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Novel Insights into Immunity, Diagnosis, and Vaccination of Avian Leukosis

Morteza Nikzad[1], Mohammad Hasan Bozorgmehrifard[2*]

[1] Department of Poultry Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2] Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

*** Corresponding author email address**: mhbfard@yahoo.com

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A B S T R A C T

Avian leukosis virus (ALV) is an oncogenic retrovirus that has caused significant damage to the poultry industry worldwide. Despite extensive research on this virus, its presence in certain parts of the world continues to attract researchers' attention. It appears that advancements in laboratory techniques toward a better understanding of the immune system's interaction with this virus and the diagnosis of the virus itself can contribute to more effective measures against it. Chickens can acquire ALV infections through natural exposure, developing virus-neutralizing antibodies that restrict viral load but have limited impact on tumor development. ALV has evolved strategies to evade the host's innate immune response, such as targeting cellular proteins involved in signaling pathways like the interferon response, which is crucial for initial antiviral defense. Recent advances in viral detection techniques, including monoclonal antibodies, proteomic analysis, and recombinase-aided amplification, have improved diagnosis and surveillance of emerging avian leukosis virus strains. The present review study has collected up-to-date information on this disease's immunity, diagnosis, and vaccination methods, aiming to provide insights for combating the virus.

Keywords: *Avian leukosis, Immunity, Diagnosis, Vaccination.*

1 Introduction

In 1958, Howard Temin and Harry Rubin took the first steps toward proving the ability of viral particles to transform normal cells into cancer cells. Avian Leukosis Virus (ALV) is a retrovirus that causes neoplastic diseases, immunosuppression, and disadvantages which cause economic loss. The first reports related to the virus were

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made in 1869 and 1896, and it was named "leukemia in birds"(1-3).

Structurally, ALV virion core contains five non-glycosylated proteins encoded by the *gag/pro* gene: MA (matrix, p19), p10, or CA (capsid, p27), which is the main gs (Gag) antigen in the core shell. NC (nucleocapsid, p12) is involved in RNA processing, and PR (protease, p15) is involved in cutting protein precursors. The virion envelope contains two glycoproteins encoded by the *env* gene: SU (surface, gp85) and knob-like structures on the viral surface that determine the subtype specificity of the ALSV viral envelope. This protein plays a vital role in virus identification and related studies. TM (transmembrane, gp37) represents a protein that crosses the membrane and connects Knob-like structures to the surface (Figure 1) (4). Avian leukosis virus is classified into ten viral subgroups from A-J. Subgroups (A-E & J) mainly infect chickens and turkeys and are classified according to the viral envelope, host range, and cross-neutralization test. Other unusual leukosis subgroups, such as F, G, H, and I, mainly represent endogenous ALVs in pheasants, partridges and quails and affect wild birds (4).

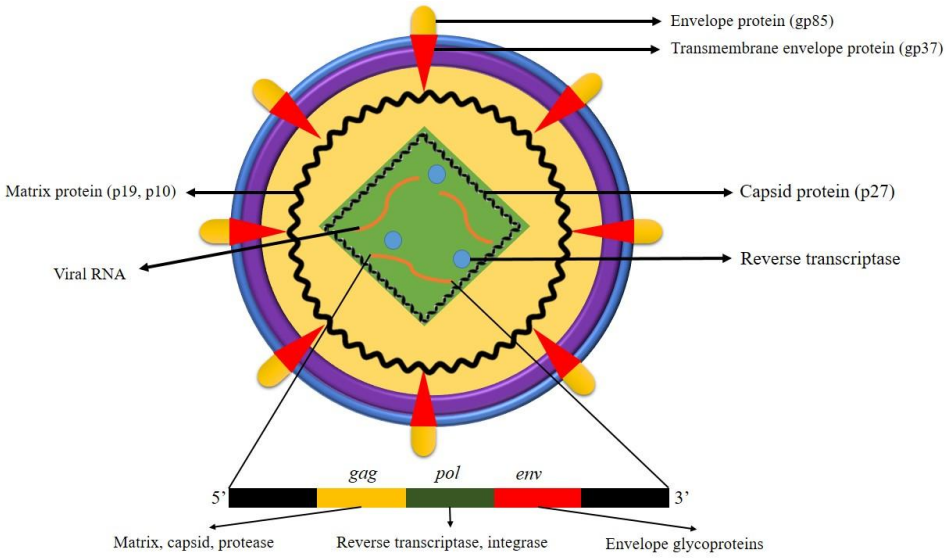

Figure 1. Structure of ALV

A new subtype of ALV, ALV-K, was recently identified in domestic Chinese chickens in 2012. The gp85 region of ALV-K strains showed more than 90% similarity with several Japanese fowl glioma viruses (FGV) and an ALV strain (TW-3593) isolated from Taiwan province (5, 6). Recent data suggest that ALV-K is closely related to subgroup A and uses the same entry receptor as that of ALV-A (7).

env and *LTR* sequences play an essential role in the virulence of ALV-K and ALV-J. In addition, *env* is mainly involved in the pathogenesis of ALV-K. After evaluating the genomic sequences of this subtype and checking the ability of the virus to replicate using the luciferase reporter method, as well as checking the rate of growth retardation and immune suppression in SPF chickens, they found that the reason for the difference in the rate of pathogenicity and virus replication is the difference in the sequences of these two genes (8).

