciences, Federal University Gashua, Gashua, Nigeria
[2]Department of Geography, Federal University Gashua, Gashua, Nigeria

Abstract

Background and Objective: Even though chicken anaemia virus (CAV) is most commonly seen in chickens, the virus can also infect other species. In this study, only free-ranging village chickens in Yobe South Senatorial District were investigated for CAV molecular detection. **Materials and Methods:** For this study, 200 samples from free-ranging village chickens were collected from apparently healthy chickens in Yobe South. Blood and thymus samples were used to extract DNA, which was carried out employing a viral nucleic acid with a high purity Roche viral nucleic acid kit (High Pure Viral Nucleic Acid Kit), Basel, Switzerland, according to the manufacturer's specifications instructions. The PCR was conducted to amplify a fragment of 713 bp from the Viral Protein 2 (VP2) gene of CAV. **Results:** The overall isolation of chicken anaemia virus from chickens in Yobe State South from 200 samples collected in the four different Local Government Areas has shown that the isolation rate is 84 (42.0%). The sex distribution of village chickens tested positive for chicken anaemia virus DNA in Yobe South showed that 39 (40.6%) isolates from female chickens, 43 (48.8%) were isolated from male chickens and 2 (12.5%) isolates from undefined chickens. The age group distribution of village chickens tested positive for chicken anaemia virus DNA in Yobe South showed that 26 (31.7%) were isolated from young chickens, 58 (49.2%) were isolated from adult chickens. the distribution of village chickens tested positive for chicken anaemia virus DNA based on sampling locations showed that 37 (74.0%) isolated from chickens in Potiskum Local Government Area, 20 (40.0%) were isolated from chickens in Fune Local Government Area,15 (30.0%) isolates from chickens in Fika Local Government Area and 12 (24.0%) isolates from chickens in Nangere Local Government Area. **Conclusion:** It's therefore, concluded that molecular studies confirmed the presence of CAV in the study area using the PCR and recommended that techniques farmer's needs to be instructed about the signs and importance of CAV and IBDV as the two viruses are similar in signs.

Key words: Chicken anaemia virus, molecular, detection, PCR, Yobe

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Corresponding Author: Ahmed Idriss Jajere, Department of Biological Sciences, Federal University Gashua, Gashua, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gyro virus is a genus of non-enveloped icosahedral viruses with circular single-stranded DNA that infects a wide range of hosts[1]. They also induce a variety of deadly diseases in animals as causative agents virus that causes chicken anaemia CAV. In young chicks, CAV infects many bone marrow-derived cells, causing severe anaemia and immunosuppression. CAV has been shown to compromise immunological responses in elderly birds[2]. CAV infection has grown pandemic among chicken populations worldwide[3]. CAV has a huge financial impact in areas where intensive poultry husbandry is practised. Vaccination is a typical method of preventing the spread of a virus[4]. In a recent investigation, a novel human gyrovirus was discovered in a skin swab and dubbed the human Gyro virus HGyV[5]. Because Circovirus and CAV share a partial similarities, the discovery of HGyV flags potential dangers to human pathophysiology further investigation is yet required[6].

Even though the negative-sense CAV genome has 2,319 nucleotides and is reproduced in a rolling-circle fashion, viral particle packing and egress remain a mystery[1,7]. VP1 is a replicase with dual-specificity phosphatase activity, whereas VP2 is also a replicase with dual-specificity phosphatase activity[8]. VP3, also known as apoptin, is a non-structural protein involved in the triggering of apoptosis and viral cytotoxicity in host cells[8].

An illness that causes no symptoms in older chickens can result in lower growth rates due to a poor feed conversion ratio. furthermore, a high prevalence of 87% was revealed in studies of the virus on live bird markets in Southeast China[3,9].

CAV infects thymic precursor T cells and bone marrow hematopoietic stem cells, causing apoptosis and cell death in these cells[10]. Lowering the development of Red Blood Cells (RBC) and White Blood Cells (WBC) produces severe immunosuppression and anemia[11].

This research reports on the molecular detection of chicken anemia virus from free-ranging village chickens using the polymerase chain reaction PCR, of whole blood, carcasses and thymus samples in Yobe South, Nigeria.

