Molecular characterization of avian infectious laryngotracheitis-isolates from Switzerland

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Five to twenty annual outbreaks of infectious laryngotracheitis (ILT) occur mainly in small, backyard and fancy breed flocks in Switzerland. In order to determine the source of ILT outbreaks and to gain data on the possible role of vaccine strains in clinical disease, 57 strains of ILT viruses (ILTV) were examined, including field isolates from Switzerland (n=44) and Southern Germany (n=11) as well as two vaccine strains of different origin. A fragment of the thymidine kinase gene was amplified by polymerase chain reaction (PCR) and digested by several restriction endonuclease (RE) enzymes. Restriction fragment length polymorphism (RFLP) analysis indicated that 39 of 44 Swiss field strains (89%) were identical to all German field strains and both vaccine strains. PCR/RFLP could therefore differentiate some, but not all Swiss field strains from the vaccine strains tested. We conclude that two clones of ILTV have been circulating in Switzerland during the last twenty years, and that PCR/RFLP has a practical application in rapid identification of ILTV from clinical samples.

Keywords: infectious laryngotracheitis; PCR/RFLP; poultry; Switzerland; thymidine kinase

Introduction

Avian infectious laryngotracheitis (ILT) is a severe clinical respiratory disease of chickens. The acute form is characterized by breathing difficulty, coughing up of blood or bloody mucus, swollen orbital sinuses, high morbidity (up to 100%), variable mortality and decreased egg production in laying hens. The chronic form of ILT may look like any other respiratory infection (Albicker-Rippinger and Hoop, 1998). The agent is gallid herpesvirus 1 (GaHV-1), which belongs to the subfamily Alphaherpesvirinae of the family Herpesviridae (Roizman, 1982). The genome of ILTV is a linear double-stranded DNA of 155 kb in size (Chang et al., 2000). Latently infected chickens are the primary source of ILT outbreaks.

In Switzerland, ILT is a notifiable disease since 1987. Outbreaks of ILT occur mainly in small, backyard and fancy breed flocks and occasionally in commercial chickens (Hoop et al., 1993; Hoop et al., 1986). Although live attenuated vaccines against ILT are available in Europe, their use is not allowed in Switzerland. The main reasons for the ban are economic considerations, negligible number of outbreaks, possible reversion of vaccine strains to virulent virus after sequential bird-to-bird passage (Guy et al., 1991; Guy et al., 1990), difficult diagnostic analysis of infections carried by vaccinated birds and tedious diagnostic differentiation between field and vaccine strains.

For identifying ILTV molecular methods have proven to be more sensitive than virus isolation, being able to detect acute as well as latent infections (Alexander and Nagy, 1997; Keam et al., 1991; Key et al., 1994; Williams et al., 1994). Several studies documented that polymerase chain reaction (PCR) technique in conjunction with restriction fragment length polymorphism (RFLP) analysis can be successfully used to distinguish field and vaccine
strains (Chang et al., 1997; Creelan et al., 2006; Graham et al., 2000; Han and Kim, 2001; Kirkpatrick et al., 2006; Sellers et al., 2004).

The objective of the present study was to characterize 44 Swiss ILT isolates from chickens, peacocks and pheasants with acute infection by PCR/RFLP in order to investigate the diversity of the strains collected over the last twenty years.

Materials and methods

A total of 44 field isolates from chickens, peacocks and pheasants with acute ILT in Switzerland, collected over the last twenty years, were analyzed. 11 strains from neighboring Southern Germany and two ILT vaccines were included in the study. The vaccine strains were of chicken embryo origin (CEO) and tissue culture origin (TCO).

DNA was prepared according to a standard protocol of Sambrook et al. (1989). Extracted DNA was used in a PCR to amplify a 2.1 kb fragment of the thymidine kinase gene of ILTV. PCR oligonucleotide sense and antisense primers were designed on the basis of the published DNA sequence of the thymidine kinase gene of ILTV by Griffin and Boursnell (1990). The resultant PCR products were digested by the RE enzymes HaeIII and MspI and separated by electrophoresis in a agarose gel. The size of RE fragments was compared with molecular weight standards.

Results and discussion

PCR/RFLP analysis indicated that 39 of 44 Swiss field strains (89%) showed the same RFLP pattern and were identical to all German field strains and both vaccine strains. The remaining five Swiss field strains showed a different cleavage pattern (Table 1).

Table 1 Summary of results of RFLP analysis of ILTV field and vaccine strains.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Enzymes</th>
<th>Field strains</th>
<th>Vaccine strains</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Switzerland</td>
<td>Southern Germany</td>
</tr>
<tr>
<td>Thymidine kinase</td>
<td>HaeIII</td>
<td>39 / 1 *</td>
<td>11 / 1</td>
</tr>
<tr>
<td></td>
<td>MspI</td>
<td>39 / 3</td>
<td>11 / 3</td>
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<td></td>
<td></td>
<td>5 / 2</td>
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<td>5 / 4</td>
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</table>

* Number of ILT strains tested / different numbers indicate different RFLP patterns.

In this paper, we describe the detection of ILTV by PCR/RFLP targeted to the thymidine kinase gene in samples collected over the last twenty years from outbreaks of acute ILT in chickens, peacocks and pheasants in Switzerland.

A full comparison of the RE cleavage products showed that two different RFLP patterns were found in the 44 Swiss field isolates and the 11 isolates from ILT outbreaks of Southern Germany. One group contained 39 Swiss and all Southern German field isolates, the other group comprised five Swiss field isolates. Using the same molecular methods as described in this report, two different RFLP patterns were also observed among field isolates from outbreaks of acute ILT in chickens in Taiwan (Chang et al., 1997).

The two vaccine strains from different origin produced identical DNA fragments, in contrast to the findings of Chang et al. (1997). They compared four different vaccine strains, one TCO vaccine from Japan and three CEO vaccines from Holland, Iowa and Kansas. As described in their protocol, the amplification and digestion of the thymidine kinase gene with the enzymes HaeIII and MspI allowed to differentiate the TCO vaccine from the CEO vaccines with identical RE cleavage products. In our study, vaccine strains, the TCO vaccine from Spain and the CEO vaccine from the Netherlands, showed the same RFLP pattern. These results suggest that TCO vaccine strains may vary in virulence.
The discrimination of virulent ILTV strains from less virulent and CEO vaccine strains by PCR/RFLP of the thymidine kinase gene and the correlation of different RFLP patterns with varying virulence of ILTV were described in a previous study from Han and Kim (2001). Other authors also reported that the virulence of herpesviruses is associated with the thymidine kinase gene (Han et al., 2002; Mettenleiter, 1991). Increased virulence for the CEO vaccine, but not for the TCO vaccine, after continuous passages in specific-pathogen-free chickens was observed in a survey from Guy et al. (1991). The authors suggested that vaccine strains could be involved in field outbreaks.

One of the aims of this project was to determine the source of ILT infections in commercial and backyard chickens, peacocks and pheasants in Switzerland and to gain data on the possible role of vaccine strains in clinical disease. Neither the classification of our Swiss field isolates in virulent and less virulent strains nor the differentiation between field and vaccine strains was possible. For a more specific characterization and reliable identification of ILTV field and vaccine strains by PCR/RFLP the examination of several gene regions with a number of RE enzymes seems to be necessary. Nevertheless, the present study showed that two various clones of ILTV have been circulating in Switzerland during the last twenty years. In fact, our findings revealed a practical application of PCR/RFLP in rapid identification of ILTV in clinical samples, but for epidemiological investigations further studies will be required.

References


