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ORIGINAL ARTICLE

Immunohistochemical and molecular detection of avian neoplastic disease viruses in layer chickens from poultry farms in Northwestern and Northcentral Nigeria

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Abstract

Viral-induced neoplasms in poultry include Marek's disease (MD), avian leukosis (AL), and reticuloendotheliosis (RE). Many times, a presumptive diagnosis can be made based on clinical history and histopathology. However, cases of avian neoplasms due to mixed infection of the viruses are common occurrences in the field, which can only be diagnosed using advanced diagnostic methods, hence the need for this study. A 7 and 10 formalin-fixed neoplastic livers from farms in Northwest (Kaduna) and Northcentral (Plateau) states of Nigeria were screened for the presence of Marek's disease virus (MDV), avian leukosis virus (ALV), and reticuloendotheliosis virus (REV) using immunohistochemistry (IHC) and polymerase chain reaction (PCR). IHC results indicate that all the 17 samples from Kaduna and Plateau States tested negative for ALV-A/B. However, all the 17 samples from the 2 states tested positive for REV. Only 14.3% of the samples from Kaduna State tested positive for MDV1, whereas all the samples from Plateau State tested negative for MDV1. The results of PCR indicate that all the 17 samples from both states tested negative for ALV-A/B/J. However, 57.1 and 50% of the samples from Kaduna and Plateau States, respectively, tested positive for REV, whereas 71.4 and 70% of the samples from Kaduna and Plateau States respectively, tested positive for MDV1. Although, immunohistochemistry and PCR did not offer complete agreement, it is clear that MDV and REV infections are endemic in the study areas. Therefore, a national program for the control and eradication of avian neoplastic disease viruses is recommended.

Keywords Avian leukosis · Immunohistochemistry · Marek's disease · Reticuloendotheliosis · PCR

Introduction

The virus-induced neoplasms of poultry include AL, RE, and MD (Payne and Venugopal 2000). Marek's disease virus is classified under the genus *Mardivirus,* comprising five species which include: *Anatid alphaherpes virus* 1, *Columbid alphaherpes virus* 1, *Gallid alphaherpes virus* 2 (MDV serotype 1 or MDV-1), *Gallid alphaherpes virus* 3 (MDV serotype 2 or MDV-2), and *Meleagrid alphaherpes virus* 1 (MDV serotype 3 or turkey herpesvirus) (ICTV 2018).

The pathogenic *Gallid alphaherpes virus* 2 or MDV-1, responsible for tumor development in tissues of infected birds, is further classified into four pathotypes: mild MDV (mMDV) which mainly causes neuropathy and is preventable with the turkey herpesvirus (HVT) vaccine; virulent MDV (vMDV) which is responsible for both neural and visceral lymphomas and also preventable by HVT vaccine;

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very virulent MDV (vvMDV) responsible for the high incidence of visceral lymphomas, and the very virulent plus MDV (vv + MDV) which causes lymphoma with very high mortality; both vvMDV and vv + MDV are preventable by vaccinating susceptible birds with a bivalent vaccine (MDV-2 + MDV-3) (Payne and Venugopal 2000). The *Gallid alphaherpes virus* 3 or MDV-2 is not pathogenic but often isolated from chickens, while the *Meleagrid alphaherpes virus* or MDV-3 is isolated from turkeys (Calnek and Witter 1997; Witter et al. 2005).

The DNA of MDV is a linear, double stranded molecule of about 170 kb in size with a unique long region flanked by a set of inverted repeat regions and a unique short region, flanked by another set of inverted repeat regions (Ross 1999). The oncogenic properties of MDV-1 are conferred by the *Meq* gene, *pp38* gene, and the *vIL*-8 (Tian et al. 2011).

ALV and REV are both retroviruses belonging to the subfamily *Orthoretrovirinae* of the family *Retroviridae* and order *Ortervirales*, however, ALV belongs to the genus *Alpharetrovirus*, the REV belongs to the genus *Gammaretrovirus* (ICTV 2018). The genome of oncornaviruses is a single-stranded RNA and replication requires the production of a DNA intermediate from the viral RNA template using the viral enzyme reverse transcriptase (RT) encoded by the pol gene region in the viral genome (Bagust et al. 2004).

