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Detection by PCR of Multiple Subgroups of Avian Leukosis Virus (ALV) in Broilers in the Sudan

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Abstract: An investigation on avian leukosis virus infection in broiler parent farm in Khartoum state, Sudan was conducted. Clinical signs, morbidity rate, mortality rate were recorded. Necropsy was performed, histopathological sections from infected livers were made, and virus isolation trials in chick embryo fibroblast and chorioallantoic membrane were performed. PCR tests were performed on DNA extracted from infected livers and spleen. Affected birds showed in-appetence, abnormal feathering, paleness of combs and wattles, loss of weight, decrease in egg production, depression, paralysis and death. Post mortem examination of affected birds revealed hepatomegaly, spleenomegaly, congestion of the lungs, enlargement of the heart and kidneys. Histopathological sections revealed diffuse infiltration of pleomorphic population of lymphocytes with mitotic figures. Virus isolation attempts failed to yield a virus. PCR tests confirmed that ALV subgroup (A-D), HPRS, and ALV-J were involved in the infection. This was the first report on avian leukosis virus infection in the Sudan.

Key words: PCR, Subgroups, Avian Leukosis, Broilers

Introduction

The commercially important avian oncogenic diseases are avian leucosis (AL) and reticuloendotheliosis (RE), which are caused by retroviruses, and Marek's disease which is caused by a herpes virus (Witter, 1999; Payne and Venugopal, 2000). These diseases are responsible for economic lossess due to both mortality and depressed performance (Davidson and Borenshtain, 1999; Payne and Venugopal, 2000 and Fadly, 2004).

Avian leukosis viruses (ALV) infecting chickens fall into six envelope subgroups A-B-C-D-E and J, classified on the basis of virus neutralization (identity of the *env* gene), receptor binding or cross interference (Payne, 1998; Benson *et al.*, 1998; Fadly, 2004, Chesters *et al.*, 2002) with subgroup J been a recently identified avian retrovirus associated with myeloid leukosis in meat type chickens (Payne *et al.*, 1991a and Smith *et al.*, 1999).

The *env* gene of the HPRs-103 strain of ALV (the prototype of the ALV J) showed several nucleotide sequence substitutions resulting in antigenic variation among the isolates (Venugopal *et al.*, 1998), the HPRs 103, differ considerably from that of other subgroups, but shows close homology to the envelope like sequences of members of the Endogenous avian leukosis viruses (EAV) family of endogenous retroviruses (Smith, *et al.*, 1999) suggesting that it might have evolved by recombination with virtually identical sequences belonging to the members of the endogenous avian retrovirus (Bai *et al* 1995a; Benson *et al.*, 1998; Venugopal, 1999 and Fadly, 2000).

Among the six envelope subgroups of avian leukosis viruses (ALVs) that infect chickens, subgroup A (ALV-A) and J (ALV-J) are the most pathogenic and wide spread among commercial chicken populations and associated with lymphoid leukosis (LL) and less frequently erythroblastosis (EB) and tumors of myeloid lineage, respectively (Fadly, 2000).

The clinical signs of various avian oncogenic viruses overlap and are of low degree of pathogonomy, such as infection with one virus might biologically alter the clinical outcome of birds that are already infected by another oncogenic virus that could influence the final outcome of the disease (Davidson, 2004).

The use of PCR was found to be rapid, specific and more sensitive than conventional diagnostic tests for detection of ALV. This technique was found to be capable of differentiating between the prototype-like viruses and the more recent isolates that show extensive antigenic and sequence variations (Smith *et al.*, 1999 and Xu *et al.*, 2004).

Moreover PCR was found to be able to detect avian oncogenic viruses either alone or in various combinations in blood and tumor DNAs of commercial birds (Davidson and Borenshtain, 1999).

In the Sudan, Marek?s disease is the only oncogenic disease been reported (Anon, 1956; Kheir *et al.*, 1992; Salim *et al.*, 2001). No reports were so far available on Avian leukosis and Reticuloendotheliosis infections, despite the wide spread nature of these diseases. In this communication we report on the detection of more than one subgroup of ALV in a broiler farm in the Sudan by PCR.