Based on the transmission mode, they can be further classified into "exogenous" and "endogenous" retroviruses. ALVs transmitted from one bird to another through eggs or contact are called "exogenous" viruses, and all carcinogenic ALVs fall into this category. However, some ALVs exist as genome-integrated proviruses and are genetically transmitted as Mendelian genes, either as complete viral genomes (such as subtype E) or, more commonly, as incomplete genomes encoding retroviral products. Several such endogenous retroviruses have been identified in the chicken genome. They are generally non-carcinogenic but may affect the bird's response to infection by exogenous ALV by inducing immune tolerance (4).

Poultry neoplastic diseases include various conditions with a common feature: neoplasia consists of one or more types of cells that, with their growth and multiplication, sometimes cause organ dysfunction. Poultry neoplastic diseases are divided into two general categories: diseases with an infectious cause and non-infectious diseases (9).

Leukosis is also one of the contagious diseases that cause neoplasia, information about which has been already recorded.

Avian leukosis can also be classified according to the type of tumor that develops: lymphoid leukosis (LL), a tumor of B lymphocytes, is one of the most common forms of leukosis. LL occurs in chicks from about four months of age and is usually caused by ALV subgroups A and B. Significant pathologic changes include diffuse or focal enlargement of the bursa of Fabricius, liver, spleen, and other organs due to the incorporation of extravascular foci of immature lymphoid cells (4).

Erythroid leukosis or erythroblastosis is an unusual tumor of erythroid cells that occurs mainly in adult chickens. In affected birds, the liver, spleen, and sometimes the kidneys are moderately and diffusely enlarged and often bright cherry red. Myeloid leukosis (ML) broadly includes both myeloblastic (myeloblastosis) and myelocytic (myelocytomatosis) forms (10, 11).

Myeloid leukosis (ML) broadly includes both myeloblastic (myeloblastosis) and myelocytic (myelocytomatosis) forms. This disease is especially prevalent in broiler breeders infected with the ALV J subtype. However, the losses caused by this disease have decreased significantly in most parts of the world following the eradication of the primary breeding flocks. ALV is also associated with various solid tumors, including fibrosarcoma, chondroma, hemangioma, histiocytic sarcoma, mesothelioma, nephroblastoma, osteoma, and osteopetrosis (11).

2 Avian immunity

The immune response of birds can only be understood by knowing its basic structure. Lymphoid tissues are either epithelial in origin (e.g., thymus and bursa of Fabricius) or mesenchymal (e.g., spleen and bone marrow) and are colonized by hematopoietic cells through the blood. The primary lymphoid organs include the thymus and the bursa of Fabricius occupied by hematopoietic stem cells. Then, the mature cells enter the blood circulation regarding immunity, and the peripheral lymphoid organs, including the spleen, cecal tonsils, payer's patches and Meckel's diverticulum, Harderian gland, and peripheral lymph nodes in the intestine, bronchi, skin, and nose are colonized. T and B-dependent regions are mainly occupied by T and B cells in peripheral tissues (4).

3 Primary lymphoid organs

3.1 Thymus:

It appears on the fifth day of embryonic life and after hatching. It continues to grow until the age of 3-4 months and regresses with the onset of sexual maturity. Its peak activity occurs at a young age. The thymus is responsible for the maturation and differentiation of stem cells into thymus-dependent or thymus-derived lymphocytes or T cells, which play a significant role in cellular immunity (12).

3.2 Bursa of Fabricius/cloacal bursa:

It is a hollow, sac-like, or oval epithelial lymphatic extension of the hindgut located in the caudal body cavity and connected to the dorsal region of the cloaca by a short duct. Bursal mucosa has 11-13 longitudinal folds. It is responsible for the maturation and differentiation of stem cells into bursa-dependent or bursa-derived lymphocytes or B lymphocytes. B cell plays a significant role in antibody-mediated immunity. On the other hand this organ can play role as a secondary lymphoid organ (13).

4 Secondary lymphoid organs

Mature B and T cells from primary or mature lymphoid organs migrate to these sites via immune migration or peripheralization. These areas include several points of the body:

4.1 Gut-Associated Lymphoid Tissues (GALT):

These tissues extend throughout the digestive tract (14). The submucosal layer contains aggregates of lymphoid cells known as the esophageal tonsil, a component of the mucosa-associated lymphoid tissue (MALT). These lymphoid cells from 6 to 8 discrete units that encircle the opening to the pre-gastric region. A delicate fibrous sheath encapsulates the esophageal tonsil, commonly called "tonsillar crypt" (15, 16). The stomach contains tubular glands situated beneath the pre-gastric mucosa. Meckel's diverticulum, a vestigial remnant of the embryonic yolk sac, is found along the ileum. Lymphatic rings, located at the junction of the jejunum and the ileum, serve important immunological functions. Similarly, cecal tonsils are positioned at the interface of the ileum and the cecum. Peyer's patches, aggregations of lymphoid tissue, are distributed throughout the intestinal mucosa and are particularly prominent in the ileum, contributing to the immune surveillance within the gastrointestinal tract. (9).

4.2 Head-Associated Lymphoid Tissues (HALT):

The harderian or supraocular gland is an endocrine and immune-related gland that mainly produces IgA and other immunoglobulins. About 80% of the population of lymphoid cells are B lymphocytes, and 20% are T lymphocytes (15). The lymphatic tissues associated with the conjunctiva are especially prominent in turkeys, and the glands in the nasal cavity and lacrimal ducts are other lymphatic tissues related to the head (17).