Avian leukosis virus in chickens is classified into 7 subgroups, designated A, B, C, D, E, J, and K, based on envelope glycoprotein, viral interference patterns, and host range (Payne et al. 1991; Silva et al. 2000; Zhao et al. 2018). Subgroups A, B, C, D, J, and K are exogenous ALVs and cause neoplasms in poultry, while subgroup E is an endogenous and non-oncogenic ALV (Payne 1992). The ALV subgroups A, B, C, and D are known to cause lymphoid leukosis (LL) majorly, while subgroups J and K are responsible predominantly for myeloid type tumors and hemangioma (Xu et al. 2004; Gao et al. 2010; Wu et al. 2010). Subgroups A, B, and J are the most prevalent of the ALVs (Lupiani et al. 2006; Dai et al. 2015), causing neoplasms in chickens, occurring as single infections (Xu et al. 2004; Fenton et al. 2005; Wang et al. 2013) or as multiple infections (Fenton et al. 2005; Zhao et al. 2012; Li et al. 2013; Dai et al. 2015).

Available literature indicates that there is an increase in outbreaks of avian neoplastic diseases in Kaduna and Plateau States, Nigeria (Wakawa et al. 2012; Jwander et al. 2013; Musa et al. 2013; Sani et al. 2017) and most of the outbreaks have been attributed to MD despite vaccination of commercial chickens. However, Jwander et al. (2012) detected Marek's disease virus (MDV) in only 50% of cases tentatively diagnosed as MD. This suggests that other causes of neoplasms in chickens, such as ALV and REV, may be involved. Although microscopic examination of neoplastic tissues can be helpful in differentiating MD from AL and RE, the problem is identifying outbreaks of avian neoplastic diseases due to mixed infection of the oncogenic viruses. Except for MDV that has been detected in tissues by PCR, other viruses responsible for avian neoplasms have not been identified by PCR and immunohistochemistry in the Northwestern and Northcentral region of Nigeria. The aim of this research was therefore to detect avian neoplastic disease viruses in formalin-fixed neoplastic livers from some layer birds in poultry farms in Northwestern and Northcentral States of Nigeria.

Materials and methods

Study area

The study was carried out in the Northwestern and Northcentral regions of Nigeria. Kaduna State in the Northwest and Plateau State in the Northcentral region were selected by convenient sampling because these states are the hive of poultry production activities in the whole of Northern Nigeria. Inhabitants of these states engage in small to large-scale livestock farming (Emaikwu et al. 2011). Kaduna State lies between latitude 9 and 12° N and longitude 6° E and 9° E., and has a population of more than 8 million, has 23 local government areas (LGAs), and occupies a land mass of 46,053 square kilometers (Emaikwu et al. 2011; Madichie and Madichie 2016; Umar et al. 2019). Plateau State is situated in the Northcentral of Nigeria. It is found between the location, latitude 10°06′12.048″N and longitude of 80°49′19.507″E. Although it is situated in the tropical rainforest zone, it has a higher altitude and a near temperate climate with an average temperature between 13 and 22 °C (Awala et al. 2019). Plateau State has 17 LGAs and occupies a total land mass of 27,147 Km^2 with a human population of 4,433,501 (NIPC 2019).

Experimental design

Seven and ten neoplastic liver samples of layers from farms in Kaduna and Plateau States, respectively, were fixed in 10% neutral buffered formalin and used for the detection of MDV, ALVs, and REV, by immunohistochemistry and polymerase chain reaction. The 7 formalin-fixed neoplastic livers from Kaduna State were labeled ZL1 to ZL7, while those from the 10-layer farms in Plateau State were labeled JL1 to JL10. The 7 cases in Kaduna State were submitted to the Poultry Clinic of the Veterinary Teaching Hospital (VTH), Ahmadu Bello University, Zaria, while the 10 cases in Plateau State were submitted to the ECWA Veterinary Clinic, Bukuru, for routine postmortem examinations.

The ethics governing the use and conduct of experiments on animals were strictly observed, and the experimental protocol was approved by the University of Abuja Ethics

Committee on Animal Use (UAECAU), with the assigned number UAECAU/2017/0020. Proper permit and consent were obtained from the management of all the farms visited before the neoplastic samples were collected and used for this experiment.

