Vet.Med.J.,Giza. Vol.54, No.2. (2006): 255-269 .

EVIDENCE FOR VARIANT AVIAN LEUCOSIS VIRUS IN CASES OF MIXED INFECTION WITH MAREK'S DISEASE VIRUS

HUSSEIN. HA*; SULTAN, HA; TANTAWI, LAILA*** and M. EL-SAFTY*****

* Department of Virology, Faculty of Veterinary Medicine, Cairo University Giza- Egypt 12211;

** Department of Poultry Diseases, Faculty of Veterinary Medicine, Minofia University;

*** Pathology Unit, Animal Health Research Institute; Dokki- Giza- Egypt

****Central laboratory for Control of Veterinary Biologics, Abassia- Cairo Egypt.

Received: 14. 2. 2006.

Accepted: 20.2.2006.

SUMMARY

In the present study, we report for the first time in Egypt the evidence for variants of avain leucosis virus subgroup J. ALV-J associated with 2 cases of mixed infection with Marek's disease virus, MDV (one case is associated with tumors and the other from homogenates of blood and tissues of chicks experienced transient paralysis syndrome of MDV)was detected. The ALV-Js might be variants as indicated by histopathological behaviour; negativity of the ALV-J specific PCR; and serological profile in the flock with mixed infection (tumor-cases of ALV and MDV) and in experimentally-infected chickens with blood and tissue homogenates of other mixed infection case of ALV-J and MDV was detected. Using specific PCR for REV and ALV groups A or B, or C and/or D, all samples of both mixed infection cases revealed negative results for amplification. However, 28.3% of sera samples collected from chickens with tumor-cases at age of 38 weeks were positive for ALV-J and negative for ALV (groups A and B) by ELISA. Testing of sera collected from the flock with tumor-cases at 45 and 55 weeks of age revealed that 38.2% and 20% of sera were positive for ALV-J and 2.9 and 3% were positive for ALV (groups A and B), respectively. In experimentally-infected chickens with homogenates of blood and spleen, 14.2 % of sera were positive to ALV-J and negative for ALV (groups A and B) when tested at 30 weeks post-inoculation. Histopathological examination revealed the occurrence of mixed infection of MDV and ALV-J with unique pathological lesions in eye and liver which were found to have heavily aggregations of myelocytes characteristic to ALV subgroup J.

INTRODUCTION

In the 1990s, after eradication programmes of exogenous avian leucosis virus (ALV) have been carried out by commercial poultry breeding companies, ALV subgroup J (ALV-J) emerged and caused myeloid leucosis (ML) in meat type chickens with economic losses worldwide in broiler breeder stocks (Payne, 1998, Fadly and Smith, 1999 and Fadly, 2000). ALV-J commonly induces myeloid leucosis (myelocytomatosis) in naturally and experimentally infected flocks (Payne et al., 1992). This is due to its ability to replicate well in blood lymphocyte than in the bursal follicle (Arshad et al., 1997). HPRS-103 strain of ALV, isolated in 1988 from meat-type chickens, is the prototype of the ALV-J (Payne et al., 1991).

HPRS-103 genome has a distinct env gene closely related (75%-97% similarity) to a novel group of endogenous retroviruses designated EAV-HP than to env gene of other ALV subgroups (40% similarity) (Bai et al., 1995; Smith et al., 1999). Such similarity support the hypothesis that ALV-J arose by recombination between unidentified exogenous ALV and an EAV (Lupiani et al., 2003). EAV-HP (Benson et al., 1998), ev/J (Smith et al., 1999) and line 0 env-like (Silva at al., 2000) are the three classes of the newly identified family of endogenous viruses. Significant antigenic variations have been observed among many isolates of ALV-J (Fadly and Smith, 1999, Hunt et al., 1999, Payne, 1998) which is probably because these isolates inherently have an unusually high frequency of mutation (Silva et al., 2000, Venugopal, 1999, Venugopal et al., 1998). Recently, many recombinants ALV-J viruses carrying a sugroup A envelope glycoprotein (gp85) have been isolated and molecularly characterized (Hunt et al., 2000, Lupiani et al., 2000). These recombinants were accidentally generated between two ALV-J isolates and a defective subgroup A ALV present in transgenic chicken line alv6 (Crittenden and Salter, 1992, Fedrspiel et al., 1991). It is well documented that ALV-J viruses may easily recombine with endogenous viruses and further genetic shift and drift may occur generating new viruses with new phenotypes such as host range, antigenicity and pathogenicity (Lupiani et al., 2000). The characterization of these variants are very important in the diagnosis of ALV-J infection by neutralization or DNA detection by PCR as they may have negative effect on the efforts to control the infections in the primary breeding stocks (Venugopal et al., 1998, Fadly and Smith, 1999, Silva et al., 2000 and Lupiani et al., 2000).

The aim of the present study is to detect and characterize avian leucosis virus associated with 2 cases of MDV infections (tumor and transient paralysis syndrome) by PCR, histopathology and serology.