MATERIALS AND METHODS

Study area: The study was carried out in Yobe South Senatorial District, Yobe State, Nigeria from January, 2021 to May, 2021 comprising all the four Local Government Areas (Potiskum, Fune, Fika and Nangere Local Government Areas). Yobe South is located on latitude 10°30"N and longitude 13°10"E. The climate of Yobe South is within a region described as tropical climate which is characterized by tropical dry and wet seasons. The temperature is uniformly high throughout the year except July to August when the clouding of the sky prevents direct isolation[12].

Sample size determination: The sample size was determined by using thrush field sample size calculation formulae[12]:

$$Z = \frac{N}{1 + Ne^2}$$

Where:

N = Population size

E = 0.05 at confidence level of 95%

Thus:

$$N = \frac{200}{1+200\times0.0025} = 133.33$$

$$\text{Approximately} = 200$$

Therefore, two hundred samples from free-ranging village chickens were collected from apparently healthy chickens in Yobe State to increase the precision of the study.

Experimental design: A total of 200 samples were obtained from chickens from four different local government areas of the Yobe South Senatorial District. The samples were collected for a period of 16 weeks from January to May, 2021. The locations for the samples collection sites are designated as follows: Fune Local Government designated as area F, Potiskum as area P, Fika as Area Fi and Nangere as Area N[12].

Sampling: Convenient sampling was conducted based on the availability and willingness of the farmers, where, samples were collected from 50 chickens in four batches from four selected Local Government Areas of Yobe South.

Polymerase chain reaction: For the Polymerase Chain Reaction (PCR), whole blood samples were collected in the heparinized tube and stored in the refrigerator (2-4°C). Carcasses, thymus samples were obtained and stored at -20°C until the experiment. Blood and thymus samples were used to extract DNA, was carried out employing a viral nucleic acid with a high purity roche viral nucleic acid kit (High Pure Viral Nucleic Acid Kit), Basel, Switzerland, according to the manufacturer's specifications instructions.

The PCR was conducted to amplify a fragment of 713 bp from the Viral Protein 2 (VP2) gene of CAV. The sequence of the primers was as follows: forward primer: 5'- GCG CAC ATA CCG GTC GGC AGT-3', reverse primer: 5'-GGG GTT CGG CAG CCT CAC ACT AT-3' [18]. Polymerase chain reaction amplification was performed in PCR buffer containing 1.5 mM $MgCl_2$, 200 µM each deoxynucleotide 5'-triphosphate, 10 pM each primer and 1.0 unit of Taq polymerase (Fermentas, Glen Burnie, MD, USA) in a 25 µL total reaction volume. The amplification was carried out in a thermal cycler (Master cycler Gradient, Eppendorf-Hintz GmbH, Hamburg, Germany) under the following conditions: Initial denaturation of 94°C for 4 min, followed by 34 cycles of denaturation, annealing, extension at 94°C for 1 min, 63°C for 1 min and 72°C for 1 min, respectively and a final extension at 72°C for 5 min. The PCR product was then analyzed by electrophoresis in 1% agarose gel and visualized under UV light after staining with ethidium bromide. In this study, Cuxhaven-1 strains of CAV (Thymovac vaccine, Lohmann Animal Health, Cuxhaven, Germany) were provided and used as positive control while deoxyribonuclease-free water was used as a negative control[12,13].

Data analysis: Data obtained from conventional PCR for CAV DNA detection and haematology in the study were analyzed using (SPSS) version 17 software, Chi-square test was used to perform categorical comparison and determine significance at 95% confidence interval. The $p \leq 0.05$ was considered statistically significant.

RESULTS

Molecular detection of chicken anaemia virus from free-ranging chickens tested PCR positive: The overall isolation of chicken anaemia virus from chickens in Yobe State south from 200 samples collected in the four different Local Government Areas is shown in Table 1 with an isolation rate of 84 (42.0%).

Sex distribution of village chickens tested positive for CAV DNA in Yobe South: Table 2 shows the sex distribution of village chickens tested positive for chicken anaemia virus DNA in Yobe South. The results showed that 39 (40.6%) were isolated from female chickens, 43 (48.8%) were isolated from male chickens and 2 (12.5%) isolates from undefined chickens.

Age distribution of chickens tested positive for CAV DNA in Yobe South, Nigeria: Table 3 show the age group distribution of village chickens tested positive for chicken anaemia virus DNA in Yobe South. The results showed that 26 (31.7%) were isolated from young chickens and 58 (49.2%) were isolated from adult chickens.

