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A report on endogenous avian leucosis virus in bantam chicken

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Abstract
In this study, a bantam chicken presented with a history of sudden death was investigated for avian pathogens by post-mortem examination and molecular diagnostic techniques. Gross pathology revealed mild hepatomegaly, suggestive of ALV or MD. PCR testing ruled out MD and the J subgroup of ALV as negative, but detected a positive amplicon for ALV subgroups A-E. Subsequent subgroup-specific PCR confirmed the presence of ALV Subgroup E. Histopathological examination revealed no lymphoid cells infiltration, indicating the presence of non-infectious endogenous ALV E. These findings underscore the importance of monitoring ALV in bantam chickens to manage its prevalence and support the breeding of ALV-free flocks.

Keywords: ALV Subgroup E, Avian lymphoid leucosis (ALV), Bantam chickens, Endogenous ALV

1. Introduction
Any small breeds of chickens in general are called as bantam chicken. The word bantam indeed originates from the name of the seaport city of Bantam in western Java, Indonesia. European sailors encountered small breeds of chickens in Southeast Asia while restocking supplies for their journeys. These chickens, due to their size and hardiness, became known as bantams, and over time, the term "bantam" came to refer to small breeds of chickens.
Globally, viral neoplastic diseases are a major economic problem for the poultry industry (Mitra *et al.,* 2013) [6]. The three diseases that produce neoplasms in chicken and are of economic importance are Reticuloendotheliosis (REV), Marek's disease (MD), and Avian leucosis (Biggs *et al.,* 1978) [1] all of which share a retroviral etiology. Avian leucosis virus (ALV) comes under the Alpharetrovirus genus and are divided into subgroups A, B, C, D, E, J, and K based on the differences in their envelope glycoprotein (Payne, 1992) [7] The traditional method for differentiating avian oncogenic viruses relies on isolating the virus and examining tumor tissues histopathologically. However, diagnosis based on virus isolation is labourious and time-consuming, and it can be additionally complicated by concurrent viral infections. Histopathological diagnosis may have the capability to differentiate between MDV and ALV-J. However, distinguishing between lymphoid tumors induced by various viruses can often be challenging due to similarities in their lesions. PCR emerges as the preferred method in diagnosing avian oncogenic viruses due to its ability to address numerous challenges encountered in the differential diagnosis. Moreover, PCR enables the detection of multiple viral infections simultaneously (Davidson *et al.,* 2009) [4]. Though ALV is reported in other species, the natural host is Chickens. This report discusses the infection of ALV in bantam chicken and subgroup specific PCR techniques for the identification of exogenous or endogenous origin of ALV.

2. Materials and Methods
A bantam chicken was presented for post-mortem examination in our laboratory with a history of sudden death. Post-mortem examination was carried out as per standard protocol (King *et al.,* 2014) [5] and tissues were collected from liver, spleen, lung, kidney, brain, proventriculus, sciatic nerve, and bursa to rule out viral diseases such as ALV and MD.

Ten percent tissue homogenate was prepared from the organs collected and DNA was extracted by phenol chloroform method. PCR was performed using the extracted DNA to diagnose MD and ALV in this bantam chicken.

2.1. Diagnosis of ALV by PCR

The published sequences (Smith *et al.*, 1998) [12] provided the source of the H5, AD1, and H7 oligonucleotide primers employed in the present case to diagnose ALV (Table 1). The primer pair of H5 and AD1 was used to rule out the subgroups A to E of ALV with an expected amplicon of 295-326bp. Similarly, H5 and H7 primers were used to diagnose only the J subgroup of ALV with a 545bp size amplicon.

The following parameters were used for the PCR amplification: an initial denaturation at 94 ℃ for 3 minutes, followed by 37 cycles comprising of denaturation at 94 ℃ for 30 seconds, primer annealing at 50 ℃ for 30 seconds, and extension at 72 ℃ for 45 seconds. A final extension step at 72 ℃ for 10 minutes was performed as per Ramya *et al*., 2024 [8]. PCR products were analyzed using 1.5% agarose gel. The samples showed positive amplification for H5 and AD1 primers were further analyzed by ALV subgroup-specific PCR for ALV-A, B/D, C, and E using the primers listed in Table 1 as described by Silva *et al.*, (2007) [10].