MATERIAL AND METHODS

Case History: First case: In 2004, chicks with transient paralysis syndrome (difficulty in move-

ments, neck and leg paralysis with mortalities which lasted for 2 weeks have been submitted for the laboratory for diagnosis of the possible causative pathogen. Using PCR and histopathology, pathogenic MDV was implicated as the cause of the problem. MDV was isolated and neurotyped as very virulent plus strain of MDV (Hussein et al., 2004). Examination of different organs from SPF chicks experimentally infected with the blood and brain and feather follicle homogenates collected from the original infected chicks by PCR and histopathology confirmed MDV infection. In a trial to evaluate the protection induced by the current MDV vaccination strategy against the isolated MDV, different groups of commercial broiler breeder chicks have been used in 2 months experiment. PCR and Histopathology examination of organs collected from those broiler breeder chicks at the end of experiment indicated lesions of MDV. Universal PCR to amplify all groups of ALVs including endogenous viruses revealed positive results in spleen collected from inoculated chicks. Therefore we kept the inoculated groups till 30 weeks of age to investigate the possibility of the occurrence of mixed infection of MDV and ALV in the original chicks. Three groups of broiler breeder chicks were kept under investigation: Group 1 and 2 were MDV vaccinated at hatch and non vaccinated chicks kept for 10 days before get challenged by blood, brain and feather follicle homogenates of the original flock via intraperitoneal route. Group 3, were kept non vaccinated and non challenged chicks as control. The chicks were kept for 208 days (almost 30 weeks). Blood, sera and different visceral organs were collected at 6 and 30 weeks and subjected for histopathology, serology and PCR. Second case: In 2005, tumor cases from 30-weeks-old, female broiler breeder flock were submitted for laboratory diagnosis. The tumors were mainly visceral form (liver and spleen). Visceral organs, brain, eye and sciatic nerves were collected from the necropsed chickens as well as blood samples with and without anticoagulants were collected from the original flock for PCR, serology and histopathology examination. Sera were also collected from the same flock at 38, 45 and 55 weeks of age for serology testing by ELISA (ALV, groups A and B and ALV-J antibodies against gp 85).

Histopathology examination: Different organs were fixed in 10% buffered neutral formalin solution, processed in normal way, paraffin sections (4-6 micron thickness) were prepared and stained with haematoxylin and eosin (Bancroft, 1996).

Extraction of viral DNA and PCR : Eight PCR assays with primers specific for MDV, REV, ALV (universal for all subgroups A to E), ALV-A, ALV-B, ALV-C, ALV-D and ALV-J were applied to detect the corresponding viruses in the collected samples (blood and spleen).

The primers used and PCR conditions were carried out as previously reported for MDV serotype 1 using MDVF and MDVR primers which could differentiate between pathogenic (yield 302bp and/or 434bp and/or 566bp) and vaccine (yield 302bp + multiple 132bp repeats) strains of MDV (Davidson and Borenshtian, 2002), REV using primers to amplify products with size 291 bp (Davidson et al., 1995), ALV (subgroups A to E) using H5 and AD-1 primers yielding 360bp products, ALV-A using H5 and CAP-A primers to amplify products with size 694 bp, ALV-B using H5 and envB primers to amplify products with size 846 bp, ALV-C using H5 and envC primers to amplify products with size 859 bp, ALV-D using H5 and envD primers to amplify products with size 797 bp, ALV-J using H5 and H7 primers to amplify products with size 545 bp (Sequences of the primers used for ALV viruses were kindly supplied by Dr. Kirk Patrick, Ross breeders, Aviagen Ltd, Scotland, UK). DNA was extracted from the samples using QIAamp DNA and DNA blood mini kits (Qiagen GmbH, Germany). The extraction protocols were carried out as recommended by manufactures. The PCR mixture reactions were ready to use master mix (ABgene, UK). The cycling conditions of each PCR assay was employed according to the type of primers used in the assay. The PCR products were analysed by electrophoresis on agarose gels stained with ethiduim bromide and photograghed using Polaroid camera.

Serology: ALV and ALV-J antibody test ELISA kits (Idexx Laboratories, Inc, USA) were used to test for the presence of antibodies against gp 85 of subgroup A and B (ALV-antibody test kit) and subgroup J (ALV antibody test kit-subgroup J) in chicken sera. ELISA was carried out as recommended by manufactures.

RESULTS

Confirmation of mixed infection of MDV and ALV in the two cases in the current study are based on the obtained results of histopathology and PCR (Figure 1, Plates 1, 2 and 3). Examination of the organs and blood of the experimentally infected commercial broiler breeder chicks (Figure 1) after 6 weeks post inoculation with the homogenates of the first case (chicks showing transient paralysis syndrome) by PCR revealed positive results for pathogenic MDV serotype 1 as shown in figure 1 E. Testing those birds 30 weeks post inoculation for ALVs by PCR assays specific to each subgroup (A, B, C, D and J) revealed negative results. Testing the same extracted DNA by PCR using universal primers that amplify fragments from all subgroup (A to E) revealed positive results. Serological testing of the same birds by ELISA at 30 weeks revealed positive results for ALV-J in a percentage of 14.2 % (2/14) and negative for ALV subgroup A and B antibodies.

Histopathological examination of the organs collected from the experimentally infected chicks revealed the occurrence of mixed MDV and ALV-J lesions with marked score lesions of the aggregated tumor cells in eye, liver and proventriculus compared with the absence of the tumor lesions in the third group (non vaccinated non infected). The observed eye and liver lesions suggested that the case is a mixed infection of MDV and ALV-J based on the presence of focal and diffuse aggregates of pleomorphic lymphocytes as well as myelocytes in the oedematous iris (Plates 1 and 2). The retina showed massive congestion with haemolysis of the choroids in addition of degeneration of the photo receptor (Plate 1). The ciliary processes in group 3 showed heavily aggregation of myelocytes with obvious mitotic division of the nuclei (Plate 2). Mean while pleomorphic lymphocytes were aggregated in the conjunctiva of gp1 (Plate 2). The liver lesions were in the form of focal parenchymal infiltration of small lymphocytes with few lymphoblasts. Perivascular aggregation of pleomorphic lymphocytes and great population of myelocytes were observed (Plate 2). Dialtation of the hepatic blood vessels and sinusoids with thrombus formation as well as hepatocellular necrosis (Plate 1). The Proventriculus exhibited hyperplasia of the mucosal epithelium and submucosal oedema (Plate 1). Submucosal and glandular infiltration of lymphocytes, lymphoblasts and reticuloendothelial cells were observed. The severity of the scors lesions of the aggregated tumor cells was high (+++) in MDV-non vaccinated than in MDV-vaccinated groups (++) challenged with the homogenates specially the eye , liver and proventriculus.

