



Review

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ONCOGENIC VIRUSES AND THEIR MOLECULAR DIAGNOSIS IN POULTRY

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Abstract


Infectious animal diseases caused by pathogenic microorganisms such as bacteria and viruses threaten the health and well-being of wildlife, livestock and human populations, limit productivity and causes significant economic losses for each. Avian oncogenic viruses are one of the most dangerous pathogenic microorganisms that threaten the poultry industry and cause damage of over billions dollars annually worldwide. These viruses include a highly contagious herpesvirus Marek's disease virus (MDV), as well as retroviruses such as avian leukosis virus (ALV) and reticuloendotheliosis virus (REV). Each group is distinguished by its nucleic acid type, antigenicity, epidemiology, host range and molecular characteristics. These viruses are cells associated with tumors and are in all organs except in the feather follicle where enveloped infectious virions egress from the body. It is difficult to detect diseases caused by these viruses and at the same time, vaccines that can provide sterile immunity against these diseases and prevent infection are incomplete. In order to be able to fight oncogenic viruses more effectively in the future, it is vital to learn more about the host immunity-oncovirus interaction and to determine powerful diagnostic techniques. In this review, oncogenic viruses and effective diagnostic techniques for these viruses are emphasized in poultry.


Keywords: Avian oncogenic viruses, Molecular diagnosis, Marek's disease virus, Avian leukosis virus, Reticuloendotheliosis virus

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1. Introduction

The poultry industry has great importance in providing the protein needed for human nutrition, and is the most consumed meat-poultry meat in the world, especially in OECD countries (OECD., 2014). The global population is expected to reach 9 billion by 2050 and the increased

income growth among the poor population will result in an unprecedented increase in animal protein demand (King et al., 2018). In this context, attention should be given to combating infectious diseases that cause various losses in the poultry industry, which play an important role in ensuring sustainable food supply. Among these

diseases, neoplastic diseases caused by viruses are a major economic problem facing the poultry industry worldwide (Mitra et al., 2012).

The oncogenic viruses causing neoplastic infections in chickens are herpesviruses comprising of Marek's disease virus (MDV), retroviruses comprising of reticuloendotheliosis virus (REV) and avian leukosis virus (ALV) (Davidson and Silva, 2008). The others, retrovirus and lymphoproliferative disease virus have caused significant losses from lymphomas in turkeys in the United Kingdom and Israel, but now appears to be rare (Payne and Venugopal, 2000) and it is not mentioned further in this paper.

Avian oncogenic viral lesions are often similar and it is difficult to make a differential diagnosis based on histopathology (Wang et al., 2014). In particular, the lesions caused by ALV, MDV and REV are very similar and there is no specific symptom in any of these three diseases (Gimeno et al., 2005). The laboratory assays for diagnosis are based on virus isolation, demonstration of specific antibodies and histopathological examination of tumor tissues (Mitra et al., 2012). In addition to their oncogenic property, these retroviruses are also immunosuppressive and may contaminate poultry vaccines (Biswas et al., 2011; Fadly and Witter, 1997). These contaminations have resulted in the large number of deadly infection (Fadly and Garcia, 2006; Li et al., 2013; Wei et al., 2012; Fadly et al., 1996).

2. Avian Leukosis Virus (ALV)

Grooming Avian leukosis virus (ALV) infection of chickens is widespread and known to be of significant economic importance; economic losses due to ALV induced tumors and reduced productivity are estimated to be in millions of U.S. dollars each year (Fadly, 2000). The term leukosis embraces several different leukaemia-like proliferative diseases of the haemopoietic system caused by ALV; the term leukosis is used because a leukaemic blood picture is not always present (De Boer, 1987; Payne and Fadly, 1997).