Immunohistochemistry

An immunohistochemical study of the neoplastic liver specimens from carcasses of layers in Kaduna and Plateau States was carried out at the Veterinary Diagnostic Laboratory, Michigan State University, East Lansing, Michigan, USA, as described by Ahmed et al. (2018).

The formalin-fixed liver specimens were embedded in paraffin and immunohistochemical labeling on the tissue sections was performed using polyclonal antibodies targeted at the *Meq* gene of MDV1, monoclonal antibodies targeted at the *p27* gene and *gp62* gene of ALV-A/B, and REV, respectively. The antibodies specific for MDV1, ALV-A/B, and REV were sourced from the Avian Disease and Oncology Laboratory (ADOL), US National Poultry Research Center, US Department of Agriculture, Agricultural Research Service, and East Lansing, Michigan, USA. Immunohistochemical labeling was done using the Dako Immunostainer 48 Automated Staining System and the Flex Detection Kit. All antibodies were applied at a concentration of 1:1000 for 32 min. Antigen retrieval was achieved using the Dako PT Link system with citric buffer 95 °C heat retrieval for 20 min. This was followed by a post-secondary antibody incubation of Dako anti-mouse antibody for 20 min. Fresh diaminobenzidine (Dako, Inc.) was applied for 5 min. Tissue sections were counterstained with hematoxylin. As for the negative controls, the primary antibodies were replaced with buffer. Tissue sections were examined under the microscope for labeling of cells by antibodies. Microphotographs of the tissues that were positive were taken, transferred to a laptop computer and labeled appropriately.

Polymerase chain reaction

The polymerase chain reaction (PCR) was carried out using specific primers to detect MDV, ALV subgroups A, B, J, and REV, at the ADOL US National Poultry Research Center, US Department of Agriculture, Agricultural Research Service, and East Lansing, Michigan, USA, as described by Ahmed et al. (2018).

The paraffin-embedded tissue blocks were sectioned into five scrolls of 5 µ each, making a total of 25 µ per tissue block. The tissues were excised from the scrolls and placed in polymerase buffer (20 mM Tris–HCl (pH 8.8), 10 mM KCl, 2 mM $MgSO_4$, 10 mM NH_4SO_4, 0.1% Triton X-100, and 1 mg/ml Bovine Serum Albumin), with 100 µg of Proteinase K and incubated overnight at 65 °C. The tissues were centrifuged at 2000 g for 10 min to pellet the paraffin. The DNA containing supernatant was then poured into a sterile microfuge tube and used for PCR analysis.

The PCR conditions involved the following: 95 °C for 3 min; 30 cycles at 95 °C for 1 min; 57 °C for 30 s; 72° for 30 s and final extension at 72 °C for 5 min. The PCR products were separated on 1.5% agarose gels and visualized with ethidium bromide using the AlphaImager™ gel documentation system. Each PCR contained positive and negative DNA controls, and all samples were analyzed for DNA quality with primers specific to the chicken glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

The 10 liver samples from Plateau State, JL1, JL2, JL3, JL4, JL5, JL6, JL7, JL8, JL9, and JL10, were tagged as samples 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, respectively, whereas those from Kaduna State, ZL1, ZL2, ZL3, ZL4, ZL5, ZL6, and ZL7 were tagged as samples 11, 12, 13, 14, 15, 16, and 17, respectively.

Data analyses

The number of samples positive for MDV, ALV subgroups A, B, and J, as well as REV by immunohistochemistry and PCR methods in the Nigerian Northwestern and Northcentral states of Kaduna and Plateau States, respectively were summarized into percentages and presented in tables.

Results

History of the cases

In the Northwestern Kaduna State of Nigeria, the commercial layer chickens had an age range of 12 to 31 weeks, with an average of 20.6 weeks. The layers in 4 (ZL1, ZL2, ZL3, and ZL7) of the 7 farms were reportedly vaccinated against MD. The commercial layer chickens from the northcentral state of Plateau had an age range of 11 to 39 weeks, with an average of 22.4 weeks. Vaccination history against MD could not be ascertained in chickens from 3 (JL2, JL5, and JL10) of the 10 farms in the northcentral state of Plateau. The clinical signs observed in most cases included anorexia, emaciation, ruffled feathers, somnolence, and shriveled combs and wattles. Other details of the affected flocks relating to flock size, morbidity, and mortality are published by Sani et al. (2021).