4.3 Skin-Associated Lymphoid Tissues (SALT):

Lymphocytes are found scattered in some tissues like skin. Lymphatic structures are accumulations of lymph cells under the skin, among other lymphatic organs (9). It is notable that some of researchers have disputed the presence of SALT in birds (18, 19)

4.4 Bronchial Associated Lymphoid Tissues (BALT):

In chickens and turkeys, the initiation of respiratory humoral immune responses involves the participation of lymphoid tissues. These tissues are vital for developing local or respiratory mucosal immunity, a fundamental immune mechanism. Key components of these tissues include the presence of lymphoid cell aggregations within the submucosal layer of the respiratory tract, the existence of lymph nodes in the lung, and the involvement of the bronchial epithelium. These components collectively contribute to establishing and maintaining a robust immune response within the respiratory mucosa, wherein secretory IgA plays a pivotal role (20).

4.5 Spleen:

The largest secondary lymphatic organ consists of white and red parts (pulps) that comprise about 80% of the spleen tissue. The white pulp surrounds the blood vessels and has morphologically distinct areas (21).

4.6 Mural lymph nodes:

These are organized accumulations of lymphoid tissue that are circular, elongated, oval, non-capsulated, and contain diffuse lymphoid tissue within which are usually found three or four germinal centers either within or closely applied to the lymphatic vessels, especially those of the limbs and neck (22).

4.7 Pineal gland:

It is located between the hemispheres of the brain and the cerebellum. Bird glands maintain the circadian rhythm, especially in laboratory conditions (23).

4.8 Bone marrow:

This organ is a primary lymphatic organ, but after immune migration, it will also act as a secondary immune organ. As a secondary lymphoid organ, it contains B lymphocytes, mononuclear cells, and T lymphocytes (24).

4.9 Cervical-thoracic nodes:

They are placed in the entrances of the chest, and their structure is relatively simple. Due to the presence of a main or central sinus that probably forms an intranodal lymphatic vessel, the lymph flow in them is relatively fast (25).

Like all animals, chickens have a robust internal defense against diseases. Air, food, housing systems, and sick or carrier animals are responsible for entering most pathogenic agents into the environment. Birds' innate and adaptive immune systems protect and maintain their health. Together, they form an effective defense against invading pathogens.

5 Immunity associated with Avian leukosis

5.1 Active immunity

Under natural circumstances, chickens acquire exogenous Avian Leukosis Virus (ALV) infections from their fellow flock members or their environment. Following an initial period of viremia, the infected chickens produce virus-neutralizing antibodies against the viral envelope antigens, which reach high levels and remain present throughout their lifespan. These virus-neutralizing antibodies restrict the viral load within the birds, subsequently limiting the occurrence of neoplastic growth. However, it is generally accepted that these antibodies have a limited direct impact on tumor development. Upon inoculation of ALV in chickens at or after four weeks of age, a transient viremic phase becomes detectable after one week, followed by the appearance of antibodies at three weeks and beyond (26). Although antibodies against the gs antigen may also be present in ALV-infected birds, they do not influence tumor growth (4). In addition, previous studies have provided evidence for the existence of cytotoxic lymphocytes that specifically target viral envelope antigens in avian species that were immunized with either ALV or

Rous Sarcoma Virus (RSV) (15). These cytotoxic lymphocytes play a crucial role in cellular immunity, and it has been suggested that the Major Histocompatibility Complex (MHC) is involved in the regression of sarcomas (11, 27).

Chickens congenitally infected by ALV do not show an immune response to the virus. Instead, they become immunologically resistant to the virus and develop persistent viremia without neutralizing antibodies (28). Infection at a young age with subtype J, in particular, is likely to be the reason for immune tolerance (29, 30). Infection by ALV can reduce primary and secondary antibody responses and cellular immunity (28, 31).

5.2 Passive immunity

The breeder transfers serum antibodies, especially IgG, to its offspring through the egg yolk, producing passive immunity that lasts 3–4 weeks. Inactivating antibodies delay infection by ALV, reduce the incidence of viremia and virus shedding, and reduce the incidence of tumors (32).

5.3 Innate immunity

A specific field of study has focused on the role of innate immunity in response to ALV infection. Innate immunity is the first line of defense against viral infections and consists of various cells and molecules that can recognize and respond to viral pathogens. Studies have shown that some innate immune cells, such as natural killer (NK) cells and macrophages, play an essential role in controlling ALV infection in chickens. The mechanisms by which ALV evades the host's innate immune response are poorly understood. Recent studies have shown that cytokine-containing protein (CIS) homology with induced Src and suppressor of cytokine signaling 3 (SOCS3) can negatively affect the innate immune response and promote viral replication. In addition, ALV-J can suppress IFN expression indirectly by targeting host proteins, such as Disruptor of Telomeric Silence 1-like (DOT1L), which inhibits the activation of the type I interferon signal transduction pathway. P53 also plays a role in the host response to ALV infection by activating the innate immune response and inhibiting the ALV-J long terminal repeat (*LTR*) activity. They are considering the unique genomic structure of ALV as a retrovirus and its persistent infection in host cells. ALV may achieve innate immune evasion by more than blocking the signal transduction pathway for type I IFN expression. However, further studies are needed to determine the important ALV viral proteins that target cellular proteins involved in signaling pathways of the host's innate immune response (33).