Table 1: Percentage of free-ranging village chickens tested PCR positive for CAV in Yobe South, Nigeria

No. of chicken tested	No. of PCR positive (%)
200	84 (42)

Table 2: Sex distribution of village chickens tested positive for CAV DNA in Yobe South

Sex	No. of chicken tested	No. of positive (%)
Male	96	39 (40.6)
Female	88	43 (48.8)
Undefined	16	2 (12.5)
Total	200	84 (42.0)

Not significant p>0.05

Table 3: Age distribution of chickens tested positive for CAV DNA in Yobe South, Nigeria

Age groups	No. of chicken tested	No. of positive (%)
Young	82	26 (31.7)
Adults	118	58 (49.2)
Total	200	84 (42.0)

Significant p<0.05

Table 4: Distribution of chickens tested positive for CAV DNA based on locations

Locations	No. of chicken tested	No. of positive (%)
Potiskum	50	37 (74.0)
Fune	50	20 (40.0)
Fika	50	15 (30.0)
Nangere	50	12 (24.0)
Total	200	84 (42.0)

Significant p<0.05

Distribution of chickens tested positive for CAV DNA based on locations: Table 4 show the distribution of village chickens tested positive for chicken anaemia virus DNA based on sampling locations. The results showed that 37 (74.0%) were isolated from chickens in Potiskum Local Government Area, 20 (40.0%) were isolated from chickens in Fune Local Government Area,15 (30.0%) isolates from chickens in Fika Local Government Area and 12 (24.0%) isolates from chickens in Nangere Local Government Area.

DISCUSSION

This study analyzed the situation of chickens concerning CAV infection. The results show that chickens have a partial high infectivity rate of 42.0% to CAV in Yobe South.This high isolation rate from free-ranging village chickens is in agreement with[2] where all the CIAV-positive samples detected in his similar study in Tunisia were from a rural farm. Molecular studies confirming CAV infection in chicken in Yobe South have not already been provided. However, during the past

decade, we observed some complications in poultry production in Yobe state comprising failure in vaccination programs, high infectivity to some bacterial diseases (e.g., colibacillosis and mycoplasmosis, salmonellosis) followed by high mortality in chickens. The hypothesis was advanced that such complication could be related to immunosuppressive agents e.g., CAV infection. Another study[14] showed that a percentage of apparently healthy chickens 24.6% may be infected with CAV at the slaughtering time[1]. The 42.0% isolation rate in this research is lower than 55.0% of similar research conducted in Southwestern Nigeria[15] and higher than seroprevalence of 36.7% found in Central African Republic[16] and 10% variant virus found in china[17]. The highest prevalence of 87% was revealed in studies of the virus on live bird markets in Southeast China[9,18].

The distribution of village chickens tested positive for chicken anaemia virus DNA based on sampling locations. The results showed that 74.0% were isolated from chickens in Potiskum Local Government Area, 40.0% were isolated from chickens in Fune Local Government Area, 30.0% were isolated from chickens in Fika Local Government Area and 24.0% isolates from chickens in Nangere Local Government Area. These findings agree with another study[13] that CAV exists and that it can no longer be overlooked in the diagnosis of poultry diseases in Nigeria. This may be attributed to fact that Potiskum is the Zonal Local Government and centre where chickens are transported from all the Local Government Areas. The differences in the results could also be due to the differences in the management practice that are used on the farm[12]. The feeds and water available for these birds could be a major source of contamination. The farm attendant and the history of contact with other avian species may also serve as major sources of contamination. These factors, collectively, appear to have contributed to differences in the results as the aforementioned factors could lead to the prevalence of the organism[12].

CONCLUSION

This study may be the first molecular study that confirmed the presence of CAV in the study area using the PCR techniques. The results showed that CAV is prevalent in all four Local Government Areas of Yobe South. The overall percentage of isolates was 42.0%. CAV79 appears not to be identical to all other isolates from this study, this is an indication that CAV79 could be a different strain of CAVs. The obtained result indicates that circulating CAVs in Potiskum shows marked variations. Village chickens provide a rich milieu for the generation of novel genotypes of CAV that may alter the epidemiologic picture of this virus in future.

SIGNIFICANCE STATEMENT

This study could shed light on the genetic feature of CAV that is circulating among free-ranging village chickens in the study area and the data gathered could be used to create diagnoses and preventive measures. Thus, cording to recent research, the role of CAV in other mammals, including humans, has to be investigated further.