PCR and histopathology examination of the tumor cases from the second case in the study revealed also mixed infection of MDV and ALV. PCR confirmed the presence of pathogenic MDV and as well as ALV (subgroups A to E) (Plate 3 G). Employing PCR assays specific for each subgroup (A, B, C, D) indicated that the samples were negative. However, the samples revealed positive amplification when universal PCR assay for all subgroups of ALV including E was applied (Plate 3H). Testing of serum samples collected from the flock at different ages post onset of tumor cases revealed positive results for ALV-J in a percentage of 28.3%, 38.2% and 20% at 38, 45 and 55 weeks of age, respectively. On the other side, testing of those sera for ALV (groups A and B) revealed low levels of antibodies 0%, 2.9% and 3% at 38, 45 and 55 weeks of age, respectively. Histopathology examination of the tumors suggested both ALV and MDV. Plate 3 represent the histopathology changes suggested that ALV is implicated. There was diffuse and massive number of proliferated and infiltrated lymphoid as well as myeloid cells which replaced most of the hepatic tissue with atrophy of the remained hepatocytes associated with sever dilatation of the hepatic sinusoids which were impacted

by massive number of erythroid cells as well as myeloid cells.(Plate3D). Also, kidney lesions showed dialation of the intertubular blood vessels and capillaries and impacted by erythroid cells in association with infiltration of the myelocytes and lymphcytes in the stromal tissue. Infiltrated myelocytes and lymphoid cells were also observed in intestine and ovary. Massive numbers of erythroid cells were seen in brain, spleen and ovaries. On the other side, MDV lesions were seen in the same tumor tissues specially in liver and spleen where perivascular in central and portal veins and sinusoids in liver as well as lymphoid cells proliferation mainly pleomorphic lymphocytes and plasma cells in liver and spleen were observed. The histopathology examination of the tumor tissue suggested the mixed infection ALV and MDV.

Figure (1)

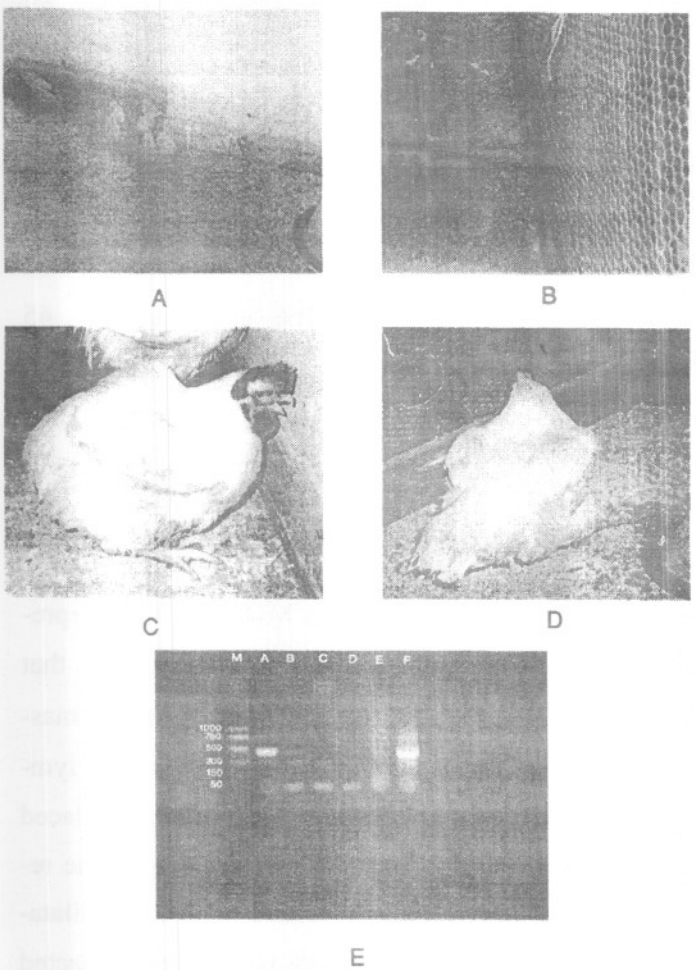

Figure 1: A and B are the groups of broiler breeder chicks used in the experimental infection with the homogenates of commercial broiler breeder chicks with acute transient paralysis syndrome. C and D, the inoculated chicks after 208 days showing some of leg problems and before they tested for histopathology and PCR. E: PCR for MDV in the spleen and blood of the inoculated chickens. Pathogenic MDV serotype 1 was detected with bands of 302bp and/or 434bp.