5.4 Immune evasion

To replicate and disseminate within a host, a virus must be able to evade the host's robust immune system. In response to the viral invasion, the host activates various signaling systems that participate in immune defense mechanisms (34). Interferon (IFN) pathway activation represents the most crucial event in the host-pathogen interaction. Interferons play a significant role in the initial immune response to viral infections, as they bind to their receptors and regulate hundreds of interferon-stimulated genes (ISGs) via the JAK/STAT pathway. These genes interfere with viral replication and provide sufficient time to establish an appropriate adaptive immune response (35, 36). Based on homology and specific receptors, interferons are classified into type I, which includes IFN-alpha and IFN-beta, type II (IFN-gamma), and type III (IFN-lambda) (37). Type I and III interferons are considered the primary antiviral cytokines, and type II interferons also possess antiviral properties (38). Each interferon type can induce unique ISGs, although some ISGs overlap (39).

6 Other aspects of ALV infection

A 2021 study by researchers found that the expression of some genes related to the immune response, such as *TLR7* and *IL-6*, was increased in ALV-infected chickens. They also found that the expression of some microRNAs, such as miR-146a, was decreased in infected chickens, which may contribute to ALV-induced immunosuppression (40).

Another study investigated the effect of ALV infection on the gut microbiome in chickens. The researchers found that ALV infection alters the composition and diversity of the gut microbiome, which may affect the chicken's immune response. These consequences include the disruption of the type and number of different bacteria, which can be considered a result of virus pathogenesis. This study studied the feces of local chickens infected with subtype J using a 16S rRNA gene sequence. An increase in bacteria and proteobacteria and a decrease in Firmicutes were observed (41).

6.1 Genetic Resistance

Recent studies have shown that genetic resistance to ALV may be possible by identifying genetic markers associated with resistance. These markers can be used in selective breeding programs to breed chickens that are more resistant to ALV infection. A mutation in the *tvb* receptor gene confers resistance to infections by subgroups B, D, or E. Similarly, deletions that disrupt the mRNA splice variant of the *tva* receptor gene lead to reduced susceptibility to subgroup A infections. A single nucleotide polymorphism (SNP) has been identified in the *SLCO1B3* gene, associated with resistance to ALV-J infection in chickens. The *SLCO1B3* gene encodes an organic anion transporter protein involved in the cellular uptake of drugs and other substances. It has been found that the identified SNP in this study is associated with decreased expression of the *SLCO1B3* gene, resulting in reduced susceptibility to ALV-J infection (42).

ALV infection depends on the presence of subtype-specific cellular receptors, and birds that lack specific receptors are genetically resistant to infection by the respective ALV subtype (43, 44). The introduction of genetic resistance to ALV subtypes such as A and B has been used by poultry companies through selective breeding as an eradication strategy. Recently, new resistance using gene editing approaches has been introduced for ALV subtype J infection (45, 46).

Inheritance of cellular resistance to infection is a simple Mendelian type. Specific autosomal independent fragments control infection responses with ALVs of subgroups A, B, and C and are designated *tva*, *tvb*, and *tvc*, respectively. Each *tv* fragment has alleles for susceptibility and resistance, called *tvas*, *tvar*. *tvbs*, *tvbr*, and *tvcs, tvcr*, respectively; susceptibility alleles dominate over resistance alleles. These genes are usually abbreviated as *ar*, etc. Each fragment likely has multiple alleles that encode for different levels of susceptibility (47, 48).

Chickens with genetic resistance to infection and tumor induction by different subtypes of ALVs are usually unable to develop antibodies (49). Genetic resistance to tumor growth has been studied mainly with sarcoma, determined by a dominant gene, *R-RS-1*, located in the chicken MHC locus (50).

Cell surface protein Tva as an entry receptor for ALV of classical subtype A and new subtype K. Since both viruses are a significant concern for the poultry industry, a frameshift deletion in the *tva* fragment was introduced in a study aimed at eliminating Tva expression and creating a virus-resistant chicken line. Tva released by CRISPR/Cas9 gene editing in chicken primordial germ cells was inoculated into the testicles of recipient roosters. Tva-negative chickens showed complete resistance to subtypes A and K of the ALV. In contrast, Tva-positive relatives were sensitive. Also, in this experiment, they found a specific impairment of cobalamin/vitamin B12 metabolism in *tva*-deleted chicks, consistent with the recently recognized physiological function of Tva as a cobalamin receptor in complex with the transcobalamin transporter (45).

The MHC class I (MHC-I) in birds, also known as the MHC-B complex, is a set of genes, many of which are polymorphic, that play a crucial role in immune responses. These genes encode proteins essential for the immune system and are called antigen-presenting molecules involved in antigen recognition and elimination. The diversity within these genes may contribute to the functional nature of their involvement in resistance against leukosis viruses (51).

In a recent study, CRISPR/Cas9 technology was employed to generate genetically modified chickens with a specific mutation in the chicken ALV-J receptor Na+/H+ exchanger type 1 (chNHE1). Recent research provides compelling evidence that this mutation offers protection against the virulent ALV-J prototype strain HPRS-103 in a commercially bred chicken line (NHE1ΔW38). It is observed that a significant impairment in the replication of HPRS-103 in NHE1ΔW38 birds and ALV-J-specific antigen was not detected in cloacal swabs at later time points. Moreover, NHE1ΔW38 chickens infected with ALV-J exhibited greater weight gain when compared to their non-transgenic counterparts (NHE1W38). Histopathological analysis revealed the development of ALV-J typical pathology in various organs of NHE1W38 chickens, while no pathological lesions were observed in NHE1ΔW38 chickens. Collectively, these findings demonstrate that this mutation has the potential to confer resistance to highly pathogenic ALV-J infection in a commercial chicken line, offering promising prospects for combating this pathogen and improving overall animal health in real-world settings (52).