Table 1: Primers used to diagnose ALV in this study

Primer	Sequence (5'-3')	Product size	Reference
ALV - A to E	H5: GGATGAGGTGACTAAGAAAG AD1: GGGAGGTGGCTGACTGTGT	295-326 bp	Smith *et al.*, 1998 [12]
ALV- J	H5: GGATGAGGTGACTAAGAAAG H7: CGAACCAAAGGTAACACACG	545 bp	Smith *et al.*, 1998 [12]
ALV-A	Fp:5'CGAGAGTGGCTCGCGAGATGG3' Rp:5'CCCATTTGCCTCCTCTCCTTGTA3'	1.3kbp	Silva *et al.*, 2007 [10]
ALV-B and D	Fp:5'CGAGAGTGGCTCGCGAGATGG3' Rp:5'AGCCGGACTATCGTATGGGGTAA3'	1.1 Kbp	Silva *et al.*, 2007 [10]
ALV –C	Fp:5'CGAGAGTGGCTCGCGAGATGG3' Rp:5'CCCATATACCTCCTTTTCCTCTG3'	1.5kbp	Silva *et al.*, 2007 [10]
ALV –E	Fp:5'CGAGAGTGGCTCGCGAGATGG3' Rp:5'GGCCCCACCCGTAGACACCACTT3'	1.25kbp	Silva *et al.*, 2007 [10]

2.2. Diagnosis of Marek's Disease by PCR

To diagnose MD in this bantam chicken, the extracted DNA was also subjected to PCR using MDV1-specific meq gene-based primers as per Chang *et al.*, (2002) [2].

2.3. Histopathology

The tissues collected were fixed in 10% neutral buffered formalin, embedded in paraffin, sections were cut into 4-6 µ thickness and stained with Haematoxylin and Eosin (H&E) as per Suvarna *et al.*, 2018[14].

3. Results and Discussion

Post mortem examination of liver showed mild enlargement, rounding of borders, grey brown appearance with multifocal petechial hemorrhages. Spleen was mildly enlarged and mottled and no tumor lesions were observed (Fig - 1).

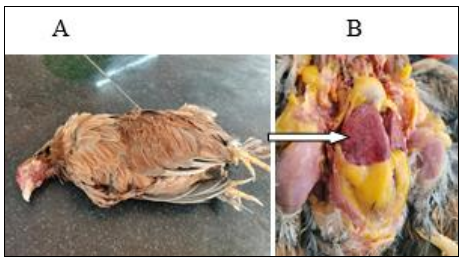

Fig 1: A- Carcass fair in body condition, B- Liver showing mild enlargement, rounding of borders, grey-brown appearance with multifocal petechial haemorrhages

The gross pathological lesions reported earlier for birds suspected of ALV and MD are soft, friable, grayish-white livers with widespread enlargement. However, hepatomegaly without the presence of a tumor is also observed in Avian lymphoid leucosis (ALV) in chickens, as reported by Soujanya *et al.*, 2019 [13]. Therefore, this flock was tested for both ALV and MD which causes hepatomegaly in affected birds.

PCR screening to rule out MD showed that the bantam chicken was negative for MD. Similarly, screening for the ALV J subgroup utilizing H5 and H7 primers did not result in any distinctive amplification. However, when employing subgroup-specific primers H5 and AD1 for subgroup A-E, a positive amplicon of 295-326 bp was observed (Fig- 2). The obtained results confirmed that the bantam chicken was positive for ALV. Additionally, PCR was conducted to determine the exact subgroup of ALV present in this bird. The findings revealed that the bantam chicken was positive for Subgroup E of ALV producing an amplicon of 1.25 kbp (Fig - 3).

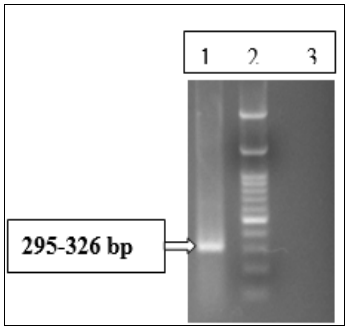

Fig 2: Avian leucosis virus subgroups A to E specific amplicon (295-326 bp) using H5 and AD1 primer

Lane 1- Positive sample of bantam chicken, Lane 2- DNA ladder 1kb, Lane 3- Negative control

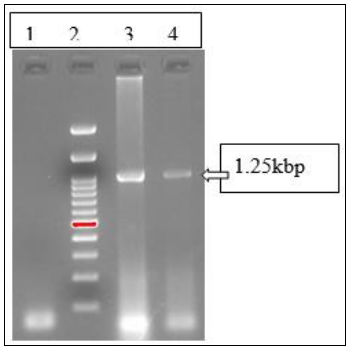

Fig 3: Avian leucosis virus subgroup E specific amplicon of 1.25 KBP

Lane 1- Negative control, Lane 2- DNA ladder 1kb, Lane 3- Positive control, Lane 4 - Positive sample of bantam chicken

Microscopically, liver revealed diffuse micro to macrovesicular fatty degeneration of hepatocytes. Multifocally, there was also mild vacuolar degeneration of hepatocytes. Spleen revealed very mild lymphoid depletion. Heart showed multifocal mild haemorrhages and congestion of blood vessels. Microscopical changes of infiltration of uniform sized lymphoid cells and lymphoblasts in the various organs specific for confirmation of Avian leucosis (Sagarika *et al*., 2017) [9] was absent in this case (Fig -4).