Detection of neoplastic disease viruses in formalin-fixed neoplastic liver samples from layers in Kaduna State using immunohistochemistry

The results of immunohistochemical labeling of *p27* gene of ALV-A/B, *gp62* gene of REV, and *Meq* gene of MDV1

Table 1 Detection of neoplastic disease viruses in formalin fixed neoplastic liver samples from layers in Kaduna State using immunohistochemistry and PCR

Sample	ALV-A/B		REV		MDV1	
	Immuno	PCR	Immuno	PCR	Immuno	PCR
ZL1	Neg	Neg	Pos	Neg	Neg	Pos
ZL2	Neg	Neg	Pos	Neg	Neg	Pos
ZL3	Neg	Neg	Pos	Pos	Neg	Pos
ZL4	Neg	Neg	Pos	Pos	Neg	Pos
ZL5	Neg	Neg	Pos	Neg	Pos	Neg
ZL6	Neg	Neg	Pos	Pos	Neg	Neg
ZL7	Neg	Neg	Pos	Pos	Neg	Pos
Summary (%)	0/7 (0.0%)	0/7 (0.0%)	7/7 (100.0%)	4/7 (57.1%)	1/7 (14.3%)	5/7 (71.4%)

ALV-A/B/J avian leukosis virus subgroups A, B, and J, *REV* reticuloendotheliosis virus, *MDV1* Marek's disease virus serotype 1, *Immuno* immunohistochemistry, *PCR* polymerase chain reaction, *Neg* negative, *Pos* positive

in formalin-fixed neoplastic liver samples (ZL1, ZL2, ZL3, ZL4, ZL5, ZL6, and ZL7) from seven chicken farms in the Northwestern Kaduna State of Nigeria were summarized in Table 1. For ALV-A/B, labeling of *p27* gene was not observed in all the seven formalin-fixed neoplastic liver samples. For REV, labeling of *gp62* gene was observed in all the seven formalin-fixed neoplastic liver samples (Fig. 1A). For MDV1, labeling of *Meq* gene was observed in one (ZL5) (Fig. 1B) of the seven-formalin fixed neoplastic liver sample. Labeling of *Meq* gene of MDV1 was not observed in the other six formalin-fixed liver samples (Table 1).

Detection of neoplastic disease viruses in formalin-fixed neoplastic liver samples from layers in Plateau State using immunohistochemistry

The results of immunohistochemical labeling of the *p27* gene of ALV-A/B, *gp62* gene of REV, and *Meq* gene of MDV1 in formalin-fixed neoplastic liver samples (JL1, JL2, JL3, JL4, JL5, JL6, JL7, JL8, JL9 and JL10) from 10 chicken farms in the Northcentral state of Plateau were summarized in Table 2.

For ALV-A/B, labeling of the *p27* gene was not observed in all the 10 formalin-fixed neoplastic liver samples. For REV, labeling of the *gp62* gene was observed in all the 10 formalin-fixed neoplastic liver samples. For MDV1, labeling of *Meq* gene was not observed in any of the 10 formalin-fixed neoplastic liver samples (Table 2).

Detection of neoplastic disease viruses in formalin-fixed neoplastic liver samples from layers in Kaduna State using polymerase chain reaction

The results of detection of ALV-A, ALV-B, ALV-J, REV, and MDV1 in formalin-fixed neoplastic liver samples (ZL1, ZL2, ZL3, ZL4, ZL5, ZL6, and ZL7) from Kaduna State