A study conducted by Zhao et al. aimed to investigate the potential influence of the PMAIP1 gene on the replication of avian leukosis virus subgroup J (ALV-J) and the underlying regulatory mechanisms. The researchers initially examined the expression of PMAIP1 using quantitative real-time PCR (qRT-PCR) both in vitro and in vivo. Furthermore, they manipulated the expression of PMAIP1 in chicken fibroblast cells (DF-1) and evaluated its impact on ALV-J infection

through qRT-PCR, immunofluorescence assay (IFA), and western blotting (WB). The results of the study demonstrated a significant downregulation of PMAIP1 in the spleen, lung, and kidney, accompanied by an upregulation in the bursa and liver of chickens infected with ALV-J when compared to uninfected chickens. Additionally, DF-1 cells infected with ALV-J exhibited a noticeable upregulation of PMAIP1 at various time points. The overexpression of PMAIP1 was found to enhance ALV-J replication, interferon expression, and the production of proinflammatory factors. Conversely, interference with PMAIP1 led to contrasting outcomes. Furthermore, the study revealed that PMAIP1 promotes virus replication by influencing mitochondrial function. In conclusion, the findings suggest that the PMAIP1 gene facilitates ALV-J replication by modulating mitochondrial function, thereby contributing to our understanding of mitochondria-related genes and their involvement in ALV-J infection (53).

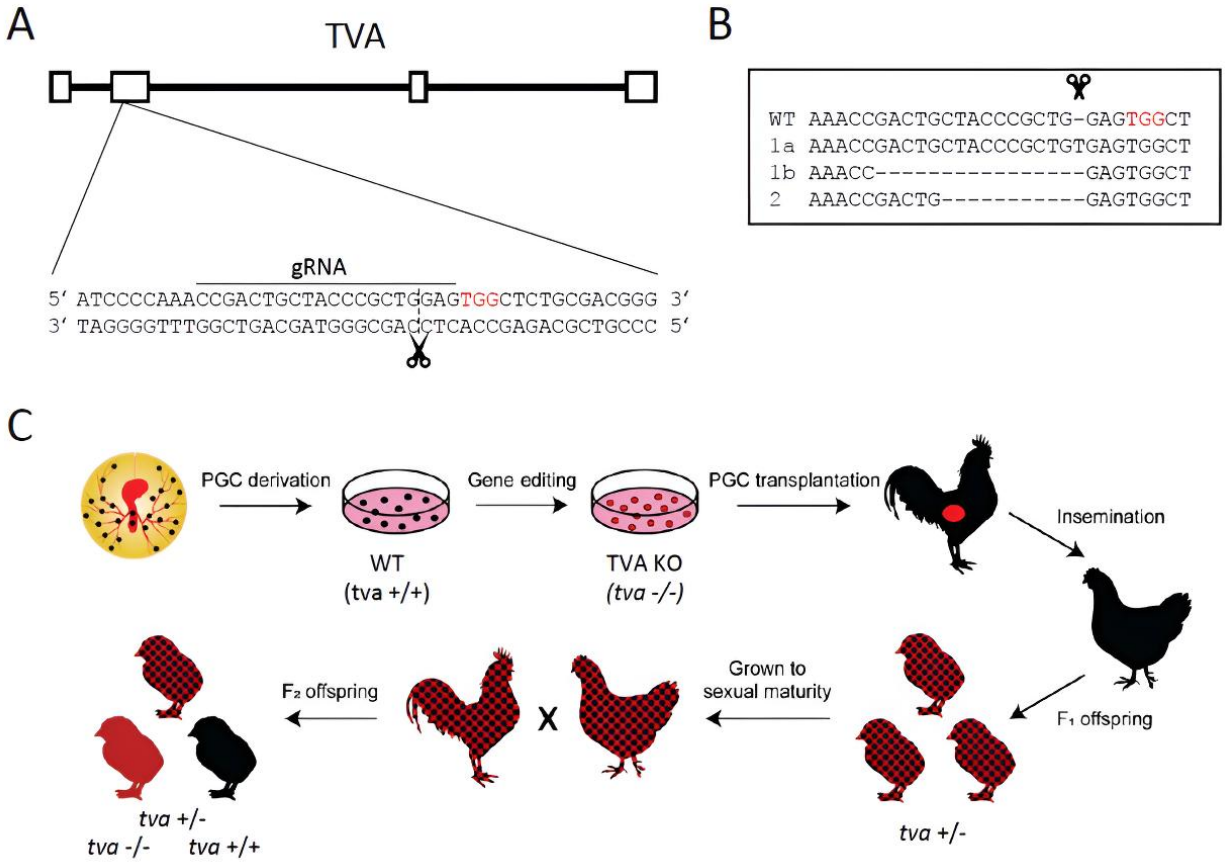

Figure 2. (A) Schematic representation of the *tva* coding sequence with exons and introns (top) and the design of gRNA used for CRISPR/Cas9 targeting of the *tva* gene (bottom). The target region of the *tva* gene is shown with the cleavage site (scissors), PAM sequence (in red), and gRNA sequence depicted in the line above. (B) Alignment of nucleotide sequences of the target region in wt and two PGC clones was used for further work with out-of-frame deletions. (C) Schematic summary of the workflow and timeline during genera -/- chickens. (45)

7 Diagnosis of Avian Leukosis

Oncogenic retroviruses vertically transmit the ALV, which is economically important and affects laying and broiler chickens, ducks, and even wild birds. They may also affect poultry vaccines during preparation (especially in eggs) to infect (54).