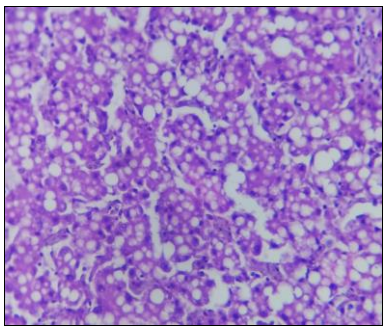

Fig 4: Histopathology of liver showing diffuse micro to macrovesicular fatty degeneration of hepatocytes. H&E - 10x

Histopathological analysis of the PCR-positive samples did not show the ALV-specific monomorphic lymphoid infiltration. This shows that the ALV detected in this bantam chicken was not infectious and it could be probably a proviral DNA of ALV E detected by PCR in this bird. Our findings are in agreement with Smith and Fadly 1988 [11] who have also reported that most of the Endogenous ALV are defective and cannot produce infectious virions. But it will enhance the infection of other exogenous viruses to induce neoplasm. Birds react to an exogenous ALV infection is influenced by the presence of endogenous ALVs (Crittenden, L.B *et al.*, 1985) [3]. Reports on ALV infection in bantam chickens are sparsely available. Therefore, it is becoming necessary to study the prevalence of ALV in these kinds of bantam chickens to control ALV at the breeding level and to produce ALV-free flocks.

4. Conclusion

The detection of non-infectious endogenous ALV Subgroup E in the bantam chicken highlights the significance of monitoring ALV in these poultry breeds. While no lymphoid infiltration was observed, indicating the non-virulent nature of the virus, its presence can still impact the susceptibility to other exogenous viral infections. Regular screening and control measures are essential to reduce ALV prevalence and ensure the production of ALV-free flocks, thereby safeguarding the poultry industry.

5. Acknowledgements

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6. Conflict of Interest

Not available

7. Financial Support

Not available

8. References

1. Biggs PM, McDougall JS, Frazier JA, Milne BS. Lymphoproliferative disease of turkeys. 1. Clinical aspects. Avian Pathol. 1978 Jan;7(1):131-139.
2. Chang KS, Lee SI, Ohashi K, Ibrahim A, Onuma M. The detection of the meq gene in chickens infected with Marek's disease virus serotype 1. J Vet Med Sci. 2002;64(5):413-417.
3. Crittenden LB, Fadly AM. Responses of chickens lacking or expressing endogenous avian leukosis virus genes to infection with exogenous virus. Poult Sci. 1985 Mar;64(3):454-463.
4. Davidson I. The knowledge that human tumor virology can gain from studies on avian tumor viruses. Adv Tumor Virol. 2009 Jan;1.
5. King JM, Roth-Johnson L, Dodd DC, Newsom ME. The Necropsy Book: A guide for veterinary students, residents, clinicians, pathologists, and biological researchers. The Internet-First University Press; c2014.
6. Mitra N, Verma R, Singh A. Molecular and pathological analysis of oncogenic virus induced multiple infections in chickens reared under Indian conditions. Nat Acad Sci Lett. 2013 Jun;36:271-277.
7. Payne LN. Developments in avian leukosis research. Leukemia. 1992 Jan;6:150S-152S.
8. Ramya R, Jayanthi N, Kalaiselvi G, Prakash Krishna B, Balakrishnan G. Co-existence of fowl cholera and lymphoid leucosis in Desi chickens. Int J Vet Sci Anim Husbandry. 2024;9(2):687-691.
9. Sagarika S, Das D, Panda SK, Das S, Jena B, Sahu RK. Avian leucosis in chickens: A clinico-pathological survey. J Entomol Zool Stud. 2017;5:1697-1701.
10. Silva RF, Fadly AM, Taylor SP. Development of a polymerase chain reaction to differentiate avian leukosis virus (ALV) subgroups: detection of an ALV contaminant in commercial Marek's disease vaccines. Avian Dis. 2007 Sep;51(3):663-667.
11. Smith EJ, Fadly AM. Influence of congenital transmission of endogenous virus-21 on the immune response to avian leukosis virus infection and the incidence of tumors in chickens. Poult Sci. 1988 Dec;67(12):1674-1679.
12. Smith LM, Brown SR, Howes K, McLeod S, Arshad SS, Barron GS, *et al*. Development and application of polymerase chain reaction (PCR) tests for the detection of subgroup J avian leukosis virus. Virus Res. 1998 Mar;54(1):87-98.
13. Soujanya S, Lakshman M, Madhuri D. Occurrence of lymphoid leukosis in chickens. The Pharma Innovation J. 2019;8(1):159-162.
14. Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. Elsevier Health Sciences; c2018 Feb 27.

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