Materials and Methods

Study area and Farm Description: This study was conducted on a broiler parent farm in Khartoum. The birds were Lohman breed imported as fertile eggs and were hatched locally and reared in a closed system. Clinical signs, morbidity rate and mortality rate were recorded.

Collection of Samples and Processing: Sick and dead chickens were brought to Virology Research Laboratory, Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum. Necropsy was performed on sick and dead chickens, postmortem lesions were recorded and liver and spleen were collected aseptically in 50% glycerol for virus isolation, 10% buffered formalin for histopathology and without a preservative for polymerase chain reaction (PCR). Specimens for virus isolation were stored at -20°C till used.

Histopathology: Liver tissues were fixed in 10% buffered formol saline, and processed according to the method of Drury and Wallington

(1980). The slides were then stained with haematoxylin and eosin (H&E) and examined under a light microscope.

Virus Isolation Trials

Preparation of samples: Liver and spleen specimens were homogenized using sterile mortars and pestles with the aid of sterile sand and physiological saline (PS). 20% suspensions were made and then centrifuged at 1000 rpm for 10 minutes. Supernate fluids were collected into sterile bottles and treated with antibiotics (1000 IU penicillin- 250mg of streptomycin) and 5.000 IU of mycostatin. The supernate fluids were left for ½ - 1 hour at 4°C and then stored at -20°C till used.

Inoculation on the Chorioallantoic Membrane: 0.2ml of liver and spleen suspension was inoculated on the chorioallantoic membrane the embryonating eggs. Two blind passages were made for each specimen before it was considered negative.

Inoculation of Chicken Embryo Fibroblast (CEF) Monolayers: 50μl of tissue homogenate (liver and spleen) were inoculated on confluent monolayers grown on 6 well plates monitored for 14 days for appearance of cytopathic effect (CPE), then two blind passages were made with cells disturbed by freezing and thawing.

Polymerase Chain Reaction (PCR)

DNA extractions: Extraction of viral DNA from specimens designated liver 1 (L1), liver 2 (L2), spleen 1 (Sp1), spleen 2 (Sp2) collected randomly from four of the affected broiler parent chickens was made using PUREGENE® DNA isolation kit (Gentra System, Minnpapolis, USA) with some modifications. Briefly, 500μl Cell Lyses Solution was added to 100mg tissue in 2ml microtube, and the mixture was homogenized then 2μl of Proteinase K solution (20mg /ml) were added to the lysate and the suspension incubated at 55°C overnight, followed by addition of 1.5μl RNaseA solution (4mg/ml). The procedure was then completed as described by the manufacturer.

Positive and Negative DNA Controls: ALV-A genome (ALV-A DNA 40ng/ul), was kindly provided by Dr. A. M Fadly the Avian Disease and Oncology Laboratory, Michigan, United States. Serial dilutions of the DNA were used to determine the optimum DNA concentration. Additionally, HPRS-103 plasmid DNA was kindly provided by Dr K, Venugopal, the Institute for Animal Health, Compton, United Kingdom. Serial dilutions were made and tested in PCR to determine the optimum DNA concentration. Reticuloendotheliosis virus genome (REV DNA 250ug/ul) was kindly provided by the Dr A, M Fadly Avian Disease and Oncology Laboratory, Michigan, United States. As for ALV-A genome serial dilutions were made and used in PCR to determine the optimum DNA concentration. DNA prepared from normal chicken liver and DDW were used as negative controls.

PCR for Avian Leukosis Viruses: The sequences of the oligonucleotide primers used in this study (Table I) were derived from the published sequences (Bai *et al.*, 1995b) and used according to Smith *et al* (1998). Primer H5 was designed that flanked the 3' region of the *pol* gene, which is, conserved across several ALV subgroups. Primer ADI when used with H5 is expected to give 292-326 bp, which was conserved among ALV subgroups (A-B-C-D-E).

Primer H2 when used with H5 is expected to give 764 bp fragments on HPRS-103 DNA. However some recent subgroup J isolates could not be amplified with this primer pair due to sequence changes in the *env* gene (Venugopal *et al.*, 1998). Primer H7 was designed from a well-conserved region of the gp85 sequence of variant viruses and expected to give 545bp product in the amplifications carried with primer H5.