Plate (1)

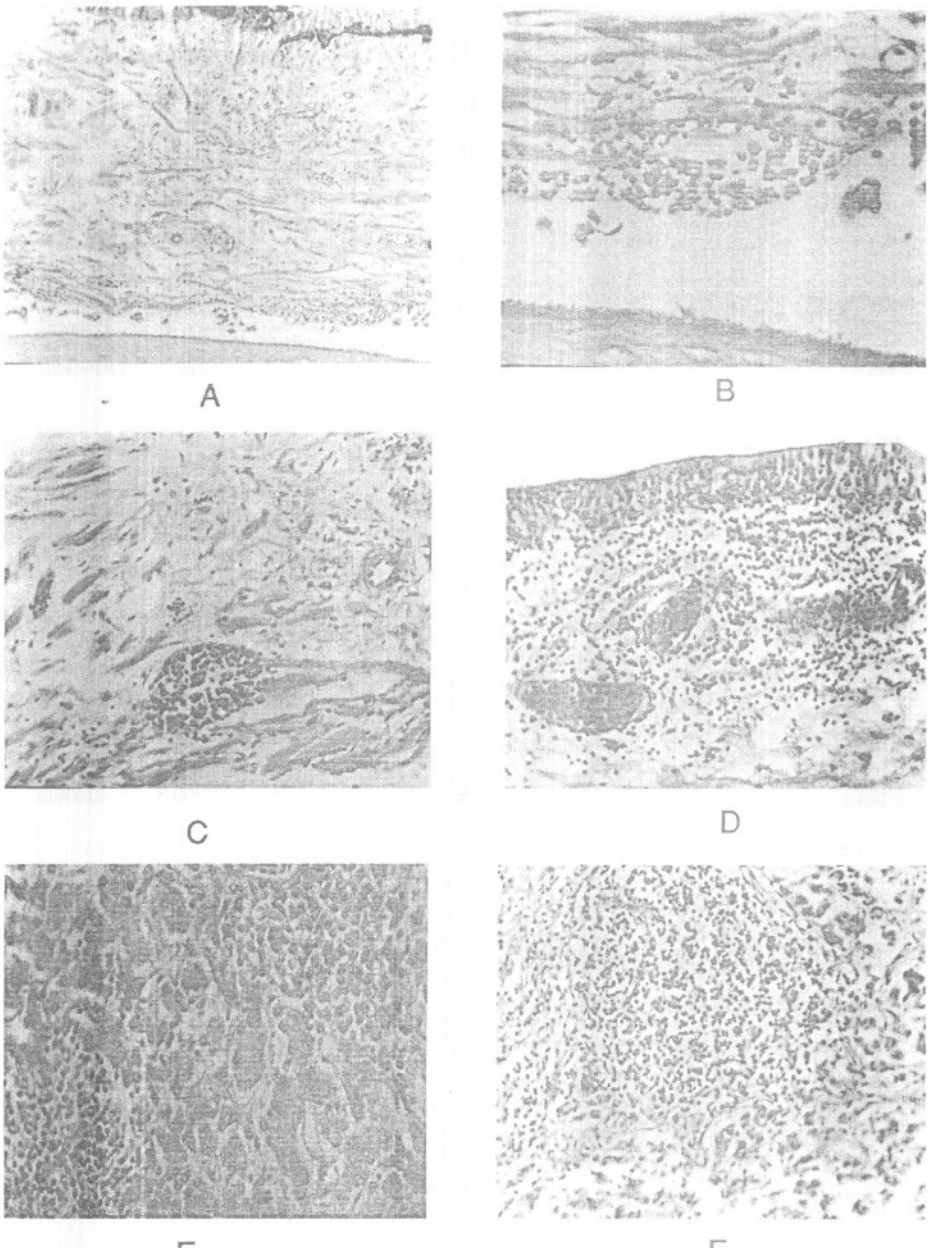

Plate 1: A , B and C: Eye lesions in group of chicks MDV-vaccinated and challenged with homogenate after 10 days post inoculation: oedema of the iris with focal aggregation of pleomorphic lymphocytes (H & E X100 and X400). D: Submucosal pleomorphic lymphocytes infiltration and hypremic blood vessels in the conjunctiva. E: Liver showing focal aggregated areas of small lymphocytes and myelocytes (H & 400X). F: Proventriculus showing glandular pleomorphic lymphocyteic aggregation (H & E 250).

Plate (2)