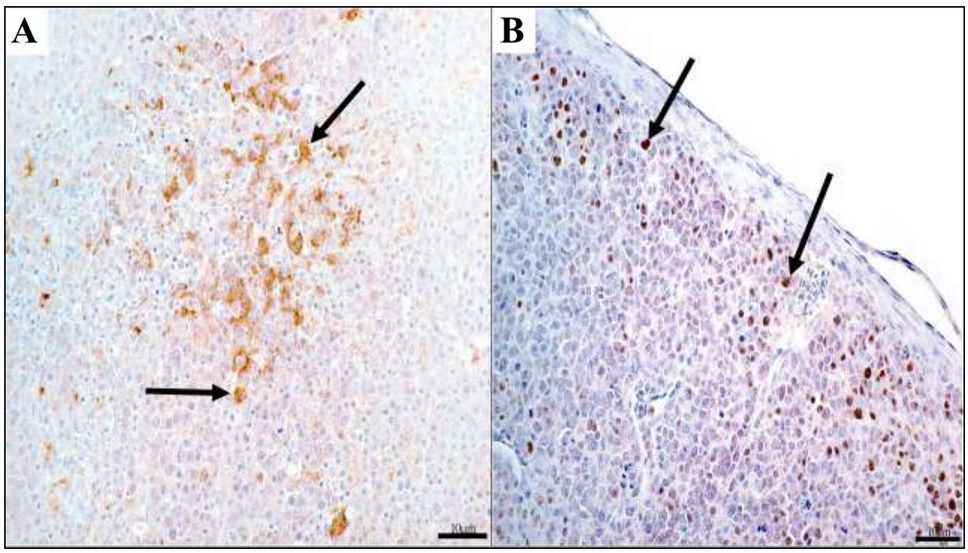

Fig. 1 Microphotograph of a section of formalin-fixed neoplastic liver of a layer (ZL5) from a farm with the neoplastic disease in Northwestern State of Kaduna, Nigeria. **A** Note the cytoplasmic labeling of the tumor cells (arrows), labeled with 11B118 (REV). **B** Note the characteristic intranuclear labeling of the tumor cells (arrows), labeled with Meq (MDV1). Size of bar: 10 µm

Table 2 Detection of neoplastic disease viruses in formalin fixed neoplastic liver samples from layers in Plateau State using immunohistochemistry and PCR

Sample	ALV-A/B/J		REV		MDV1	
	Immuno	PCR	Immuno	PCR	Immuno	PCR
JL1	Neg	Neg	Pos	Pos	Neg	Pos
JL2	Neg	Neg	Pos	Neg	Neg	Pos
JL3	Neg	Neg	Pos	Pos	Neg	Pos
JL4	Neg	Neg	Pos	Pos	Neg	Pos
JL5	Neg	Neg	Pos	Neg	Neg	Pos
JL6	Neg	Neg	Pos	Neg	Neg	Neg
JL7	Neg	Neg	Pos	Pos	Neg	Pos
JL8	Neg	Neg	Pos	Neg	Neg	Neg
JL9	Neg	Neg	Pos	Neg	Neg	Pos
JL10	Neg	Neg	Pos	Pos	Neg	Neg
Summary (%)	0/10 (0%)	0/10 (0%)	10/10 (100%)	5/10 (50%)	0/10 (0%)	7/10 (70%)

ALV-A/B/J avian leukosis virus subgroups A, B, and J, *REV* reticuloendotheliosis virus, *MDV1* Marek's disease virus serotype 1, *Immuno* immunohistochemistry, *PCR* polymerase chain reaction, *Neg* negative, *Pos* positive

were summarized in Table 1. No PCR product was amplified using ALV subgroups A, B, and J specific primers in all the seven formalin-fixed neoplastic liver samples. For REV, PCR product was amplified using SNV LTR-specific primers in four (ZL3, ZL4, ZL6, and ZL7) of the seven formalin-fixed neoplastic liver samples. No PCR product was amplified using SNV LTR specific primers in three (ZL1, ZL2, and ZL5) of the seven formalin-fixed neoplastic liver samples (Fig. 2A). For MDV1, PCR product was amplified using MDV gB-specific primers in five (ZL1, ZL2, ZL3, ZL4, and ZL7) of the seven formalin-fixed neoplastic liver samples (Fig. 2B). No PCR product was amplified using MDV gB-specific primers in two (ZL5 and ZL6) of the seven formalin-fixed neoplastic liver samples (Table 1).

Detection of neoplastic disease viruses in formalin-fixed neoplastic liver samples from layers in Plateau State using a polymerase chain reaction

The results of detection of ALV-A/B/J, REV, and MDV1 in formalin-fixed neoplastic liver samples (JL1, JL2, JL3, JL4, JL5, JL6, JL7, JL8, JL9, and JL10) by PCR from 10 chicken farms in Plateau State were summarized in Table 2. No PCR product was amplified using ALV subgroups A, B, and J specific primers in all the 10 formalin-fixed neoplastic liver samples from Plateau State (Fig. 3A−C). For REV, PCR product was amplified using SNV LTR-specific primers in five (JL1, JL3, JL4, JL7, and JL10) of the 10 formalin-fixed neoplastic liver samples. No PCR product was amplified using SNV LTR-specific primers in five (JL2, JL5, JL6, JL8, and JL9) of the 10 formalin-fixed neoplastic liver samples. For MDV1, PCR product was amplified using MDV gB-specific primers in seven (JL1, JL2, JL3, JL4, JL5, JL7, and JL9) of the 10 formalin-fixed neoplastic liver samples. No PCR product was amplified using MDV gB-specific primers in three (JL6, JL8, and JL10) of the 10 formalin-fixed neoplastic liver samples (Table 2).

