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Recent trends in the molecular diagnosis of infectious bursal disease viruses

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Abstract

Infectious bursal disease virus (IBDV) causes an immunosuppressive disease in young chickens. Two serotypes of this double-stranded RNA virus exist but only serotype 1 viruses cause disease in chickens. Detection and strain identification of IBDV is important because antigenic subtypes found within serotype 1 make it necessary to tailor vaccination programs to the antigenic type found in the bird's environment. Because conventional virus isolation and characterization are not practical for routine diagnosis of IBDV, antigen-capture enzyme-linked immunosorbent assay (ELISA) and molecular assays based on reverse transcription–polymerase chain reaction (RT-PCR) technology were developed. Compared with antigen-capture ELISA, RT-PCR assays have greater versatility and are more sensitive and specific. Strain identification has been accomplished using a variety of post-RT-PCR assays, including restriction enzyme digestion of the RT-PCR products. The resulting restriction fragment length polymorphisms (RFLP) are used to differentiate viruses into molecular groups that correlate with antigenic and pathogenic types. Recently, two types of real-time RT-PCR have been used to identify and differentiate strains of IBDV. Both methods use distance-dependent interaction between two dye molecules, known as fluorescence resonance energy transfer (FRET). The dye molecules are attached to one or more nucleotide probes that detect specific nucleotide sequences of the virus. Our laboratory has used a two-probe assay to identify single-nucleotide mutations among IBDV strains. A mutation probe is used in this assay to detect substitution mutations in a region of the viral genome that encodes a neutralizing epitope of the virus. These assays are accurate, reliable and inexpensive compared with conventional RT-PCR because they do not require RFLP or other labor-intensive post-RT-PCR assays to distinguish viral strains.

Keywords: infectious bursal disease virus; molecular diagnosis; RT-PCR; real-time PCR

Introduction

Two serotypes of infectious bursal disease virus (IBDV) have been identified but only serotype 1 viruses have been found to cause disease in chickens (Jackwood *et al.*, 1985; Ismail *et al.*, 1988). At least six antigenic subtypes of IBDV serotype 1 viruses have been identified using conventional virus-neutralization in cell culture (Jackwood and Saif, 1987). Antigenic variant IBDV strains have been isolated from commercial flocks of chickens with high levels of maternal antibodies to IBDV (Rosenberger and Cloud, 1986; Ismail *et al.*, 1990). Ismail and Saif (1991) demonstrated that vaccination with one subtype of serotype 1 did not always protect chickens from challenge with another serotype 1 subtype, particularly when a vaccine dose containing low virus titers was used. Differences in the virulence of IBDV strains have also been documented. Classic IBDV strains cause clinical disease, and mortality can range from 0 to 25%. Variant strains of the virus generally do not cause inflammation or clinical disease but they do infect lymphocytes and cause immunosuppression. In the mid 1980s a very virulent strain of IBDV was identified that can cause mortality up to 70% in susceptible

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birds (van den Berg *et al.*, 1991). The antigenic and pathogenic variation among IBDV strains makes the diagnosis of these viruses very important for establishing the best control measures.

Molecular diagnosis of IBDV strains

Virus neutralization assays to identify antigenic subtypes of serotype 1 viruses are time-consuming and expensive and require the IBDV strain in question to replicate in cell culture. Pathogenicity studies must be conducted in specific pathogen-free chickens. Researchers have turned to molecular diagnostic assays for the identification of these different IBDV strains.

RT-PCR

The reverse transcriptase–polymerase chain reaction (RT-PCR) assay has been used by several laboratories to identify IBDV. Most researchers have focused on a variable sequence region of the VP2 gene that is known to encode one or more neutralizing epitopes of the virus. At least one of these epitopes appears to be located in hydrophilic peak B of the VP2 protein. Several neutralizing monoclonal antibodies have been mapped to specific amino acid sequences in the hypervariable region of VP2. Two of these recognize different epitopes in hydrophilic peak B that are clustered around amino acids 321 and 324, respectively (Eterradossi *et al.*, 1998). Another monoclonal antibody, 17–82, recognizes a conformational-dependent epitope in hydrophilic peak B; amino acids 318 (glycine) and 323 (aspartic acid) are critical for binding of this monoclonal antibody (Heine *et al.*, 1991). Frequent mutations in hydrophilic peak B between amino acid positions 317 and 323 have been documented by nucleotide sequencing of this region (Bayliss *et al.*, 1990; Heine *et al.*, 1991; Lin *et al.*, 1993; Schnitzler *et al.*, 1993; Vakharia *et al.*, 1994; Dormitorio *et al.*, 1997; Eterradossi *et al.*, 1999; Proffitt *et al.*, 1999; Jackwood *et al.*, 2001).