PCR was performed in a final volume of 50μl including 1μl of template DNA, 2.5U Taq polymerase (Invitrogen), 2μl of each primer (H5 and AD1), 1.0 mM dNTP, 5ul 10X PCR buffer (-$MgCl_2$), 1mM $MgCl_2$ (I). As a positive control 1μl of the original ALV-A genome was included and as negative controls DNA prepared from an uninfected bird and DDW were included in each test.

For detection of HPRS-103 and ALV-J the same PCR conditions were followed using primer H5, primer H2, primer H7 (Table 1) and 1μl of the HPRS-103 plasmid as positive control for both HPRS-103 and the recently isolated ALV-J.

A touch down PCR program (Smith *et al.*, 1998), was performed in a TP3 Thermocycler (BIOMETRA, Germany) as follows: Denaturation at 93°C for 1 min, annealing at 60°C for 1min decreased by 1 min in each cycle of the following cycles and extension at 72°C for 1 min 30s, with a final extension at 72°C for 10 min.

PCR for Reticuloendotheliosis Virus (REV): Specific primers were used for the amplification of REV provirus envelope gene were that derived from the published sequences and were used according to (Kim *et al.*, 2003) (Table 1).

PCR was carried out in a final volume of 50μl including 1μl of DNA template, 2.5U Taq polymerase (INVITROGEN), 2 μl of primer REV-F and primer REV-R (SIGMA), 1.0 mM of dNTP, 5ul 10X PCR buffer (-MgCl2), 1mM MgCl2 (INVITROGEN). As a positive control 1μl of the original REV genome was included and as negative controls. DNA prepared from an uninfected bird, and DDW were included in each test.

The amplification was performed in a TP3 Thermocycler (BIOMETRA, Goettingen, Germany), as follows: after initial denaturation for 30s at 94°C, the DNA was amplified for 35 cycles of 30s of denaturation at 94°C, 30s of annealing at 56°C, and 9 min of extension at 68°C, with a final extension step of 10 min at 68°C.

Analysis of PCR Products: The PCR products (amplicons) were separated electrophoretically in 1% agarose gels (SIGMA) (Appendix 8.1) containing ethidium bromide (1μl/40 ml agarose) (PROMEGA, Madison, USA) (Appendix 8.5). 10μl of 100 bp DNA ladder (INVITROGEN) (Appendix 8.4) were loaded in the first slot of the gel. Then 10μl of the PCR products were mixed with 5μl of loading dye (Appendix 8.3) and loaded on the rest wells. Electrophoresis was performed in a Mini gel electrophoresis (BIOMETRA) using 75 volt for 45 min after the gel was covered with TAE buffer (Appendix 8.2) using Standard Power Pack P25 (BIOMETRA). DNA bands were visualized using the BIODOC ANALAYZ gel documentation system (BIOMETRA).

Results

Morbidity and Mortality Rates: The clinical disease appeared in a flock of broiler breeder at 26 weeks of age with 10% morbidity and 0.2-1% mortality per week that increased at 27-40 weeks of age to 2-3% per week with a total mortality of 60%. Culling of the affected flocks was done at the same period, in stead of total culling at 64 weeks of age.

Clinical signs: Affected birds showed in-appetence, abnormal feathering, paleness of combs and wattles, loss of weight, decrease in egg production, depression paralysis and death.

Postmortem Findings: Post mortem examination of affected birds revealed hepatomegaly up to three times of the normal size, spleenomegaly, congestion and consolidation of the lungs. The heart was enlarged with hydropericardium. The kidneys were enlarged, congested and pale.

Histopathological Findings: The most striking lesions in liver sections were diffuse infiltration of pleomorphic population of lymphoid cells with mitotic Figures (Fig. 1). These cells consisted of small and large lymphocytes and some cells with plasmocytoid differentiation in which the nuclei were densely stained and eccentrically placed in abundant of eosinophilic cytoplasm (Fig. 2). The majority of these cells were large lymphocytes with centrally lobulated nucleus. The cytoplasm of these cells varied from abundant to scanty faintly stained with indistinguishable borders. Many of these cells containing abundant eosinophilic cytoplasm were in mitosis.
Sectioned liver also revealed intense diffusion of lymphoid cells packing the congested dilated sinusoids resulting in pressure-induced atrophy to the hepatocytes. In severely affected areas the hepatic parenchyma was replaced by the accumulation of the infiltrated lymphoid tissue.