Based on properties of viral envelope glycoproteins, ALV from chickens, a member of the leukosis/sarcoma (L/S) group of avian retroviruses are classified into six subgroups: A, B, C, D, E and J (Coffin, 1992; Payne and Fadly, 1997) and are classified as exogenous ALVs (A, B, C, D, J) and endogenous ALV (E) based on the viral envelope glycoprotein properties (Fadly, 2000). Particularly, the viral envelope glycoprotein is responsible for attachment and receptor specificity as well as the production of neutralizing antibodies (Coffin, 1992; Payne et al., 1992). Of the viral subgroups so far identified, subgroups A, B, and J are considered most prevalent and more economically important (Dai et al., 2015). Exogenous ALVs are capable of inducing lymphoid leukosis and subgroup A (ALV-A) is more commonly isolated than any other subgroup (Payne and Fadly, 1997). While exogenous ALVs have been shown to cause several neoplastic diseases in infected chickens (Cooper

et al., 1968; Crittenden et al., 1980), and nonneoplastic diseases such as myocarditis (Gilka and Spencer, 1990), and osteopetrosis (Smith, 1982), ALV-E is not known to be pathogenic to chickens (Crittenden et al., 1979; Linial and Neiman, 1976). Subgroup J was first isolated in meat-type chicken in the United Kingdom in 1989 but it is currently causing devastation in the poultry industry worldwide (Payne et al., 1991) and subgroup J associated primarily with myeloid leukosis in meat-type chickens (Fadly and Smith, 1999). Recent laboratory observations provided evidence for recombination between subgroup A and J ALV (ALVA/J), a recombinant ALV with the envelope of subgroup A and long terminal repeat (LTR) of subgroup J; this recombinant ALV resulted from passing ALV- J in cells expressing subgroup A envelope (Lupiani et al., 2003). As a potential contaminant of live-virus vaccines of poultry, ALV can also cause significant losses if contaminated vaccines were used in susceptible flocks (Fadly, 2016). Most recently, a recombinant subgroup A ALV containing envelope of ALV-A and LTR of ALV-E was isolated from commercial Marek's disease vaccines (Fadly et al., 2006; Silva et al., 2007). To date, because no commercial vaccines are available for control of ALV infection, eradication of virus infection at the primary breeder level remains to be the principal method for controlling ALV infection in chickens (Nair and Fadly, 2013).

3. Marek's Disease Virus (MDV)

Marek's disease virus (MDV), or Gallid herpesvirus 2 (GaHV-2) is the etiological agent responsible for Marek's disease (MD) in the chicken, a multifaceted disease most widely recognized by the induction of a rapid and extensive malignant T-cell lymphoma (Couteaudier and Denesvre, 2014), and MD results in substantial economic losses estimated at more than 1 billion per year (Morrow and Fehler, 2004).

Although MD was described in 1907 by Joseph Marek, the virus (MDV) was only isolated in 1967 in the United Kingdom (Churchill and Biggs, 1967) and the United States (Nazerian et al., 1968) independently. MDV is cell associated in body organs and tumors, it replicates and exists as enveloped free form in the feather follicles making feathers particularly dander, dust and litter materials loaded with MD virus, thus facilitating virus transmission by air borne route (Calnek and Witter, 1997; Adene and Akpavie, 2004; Frank, 2001). Susceptible chickens infected with the pathogenic MDV suffer cytolysis of the lymphoid organs and a concomitant immunosuppression (Gordon, 1979; Frank, 2001). Such birds mainly die as a result of tumour development in the visceral organs and peripheral nerves (Frank, 2001). Small RNA profiling studies have revealed that some viruses, particularly large DNA viruses such as Marek's disease virus (MDV) encode their own set of miRNAs (Hicks and Liu, 2013), and thus it is thought to silence the tumor suppressor genes of MDV.

The ability of MDV to replicate in the host is related to its

pathogenicity and the MDV genome load in infected chickens contributes to our understanding of the pathogenesis of MDV infection (Baigent et al., 2005; Islam et al., 2006). In addition, several MDV encoded genes, including meq (MDV EcoRI-Q-encoded protein) (Brown et al., 2009; Lupiani et al., 2004), pp38 (MDV phosphoprotein 38) (Cui et al., 1991; Reddy et al., 2002), vIL-8 (MDV-encoded CXC chemokine viral interleukin 8) (Cui et al., 2004; Parcels et al., 2001), and ICP4 (MDV infected-cell peptide 4) (Strasheim et al., 2012; Gennart et al., 2015), play important roles in MDV pathogenesis. Vaccination with MD vaccines is the primary approach used to protect chickens against MD (Sun et al., 2017). Although the immune-protection mechanisms induced by MD vaccines are not fully understood currently, it is recognized that effective immunity to MD requires the involvement and coordinated activation of innate and adaptive immune responses (Haq et al., 2013). Nevertheless, MD outbreaks have continued around the world in recent years, which is likely due to MDV evolution and co-infection with other viruses (Walkden-Brown et al., 2013b; Zhang et al., 2016).