To differentiate the antigenically different strains of IBDV, researchers have used the variable region of VP2. Initial RT-PCR assays were followed by digestion with multiple restriction enzymes (Wu *et al.*, 1992; Jackwood and Jackwood, 1994; Liu *et al.*, 1994). These assays met with limited success and were soon replaced by RT-PCR followed by restriction enzyme fragment length polymorphism (RFLP) assays (Lin *et al.*, 1993; Jackwood and Sommer 1997; Zierenberg *et al.*, 2001). We have used this assay to detect and identify IBDV strains in our laboratory (Jackwood and Sommer, 1997, 1998, 1999). This assay has been useful in placing vaccine strains of the virus into molecular groups. Within a molecular group, IBDV strains are related by ancestry (Jackwood *et al.*, 2001). Furthermore, viruses within a molecular group have nucleotide and amino acid sequences that are relatively more alike compared with viruses in different molecular groups. The results indicate that RFLP profiles can be used to predict the relative similarities and differences among unknown IBDV strains, but determining the actual antigenic relatedness among IBDV strains still requires *in vivo* testing.

The RT-PCR–RFLP procedures used to generate molecular groups of IBDV are designed to assess the nucleotide similarity or diversity among viruses. If the gene sequences responsible for specific phenotypic differences are known, it should be possible to devise a method to detect the genetic differences controlling these traits. Even when the gene sequences controlling a trait are not known, it has been possible to identify a genetic marker that is highly correlated with a particular phenotype. An example of this is the identification of a molecular marker for very virulent IBDV strains by Lin *et al.* (1993). These scientists found that all known very virulent IBDV strains contain an *Ssp*I restriction site in the variable sequence region of VP2, a finding we have confirmed and extended (Jackwood and Sommer 1999).

Although the RT-PCR–RFLP assay was an excellent diagnostic assay to rapidly identify the relative relatedness among IBDV strains, this assay has several limitations. It required two days to complete and the cost of each assay was too high to permit wide use of the test. Moreover, most diagnostic laboratories were not amplifying the same VP2 gene fragment, so results could not be compared between the different laboratories. Although the RFLP assay was successful because restriction enzymes were used to target sequences associated with, or involved in, determining the antigenic or pathogenic characteristics of the virus, some important nucleotide sequences could not be targeted because a restriction enzyme site in that region was not present. To circumvent some of these limitations, real-time RT-PCR was used to diagnose and differentiate IBDV strains (Moody *et al.*, 2000; Raue and Muller, 2001; Jackwood and Sommer, 2002; Jackwood *et al.*, 2003; Raue and Mazaheri, 2003).

Real-time RT-PCR

Moody *et al.* (2000) used the TaqMan real-time RT-PCR assay to quantify viral load in the blood of IBDV-infected chickens. Although very rapid and sensitive, this assay was not used to differentiate different strains of IBDV. We recently reported on the use of the LightCycler (Roche Diagnostics, Mannheim, Germany) and hybridization probe system (Roche, Molecular Biochemicals, Almeda, CA) to differentiate among IBDV strains. This real-time RT-PCR probe system employs fluorescence resonance energy transfer (FRET) to identify the RT-PCR products. There are two probes, one labeled with fluorescein isothiocyanate and the other with a Red

