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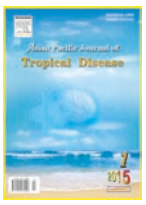

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Novel insights into identification of shedders and transmitters of avian leukosis virus

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ABSTRACT

Avian leukosis virus (ALV) infection is one of the most common infections in domestic poultry. Even though loss due to mortality is only 1%-2%, it causes drastic decline in production because of subclinical infections. Exogenous ALV infected birds follow a complex pattern of transmission. Transmission is strongly related to immune status of the birds against ALV. Based on the ability to transmit ALV, infected birds can be classified to either shedders or transmitters. The knowledge on shedders and transmitters of exogenous ALV will facilitate in implementing an effective strategy to eliminate ALV. Identification of these shedders and transmitters is of absolute necessity at breeding stock to achieve ALV free status. Presently group-specific antigen used based ELISA cannot differentiate endogenous and exogenous ALV infections. Advances in molecular diagnosis make better differentiation of various subgroups and few tests like loop mediated isothermal amplification, can be used as pen-side tests. Besides this identification of infection, selection of genetically resistant strains of birds to ALV also holds a key role in elimination of ALV. Thus, combination of different strategies in the breeding programs may facilitate eradication of ALV infection. Here in this review, we have discussed about biology of ALV shedders and transmitters, with reference to eradication programs.

1. Introduction

Infection of chickens with avian leukosis virus (ALV) is the most common among avian leukosis/sarcoma virus (AL/SV) infections encountered in the field flocks. It causes neoplastic, non-neoplastic and subclinical infections, thereby significantly affects the economy of poultry industry throughout the world[1,2]. Even though neoplastic conditions causes mortality in 1%-2% of birds infected with AL/SV[3], ALV can cause mortality up to 20%[4]; losses due to reduction in economic traits are tremendous, mainly due to non-neoplastic and subclinical infections of birds[3]. ALV is considered one of the most common immunosuppressive viruses in chickens[5-7].

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ALV infection spreads in the flock by three modes: i) horizontal transmission, ii) vertical or congenital transmission and iii) genetic transmission[8,9]. Based on the transmission of virus to the progeny birds are categorized as shedders and transmitters. ALV shedders are those birds, which are infected with ALV, shed virions or the group-specific antigen (Gag) into cloacae or egg albumen. ALV infected birds that transmit virions to progeny are referred to as transmitters[10]. These shedder and transmitter birds are major threat to breeding stock, thus eradication programs target towards identification and elimination of both shedders and transmitters in the breeding flocks; thereby, breaking the vertical transmission of virus from dam to progeny and prevention of reinfection of poultry flock through the progeny chicks. For designing a better program to eliminate shedders and transmitters, it is of almost importance to have a clear knowledge on biology of these birds, thus loss of good genetic stock can be avoided because of misinterpretation. Here we review behaviour of ALV infection in terms of Gag or virion shedding/transmission to their progenies.

2. AL/SV

AL/SV are classified under genus *Alpharetrovirus* in subfamily Orthoretrovirinae and family Retroviridae[11]. ALV is the type species of the genus *Alpharetrovirus* and causes lymphoid leukosis (LL) in many species of birds including domestic chickens, responsible for causing significant economic losses due to various neoplastic/non-neoplastic conditions, morbidity and subclinical production losses.

AL/SV has an outer viral envelope and inner core consisting of capsid, matrix, nucleocapsid, with two molecules of linear positive-sense single stranded RNA arranged as an inverted dimer. The size of genome is about 7.3 kb. Sequence of the structural genes of ALV, from 5'-3' end of RNA molecule, is gag/pro-pol-env, flanked by long terminal repeats (LTR) region in the proviral DNA (Figure 1). The virion core contains 5 non-glycosylated proteins encoded by the *gag/pro* gene: matrix proteins (p19, p10) and capsid protein (p27), which is the major Gag in the core; nucleocapsid (p12), and protease (p15). The *pol* gene encodes reverse transcriptase (RT) and integrase (p32) enzyme. The *env* gene encodes two viral envelope glycoproteins: surface (gp85) and transmembrane (gp37). These two envelope proteins are linked to form a dimer, termed virion glycoproteins[3,12].