4. Reticuloendotheliosis Virus (REV)

Reticuloendotheliosis virus (REV) is an oncogenic and immunosuppressive retrovirus that causes reticuloendotheliosis (RE) (Niewiadomska and Gifford, 2013), an avian disease mainly characterized by immunosuppression, runting-stunting syndrome and chronic lymphomas (Walker et al., 1983, Purchase et al., 1973). REV has extensive avian hosts including chickens, turkeys, ducks, mallards, geese, peafowl, pheasants, pigeons, Hungarian partridges, Chinese partridges, Attwater's prairie chickens and many other wild birds (Bohls et al., 2006; Jiang et al., 2013). REV infection of susceptible hosts such as chicks usually causes atrophy of the thymus and bursa of Fabricius, impairing the development and immune system functions of infected hosts, resulting in the suppression of host immune responses to some avian vaccines (Bulow, 1977; Yang et al., 2016).

The genomic structure of REV consists of a group-specific antigen (gag), protease (pro), polymerase (pol) and envelope (env) regions flanked by long-terminal repeats (LTRs) (Witter and Fadly, 2003). The gag gene encodes five structural proteins p10, p12, pp18, pp20 and p30. The p30 (30 kDa) protein is the major REV group-specific antigen (Tsai et al., 1985). The env gene encodes two envelope glycoproteins gp90 and gp120 (Tsai et al., 1986). The pol gene encodes a reverse transcriptase similar to those of mammalian type retroviruses and differs from the Avian Leucosis- Sarcoma Virus (Moelling et al., 1975; Bauer and Termin, 1980).

The wide range of host species and potential for contaminations with REV contribute to viral transmission (Sun et al. 2017). REV can be present as a contaminant in a variety of poultry biologics and vaccines (Fadly and Garcia, 2006; Li et al., 2015) and usually REV

can integrate into the genome of large DNA viruses including Marek's Disease and fowlpox (Isfort et al., 1992). Previous serological surveys have revealed that the positive rate of REV was approximately 2.3–23.5% among commercial chicken and turkey flocks in the United States (Witter et al., 1982). Usually, REV infection in chicken flocks is mainly due to REV contamination in poultry vaccines (Fadly and Garcia, 2006; Li et al., 2013; Wei et al., 2012; Fadly et al., 1996).

5. Molecular Diagnosis of Oncogenic Viruses

The frequent overlap of lesions caused by avian oncogenic virus infections requires specific laboratory diagnosis (Davidson, 2001). Nucleic acid amplification, hybridization technology and immunological tests have been adapted to diagnostic applications and identification of agents responsible for a wide variety of infectious diseases (Hafez and Hess, 1999). Different methods have been established for detecting avian oncogenic viruses, including traditional virus isolation plus an antigen-capture enzyme-linked immunosorbent assay (ELISA) for group-specific antigen of virus, immunofluorescence assay (IFA), loop-mediated isothermal amplification (LAMP) and quantitative reverse transcription PCR (Q-RT-PCR) (Kim and Brown, 2004; Zhang et al., 2010). However, each of these methods has limitations (Dai et al., 2015). For instance, ELISA and IFA are both time-consuming and quantitative data can't be acquired by the current LAMP method (Dai et al., 2015).

The PCR has become a routine technique in many research and diagnostic laboratories and real-time PCR has since its introduction in the mid-1990s removed many limitations of the standard PCR, which is significantly limited by its sensitivity (Edwards et al., 2005). In addition to enhanced sensitivity, the benefits of real-time PCR assays over conventional endpoint detection methods include their large dynamic range, a reduced risk of crosscontamination, an ability to be scaled up for highthroughput applications and the potential for accurate target quantification (Nazarenko et al., 1997; Schweiger et al., 2000; Black et al., 2002).