Virus Isolation Trials
Egg Inoculation: No lesions were produced by inoculation of the CAM of chicken embryonated eggs with tissue all homogenates.

Cell culture: No lesions or any morphological changes were seen in chicken embryo fibroblast cell culture after inoculation with tissue suspension in two passages.

Polymerase Chain Reaction (PCR)
PCR with primer pair H5/AD1: No amplification product was detected when control negative DNA was used as template, while the control DNA (ALV A genome) gave positive result. The 4 DNA samples under test gave positive result for the *Pol* gene. Strong bands were detected in the ethidium bromide stained gel that correspond exactly to the expected DNA band size of the control positive DNA (326) (Fig. 3).

PCR with Primer Pair H5/H2: No amplification product was detected when control negative DNA was used as template while the control positive gave positive result. Of 4 DNA samples under test three samples (two livers and one spleen gave positive result for the *env* gene. Strong bands were detected in the ethidium bromide stained gel that corresponds exactly to the expected DNA band size of the control positive plasmid ALV-J (764 bp) (Fig. 4).

PCR with Primer Pair H5/H7: No amplification product was detected when control negative DNA was used as templates while the control positive DNA gave positive result. All the 4 DNA samples under test gave positive results for *gp*85 gene. Strong bands were detected in the ethidium bromide stained gel that corresponds exactly to the expected DNA band size of the control positive ALV-J plasmid (545 bp) (Fig. 5).

PCR with Primer Pair REV-F/REV-R: No amplification product was detected when control negatives (DNA prepared from normal liver and DDW) were used as templates while the control DNA gave positive result, while the 4 DNA samples under test gave negative result (not shown).

Discussion

In the Sudan, Marek?s disease is the only oncogenic disease been reported (Anon, 1956; Kheir *et al.*, 1992; Salim *et al.*, 2001). No reports were so far available on Avian leukosis and Reticuloendotheliosis infections, despite the wide spread nature of these diseases. Reason could be lack of reliable techniques to detect these oncogenic viruses in the country.
During the last years increased mortality in broiler parent farms that associated with tumor formation on liver and spleen were observed (Khalafalla, personal communication). In this investigation epidemiological data were collected from one of the big broiler farms in Khartoum. The broiler breeder flock under investigation suffered from 10% morbidity rate, 0.2-0.1% mortality rate per week at 26 weeks of age, increased up to 2-3% at 27- 40 weeks of age, with total mortality reaching 40% of the total number. Culling of the affected flocks was done daily at the same period, instead of total culling at 64 weeks of age.
Clinical signs in the affected birds included dullness, inappetence, abnormal feathering, paleness of comb and wattle, loss of weight, decrease in egg production depression, paralysis and death. Palpation often revealed enlarged liver. Necropsies were performed on sick and recently dead chickens and revealed hepatomegaly, spleenomegaly and enlagement of kidneys, enlarged heart with hydropericardium and congested lungs.
These clinical and pathological findings were not adequate in making definite diagnosis. Aiello and Mays (1998) and Davidson (2004), stated that clinical signs of various avian oncogenic viruses overlap and they are not so pathogenomic. Furthermore co-infection between different oncogenic viruses may cause modification in the final out come of the disease (Davidson and Borenshtain, 2002). According to Murphy *et al* (1999) several avian leukosis overlapping syndromes were produced. The overlapping may be attributed to several factors such as co-infection between several avian oncoretroviruses, immunosuppression accompanying oncoretrovirus infection (Davidson,

2004; Fadly, 2004). Accordingly, we are unable to relate these finding to certain oncoretrovirus.