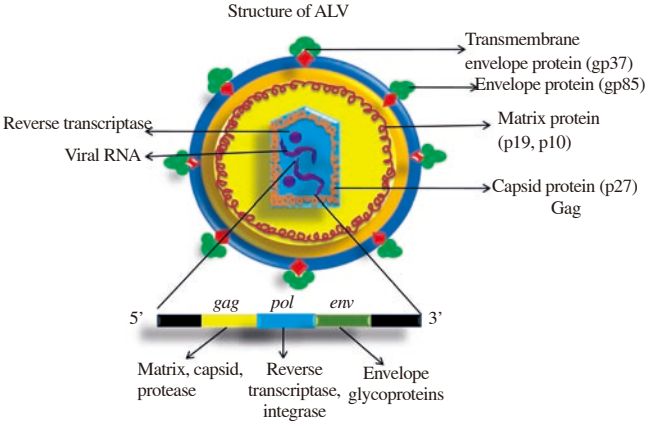

Figure 1. Structure of ALV.

2.1. AL/SV subgroups

ALVs that affect chickens have been classified into six well defined subgroups A, B, C, D, E, and J[13-15] based on differences in their envelope glycoproteins, interference patterns, host range in chicken cells of varying phenotypes[16] and serum neutralization[6]. Other subgroups, *viz.*, F, G, H, and I, represent endogenous ALVs that infect pheasants, partridge and quail[17]. Genes encoding gp85 of ALV subgroups A-E have 2 hypervariable regions, hr 1 and hr 2 and; 3 less variable regions, vr 1, vr 2 and vr 3, which are responsible for the differences between the subgroups. But, the recently evolved subgroup J differs extensively from other subgroups, notably at hr 1, hr 2, vr 2 and vr 3[18,19].

2.2. AL/SV forms

2.2.1. Exogenous viruses

Subgroups A, B, C, D, and J that infect chickens are exogenous ALVs. Based on genome sequences, exogenous viruses can be divided into two types: 1) replication competent, which have complete genomic sequence 5' gag/pro-pol-env 3'[1] and 2) replication defective, which lack any of the *gag/pro*, *pol* or *env* genes, but usually acquire oncogene(s) from any cellular oncogenes to produce malignant tumours rapidly.

2.3. Endogenous viruses

Normal chicken genome also contains at least four families of avian retrovirus-like elements that are transmitted genetically in a Mendelian fashion to their progeny by both sexes[8]: i) endogenous viral (ev) loci; ii) endogenous avian retrovirus (EAV); iii) avian retro-transposon from chicken genome and iv) chicken repeat 1 (CR 1).

Ev loci-genetic sequences of ev loci are related to subgroup E ALVs and are present as either complete or defective genomes in almost all normal chickens[20]. ALV-E proviruses entered the chicken germline after speciation but before domestication. All chickens that possess *env* gene exhibit a common subgroup specificity designated as 'E' regardless of the ev loci, since sequence homology of *env* gene expresses identity of approximately 85%-90%. ALV-E proviruses, if complete and transcriptionally active, are able to produce infectious viral particles, either spontaneously or after induction[21].

EAV-EAVs are not expressed as infectious particles, but its reverse transcriptase activity may be expressed and found in live viral vaccines. EAV-HP (ev/J) elements play an important role in emergence of ALV-J, showing uniquely close relationship to the ALV-J prototype clone HPRS-103 env region[20,8].

Avian retro-transposon from chicken genome was found to be a recently acquired component of the chicken genome, since it is not found in the genome of other bird-species, including such closely related species as turkeys and quails. Though, avian retrotransposons are transcriptionally active and so far no protein products are found to be encoded by these elements[8].

CR 1 is a short interspersed repetitive DNA element belonging to the non-LTR class of retrotransposons[22]. CR 1 elements have been identified in several avian and reptilian species, demonstrating that they are ancient sequences that arose before the divergence of birds and reptiles and are not functionally expressed[23].

2.4. AL/SV transforming types

Additionally, based on rapidity of tumor induction, AL/SV can also be classified as: i) acutely transforming viruses, which induce neoplasm within few days of infection (all acute ALVs are defective in their genomes, but carry viral oncogenes and); ii) slowly transforming viruses, which are non-defective and do not carry any oncogenes. They induce tumors by insertional mutagenesis, and development of tumors takes many weeks to months[24]. Even though Rous sarcoma virus is an acutely transforming virus, it has complete genome.

3. Replication of virus

Replication of ALV is characterized by formation of a DNA provirus that becomes linearly integrated into the host cell genome under direction of reverse transcriptase. It is as similar as other retroviruses, with exception of specific receptors used for attachment to the target cell by specific subgroups viral envelope proteins.