Zeng et al. (2015) reported the advantages of the GeXP-multiplex PCR assay to include its specificity and its high-throughput ability to immunosuppressive viruses. These advantages stem from the use of chimeric and universal primers in a 3-step PCR procedure with different annealing temperatures: the first step amplifies genespecific sequences within specific regions of the chimeric primers; the second step utilises the entire chimeric primer; and the last step uses universal primers for amplification (Zeng et al., 2015). Abdul-Careem et al. (2006) have shown that realtime PCR is 2.5-10 times more sensitive than conventional PCR techniques used for MDV detection. Moreover, PCR methods allow the detection and quantification of viral DNA in dust

collected and concentrated on filters (Islam et al., 2006; Baigent et al., 2013; Walkden-Brown et al., 2013a).

When real-time PCR is widely used to quantify viral genes, a host gene expressed steadily in a host cells or tissue samples as an internal control becomes one pivot point for calculating the copy number of specific viral genes (Dai et al., 2015). And the sensitivity of real-time PCR assay is at least 100 times higher than that of the routine PCR assay (Dai et al., 2015). Also, it has been possible to combine several assays in a single tube with the development of multicolor real-time PCR cyclers and and "ready-to-use" commercial multiplex real-time PCR kits (Hoffmann et al., 2009). Major advantages of multiplexing include a reduced sample requirement, which is especially important when sample material is scarce (Persson et al., 2005; Belak, 2007), and the ability to combine assays with an internal control system (Hoffmann et al., 2006).

6. Detection formats with Real Time PCR

6.1. Detection Formats without Sequence Confirmation of the PCR Product

Fluorescent dyes such as ethidium bromide (Higuchi et al., 1993; Wittwer et al., 1997a; Le Pecq and Paoletti, 1966) that are specific for double-stranded DNA (dsDNA) were the first systems employed in real-time PCR assays. Other intercalating dyes such as YO-PRO-1 have also been used (Ishiguro et al., 1995; Tseng et al., 1997). SYBR Green I is currently the most frequently used intercalating dye in real-time PCR and it has a 100 times higher binding affinity than ethidium bromide and the fluorescence of bound dye is more than 1000-fold higher than that of free dye (Hoffmann et al., 2006).

These properties make SYBR Green I highly suitable for monitoring product accumulation during PCR (Wittwer et al., 1997b; Morrison et al., 1998). Another detection technology known as 'LUX (Light Upon eXtension)' utilizes a modification to one of the two primers such that it possesses a fluorophore located near the 3' end in a hairpin structure (Nazarenko, 2006; Kusser, 2006). The Amplifluor Quantitative PCR detection system uses a similar approach with labelled and unlabelled primers (Nazarenko et al., 1997; Nuovo et al., 1999; Khripin, 2006).

6.2. Detection Formats with Increased Target Specificity

Fluorophorelabelled oligonucleotide probes are most commonly used for the specific detection of target sequences (Cardullo et al., 1988; Clegg, 1995; Wu and Brand, 1994). In these assays, an increase in fluorescence signal proportional to the accumulation of PCR product arises as a consequence of fluorescence resonance energy transfer (FRET) between separate fluorogenic labels (known as the reporter and quencher) conjugated to the probe (or primers). FRET, also called Förster transfer, is a spectroscopic process by which energy is passed over a maximum distance of 70Å between reporter and acceptor molecules possessing overlapping

emission and absorption spectra (Selvin and Hearst, 1994). The most commonly used fluorogenic quenchers are TAMRA and DABCYL, while Black Hole Quencher (BHQ) is also widely used and disperses energy from the reporter as heat rather than fluorescence (Didenko, 2001).

6.3. Hybridisation Probes

Hybridisation probes, also known as 'HybProbes', use a pair of adjacent, fluorogenic hybridisation oligos and are the only detection format that directly measures FRET (Cardullo et al., 1988). These probes have become the preferred chemistry of the manufacturer for the capillary-based LightCycler system (Wittwer et al., 1997a, b) with special filters for the detection of the acceptor fluorophores Red 640 and Red 705.