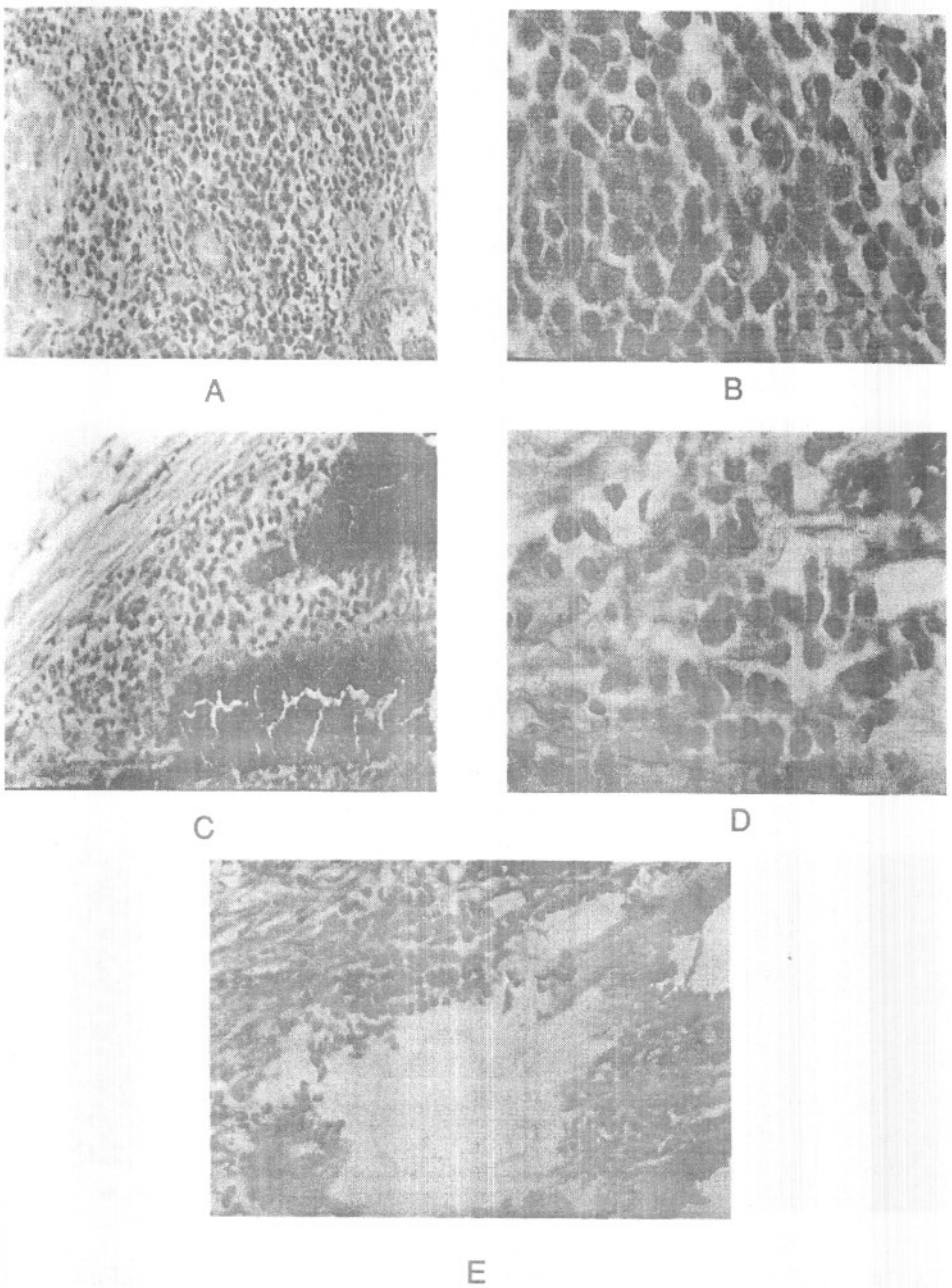

Plate 2: A and B: Liver of the chicks (MDV non-vaccinated and challenged with the homogenates) showing perivascular infiltration of myelocytes (H & E X 400 and 1000). C, D and E: Eye lesions showing the myelocytes infiltration in the ciliary processes (H & E X400 and X1000).

Plate (3)

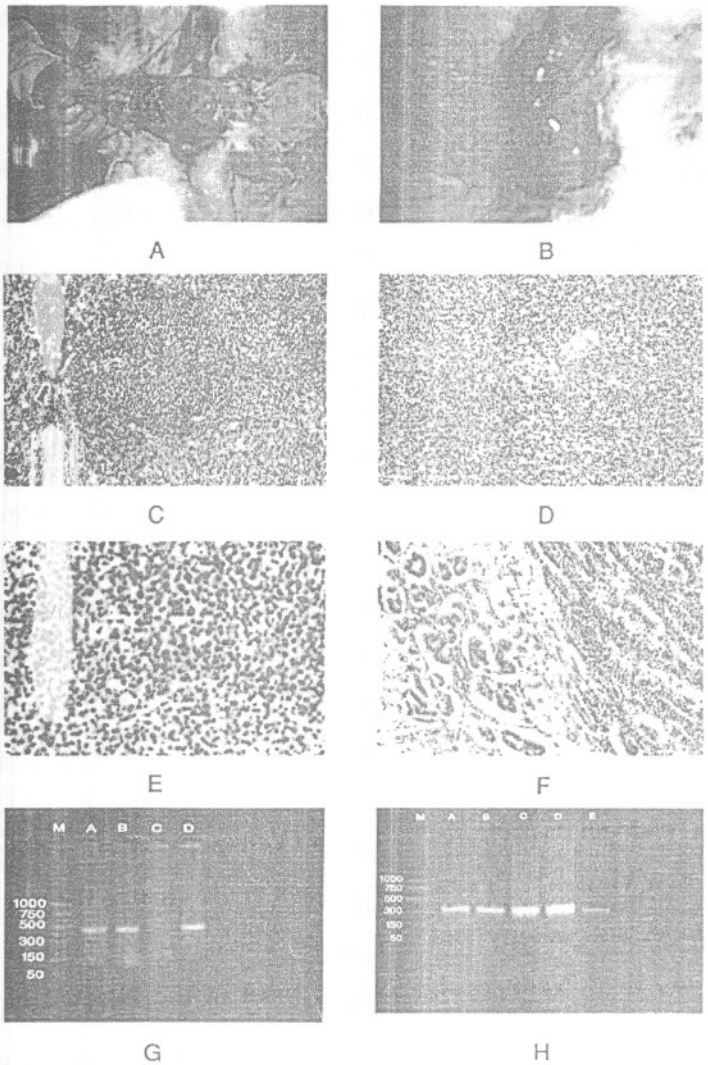

Plate 3: A and B: Thirty weeks old female chickens with visceral tumors in liver and spleen. C and E: Liver focal an predominant lymphoblast cells replacing the hepatic tissue in focal circumscribed round manner. D: Spleen showing focal necrotic areas with massive numbers of infiltrated myelocytes and erythrocytes in the pulp. F: Proventriculus showing lymphoid cells infiltration in the lamina propria with necrosis of the glandular structure. G: PCR for MDV, pathogenic MDV serotype 1 was detected in spleen tumors of the chicken (434bp; lanes A,B and D or 366bp in lane C of the photo). H: PCR universal for all ALV subgroups A, B, C, D and E; 360bp bands in spleen (lanes A to D) and blood (lane E).