Different ALV subgroups have different receptor specificities. The receptor for ALV subgroup A (ALV-A), designated as TVA, encoded by tva gene, is related to the family of human low-density lipoprotein receptors. The chicken tvbs3 and tvbs1 alleles encodes cellular receptors (TVB) for subgroups-B (ALV-B), -D (ALV-D), and -E (ALV-E), and for ALV-B and ALV-D, respectively. TVB proteins are members of the tumor necrosis factor receptor (TNFR) family and are most likely the

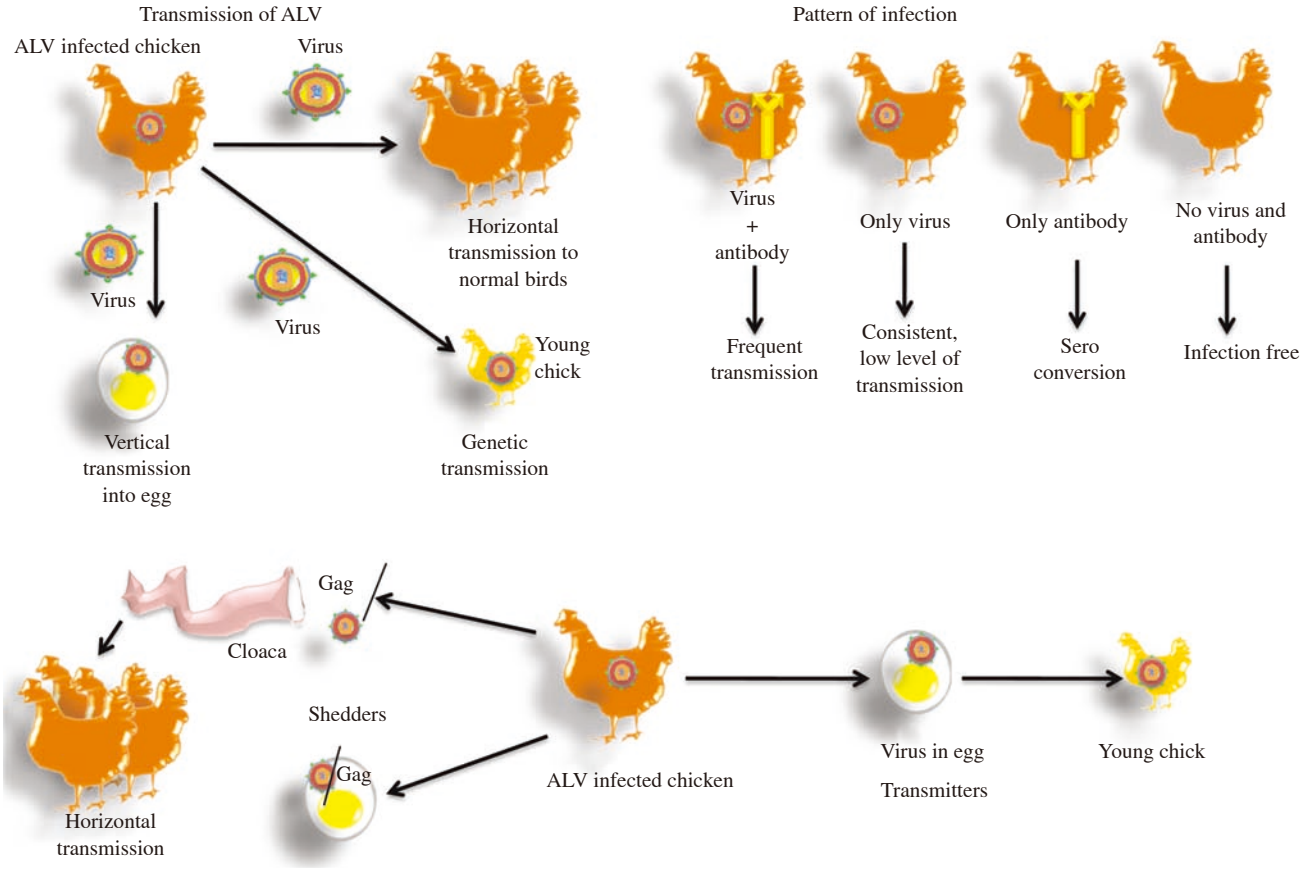

Figure 2. Complex phenomenon of shedders and transmitter of ALV.

avian homologs of the mammalian TNF- related apoptosis inducing ligand (TRAIL) receptors. The receptor for subgroup-C (ALV-C) TVC, encoded by *tvc* gene, is related to mammalian butyrophilins, members of the immunoglobulin superfamily[25]. The host cell receptor used by subgroup-J (ALV-J) is chicken N+/H+ exchanger type 1 (chNHE1) protein[4].