Discussion

Although IHC and PCR techniques have been applied in the differential detection of avian neoplastic disease viruses in formalin-fixed paraffin-embedded tissues (Cheng et al. 2010; Wang et al. 2013; Ahmed et al. 2018), the successful application of these techniques in field cases of avian neoplasms as well as the effect of prolonged preservation of tissue samples in formalin on accurate detection of antigens using immunohistochemistry and PCR techniques are still undergoing evaluation.

There was no labeling of the *p27* antigen in all the tested formalin-fixed neoplastic liver samples from layer farms in Kaduna and Plateau States using immunohistochemistry and PCR methods. It is believed that birds that acquire infection by contact after hatching are most likely to develop immunity to infection, are likely to constitute the majority of a flock, and are less likely than viraemic birds to develop neoplasms (Payne and Venugopal 2000). Tolerant viraemic chickens express a high level of antigen in tissues, while the non-viraemic chickens hardly express the antigens in their tissues (Pandiri et al. 2008). Viraemia, antibody production, and shedding of ALV are dependent on how early the chicken was infected (Mays et al. 2006).

There was the labeling of the *gp62* gene in all the formalin-fixed neoplastic livers from all the layer farms screened in Kaduna and Plateau States. This finding suggests that REV infection may be endemic and widespread in layer flocks in Kaduna and Plateau States. The reasons for this widespread infection of chickens with REV are unknown. Recently, Shittu et al. (2018) reported that some vaccines imported into Nigeria for use in the prevention