DISCUSSION

The great similarity in envelop gene of both ALV-J and EAV viruses support the hypothesis that ALV-J arose through recombination event between an exogenous ALV and endogenous EAV viruses (Bai et al., 1995b, Benson et al., 1998). The eradication programme for ALV is based on the removal of group specific antigen (GSA)-shedder birds from ALV-J infected flocks (Spencer, 1984). Diversity among retroviruses can occur through either recombination or polymerase errors followed by antigenic selection (Dorner and Coffin, 1986, Nichol, 1996). A significant antigenic variation among ALV-J isolates has been reported (Venugopal et al., 1998, Fadly and Smith, 1999, Hunt et al., 1999). Studies have suggested that ALV-J viruses may have unusual high frequency of mutation and also the viruses can easily recombine with endogenous viruses leading to the generation of new viruses with new phenotypes such as host range, antigenicity and pathogencity (Venugopal et al., 1998, Venugopal, 1999, Silva et al., 2000).

In the present study, we reports the presence of ALV-J variant strains circulation in the field. Interestingly is the occurrence of these variants in cases mixed with MDV infections (with and without neoplasms). ALV-J variants have been previously detected and characterized in Europe and USA (Venugopal et al., 1998, Silva et al., 2000). The origin of these viruses can be traced by sequencing the detected viruses in the field cases. The absence of tumors in the first case in the study is normal and support our suggestion that the circulating ALV-J is differed from the previously reported ALV-J infection in Egypt (Ahmed et al., 1999, Aly, 2000). The observed decrease in the number of tumor or clinically affected cases may be due to the eradication programme initiated by the major breeding companies in their home countries. However, certain meat-type chickens are capable of developing persistence viremia and virus shedding following infection post-hatching possibly because of the immune tolerance (Payne et al., 1992). Most of the variant strains of ALV-J have been isolated during the eradication programme which involved testing and removal of virus shedders. This approach had helped in reducing the incidence of ALV-J infection at low levels (Venugopal et al., 1998). Conversely, this approach could change the status of immunocompetence among susceptible population of birds where most of the infected birds mount the immune response with an increase in selection pressure on the virus lead to neutralization-sensitive population which could be eliminated giving ways to the emergence of variant viruses which are able to escape neutralization and survive in host population (Venugopal et al., 1998). In study on the selection pressures on the env gene and in particular, the hr1, hr2, vr3 regions of the gp85, a conclusion has been made by the authors that

there were some selection pressures on the env gene of ALV-J in hr2 and vr3 where the NS/S ratios were as high as 7.0 and 4.50, respectively (Venugopal et al., 1998). Others have indicated more mutations and stronger selection pressures on specific regions of gp85 during the ALV-J evolution in the last several years (Cui et al., 2003). While it is not known that these variant viruses exhibit alteration in other regions of their genome, there was an alteration on the long terminal repeat (LTR) in some of the examined isolates (Venugopal et al., 1998). Studies in USA reported that ALV-J USA strains may be continuing to mutate based on the phylogenetic pattern of such isolates (Silva et al., 2000). If an additional mutation with EAV or other sequences occurs it could result in the emergence of a new strains with new pathogenicity. Alteration in the genomic or antigenic epitopes will have negative effect on the currently used test for diagnosis in ALV-J. The PCR employed in the current study for characterization of ALV-J is currently used in many laboratories specially in UK with great success in detecting most of ALV-J viruses isolated (Venugopal, 1999). The continuous improvement in the primer design is critical in the success of the PCR assay. The failure of the primers used to detect ALV-J in the presence study may be due to these viruses possesing an alteration in their genome. The sequence analysis of these viruses will explain such failure. Conversely, ELISA used in the study was able to detect antibodies to ALV-J in the two studied cases confirming that the ALV-J is implicated in these cases. Detecting antibodies specific to ALV-J in serum consider one of the identification tool of diagnosis (Venugopal, 1999). Binding of antibodies detected in ELISA against ALV-J appeared to be more cross reacting among variant strains of ALV-J than the more neutralizing antibodies (Venugopal, 1999). Hence, ELISA results in the study confirm the implication of ALV-J in the two cases of the study.

The presence of the histopathological lesions for both MDV and ALV-J in the 2 cases under study confirmed that both flocks were affected by both viruses. However, the situation may be vary. The typical lesions characteristic for ALV-J was observed in both cases in the form of mixed aggregations of lymphoblasts and myelocytes (Payne, 1998). The histopathological lesion seen in the study in both tumor tissues and experimentally infected chicks in accordance with those previously reported with ALV-J infection confirmed the behaviour of ALV-J (Arshad et al., 1997). Moreover, the study reports eye lesions induced by ALV-J and these lesions are of added interest and experess the changeable behaviour of ALV-J in its tissue tropism. Arshad et al., (1997) who studied the tropism of ALV-J to different tissue concluded that ALV-J did not show correlation with the incidence of tumors through their studies on the gene expression level of ALV-J in different tissues. Therefore, the presence of ALV-J infec-