4. Transmission of AL/SV

Under natural conditions, ALVs spread in the flock by three modes: i) horizontal transmission, when the virus is passed from bird to bird by direct or indirect contact; ii) vertical or congenital transmission, when the virus is passed from hen to offspring through egg albumen to embryo and iii) genetic transmission, when endogenous viral genome is transmitted from parents to offspring[8].

Pattern of infection has strong impact on mode of transmission. Depending on the presence or absence of viremia, antibodies, or both, birds can be classified into: i) viremic, antibody-positive/ immune (V+A+); ii) viremic, antibody-negative/ non-immune (V+A-); iii) non-viremic, antibody-positive/ immune (V-A+) and iv) non-viremic, antibody-negative/ non-immune (V-A-)[26]. V-A- birds belong to ALV infection free flock or genetically resistant stock[3,8].

5. Shedders and transmitters

Infected birds that shed virions or Gag into cloacae or egg albumen are classified as 'shedders'[27]. ALV infected birds that transmit virions to progeny are referred to as 'transmitters'. More than 60% shedders transmit the virus vertically to their progeny[28]. The shedders release virus in large amount into the environment hence the hatchmates are infected horizontally[27]. ALV infected birds present a complex pattern of shedders and non-shedders. Based on presence or absence of viremia, antibody and shedding of virions or Gag into egg albumen or cloacae, shedders (S+) and non-shedders (S-) may be classified as: i) V+A+S+ ii) V+A-S+ iii) V-A+S+ iv) V-A+S- v) V-A-S-[10](Figure 2).

Viremic tolerant (V+A-) chicken shed Gag persistently into cloacal swabs and albumen, in turn, transmitting virus congenitally to their progeny, however, at a much lower rate. A small proportion of V+A+ birds shed Gag in cloacal swabs but intermittently into egg albumen and transmit ALV congenitally to their progeny more intermittently. At later stages of infection V+A+ birds, sero-convert from a transient viremic state to an V-A+ state and these birds, shedding Gag at low rates in cloacae and albumen, are considered as non-shedders[29]. There is tendency for congenital transmission in V+A+ hens which are more frequent in the hens with low antibody titre[30].

Virus budding occurs in various cell types in the ovary but not in the follicular cells or ovum, thus precludes transovarial transmission[31]. Shedding of ALV into egg albumen and transmission to embryo is a consequence of virus production by albumen secreting glands of the oviduct. As the ovum passes through the parts of oviduct it acquires the ALV infection in oviduct, where shedding of virus is more. Embryo-infection is closely correlated with ALV produced at the oviduct, but not with ALV transferred from other parts of the body[27]. Not all the eggs that have ALV in the albumen give rise to infected embryos or chicks[31,30]. In contrast, embryos positive for virus were obtained from the eggs that were negative for albumen Gag[28,32]. Persistent infection is strongly associated with tumor mortality and virus transmission. But, occasionally non-viremic immune hens also transmit virus to their progeny[33]. Virus-producing *ev* genes were a predisposing factor for shedding ALV[34]. Endogenous viruses were transmitted to a high

percentage of embryos regardless of whether or not the hens shed Gag into eggs[35]. Moreover, endogenous viruses present in chicken genome influence exogenous virus infection and disease progression[36].

Horizontal transmission occurs among birds through direct or indirect contact of secretions, excretions, remnants of cornified cells from skin and contaminated feed/ water *etc*., of infected birds[33]. In cocks, virus budding has been seen on all the structures of reproductive organs except germinal cells[37], thus cock acts as a virus carrier and source of venereal infection to other birds[38].

6. Clinical effects of AL/SV

AL/SV produces neoplastic diseases, non-neoplastic conditions and subclinical infections resulting in tumor mortality, non-specific mortality and production losses.

6.1. Neoplastic diseases

Acutely transforming viruses carry a viral oncogene responsible for rapid malignant conversion of target cells. Slowly transforming viruses *i.e.* ALV, which do not possess an oncogene, are able to induce a broad spectrum of tumors after a long latency by a mechanism of insertional mutagenesis, provirus integration into the genome near a cellular proto-oncogene[26]. The cellular proto-oncogene becomes activated by the promoter or enhancer sequence of the LTR, leading to abnormal expression of the oncogene and neoplasia. Both the *LTR* and *env* genes of ALV are known to play important roles in the oncogenicity and tissue tropism of virus[39]. All the avian acutely transforming viruses are genetically defective and require a helper leukosis virus to complement them and enable replication (*eg.* BH-RSV). Endogenous viruses have little or no oncogenicity, because of its weak promoter activity of LTRs.