Leukosis in different species of birds causes different clinical symptoms such as ruffled feathers, emaciation, grounding, pale color of the crown and beard, and reduced egg production in laying hens (55). Also, infection can lead to disruption of cell growth and the occurrence of neoplastic changes, and it is primarily related to myeloid leukosis (56). Most infected farm chickens show subclinical symptoms such as reduced egg production, immunosuppression, or

growth retardation (57). Subclinical leukosis infection causes economic problems much more than its clinical form, which causes tumors and death. The prevalence of leukosis in chicken flocks has been reduced and controlled in most countries with an advanced poultry industry (58).

The host specificity of each viral subgroup is determined by the gp85 protein, which forms spherical structures on the viral surface and plays a role in the viral attachment process (59, 60)

There are many techniques for viral sample identification. Conventional methods for virus detection are time-consuming, expensive, and often poor, requiring specialized facilities and trained personnel. Simple instrumentation and fast and low-cost virus detection can prevent the spread of the virus (61). In the last decade, the occurrence of avian leukosis due to the international ALV eradication program has been reduced (58). Because new recombinant strains and even new subtypes have emerged frequently in recent years, the risk of developing leukosis still exists (62). K strain, as mentioned, is one of the recently identified strains. However, these isolated strains of ALV-K had no or little pathogenicity (63). A specific type of ALV-K named HB2015032 was found in Hubei Province, China, in 2015, which showed tumorigenicity (64). These new ALV-K strains indicate an increased prevalence and pathogenicity of ALV-K in chicken flocks in China. (65).

There is still no specific and rapid diagnostic method for ALV-K. For diagnosis, a specific antibody that recognizes ALV-K is needed. In a study, a monoclonal antibody (Mab) called Km3 against the GP85 protein gene of ALV-K was prepared, and the antigenic epitope was defined as Mab. It may serve as a useful tool for diagnosing ALV-K in the future. gp85 gene of ALV-K HB2018003 is amplified with a list of specific primer pairs. the gp85 gene was cloned into prokaryotic expression vectors and transformed into E. coli BL21 (DE3). Gp85 production was induced and purified using affinity chromatography. Various methods, including ELISA and immunofluorescent antibody, were used for identification. The epitope recognized by Mab Km3 was determined by dividing the gene into fragments, amplifying them through PCR, and analyzing their interaction with Km3 through western blot analysis. The analysis of the obtained data revealed specific peptide segments of the Gp85 protein associated with subgroup K (66).

Seminal extracellular vesicles (SE) were isolated and purified from the seminal plasma of ALV-J infected roosters (SE-ALV-J), which were shown to contain ALV-J genomic RNA and proteins by RNA sequencing, quantitative PCR, and western blotting methods. In addition, SE-ALV-J was shown to transmit ALV J infection to host cells and establish infection from the mother herd. Furthermore, artificial inoculation experiments showed that SE-ALV-J transmitted ALV-J infection to SPF chickens and subsequently caused vertical transmission of ALV-J from SPF chickens to offspring (67).

One recently used method is proteomic analysis. In this method, protein samples are digested with trypsin solution at 37 degrees Celsius for 4 hours with an enzyme: protein ratio of 1:20 by weight. The resulting peptide mixture is extracted, desalted, and dried. Nano LC-MS/MS performs proteomic analysis. Finally, this peptide is ionized by the nanoESI source and then enters the Q-Exactive HF X mass spectrometer to detect the dependent data mode (DDA) (68).

In a study with histopathological analysis of samples collected from chickens and ducks with tumors and molecular analysis of gp85 surface protein antigen by determining the recombination between different strains, the relationship between ALV-J and tumor formation was investigated, and the results of greater enlargement internal organs and in some cases, nodular enlargement was observed. Histopathological examinations indicated the penetration of myeloid cells in the studied tissues (69).

Subgroup E of leukosis virus, endogenous retroviruses are limited to domestic birds and their wild offspring. In industrial chickens, ALV-E has a harmful effect on production and is a source for recombining exogenous retroviruses. Researchers identified 974 avian leukosis virus subgroup E (ALV-E) strains from 407 sampled chickens in Ethiopia, Iraq, and Nigeria. Advanced bioinformatics methods were employed to analyze the whole genome sequencing data and identify ALV-E insertions. Confirmation was done using PCR assays, and a binary matrix was created to indicate the presence or absence of each ALV-E in each bird. The data was visualized using t-SNE and hierarchical clustering. The genotypes were associated with geographic, phenotypic, and epidemiological data. The distribution of ALV-E genomes was analyzed and compared to a simulated dataset. The study revealed over 850 novel ALVEs, significantly expanding the known diversity of these retroviral elements (70).

In a novel approach, a real-time reverse transcription recombinase-aided amplification (RT-RAA) assay was developed to detect ALV. Specifically, a 114-bp target sequence from the ALV P12 gene was amplified using reverse transcription recombinase-aided amplification. This

method demonstrated rapid, highly specific, cost-effective, and simple operational characteristics. It could detect as low as 10 copies of ALV RNA molecules per reaction without cross-reactivity with other avian viruses. Furthermore, the RT-RAA assay exhibited a high level of concordance with reverse transcription polymerase chain reaction (RT-PCR) for clinical samples, indicating its potential as a valuable diagnostic method for ALV detection (71).