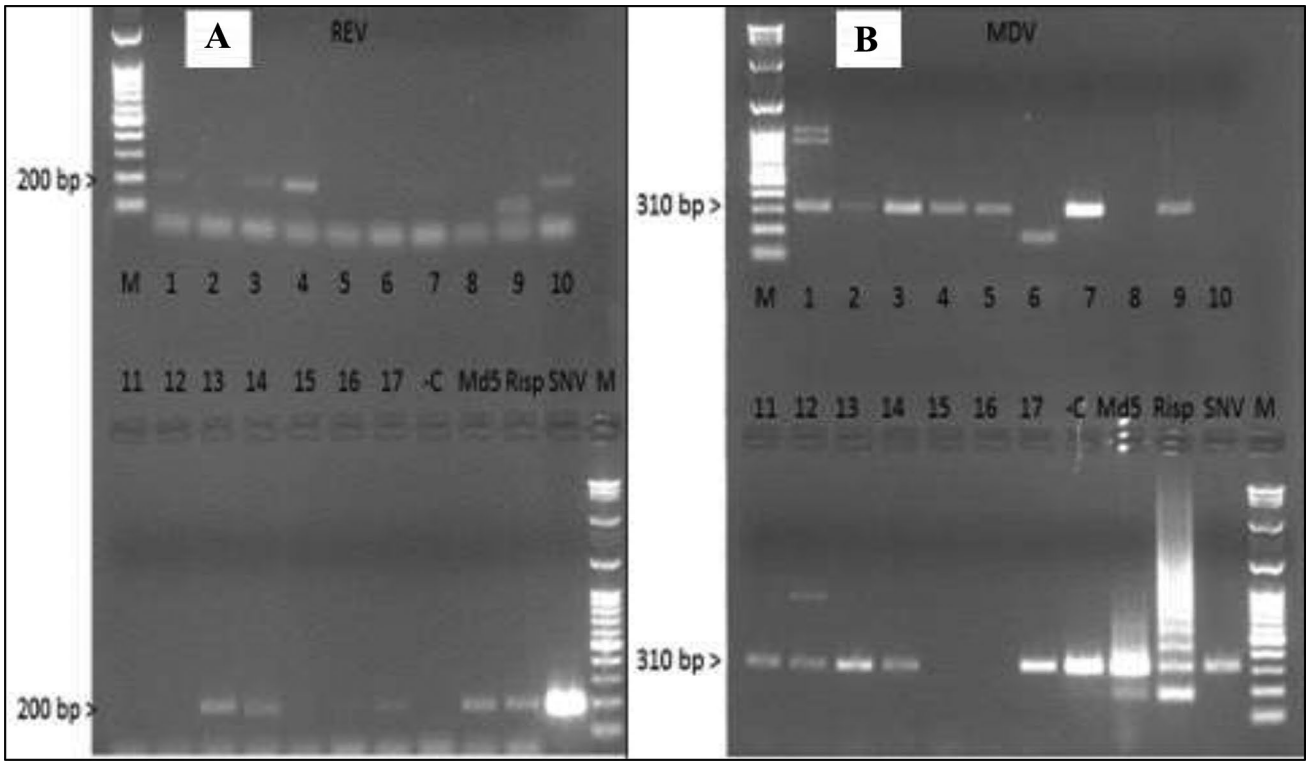

Fig. 2 **A** Amplification of 200 bp of the SNV LTR of REV in 1, 3, 4, 7, 10, 13, 14, 16, and 17 from samples JL1, JL3, JL4, JL7, JL10, ZL3, ZL4, ZL6, and ZL7, respectively. **B** Amplification of 310 bp of the gB gene of MDV1 in 1, 2, 3, 4, 5, 7, 9, 11, 12, 13, 14, and 17 from samples JL1, JL2, JL3, JL4, JL5, JL7, JL9, ZL1, ZL2, ZL3, ZL4, and ZL7, respectively