AL/SVs induce leukoses affecting the erythroid, lymphoid, and myeloid series of hematopoietic cells, and a number of solid tumors[40]. These neoplastic conditions include LL, myeloblastosis, myelocytomatosis, haemangiomas, erythroblastosis, osteopetrosis, sarcomas, granulose cell tumours, epitheliomas and nephroblastomas. The oncogenic spectrum of virus strains is wide, though they produce tumors characteristic of that particular strain. The type of ALV-induced tumor is influenced by strain of virus, exposure dose, host genotype and sex, route and age at exposure[3].

The commonest neoplasm induced by slowly transforming ALV is LL. The incubation period is rarely less than 14 weeks, LL is usually a neoplastic disease of breeders and commercial egg-layers[41]. LL is a bursal lymphoid (B) cell lymphoma of chickens that originates in the follicles of bursa of Fabricius and metastasizes to the liver, spleen, and other visceral organs[42]. ALV integration to the *c-myc* gene (or also c-myb experimentally), results in activation of B cell[43]; formation of metastatic bursal lymphomas by inhibition of cellular differentiation and insensitization to apoptosis[43,44]. Differentiation of neoplastic B cell is blocked at the IgM-producing stage. ALV-J induced mainly myelocytomatosis and nephromas in white meat-type chickens[45]. MHC *B-G* genes plays significant role in ALV-J infection and tumorigenesis[46]. Proteins related to cell signaling pathways, cytoskeleton, metabolic process and miRNA play role in ALV-J infection and tumorigenesis[47,48].

6.2. Non-neoplastic conditions

A number of non-neoplastic conditions have been observed, mostly in experimental infections. When young chickens, turkeys and jungle fowl exposed, to certain ALVs [RAV-1, RAV-60, MAV-2(0)] and ALVs of subgroup B and D develop anaemia, hepatitis, immunodepression[49]. In the acute phase of infection, particularly in congenitally infected chickens, a non-neoplastic syndrome, the wasting disease, develops with a latency of 2 to 3 weeks after hatching[50]. Basopholic intracytoplasmic viral matrix inclusions (MIs) are found in organs rather than muscular systems in the chickens naturally affected with ALV- associated tumors[51].

6.3. Subclinical infection

Subclinical infection with ALV may be of far greater importance in the intensive poultry industry because of its detrimental effect on many production traits of chickens[52]. ALV shedding was strongly associated with delayed onset of sexual maturity. Eggs from Gag shedding hens are characteristically lower in quality, fertility, and hatchability than eggs from non-Gag shedding hens[53,54]. Congenitally infected chickens tend to have lower egg production and survivability than chicks which are horizontally infected with exogenous ALV[55,56]. In addition to economic losses from tumors caused by ALV-J, broiler breeders have observed an increased number of small sized eggs in infected flocks[57]. The presence of ALV in semen affects fertility[58].

7. Diagnosis of AL/SV

7.1. Detection of ALV induced LL

Predominant neoplasm caused by AL/SV is LL, which is a disease of adult birds of more than 16 weeks of age. LL can be diagnosed by the presence of gross lesions in affected flock and confirmed by histopathology[3].

7.2. Detection of ALV infection

ALV infection is widespread in chicken, and can be detected by various biological, serological and molecular tests. Most of these assays are based on detection of virions or part of virions. However, detection of exogenous and endogenous AL/SV infections is yet a major challenge. Various assays may be summarized, as following:

i) virus isolation: virus isolation is generally an ideal method ('gold' standard) for detection of ALV infection[29]. Materials commonly used for isolation include serum, buffy coat cells and tumor tissue, cloacal or vaginal swabs, egg albumen, embryos and meconium. Virus can be cultivated in chicken embryo fibroblasts (CEF) and embryonated chicken eggs through chorio allantoic membrane route[59].

ii) Serological assay: serological assays are based mainly on detection of common major Gag or viral capsid protein p27, and/or antibody against p27. Most commonly ELISA has been used. But, false positive results due to common Gag shared between endogenous and exogenous viruses are main disadvantage, besides lack of subgroups differentiation[60]. Still, the p27 based serological assays are most commonly used tools currently available, as on date.

Thus, for detection of ALV infection, detection of p27 antigen may be done by sandwich ELISA and anti-p27 antibody by indirect ELISA[61].

Recently, however, prokaryotically expressed and affinity purified p27 was developed, and used for detection of ALV specific antibodies in chicken sera[62]. Further, for detection of p27 antigen using sandwich ELISA, the samples include serum[63], vaginal or cloacal swabs, egg albumen[64,65] and embryo[27]. The test is particularly used in ALV eradication program, and for detecting ALV in cultured CEFs[29].