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640 fluorophore. These probes are not destroyed during amplification, as they are in the TaqMan system. Thus, they can be used after RT-PCR amplification to generate a melting temperature (T_m) for each IBDV strain. The T_m is the temperature at which one of the probes (usually identified as the mutation probe) will dissociate from the RT-PCR product. Sequence identity or mutations can be assessed by comparing the T_m of the mutation probe for each viral RT-PCR product (Bernard *et al.*, 2001). A T_m for the homologous virus (exact sequence match) is determined first. This value is then compared with the T_m values determined for unknown viruses. A T_m within two standard deviations of the homologous T_m indicates that the sequences of the mutation probe and test virus are identical (Jackwood *et al.*, 2003). If a T_m for the test virus is lower than two standard deviations from the homologous T_m, the sequences are considered to be different in at least one or more nucleotides (Jackwood and Sommer, 2002; Jackwood *et al.*, 2003). Since these studies were conducted with a mutation probe to a region of VP2 that encodes a neutralizing epitope (Heine *et al.*, 1991; Eterradossi *et al.*, 1998) the results provided information on the antigenic relatedness of the viruses being tested.

Melting temperature analysis following real-time RT-PCR has the potential to identify mutations in any region of the IBDV genome and to provide a relative value for the amount of sequence homology between wild-type IBDV and any vaccine strain, and can be used to help in the selection of candidate wild-type viruses for autogenous vaccines. The use of autogenous vaccines has helped control infectious bursal disease in local regions of the USA where outbreaks are not controlled by commercially available vaccines. Selecting the field virus to use in an autogenous vaccine is difficult and expensive. Screening viruses from outbreaks using real-time RT-PCR will greatly increase the speed of this process and significantly lower the cost. The greatest power of real-time RT-PCR is in reducing the number of vaccine virus candidates being considered for use in a flock or for development into an autogenous vaccine. This will substantially reduce the number of bird experiments needed to select the best commercial vaccine or autogenous vaccine candidate.

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References

Bayliss CD, Spies U, Shaw K, Peters RW, Papageorgiou A, Muller H and Boursnell MEG (1990). A comparison of the sequences of segment A of four infectious bursal disease virus strains and identification of a variable region in VP2. *Journal of General Virology* **71**: 1303–1312.

Bernard PS, Reiser A and Pritham GH (2001). Mutation detection by fluorescent hybridization probe melting curves. In: Meuer S, Wittwer C and Nakagawara K-I, editors. *Rapid Cycle Real-Time PCR Methods And Applications.* Berlin: Springer-Verlag, pp. 11–20.

Dormitorio TV, Giambrone JJ and Duck LW (1997). Sequence comparisons of the variable VP2 region of eight infectious bursal disease virus isolates. *Avian Diseases* **41**: 36–44.

Eterradossi N, Arnauld C, Toquin D and Rivallian G (1998). Critical amino acids changes in VP2 variable domain are associated with typical and atypical antigenicity in very virulent infectious bursal disease viruses. *Archives of Virology* **143**: 1627–1636.

Eterradossi N, Arnauld C, Tekaia F, Toquin D, Le Coq H, Rivallan G, Guittet M, Domenech J, van den Berg TP and Skinner MA (1999). Antigenic and genetic relationships between European very virulent infectious bursal disease viruses and an early West African isolate. *Avian Pathology* **28**: 36–46.

Heine H-G, Haritou M, Failla P, Fahey K and Azad A (1991). Sequence analysis and expression of the host-protective immunogen VP2 of a variant strain of infectious bursal disease virus which can circumvent vaccination with standard type 1 strains. *Journal of General Virology* **72**: 1835–1843.

Ismail NM and Saif YM (1991). Immunogenicity of infectious bursal disease viruses in chickens. *Avian Diseases* **35**: 460–469.

Ismail NM, Saif YM and Moorhead PD (1988). Lack of pathogenicity of five serotype 2 infectious bursal disease viruses in chickens. *Avian Diseases* **32**: 757–759.

Ismail NM, Saif YM, Wigle WL, Havenstein GB and Jackson C (1990). Infectious bursal disease virus variant from commercial leghorn pullets. *Avian Diseases* **31**: 141–145.

Jackwood DH and Saif YM (1987). Antigenic diversity of infectious bursal disease viruses. *Avian Diseases* **31**: 766–770.

Jackwood DJ and Jackwood RJ (1994). Infectious bursal disease viruses: molecular differentiation of antigenic subtypes among serotype 1 viruses. *Avian Diseases* **38**: 531–537.