REFERENCES

1. Cheung, A.K., 2012. Porcine circovirus: Transcription and DNA replication. Virus Res., 164: 46-53.
2. di Francesco, A., G. Quaglia, D. Salvatore, S. Sakhria and E. Catelli *et al*., 2022. Occurrence of chicken infectious anemia virus in industrial and backyard Tunisian broilers: Preliminary results. Animals, Vol. 12. 10.3390/ani12010062.
3. Eltahir, Y.M., K. Qian, W. Jin, P. Wang and A. Qin, 2011. Molecular epidemiology of chicken anemia virus in commercial farms in China. Virol. J., Vol. 8. 10.1186/1743-422x-8-145.
4. Varela, A.P.M., H.F. dos Santos, S.P. Cibulski, C.M. Scheffer and C. Schmidt *et al*., 2014. Chicken anemia virus and avian gyrovirus 2 as contaminants in poultry vaccines. Biologicals, 42: 346-350.
5. Sauvage, V., J. Cheval, V. Foulongne, M.A. Gouilh and K. Pariente *et al*., 2011. Identification of the first human *Gyrovirus*, a virus related to chicken anemia virus. J. Virol., Vol. 85. 10.1128/JVI.00639-11.
6. Natesan, S., J.M. Kataria, K. Dhama, S. Rahul and N. Baradhwaj, 2006. Biological and molecular characterization of *Chicken anaemia virus* isolates of Indian origin. Virus Res., 118: 78-86.
7. Prasetyo, A.A., T. Kamahora, A. Kuroishi, K. Murakami and S. Hino, 2009. Replication of chicken anemia virus (CAV) requires apoptin and is complemented by VP3 of human torque teno virus (TTV). Virology, 385: 85-92.
8. Peters, M.A., D.C. Jackson, B.S. Crabb and G.F. Browning, 2002. Chicken anemia virus VP2 is a novel dual specificity protein phosphatase. J. Biol. Chem., 277: 39566-39573.
9. Miller, M.M., K.A. Ealey, W.B. Oswald and K.A. Schat, 2003. Detection of chicken anemia virus DNA in embryonal tissues and eggshell membranes. Avian Dis., 47: 662-671.
10. Todd, D., 2000. Circoviruses: Immunosuppressive threats to avian species: A review. Avian Pathol., 29: 373-394.
11. Schat, K.A., 2009. Chicken Anemia Virus. In: Curr. Top. Microbiol. Immunol., Ahmed, R., S. Akira, A. Casadevall, J.E. Galan and A. Garcia-Sastre *et al*., (Eds.), Springer, Berlin, Heidelberg, Pages: 151–183.
12. Jajere, A.I., M. Samuel, A.B. Mustapha and R.A. Mashood, 2020. Phenotypic characterization of *Salmonella enterica* from chickens in some selected local governments of Yobe State. Int. J. Res. Rev., 7: 76-94.

13. Oluwayelu, D.O., D. Todd, N.W. Ball, A.N.J. Scott and O.A. Oladele *et al*., 2005. Isolation and preliminary characterization of chicken anemia virus from chickens in Nigeria. Avian Dis., 49: 446-450.
14. Ducatez, M.F., A.A. Owoade, J.O. Abiola and C.P. Muller, 2006. Molecular epidemiology of chicken anemia virus in Nigeria. Arch. Virol., 151: 97-111.
15. Owoade, A.A., D.O. Oluwayelu, O.A. Fagbohun, W. Ammerlaan, M.N. Mulders and C.P. Muller, 2004. Serologic evidence of chicken infectious anemia in commercial chicken flocks in Southwest Nigeria. Avian Dis., 48: 202-205.
16. Snoeck, C.J., G.F. Komoyo, B.P. Mbee, E. Nakouné, A. Le Faou, M.P. Okwen and C.P. Muller, 2012. Epidemiology of chicken anemia virus in Central African Republic and Cameroon. Virol. J., Vol. 9. 10.1186/1743-422X-9-189.
17. Zhang, X., Y. Liu, J. Ji, F. Chen and B. Sun *et al*., 2014. Identification of a chicken anemia virus variant-related *Gyrovirus* in stray cats in China, 2012. BioMed Res. Int., Vol. 2014. 10.1155/2014/313252.
18. Ducatez, M.F., H. Chen, Y. Guan and C.P. Muller, 2008. Molecular epidemiology of chicken anemia virus (CAV) in Southeastern Chinese live birds markets. Avian Dis., 52: 68-73.