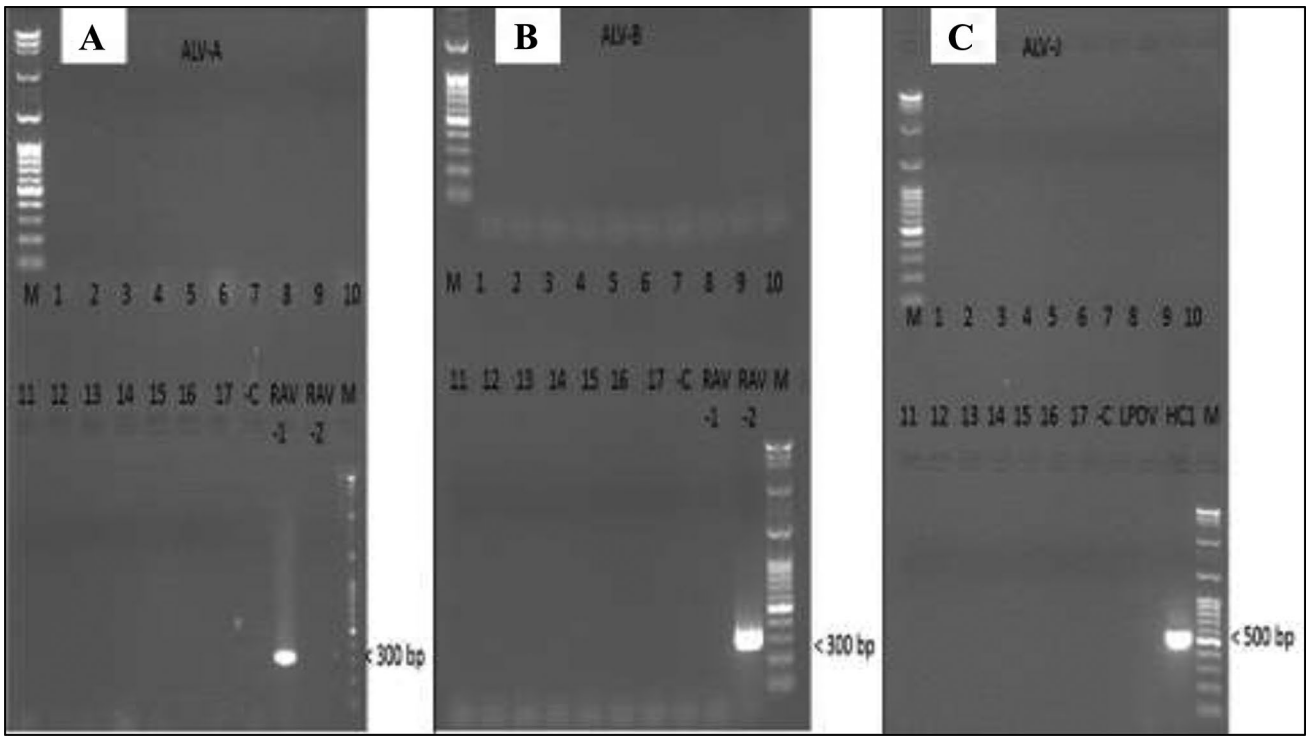

Fig. 3 **A** No amplification of 300 bp of RAV-1 gene of ALV-A in all the samples tested. **B** No amplification of 300 bp of RAV-2 gene of ALV-B in all the samples tested. **C** No amplification of 500 bp of HC 1 gene of ALV-J in all the samples tested

of diseases in poultry were found to be contaminated with ALV subgroup J. However, the vaccines were not screened for possible contamination with REV.

Only 14.3% (1/7) of all the formalin-fixed liver samples from Kaduna State tested positive for both REV and MDV1 using immunohistochemistry, while no sample from Plateau State tested positive for both REV and MDV1. However, 42.9% (3/7) of samples tested from Kaduna State were positive for both REV and MDV1 using the PCR method. Also, 40% (4/10) of the samples from Plateau State tested positive for both REV and MDV1 using the PCR method. This is in line with previous reports that multiple infections with two or more avian neoplastic disease viruses are common in affected birds (Mitra et al. 2013; Gopal et al. 2012). It is believed that co-infection of REV with MDV in chicken increases the severity of disease outcomes, including high mortality and increase tumor development, especially in flocks that were not vaccinated against MD (Chacón et al. 2019; Thontiravong et al. 2019; Sun et al. 2017). Co-infection with avian neoplastic disease viruses has also been reported to cause poor vaccine response and increased susceptibility to secondary bacterial infection in infected chickens (Zeng et al. 2015). In an experiment by Cui et al. (2009), co-infection with REV and ALV subgroup J in chickens delayed the production of antibodies against ALV subgroup J for up to 7 weeks. Sun and Cui (2007) reported that immunosuppression induced by REV infection could last for at least 4 months and dramatically decreased the protective efficacy of an inactivated vaccine against H5N1 highly pathogenic avian influenza. Co-infection of chickens with REV and MDV significantly reduced the protective efficacy of MD vaccines compared to chickens infected with MDV alone (Sun et al. 2017). This may be one of the reasons why outbreaks of MD were observed in flocks vaccinated against MD in this study. It is also suggested that co-infection with REV and MDV in chicken might increase disease severity by increasing the expression of pathogenic genes. Four MDV pathogenic genes, *Meq, pp38, vIL-8,* and *ICP4,* were upregulated significantly in the MDV and REV co-challenged group, compared to the group that was challenged with MDV only (Sun et al. 2017). The detection of AND viruses in tissues by PCR is not confirmation that the viruses were responsible for the tumors. However, IHC determines if a virus is being expressed in tumor cells and therefore provides more diagnostic value for the cause of the tumor.

To the best of our knowledge, this is the first report on the detection of MDV and REV in formalin-fixed neoplastic livers from layers by immunohistochemistry and PCR in Nigeria. Further study to determine the strains of avian neoplastic disease viruses, especially MDV, that are endemic in Kaduna and Plateau States, Nigeria, is recommended. A national program for the control and eradication of avian neoplastic disease viruses is also recommended.

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Author contribution Oladele, S.B., Abdu, P.A., Njoku C.O.I., and Nuhu, N.A conceived the idea, Nuhu, N.A and Dunn J.R carried out the research, Nuhu, N.A wrote the original draft, while I.C.I. Ugochukwu., S.E. Abalaka., Saleh, A., and S.I. Idoko wrote, edited, and reviewed the manuscript. All authors read and approved the manuscript for submission.

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Declarations

Ethics approval The ethics governing the use and conduct of experiments on animals were strictly observed and the experimental protocol was approved by the Ahmadu Bello University, Zaria Senate committee on Medical Research ethics. Proper permit and consent were obtained from the management of all the farms visited, before the neoplastic samples were collected and used for this experiment.

Conflict of interest The authors declare no competing interests.

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