The gp85 protein is recognized as the primary envelope protein of avian leukosis subgroup J (ALV-J) and plays a crucial role in virus neutralization. In a study, researchers aimed to map the specific epitope within the ALV-J gp85 protein using synthetic peptides and develop diagnostic methods based on this epitope for detecting ALV-J infection. The study results revealed that the monoclonal antibody JE9 recognized a specific motif, ^{83}WDPQEL^{88}, within the gp85 protein. Through homology analysis, it was determined that this motif was highly conserved among various ALV-J strains. Furthermore, the performance of the epitope-based peptide ELISA was evaluated using 240 serum samples obtained from different chicken farms. Notably, the epitope-based peptide ELISA demonstrated significantly higher sensitivity for antibody detection of ALV-J compared to a commercially available ELISA kit. In conclusion, a novel B-cell epitope recognized by the JE9 monoclonal antibody was identified in the study. The peptide-based ELISA developed based on this epitope holds potential for laboratory viral diagnosis, routine surveillance in chicken farms, and advancing our understanding of the pathogenesis of ALV-J (72).

8 Control and Eradication methods

ALV can be transmitted vertically (congenital or oocyte) or through contact via horizontal routes. In vertical transmission, eggs are infected with the virus in the oviduct, which leads to embryo infection during development. This route of congenital infection leads to birds becoming viral carriers by shedding the virus in vaginal swabs, egg albumin, and embryos, whose detection provides the basis for ALV eradication programs. Congenitally infected chickens Tumors of the avian immune system are important sources of contact infection in the hatchery and during the rearing period, and the meconium and feces of congenitally infected chickens contain high concentrations of ALV. The horizontal transmission mode is responsible for the high incidence of infection in the flock (11).

A 2022 research study in China found that ALV infection was widespread among all five chicken breeds, including three specific Chinese local chicken species. The local breeds had a higher incidence of ALV infection, highlighting the need for intensified eradication efforts in these breeds. Current diagnostic methods, such as the ELISA kit and test strip, cannot differentiate between exogenous and endogenous ALV strains. However, clinical investigations revealed typical Avian Leukosis symptoms in diseased birds from positive flocks, including abdominal enlargement, liver tumors, shank protuberances, and hemangiomas. These findings provide valuable insights into assessing virus infection status and associated risks in poultry farms. Future research should focus on isolating and studying the subtypes of exogenous epidemic ALV strains using DF-1 cells, which exclude the growth of the endogenous virus. Long-term and continuous ALV eradication efforts are crucial for conventional and local chicken breeds, supported by real-time epidemiological monitoring, to effectively prevent and control this poultry tumor disease (73).

ALV is known to cause B-cell lymphomas and other malignancies in chickens by activating oncogenes through insertional mechanisms. Activation of the *c-myc* oncogene has been frequently observed in ALV-induced tumors. In a study performed in 2022, Roy et al. utilized the HP45 cell line, derived from ALV-transformed B-lymphoma, and employed in situ CRISPR-Cas9 editing to specifically target and delete the integrated proviral long terminal repeat (LTR). The aim was to investigate the effects of LTR deletion on gene expression and cell proliferation. They found that targeted deletion of the LTR resulted in a significant decrease in the expression of multiple LTR-regulated genes, including *c-myc*. Furthermore, LTR deletion induced apoptosis in HP45 cells, impacting their proliferation and highlighting the importance of LTR-mediated regulation of crucial genes. While the deletion of multiple LTR-regulated genes had global effects on gene expression and cellular functions, the deletion of *c-myc* pronounced impacted HP45 cell proliferation, resembling the phenotype observed with LTR deletion. This underscores the significance of *c-myc* expression in ALV-induced lymphomagenesis. Overall, these findings demonstrate the potential of targeted LTR editing for inhibiting retrovirus-induced transformation on a global scale and provide insights into the roles of LTR-regulated genes in ALV-induced neoplastic transformation (74).

The successful eradication of ALV hinges upon interrupting the vertical transmission of the virus from

infected hens to their offspring, as well as preventing subsequent reinfection. Eradication strategies primarily revolve around identifying and removing hens that serve as sources of ALV transmission to their egg albumin, thereby safeguarding the embryos and resulting chicks. By targeting these key transmission pathways, efforts can be directed towards effectively mitigating the spread of ALV within poultry populations. Such hens that are avirulent and shedding virus are usually detected by ELISA testing their cloacal/vaginal swabs, semen, or egg albumin for high levels of gs-ALV antigen. Continuous monitoring and removal of infected birds breaks the virus transmission cycle and eradicates ALV (74).