Histological sections from affected liver revealed diffuse infiltration of pleomorphic lymphoid cells, the majority of these cells were large lymphocytes with centrally lobulated nucleus; some of these cells were in mitosis with indistinguishable borders. It also revealed atrophy of hepatocytes, replaced with lymphoid cells. These features were similar to that of lymphoid leukosis described by Cooper *et al* (1968), Calnek and Adldinger (1971), and to some extent to Myelocytomatosis described by Mldenov *et al* (1967) and pleomorphic cells may be as a consequence of mixed ALV infection.

Virus isolation was tried by inoculation of chicken embryos and chick embryo fibroblast cells but failed to propagate the virus, though the ALVs were previously reported to induce high titer pocks on the CAM of susceptible embryos (Coates *et al* (1968). The failure to isolate the virus may be attributed to the presence of replication defective viruses (Murphy *et al.*, 1999; Fadly, 2000) or to the low titer of the virus in specimen or to individual variation in susceptibility of the embryos.

Inoculation of chick embryo fibroblast with tissue suspension showed no morphological changes in the cells. These findings were in line with those described by Davidson (2004), that replication of avian retroviruses in cell culture revealed no effect on cell morphology. According to Haguenau and Beard (1962) and Calnek (1964), most of the leukosis viruses do not produce a cytopathic effect in cell culture.

Nucleic acid amplification and the detection methods developed in the past decades are useful for the diagnosis and management of a variety of infectious diseases. PCR assay is one of these methods, which can detect rapidly

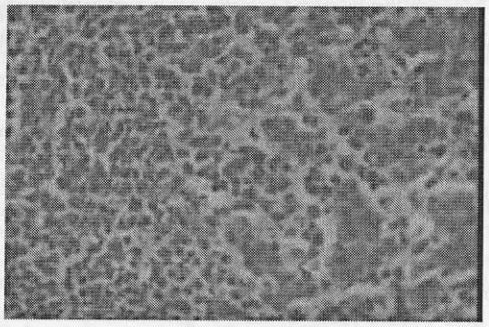

Fig. 1: Histopathological section of infected liver showing diffusion of pleomorphic lymphoid cells, some of them in mitosis (arrows). (H&E) x 40

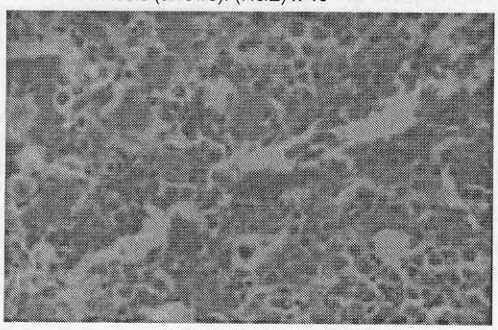

Fig. 2: Histopathological section of infected liver showing infiltration of small and large lymphocytes with plasmocytes with plasmocytoid differentiation (arrows). (H&E) x 40.

and accurately the infectious agent directly from the clinical specimen (Louie *et al.*, 2000). This technique allows the amplification and detection of minute amounts of nucleic acid sequences from tissues or body fluids. The PCR technique dramatically changed approach to the laboratory diagnosis of many diseases where routine laboratory investigations are likely to be not diagnostic or will provide results in a timely manner (Saiki *et al.*, 1985).

In this study we confirmed the presence of mixed ALV subgroups (A-D) and ALV-J infection in broiler parent farm in Khartoum using PCR. Avian leukosis virus subgroup A-D was detected using pair of primers targeting conserved *Pol* gene sequence among

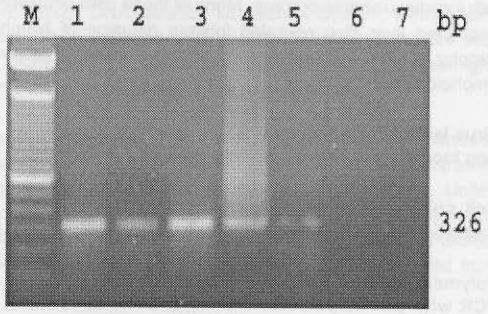

Fig. 3: Ethidium bromide stained agarose gel (1%). PCR was carried out on DNA samples extracted from liver and spleen of infected birds using primers H5/AD1. Lane M: 100 bp ladder, lane 1 control positive,lane 2 liver 1, lane 3 liver 2, lane 4 spleen 1, lane 5 spleen 2, lane 6 control negative (DDW), lane 7 control negative DNA

