Differential Detection of Avian Oncogenic Viruses in Poultry Layer Farms and Turkeys by Use of Multiplex PCR

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Avian oncogenic viruses include Marek’s disease virus (MDV), a highly contagious herpesvirus, as well as retroviruses such as avian leukosis virus (ALV) subgroups A to J and reticuloendotheliosis virus (REV). In this study, we examined the incidence of these viruses in suspected samples collected from poultry layer farms of South India, mainly in the Namakkal district of Tamil Nadu, a highly dense poultry-growing area in India. The histopathology-positive tissue sections were identified and further confirmed by immunohistochemistry using virus-specific antibodies. The viruses belonging to all 3 groups (MDV, ALV, and REV) were isolated in a cell culture system and confirmed by immunofluorescence using virus-specific antibodies. PCR appeared to be the method of choice for rapid and accurate diagnosis of these viruses. The multiplex PCR primers specific to MDV, ALV, REV, and chicken DNA were designed for rapid differential diagnosis. The specificity of the primers was checked by amplification of DNA from virus-infected cell culture in comparison with uninfected samples, and sensitivity was evaluated by calculating the minimum copy number at which amplification occurs in the cloned PCR products. The sequences of the amplicons were compared by BLAST analysis. PCR tests demonstrated the presence of single, dual, or triple viruses in some of the samples. Of 169 samples screened by multiplex PCR, 9 samples were positive for MDV, 17 samples were positive for ALV, 12 samples were positive for REV, and 17 samples were positive for both ALV and REV. Three samples were positive for all three viruses. ALV-positive samples were further subjected to subgroup-specific PCR, which gave positive results for subgroups B and D but not for subgroup J. Multiplex PCR appeared to be a useful technique for rapid differential diagnosis of avian oncogenic viruses and detection of multiple infections of avian oncogenic viruses under field conditions.

Avian oncogenic viruses are considered significant pathogens of poultry, with huge economic significance to the poultry industry. These viruses include Marek’s disease virus (MDV) (35), avian leukosis virus (ALV), containing subgroups A to J, and the reticuloendotheliosis virus (REV) (34). MDV is classified in the Alphaherpesvirus genus and transforms T lymphocytes, not only resulting in the formation of skin and visceral tumors but also causing immunosuppression, neurological symptoms, and ocular lesions until tumors become visible (25). ALV subgroups belong to the Alpharetrovirus genus and are generally associated with lymphoid leukemia, with tumors primarily in the bursa of Fabricius and visceral organs (14), but ALV subgroup J (ALV-J) targets cells of the myeloid lineage, inducing late-onset myelocytomatosis (32). REV is in the Gammaretrovirus genus and causes a group of disease syndromes that are unrelated to those caused by the leukosis/sarcoma (L/S) group of viruses; it transforms pre-B and pre-T lymphocytes, causing bursal and T-cell lymphomas in chickens and turkeys (7). Reports on multiple oncogenic virus infections are available, and commercial poultry flocks surveyed in Israel between 1993 and 2004 showed multiple oncogenic virus infections in 25% of commercial chicken and turkey flocks (7). At times ALV and REV have been detected as contaminants in Marek’s disease vaccines (18, 23, 37).

The classical differential diagnosis of avian oncogenic viruses is based on virus isolation and histopathological examination of tumor tissues. Diagnosis based on virus isolation is laborious and time-consuming and can be further complicated by multiple virus infections in which adaptation of each virus to cell culture is difficult and involves different systems. Although histopathological diagnosis may be able to distinguish between MDV and ALV-J tumors, the lesions of lymphoid tumors induced by different viruses are often difficult to distinguish. Immunohistochemistry can be used for differential diagnosis of avian oncogenic viruses, but virus-specific antibodies are needed (9). PCR appears to be the method of choice for the diagnosis of avian oncogenic viruses because it overcomes many of the challenges encountered in the differential diagnosis and enables the detection of multiple viral infections (7). Hence, a rapid and precise multiplex PCR was developed to differentiate avian oncogenic viruses circulating in south Indian states, an area of India with a very high poultry population.

MATERIALS AND METHODS

Sample collection. A total of 169 suspected tissue samples, i.e., liver, spleen, bursa, and kidney from commercial layer chickens and liver, spleen, and intestine from turkeys, were collected during necropsy in and around the Indian states of Kerala, Karnataka, and Tamil Nadu. One portion of the tissue was stored at −80°C, and the second portion was collected in formalin for histopathological studies. Gross pathological lesions were recorded at the time of necropsy.

Histopathological examination and immunohistochemistry. Forty-seven samples were initially screened for histopathological examination by using paraffin-fixed sections. Thin sections (4 to 6 mm) were cut by microtome and stained with hematoxylin and eosin using a standard pro-