In a recent study in Egypt, a comprehensive investigation of avian leukosis virus subgroup J (ALV-J) was conducted, involving the collection and analysis of 117 blood samples and 57 tissue specimens from various organs. The study aimed to determine the prevalence of this viral tumor disease in broiler flocks located in the El-Sharqia, El-Dakahliya, and Al-Qalyubiyya Egyptian governorates from 2021 to 2023, employing different diagnostic techniques and genetic diversity determination of the ALV-J gp85 gene. ALV-J was successfully isolated using chicken embryo rough cell culture, leading to observable signs of aggregation, rounding, and degeneration. Notably, embryonic death, stunting, and curling were observed upon egg inoculation. Out of the 117 serum samples tested using ELISA, 79 samples (67.52%) were positive for ALV-J. Histopathological examination revealed the presence of tumors characterized by well-differentiated myelocytes, lymphoid cells, or both, primarily observed in the liver, spleen, and kidneys. Immunohistochemical analysis confirmed the presence of myelocytomatosis-positive signals in these organs. Polymerase Chain Reaction (PCR) analysis targeting the ALV-J gp85 gene confirmed the presence of a 545-base pair fragment in 43 out of the 57 positive samples (75.4%). Additionally, two of the positive samples were sequenced and deposited in the Genbank with accession numbers OR509852-OR509853. Phylogenetic analysis based on the gp85 gene demonstrated that the ALV-J Dakahlia-2 isolate shared genetic relatedness with ALV-EGY/YA 2021.3, ALV-EGY/YA 2021.4, ALV-EGY/YA 2021.14, and ALV-EGY/YA 2021.9, exhibiting amino acid identity percentages of 96%, 97%; 96%, and 96%, respectively. This study provides important insights into the prevalence and genetic characteristics of ALV-J in Egyptian broiler flocks, contributing to the understanding and management of this viral disease (75).

9 Vaccination

A research study conducted in Nigeria aimed to assess the presence of ALV subgroups in commercial live and inactivated viral vaccines. Forty-four vaccines were collected from various distributors and veterinary sales centers in Nigeria, and PCR screening was performed to detect ALV subgroups A, B, C, D, and J. The results showed that 9% of the tested vaccines were positive for ALV-J, while no other subgroups were detected. These findings indicate contamination of ALV-J in commercially sold poultry vaccines in Nigeria, emphasizing the urgent need for comprehensive screening and quality control measures in vaccine production and distribution within the poultry industry (76).

Autophagy represents a vital cellular process implicated in the degradation of proteins and damaged organelles, serving a fundamental role in maintaining cellular homeostasis within eukaryotic organisms. Moreover, autophagy plays a significant role in the immune response by protecting the host against pathogenic invasions. However, it has been observed that avian leukosis virus subgroup J (ALV-J) infection impedes the autophagic process within infected cells. Researchers embarked on a recent investigation to explore potential strategies for the development of ALV-J vaccines. In one field of study, autophagy induction was achieved using rapamycin, a pharmacological agent known to stimulate autophagy. A DNA vaccine was also developed, targeting autophagy and the major antigens associated with ALV-J. Laboratory experimentations confirmed the successful expression of the fusion protein under controlled conditions. The study demonstrated that combining the vaccine with rapamycin enhanced its effectiveness in combating ALV-J infection, primarily by promoting autophagy induction. These findings suggest that developing targeted vaccines capable of modulating autophagy represents a promising avenue for ALV-J vaccine research and development (77).

In a study on avian leukosis virus subgroup J (ALV-J) vaccines, researchers investigated using genetically modified *Lactobacillus plantarum* NC8, an invasive bacterial strain, as a delivery vehicle for ALV-J antigens. The modified strain expressed the gp85 protein from ALV-J and was administered orally to chicks. The immune response was assessed through various parameters, including T lymphocyte quantification, splenocyte proliferation, cytokine levels, antibody measurements, and examination of mucosal antibodies. The study found that the modified *L.*

plantarum strain significantly enhanced chicks' cellular, humoral, and mucosal immune responses. This approach shows promise for developing live ALV-J vaccines, highlighting the potential of invasive bacterial strains as effective delivery platforms for ALV-J antigens (78).

Researchers have developed a multi-epitope vaccine targeting avian leukosis virus subgroup J (ALV-J) by identifying a neutralizing epitope called hr1 within the hypervariable region of ALV-J. The multi-epitope gene was expressed in *Escherichia coli* BL21, and the resulting protein specifically reacted with ALV-J-positive sera. The vaccine demonstrated strong immunogenicity, with high antibody titers achieved within 42 days of immunization in chicks, and the immunity provided by the vaccine persisted for at least 126 days. Importantly, all vaccinated chicks were effectively protected against the ALV-J challenge. These findings highlight the potential of multiepitope vaccines as a promising strategy for combating highly variable viruses and provide valuable insights into developing effective ALV-J vaccines using epitope-based approaches (79).

10 Conclusion

Ongoing research on ALV has revealed important insights into its impact on poultry populations, helping to develop better control measures and reduce economic losses. Recent studies have focused on identifying viral subgroups, with specific attention given to the gp85 protein and its role in determining host specificity. Additionally, advancements in diagnostic methods, such as proteomic analysis and real-time reverse transcription recombinase-aided amplification (RT-RAA) assays, have provided more efficient and rapid means of detecting ALV infections. Furthermore, investigations into the transmission routes, both vertical and horizontal, have contributed to the development of control and eradication strategies, targeting the interruption of viral spread and the removal of infected individuals. These newer research findings have expanded our understanding of ALV pathogenesis and epidemiology and hold promise for improved diagnostics and management practices in the poultry industry, paving the way for more effective control and prevention measures in the future.

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Conflict of Interest

The authors declare no competing interests.

Author Contributions

Morteza Nikzad and Mohammad Hassan Bozorgmehri Fard were involved in the idea, design, data collection, and paper preparation. Mohammad Hassan Bozorgmehri Fard contributed to the study's supervision, as well as the manuscript's drafting. All authors have approved the final version of the article.

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