6.4. Hydrolysis Probes (50-exonuclease Assay)

Hydrolysis probes (commercially called TaqMan1 probes) are dual-fluorophore-labelled oligonucleotides, with a 50-terminal reporter (e.g. FAM) and a 30-terminal quencher (e.g. TAMRA). (Hoffmann et al., 2009). Once the labels are separated by destroying the TaqMan1 probe based on the 50-exonuclease activity of the DNA polymerase (e.g. Taq polymerase), the increase in reporter fluorescence caused by the removal of the adjacent quencher is monitored by a realtime PCR instrument. (Heid et al., 1996; Livak et al., 1995; Gibson et al., 1996). A modification of this strategy exploits the so-called minor groove binding (MGB) probes. MGB probes form extremely stable duplexes with singlestranded DNA targets mediated via van der Waals forces (Afonina et al., 2002): as a consequence shorter-length probes are required for hybridisation. In comparison with unmodified DNA, MGB probes have higher T_m and are reported to hybridize with greater sequence specificity (Afonina et al., 1996; Kutayavin et al., 2000). These short MGB probes are ideal for allele discrimination studies or for detection of single-nucleotide polymorphisms (SNPs) because they are more significantly destabilised by nucleotide changes within the hybridisation site compared with probes of longer length (De Kok et al., 2002; Belousov et al., 2004; Itabashi et al., 2004).

6.5. Molecular Beacons

Molecular beacons are hairpin-shaped oligoprobes terminally labelled with a reporter and a quencher fluorophore (Tyagi and Kramer, 1996; Tyagi et al., 1998; Vet et al., 2002).

6.6. Scorpion Primer

The Scorpion technology is mainly used in allelic discrimination (Whitcombe et al., 1999; Thelwell et al., 2000) and in SNP genotyping (Roberts, 2000).

6.7. Locked Nucleic Acid (LNA) Probes

Incorporation of LNA residues increases the T_m of the oligonucleotide sequence, allowing the use of markedly shorter probes as allele-specific tools in genotyping assays (Costa et al., 2004; Latorra et al., 2003; Braasch and Corey, 2001).

7. Conclusion

This study is primarily concerned with avian oncogenic viruses, the economically most important viruses such as MDV, ALV and REV. And more focused on some of the molecular techniques most commonly used to improve avian oncogenic virus detection for diagnosis and disease control. We showed some advanced biotechnological approaches that allow early detection of pathogens that affect poultry. Conventional diagnostic techniques are frequently time consuming, labor intensive and require to be performed on sophisticated equipment. Real Time PCR is one of the most important of these biotechnological methods. The high sensitivity and ability to quantify viral targets, the substantial gain in specificity and the reduced risk of cross-contamination are important features of this technology. Also, applications of Real-Time PCR include measurements of viral load, gene expression studies, clinical diagnostics and various pathogen detection.

Since it is suitable for the diagnosis of multiple viral infections, molecular techniques such as PCR and/or Real Time PCR are used for the diagnosis of avian oncogenic viruses (Silva et al., 2007). Nevertheless virus isolation is considered the "gold standard" for diagnosis and is often the starting point for more detailed studies (Nair, 2013). Virus-specific amplifications are confirmed by sequencing of precipitated PCR products. Using molecular methods can be useful for the rapid differential diagnosis of avian oncogenic viruses and for the detection of multiple infections (Gopal et al., 2012). Likewise, multiplex PCR primers can be useful in detecting the presence of ALV-J by modification in ALV primer sequences (Gopal et al., 2012). PCR has emerged as a chosen method for rapid and accurate diagnosis of viruses that appear in poultry. Multiplex PCR primers specific to MDV, ALV, REV and chicken DNA can be designed for rapid differential diagnosis (Gopal et al., 2012). Preferred amplification frequently occurs in multiplex PCR as a result of the various efficiencies of different primer pairs and due to the layout of the primer dimers. This is more likely as the number of primers increases (Elnifro et al., 2000), and the problem of amplification failure disappears when the template DNA contains several copies. This problem can be solved by optimizing multiplex PCR by changing the primer sequences, concentrations and cycle conditions (Frumkin et al., 2008).

Conflict of interest

The authors declare that there is no conflict of interest.

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