tions are not usually associated with tumors and this confirms the decreased cases of tumors noticed in the last 2 years. The present study raises the possibility that the current circulating ALV-Js are vairants and their histopathological behaviour is also changeable. Employing PCR on organs in the study confirmed mixed MDV (by PCR and histopathology) and ALV (by ALV universal PCR, histopathology and serology for ALV-J) infection. Based on the histopathological lesions seen in the examined organs, it is likely that the virus in both cases is a ALV-J. PCR conducted in the study could not detect any of these viruses. Hence, such negativity in PCR and the presence of ALV-J antibodies in sera of the affected chickens highly suggest that the ALV in such cases is a variant ALV-J. The susceptibility of cultured monocytes and bone marrow cells from different chicken lines for in vitro transformation by an acute transforming variant strain of ALV-J HPRS-103 (966) showed a correlation with the in vivo susceptibility to myloid leucosis and other tumors (Arshad et al., 1997). The mortalities from tumors seen in the field suggested that these chickens generated from infected breeding stocks (Venugopal, 1999). Many factors play a role in the existence of ALV-J variants strains. First, the congenital and horizontal transmission of the virus. Second, the environment which always modified and may enhance the expression and shedding of ALV-J infections. Such stress factors, specially high bird density, excessive or rough handling, improper beak trimming, deficiencies in feeder or drinker space availability, inadequate male/female ratios, vaccine immunosuppression, vaccine overload, concomitant immunosuppressive disease and nutritional deficiencies or imbalance, can contribute in the enhancement of the ALV-J genes expression and possible shedding of viruses (Zavala, 2000). Also, MDV, IBD, CAV are synergetic with ALV-J (Zavala, 2000). It is not clear in the cases of mixed infection reported in the current study that the primary infection of the chicks under study in both cases (with tumor or experimentally inoculated chicks) was with ALV-J followed by MDV or MDV followed by ALV. The transmission of all exogenous ALVs occurs either by vertical or horizontal and the ALV infection status is expressed by the absence (-) or presence (+) of viremia (V), serum antibodies (A) and virus shedding (S). In case of vertical transmission, the chicks (infected from hens) became immunotolerant and express V+ A- S+. These chicks more likely develop tumors. These chicks and their meconium in hatchs are the source of virus horizontal transmission. Horizontal transmission post hatch with only ALV-J differs in its outcome. As in case of ALV subgroups other than J, the outcoming chicks considered as immuno non-shedders (V- A+ S-). In case of ALV-J, the resulted chicks from the horizontal transmission either immunotolerant (V+ A- S+) or immune status with transient viremia. Some of these birds may also shed (V- A+ S+). The tol-

erance in some of these bird are not complete and plasma cells can be detected in their spleen in spite of the absence of neutralizing antibodies in the serum of these chicks (Russel et al., 1997). In the present study, plasma cells were seen in the spleen during the histpathology examination of the organs in both cases. It is not clear if these cells are due to ALV-J infection or any other pathogens. Even more, the factors that determine the tolerance or immune status of ALV-J are so many and not clearly understood (Venugopal, 1999). The expression of EVA-HP which constitutes the genetic backbone of the ALV-J env sequences may play a role in postulating the induction of tolerant status (Smith et al., 1999). The presence of anti-ALV-J antibodies in sera collected from the birds in the current study may be attributed to change in the immune status of the ALV-J infected birds and these changes may confirm that these viruses are variant ALV-Js.

Changing behaviour was seen before with the increase occurrence of ALV-J variants with sequence changes in variable regions specially in flocks undergoing eradication programmes (Venugopal et al., 1998, Venugopal, 1999).

In conclusion, the study reports the existence of mixed infection of MDV and ALV in the examined chicks. The negativity of the employed PCR for ALV-J and the positively of these samples in histopathology and serology for ALV-J indicate the need for continuous update the primers used in the specific PCR and the urgent combination between PCR, virus isolation and histopathology in the diagnosis of ALV-J cases. The histopathology behaviour of the detected ALV-J and lesions seen in eye represent new finding and may indicate the presence of circulating variant viruses.

AKNOWLEDGMENTS

The authors thank dr. Assia El-Sawi, Head of histopathology unit, Animal Health Research Institute, Dokki, Egypt for her kindley help in the histopathology work .

REFERENCES

Ahmed, A. S.; Amin, A. Afaf; Hassan, M. K. and Abd El-Taher, A. (1999). A preliminary report of subgroup J avian leukosis virus-infection in imported broiler parent chickens. Vet. Med. J. Giza, 47 (4): 605-610.

Aly, M. Mona (2000). Proceedings of International Symposium on ALV-J and other Avian Retroviruses (pp. 165-176). Rauischholzhausen, Germany.

Arshad, S. S.; Howes, K.; Barron, G. S.; Smith, L. M.; Russel, P. H. and Payne, L. N. (1997). Tissue tropism of the HPRS-103 strain of J subgroup avian leukosis virus and a derivative acutely transforming virus. Veterinary Pathology, 34: 127-137.

Bai, J.; Howes, K.; Payne, L. N. and Skinner, M. A. (1995b). Sequence of host range determinants in the env

gene of a full-length, infectious proviral clone of exogenous avian leukosis virus HPRS-103 confirms that it represents a new subgroup (designated J). Journal of General Virology, 76: 181-187.

Bai, J.; Payne, L. N. and Skinner, M. A. (1995). HPRS-103 (exogenous avian leucosis virus subgroup J) has an env gene related to those of endogenous elements EAV-0 and E51 and has an E element found previously only in sarcoma viruses. Journal of Virology, 69: 779-784.

Bancrot, J. D.; Stevens, A. and Turner, D. R. (1996). Theory and practice of histological techniques. 4th ed. Churchill Livingstone, New York.

Benson, S. J.; Ruis, B. L.; Fadly, A. M. and Conklin, K. F. (1998). The unique envelope gene of the subgroup J avian leukosis virus derives from ev/J proviruses, a novel family of avian endogenous viruses. Journal of Virology, 72 (12): 10157-10164.