Assay of anti-p27 antibodies using indirect ELISA may be carried out to detect presence or absence of exogenous ALV infection for flock surveillance[27]. The samples include serum, plasma and egg yolk. Other tests for detection of anti-p27 antibodies may include virus neutralization tests and flow cytometry.

iii) Molecular assay: PCR assays can detect various ALV subgroups specifically[66,67]. Most sequences used for developing PCR primers are based on *env* gene and LTR region and are highly specific for each subgroup[68]. PCR can be performed on DNA extracted from infected liver, spleen[69], dried feather shafts, feather tips[70] and blood, comb and toe[71] to detect various subgroups[60]. The PCR designed to amplify subgroup B viruses also amplify subgroup D viruses[68], since subgroup B and D envelopes are similar and share the same cellular receptor[72]. A multiplex PCR (mPCR) method for the detection of ALV subgroups A, B, and J has been developed and optimized[73]. These tests are sensitive, rapid, and may be used to detect ALV proviral sequences in tumor material or cultured CEFs. However, a problem with PCR methods is that the primer pairs used may not detect all variant viruses, necessitating continued redesigning of primers[29].

RT-PCR is more specific, sensitive and faster in comparison to serological diagnostic techniques[74]. RT-PCR assay in conjunction with restriction endonuclease digestion has been described to detect ALV contamination of vaccines[75]. ALV viral RNA can be isolated directly from albumen of unfertilized eggs from chickens, and also from blood, feather pulp, and different organs of chicken[76]. Use of albumen from unfertilized eggs facilitated development of RT-PCR assay without interference of parental genes found in fertilized eggs, maternal genes found in the vitelline membrane surrounding the egg yolk, or maternal IgY antibodies found in the egg yolk. Testing of egg albumen for presence of RNA genome of ALV could facilitate the identification of shedders[77].

7.3. Recent advances in diagnosis

A novel method, loop mediated isothermal amplification has been developed for rapid detection of ALV subgroup A and distinguishing from other subgroups of the ALV[78]. A duplex quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) assay was developed to detect and quantify ALV subgroups A and B (ALVA/B)[79]. To facilitate better diagnosis of ALV A, two monoclonal antibodies (MAbs-A5C1 and A4C8) to ALV-A are developed[80]. Recently a one-step, single-tube reverse transcription loop-mediated isothermal amplification test for detecting ALV A and J separately has been developed[81]. Glycoprotein 85 specific for ALV A was expressed in baculovirus expression system and characterized to detect antibodies against ALV A by indirect ELISA[82]. Recently two monoclonal antibodies were developed targeting the gp85 of ALV A which was found to be useful for diagnosis of ALV A[83]. Antigen capture ELISA based on monoclonal and polyclonal antibodies against p27 antigen was developed which showed good results for diagnosis of ALV[84]. Differentiation of oncolytic diseases is important and a difficult task by histopathology hence an oligonucleotide microarray was developed which differentiates ALV, Marek's disease and also reticuloendotheliosis virus[85].

8. Identification of shedders and transmitters

Shedders hens are V+A+S+, V+A-S+ or V-A+S+; and major transmitters of ALV are V+A+S+, V+A-S+ transmitting virion through egg albumen to embryo[27,32]. However, a small proportion of V-A+S+ are intermittent transmitters since they transmit virus to progeny, rather, in an infrequent manner, and cannot be identified even by screening of embryos[27,86-88]. Serum from adult flocks can be tested for ALV virions or Gag, and also for antibodies against ALV, in addition to testing of albumen Gag. Additionally, serum from day-old chicks can also be tested for the presence of antibodies to ALV. Positive results indicate presence of the infection in the dam. Virus isolation from plasma at hatch is insufficient in detecting transmitters but, the virus isolated at 4 weeks of age detected 90% transmitter hens and also identified 88% non-transmitter hens[32].

Recently, we have used PCR, RT-PCR and Gag based ELISA for identification of true exogenous ALV transmitters. Initially, dams were screened by both molecular and ELISA, which found positive for ALV-A in PCR were taken into study. Subsequently, dams were artificially inseminated and fertile eggs were collected. After 21 days of incubation before hatching embryos were sacrificed and tested for presence of ALV-A similar their dam. Results, indicated that testing of dams and their day-old chicks will facilitate in differentiating shedders and transmitters. Application of both molecular and serological assays clearly demarcate the infections from different subgroups of ALV, especially to circumvent problems of common Gag, which leads to cross-reaction between endogenous and exogenous ALVs in case of Gag based ELISAs[10].