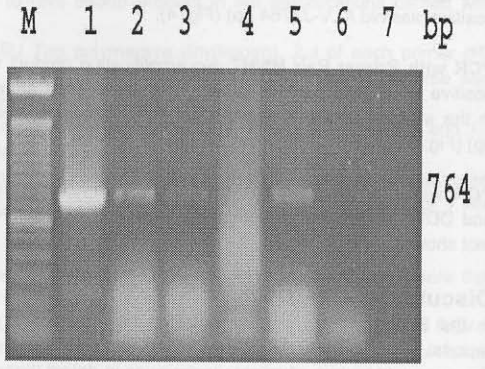

Fig. 4: Ethidium bromide stained agarose gel (15). PCR was carried out on DNA samples extracted from liver and spleen of infected birds using primers H5/H2. Lane M: 100bp ladder, lane 1 control positive, lane 2 liver 1,k lane 3 liver 2, lane 4 spleen 1, lane 5 spleen 2, lane 6 control negative (DDW), lane 7 control negative DNA

these subgroups (A-D). Although these primers can also detect ALV-E this subgroup is excluded since it causes no tumors (Fadly, 2000). Avian leukosis virus subgroup-J was also detected using primer pair targeting all ALV-J isolates (both original and recent isolates). All samples gave positive result for ALV-J, and when primer pairs targeting only the original ALV-J were used only three s amples (two livers and spleen) gave positive results

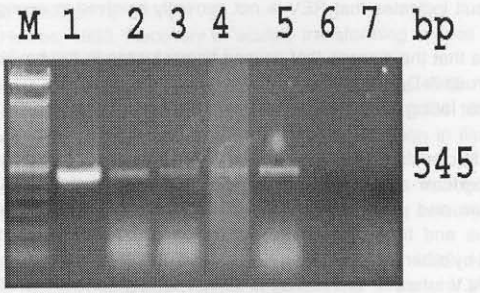

Fig. 5: Ethidium bromide stained agarose gel (1%). PCR was carried out on DNA samples extracted from liver and spleen of infected birds using primers H5/H7. Lane M: 100 bp ladder, lane 1 control positive, lane 2 liver 1, lane 3 liver 2, lane 4 spleen 1, lane 5 spleen 2, lane 6 control negative (DDW), lane 7 control negative DNA

Table 1: Sequence of the oligonucleotide primers used in this study and targets and expected PCR product sizes

Primer	Sequence 5-3	Product size with H5 (bp)	Aplification targets
H5	GGATGAGGTGACTAAGAAAG	-	-
AD1	GGGAGGTGGCTGACTGTGT	295-326 with H5	SubgroupsA-E ALV
H2	ACTGGTGAATCCACAATATCTACG	764 with H5	HPRS-103 ALV
H7	CGAACCAAAGGTAACACACG	545 with H5	Subgroup J ALV
REV-F	GAAGCAGACAATAGGACTGG	-	-
REV-R	CCTCGAGGTCAAATCATTGACCTAGG	900 with REF-F	REV

indicating the presence of two types of ALV-J virus both original and recent ALV-J viruses which could not be amplified with primers targeting original isolates. Recent ALV-J isolates were evolved from the original viruses by transduction of cellular oncogenes accompanied with deletion in the virus genome causing rapid onset of tumors in meat type chickens (Chesters *et al.*, 2001).

The detection of more than one subgroup of ALV is not unexpected since Davidson (2004), stated that one flock or even individual bird, and may commonly be infected with multiple oncogenic viruses. Detection of mixed infection was also supported by the histopathological feature in which we observed the infiltration of pleomorphic cells instead of homogenous cells in the single infection, which suggest mixed infection (Aiello and Mays, 1998).

For definite diagnosis we used different pairs of primers targeting avian oncoretroviruses in PCR according to the method described by Smith *et al* (1998). This PCR is rapid, specific and more sensitive than conventional diagnostic tests and capable of differentiating the prototype- like viruses and more recent isolates that show extensive sequence variations (Smith *et al.*, 1998 and Xu *et al.*, 2004). Moreover, sequence data from different exogenous and endogenous ALVS allowed the selection of conserved set of primers targeting exogenous viruses (Garcia *et al.*, 2003). It was also known that PCR was found to be able to detect avian oncogenic viruses either alone or in various combinations in tumor DNAs of commercial birds (Davidson and Borenshtain, 1999).