Crittenden, L. B. and Salter, D. W. (1992). A transgene, alv6, that expresses the envelope of subgroup A avian leukosis virus reduces the rate of congenital transmission of a field strain of avian leukosis virus. Poultry Science, 71: 799-806.

Cui, Z.; Du, Y.; Zhang, Z. and Silva, R. F. (2003). Comparisonof chineses field strains of avian leukosis subgroup J viruses with prototype strain HPRS-103 and united states strains. Avian Diseases, 47: 1321-1330.

Davidson, I and Borenshtian, R. (2002). The feather tips of commercial chickens are a favorable source of DNA for the amplification of Marek`s disease virus and avian leukosis virus, subgroup J. Avian pathology, 31: 237-240.

Davidson, I.†; Borovskaya, A.†; Perl, S. and Malkinson, M. (995). Use of the polymerase chain reaction for the diagnosis of natural infection of chickens and turkeys with Marek`s disease virus and reticuloendotheliosis virus. Avian Pathology, 24: 69-94.

Dorner, A. J. and Coffin, J. M. (1986). Determinants for receptor interaction and cell killing on the avian retrovirus glycoprotein gp85. Cell, 45: 365-374.

Fadly, A. M. (2000). Isolation and identification of avian leukosis viruses: a review. Avian Pathology, 29: 529-535.

Fadly, A. M. and Smith, E. J. (1999). Isolation and some characteristics of a subgroup J-like avian leucosis virus associated with myeloid leukosis in meat-type chickens in the United States. Avian Diseases, 43: 391-400.

Federspiel, M. J.; Crittenden, L. B.; Provencher, L. P. and Hughes, S. H. (1991). Experimentally introduced defective endogenous proviruses are highly expressed in chickens. Journal of Virology, 65: 313-319.

Hunt, H. D.; Lee, L. F.; Fister, D.; Silva, R. F. and Fadly, A. M. (1999). A genetically engineered cell line resistant to subgroup J avian leukosis virus infection. Virology, 264 (1)i 205-210.

Hunt, H. D ; Lupiani, B. and Fadly, A. M. (2000). Recombination between ALV-J and endogenous subgroup E viruses. In: Proc. International Symposium on ALV-J and other retroviruses. E. F. Kaleta, L. N. Payne and U. Hefffels-Redmann, eds. Institut f¸r Geflügelkrankheiten, Justus Liebig University, Giessen, Germany. pp. 50-60. June 5-8, 2000.

Hussein, H. A.; Sultan, H. and El-Safty, M. (2004). A very virulent Marek`s disease virus (EgMD-C) associated with an acute transient paralysis and the protection induced by bivalent vaccination against the isolated virus

in broiler breeder chickens. Proceeding of the 6th Conference of the Egyptian Veterinary Poultry Association, Sept. 25-27, 2004. pp. 51-62.

Lupiani, B.; Hunt, H.; Silva, R. and Fadly, A. (2000). Identification and characterization of recombinant subgroup J avian leukosis viruses (ALV) expressing subgroup A envelope. Virology, 276: 37-43.

Lupiani, B.; Williams, S. M.; Silva, R. F.; Hunt, H. D. and Fadly, A. M. (2003). Pathogenicity of two recombinant avian leukosis viruses. Avian Diseases, 47: 425-432.

Nichol, S. (1996). RNA viruses. Life on the edge of catastrophe (news). Nature, 384 (6606): 211-219.

Payne, L. N. (1998). HPRS-103: a retrovirus strikes back. The emergency of subgroup J avian leukosis virus. Avian Pathology, 72 (Supp. 1). S36-S45.

Payne, L. N.; Brown, S. R.; Bymstead, N.; Howes, K.; Frazier, J. A. and Thouless, M. E. (1991). A novel subgroup of exogenous avian leucosis virus in chickens. Journal of General Virology, 72: 801-807.

Payne, L. N.; Gillespie, A. M. and Howes, K. (1992). Myeloid leukaemogenicity and transmission of the HPRS-103 strain in avian leucosis virus. Leukemia. 6: 1167-1176.

Russell, P. H.; Ahmed, K.; Howes, K. and Payne, L. N. (1997). Some chickens when are viraemic with the subgroup J avian leukosis virus have antibody forming cells, but no circulating antibody. Research in Veterinary Science, 63: 81-83.

Silva, R. F.; Fadly, A. M. and Hunt, H. D. (2000). Hypervariability in the envelope genes of subgroup J avian leukosis viruses obtained from different farms in the US. Virology, 272: 106-111.

Smith, L. M.; Toye, A. A.; Howes, K.; Bumstead, N.; Payne, L. N. and Venugopal, K. (1999). Novel endogenous retroviral sequences in the chicken genome closely related to HPRS-103 (subgroup J) avian leukosis virus. Journal of General Virology, 80: 261-268.

Spencer, J. L. (1984). Progress towards eradication of lymphoid leukosis viruses - A review. Avian Pathology, 13: 599-607.

Venugopal, K. (1999). Avian leukosis virus subgroup J: a rapidly evolving group of oncogenic viruses. Research in Veterinary Science, 67: 113-119.

Venugopal, K.; Smith, L. M.; Howes, K. and Payne, L. N. (1998). Antigenic variants of J subgroup avian leukosis virus: sequence analysis reveals multiple changes in the env gene. Journal of General Virology, 79: 757-766.

Zavala, G. (2000). Myeloid leukosis. Zootecnia International: 46-50.