8.1. Screening of eggs

Initially, albumen samples, can be tested by the Gag ELISA, further, the positive samples can be tested by RT-PCR to detect exogenous ALV. All of the transmitters could not be detected by testing albumen alone for Gag[33]. Presence of infectious ALV in albumen from a newly laid egg per hen is effective to identify the transmitters to some extent[27]. Detecting infectious ALV is more effective in identifying[89] congenitally transmitting hens than that for ALV antigens[30]. Virus can be detected in eggs negative for albumen Gag. Albumen tests may be more valuable than cloacal swabs for detection of transmitters, when used in combination with plasma testing for virus[32].

9. Eradication of ALV

Eradication of exogenous ALV from a flock depends on breaking the vertical transmission of virus from dam to progeny and prevention of reinfection of the progeny. These procedures depend on the identification and elimination of hens that shed ALV to the egg albumen and hence to the embryos and chicks[31,33]. These hens, belong to V+A-S+ and V-A+S+, usually identified by using ELISA to test cloacal or vaginal swabs, or albumen from eggs, for the presence of high levels of ALV Gag. One limiting factor is the efficiency of detection of transmitter hen. Thus, various procedures applied to dams may not detect all the hens that are capable of transmitting virus to their embryos. Another limiting factor involves post-hatch transmission of virus. Even a small number of infected chicks 'leaking through' from screened parental flocks

may infect hatch-mates by horizontal exposure, replenishing at least in part, the transmitter hen pool[32]. Small group hatching and rearing procedures allowed identification and removal of groups of infected chickens prior to hatching and prevented horizontal transmission of ALV-A in egg-type chicken[90] and ALV-J in meat-type chicken[91]. Eradication programs based solely on dam testing, may be less efficient than those, where combined procedures which mitigate early horizontal transmission to chicks[32].

A programme of testing elite pedigree birds, currently in use for eradication of subgroup J ALV in meat-type birds, involves following procedure: i) when birds are 20-weeks old, cloacal swabs from both sexes are tested for Gag by a commercial ELISA kit and positive birds are rejected; ii) at 22-weeks, serum samples from both sexes are tested for viremia by virus isolation and positive birds are rejected (viruses isolated may be tested by PCR to confirm subgroup specificity); iii) at 23-weeks or over, egg albumen from the first two eggs from all hens is tested for Gag, and positive hens are rejected; iv) at 26-weeks or over, meconium samples from the first chicks of all dams are tested for Gag, and positive dam families and dams are rejected; v) at approximately 40-weeks, when replacements are to be taken, repeat samples of albumen and meconium are tested for Gag, and positive dam families and dams are rejected. These five test procedures are aimed at ensuring that all shedding hens and roosters are progressively detected, allowing for any false negative results and for late shedders to appear[29].

9.1. Vaccination

No vaccines are available against ALV infection[73]. Relatively little research has been performed on vaccine development because vaccination is unlikely to be effective against a vertically transmitted virus which induces immunological tolerance. However, the propensity of subgroup J ALV to induce tolerant rather than immune infections following early contact infection has stimulated interest in developing vaccines against subgroup J ALV to protect chicks against early exposure[29]. A recombinant AL/SV expressing subgroup A and ALV-J envelope glycoproteins that could be used as vaccines to protect against horizontal transmission have been produced[92]. Various studies are carried out to develop an effective vaccine for ALV. Inactivated ALV B vaccine was prepared which was found to produce good antibody titre[93].

9.2. Selection for genetic resistance

Two principal types of genetic resistance to leukosis have been recognised, namely, genetic resistance to ALV infection and genetic resistance to development of leukotic tumors[94]. Three autosomal loci, tva, tvb and tvc independently control susceptibility to infection by ALV of subgroups A, B and D, and C, respectively, with dominant susceptibility genes encoding the presence of virus receptors and recessive resistance genes encoding the absence of these receptors. The tvb receptor has also been reported to control infection by exogenous subgroup E ALV. Approaches for selection of resistant chicken lines such as use of resistance to ALV, a recessive genetic trait, and polymerase chain reaction techniques are being introduced[50]. Single nucleotide polymorphism on *tva* and *tvb* genes have to be studied in various strains of birds to establish ALV resistant chicken lines[95]. Some of inbred lines of white leghorn has 4 different genetic resistant loci (tvar1, tvar2, tvar3, and tvar4) in *tva* receptor gene while *tvb* receptor gene has a single genetic resistant locus tvbr. Single amino acid changes[96], splicing mechanisms[97], premature stop codons[98] can change the susceptibility of birds to different subgroups of ALV.