First we performed PCR using primer pair H5/AD1 amplified about 326 bp region flanking the 3` region of the *Pol* gene which is conserved across several ALV subgroups A-B-C-D and E according to the published sequences (Bai *et al.*, 1995b). The test performed well since positive result using control ALV-A genome and negative result when DNA prepared from normal liver tissue and DDW were obtained. Positive results from all DNAs extracted from infected livers and spleens, collected from sick birds, were obtained. This result points to infection with ALV subgroups A-B-C-D. The ALV-E was excluded because it is non pathogenic and it does not induce neoplasms (Fadly, 2000).

Additionally, we performed PCR test using primer pair H5/H2 according to the published sequences (Bai *et al.*, 1995b), that amplified 764 bp region of all the original ALV-J isolates designated (HPRS-100, HPRS-101, HPRS-102, HPRS-103, HPRS-104). HPRS-103 plasmid, which was used as positive control, gave positive result and a DNA prepared from normal liver tissue and DDW, which used as negative controls, gave negative results. The DNAs prepared from only three specimens (two livers and one spleen) out of 4 gave positive result. This result shows the presence of the original ALV J subgroup in affected birds.

In order to detect all ALV-J isolates (both original and recently isolated ALV-J isolates) we used a new PCR test which was designed using a new primer H7 derived from a highly conserved region of the env gene of all the ALV-J isolates sequenced to date (Venugopal, 1998). This new PCR test was performed using the primer pair H5/H7. HPRS-103 plasmid that was used as positive control gave positive result while normal liver tissues and DDW gave negative result. All DNA templates used in the previous PCR tests gave the expected product size (545 bp) fragment. This result indicates that the recently emerging ALV-J was also involved in the infection.

It was reported that, ALV-J (recent isolate) was recovered from a myeloid tumor induced experimentally by HPRS-103 (Payne *et al.*, 1993). The recent ALV-J may emerged as a consequence of deletions in the prototype (HPRS-103), replaced by cellular oncogene (*c-myc*) gene (Chesters *et al.*, 2001) or selection pressure, probably from the immune response, which may drive sequence variation among these isolates (Venugopal *et al.*, 1998).

Furthermore, since primer pair H5/H7 detects both original and recent ALV-J, while primer pair H5/H2 detect only the original ALV-J isolates our results confirm the presence of two types of ALV J.

According to Smith *et al* (1998) the use of both tests may provide useful epidemiological data on the types of virus isolates prevalent in the infected flocks.

Using primer pairs REV-F/REV-R according to the published sequences (Kim *et al.*, 2003) that specifically amplify reticuloendotheliosis

virus provirus *env* gene resulted in the amplification of 900 bp from the control positive (REV genome) but not from DNA prepared from livers and spleens of affected birds. This result indicates that REV is not currently involved in oncogenic diseases of broilers in the Sudan.

Results of the present work provide evidence that the disease that caused heavy losses in the broiler parent flock we investigated is caused by three avian leukosis subgroups (ALV subgroup A-D, HPRS and ALV-J).

ALV-J becomes one of the major pathogens facing broiler meat industry worldwide (Venugopal, 1999; Smith *et al.*, 1999 and Chesters *et al.*, 2001).

This study showed that PCR using DNA extracted from liver and spleen can be used for the diagnosis of avian oncoretrovirus infections and for sub grouping since it is easy to perform and rapid and can be performed in 1-2 days to obtain a result including the DNA extraction step. In comparison, clinical picture and post mortem findings are not characteristics and the histopathology is not sufficiently reliable. Besides, virus isolation is laborious and time consuming and requiring 7-14 days for one passage, and it may need further serological identification or it may be hindered by other factors.

To our knowledge this is the first report on ALV infection in the Sudan. Efforts should continue to study the impact of this newly diagnosed disease on poultry industry in the country.

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