Jackwood DJ and Sommer SE (1997). Restriction fragment length polymorphisms in the VP2 gene of infectious bursal disease viruses. *Avian Diseases* **41**: 627–637.

Jackwood DJ and Sommer SE (1998). Genetic heterogeneity in the VP2 gene of infectious bursal disease viruses detected in commercially reared chickens. *Avian Diseases* **42**: 321–339.

Jackwood DJ and Sommer SE (1999). Restriction fragment length polymorphisms in the VP2 gene of infectious bursal disease viruses from outside the United States. *Avian Diseases* **43**: 310–314.

Jackwood DJ and Sommer SE (2002). Identification of infectious bursal disease virus quasispecies in commercial vaccines and field isolates of this double-stranded RNA virus. *Virology* **304**: 105–113.

Jackwood DJ, Saif YM and Moorhead PD (1985). Immunogenicity and antigenicity of infectious bursal disease virus serotypes 1 and 2 in chickens. *Avian Diseases* **29**: 1184–1194.

Jackwood DJ, Sommer SE and Knoblich HV (2001). Amino acid comparison of infectious bursal disease viruses placed in the same or different molecular groups using RT-PCR-RFLP. *Avian Diseases* **45**: 330–339.

Jackwood DJ, Spalding BD and Sommer SE (2003). Real-time reverse transcriptase–polymerase chain reaction detection and analysis of nucleotide sequences coding for a neutral-

izing epitope on infectious bursal disease viruses. *Avian Diseases* **47**: 170–176.

Lin Z, Kato A, Otaki Y, Nakamura T, Sasmaz E and Ueda S (1993). Sequence comparison of a highly virulent infectious bursal disease virus prevalent in Japan. *Avian Diseases* **37**: 315–323.

Liu H-J, Giambrone JJ and Dormitorio T (1994). Detection of genetic variations in serotype 1 isolates of infectious bursal disease virus using polymerase chain reaction and restriction and nuclease analysis. *Journal of Virological Methods* **48**: 281–291.

Moody A, Sellers S and Bumstead N (2000). Measuring infectious bursal disease virus RNA in blood by multiplex real-time quantitative RT-PCR. *Journal of Virological Methods* **85**: 55–64.

Proffitt JM, Bastin DA and Lehrbach PR (1999). Sequence analysis of Australian infectious bursal disease viruses. *Australian Veterinary Journal* **77**: 186–188.

Raue R and Mazaheri A (2003). Eine real-time RT-PCR zum Nachweis des Virus der infektiosen Bursitis auf der Grundlage des Genomsegments B. *Archiv für Geflügelkunde* **67**: 22–27.

Raue R and Müller H (2001). Detection and quantification of IBDV by real-time RT-PCR. In: *Proceedings, II International Symposium on Infectious Bursal Disease and Chicken Infectious Anaemia, Rauischholzhausen, Germany*, pp. 271–277.

Rosenberger JK and Cloud SS (1986). Isolation and characterization of variant infectious bursal disease viruses [abstract]. *Journal of the American Veterinary Medical Association* **189**: 357.

Schnitzler D, Bernstein F, Müller H and Becht H (1993). The genetic basis for the antigenicity of the VP2 protein of the infectious bursal disease virus. *Journal of General Virology* **74**: 1563–1571.

Vakharia VN, He J, Ahamed B and Snyder DB (1994). Molecular basis of antigenic variation in infectious bursal disease virus. *Virus Research* **31**: 265–273.

van den Berg TP, Gonze M, Meulemans G (1991). Acute infectious bursal disease in poultry: isolation and characterization of a highly virulent strain. *Avian Pathology* **20**: 133–143.

Wu CC, Lin TL, Zhang HG, Davis VS and Boyle JA (1992). Molecular detection of infectious bursal disease virus by polymerase chain reaction. *Avian Diseases* **36**: 221–226.

Zierenberg K, Raue R and Müller H (2001). Rapid identification of very virulent strains of infectious bursal disease virus by reverse transcriptase–polymerase chain reaction combined with restriction enzyme analysis. *Avian Pathology* **30**: 55–62.