10. Impacts of ALV

ALV infection in commercial chicken is a major issue of concern for economic reasons, public health concerns and newer isolate development, contaminants of vaccines and mixed infections.

10.1. Economic losses

It is considered as one of the important cause of economic losses in the poultry industry by inducing a variety of tumors, increased mortalities, growth retardation, decreased egg and meat production and production of eggs of reduced size and quality, immune suppression and decreased efficiency of progeny from infected birds that led to serious economic losses in affected flocks[3,99].

10.2. Public health concerns

There are few reports on the public health concern due to ALV infection. Presence of antibodies against ALV was detected in poultry farm workers and in persons not in contact with birds[100-102]. Low levels of reverse transcriptase activity was reported in human live vaccines (measles, mumps and yellow fever) grown in specific pathogen free avian cells was investigated, but no evidence was found for presence of infectious virus of ALV or EAV sequences in vaccine recipients[103,104]. Reports also indicated possibility of proviral DNA integration into the human genome activating the cellular oncogenes by insertional mutagenesis[102]. Laboratory and *in vivo* studies in primates and serological evidence in humans indicated that food animal oncogenic viruses revealed potential for causing cancer in humans[105].

10.3. Newer isolate development

ALV-A, ALV-B, and ALV-J are not only the most common but also the most dangerous viruses to the poultry industry[73]. In addition, ALV-A and ALV-J can infect the same chicken, and ALV-A and ALV-B have also been detected in the same commercial laying hens[107,108]. These types of co-infection provide a potential opportunity for recombination between different ALV subgroups. Molecular characterization of subgroup J has shown that its *env* genes might have arisen from multiple recombination events between one or more endogenous and exogenous viruses[106,109].

10.4. Contaminants of vaccines

It is important to assure that live virus vaccines of poultry, animals and humans that are produced from chicken source ingredients should be free from exogenous and endogenous ALVs. Concerns have been raised on contamination of chicken origin vaccines with exogenous and endogenous ALVs for use in humans, animals and birds and subsequent transmission of these retrovirus to vaccine recipients, especially the poultry breeding stocks[99]. Reports indicated even contamination of commercial Marek's disease vaccine[15,99,109-111], and Newcastle disease virus vaccine[112]. RT-PCR assay in conjunction with restriction

endonuclease digestion has been described to detect ALV contamination of vaccines produced in embryonated eggs and cell cultures derived from chicken[75]. Humans immunized with vaccines prepared from ALV-contaminated sources did not become antibody- or virus-positive for ALV[113].

10.5. Mixed infections

In recent years, co-infections with immunosuppression viruses and bacteria in chicken embryos occur with an increased frequency[114,115]. Recent reports indicated about the mixed infections of ALV with various immunosuppressive microorganisms, *viz*., *Salmonella pullorum*[116,117]; *Bordetella avium*[115], Marek's disease virus[118], reticuloendotheliosis virus[119,120]. ALVs increase susceptibility of chickens to secondary infections and inhibit immune functions. Infection with a virus or immunosuppressive agent may biologically alter the clinical outcome of birds that are already infected by another oncogenic virus and consequently they may even have a more profound molecular interaction aggravating final outcome of the disease. Reports also indicated about joint ability of MDV-1 and ALV to cause tumors and to increase mortality in experimental dually infected chickens; and Marek's disease vaccine of serotype 2 MDV enhances spontaneous occurrence of LL[121]. For simultaneous detection of MDV1, ALV and reticoloendotheliosis virus in tumour tissues of naturally infected chickens, a multiplex PCR has been developed[122].

11. Conclusions

ALV infected birds show different patterns of shedding and transmission of virion or Gag to progeny/environment. The phenomenon of shedders and transmitters is highly complicated, which makes it difficult to identify these birds. These shedder and transmitter birds are major threat to breeding stock, thus eradication programs target towards identification and elimination of both shedders and transmitters; thereby, breaking the vertical transmission of virus and prevention horizontal infection. Screening of both dams and their progeny is necessary to identify the true transmitters of infection. Thus loss of valuable genetic stock can be avoided. Knowledge on genetic make of up birds will certainly help to establish genetically resistant stock of breeders. An effective combination of selection and screening protocols at various ages of birds might help to eliminate the susceptible birds, will facilitate in establishing ALV free stock of breeders. Recent emergence of ALV J subgroup and subsequent impact on the poultry industry raises concerns over further emergences of new subgroups. Evidence of public health issue is a worry, since introduction of avian retroviral genes by contaminated vaccines into human may have an impact on oncogenesis.

Conflict of interest statement

We declare that we have no conflict of interest.

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