

Detection and Characterization of Infectious Laryngotracheitis Virus from an Outbreak of Respiratory Disease in Ecuador

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ABSTRACT

Infectious laryngotracheitis (ILT) is an acute respiratory disease transmitted through respiratory secretions or fomites from naturally infected birds or the use of live vaccines. We investigated an outbreak of respiratory disease occurred in 2011 in Ecuador, a country where the disease was regarded as exotic. Viral detection was carried out by PCR (genes ICP4 and TK), and partial viral characterization was accomplished by amplicon sequencing. Our results suggested

that the outbreak was caused by a field strain of ILTV.

INTRODUCTION

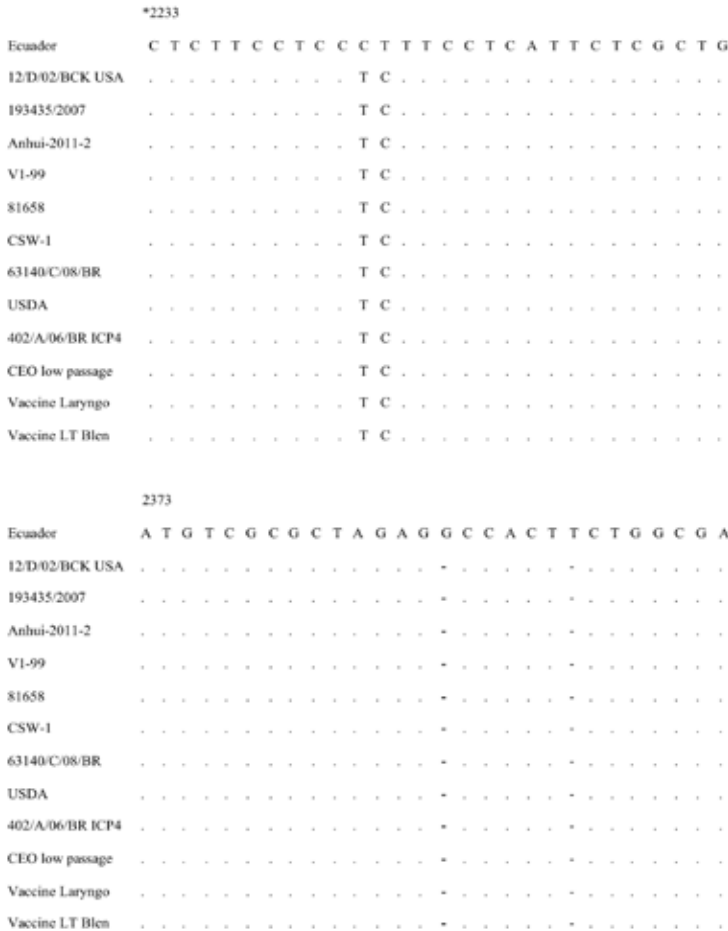
Avian infectious laryngotracheitis (ILT) is an avian respiratory disease caused by a virus of the family Herpesviridae, Gallid herpesvirus I (Williams et al., 1992). The severity of the disease depends on the viral strain; highly virulent strains produce severe respiratory signs such as bloody respiratory tract discharges and high mortality, whereas other strains cause a milder symptoms such as sinusitis, watery eyes, depression, and low mortality (Fuchs et al, 2007; Ojkic et al, 2006; Hughes et al, 1987). This virus can also cause lifelong persistent infections, which also contribute to viral dissemination

(Williams et al., 1992). Although the ILTV has been described primarily in poultry, the virus circulates in other birds such as pheasants, partridges, and peafowl (Samber et al, 1969).

Outbreaks of ILTV could originate from direct contact with secretions (or fomites) from acutely or persistently infected animals with either field or vaccinal strains (Shehata et al, 2013; Han et al, 2002; García & Riblet, 2001; Cover, 1996; Keller et al, 1992; Hughes et al, 1991; Guy et al, 1991).

Figure 1. Nucleic acid sequence of ICP4 gene from field and vaccine strains. All DNA sequences were obtained from GenBank except for the Ecuadorian one. Letters indicate single nucleotide substitutions.

*Sequence numbers correspond to a previously published ILTV sequence (Zhao et al., 2013).



Detection of the source of viral strains could be crucial for disease control (Chacón et al, 2010; Neff et al, 2008; Oldoni & García, 2007; Creelan et al, 2006; Ojkic et al, 2006). Efforts to discriminate vaccine strains from field isolates have focused in nucleotide differences, causing restriction length fragment polymorphism (RLFP) in the ICP4 gene (Oldoni & García, 2007; Creelan et al, 2006; Graham et al, 2000).

Due to their potential infectious nature, live vaccines are often prohibited in countries (such as Ecuador), where ILT is considered exotic. In Ecuador, despite the local regulations live vaccines are often smuggled, and may be causing outbreaks of respiratory disease. In this study, we used a simple approach consisting of PCR and amplicon sequencing to detect and characterize ILTV in an outbreak of respiratory disease occurred in 2011 in Ecuador.

MATERIALS and METHODS

Sampling

In 2011, 219 tracheal samples from acutely ill animals were obtained from 26 poultry farms (16 broiler and 10 laying hens operations); nine farms were located in the Ecuadorian Coast, and the rest were located in the Sierra region. All the samples came from animals that exhibited dyspnea, con-

conjunctivitis, sneezing, and nasal and ocular discharges. Samples were transported on ice in viral transport medium Remel M4RT and preserved at -20°C until analyzed.

DNA Extraction

A modified CTAB method was used for viral DNA isolation from samples. Tracheal epithelial cells were obtained using a sterile Papanicolaou brush; cells were suspended in 500 µl of PBS 1X buffer. Cell lysis was performed by adding 700 µl of CTAB solution (Doyle & Doyle, 1987). The solution was then treated with an equal volume of chloroform:isoamylalcohol (24:1). DNA was precipitated in sodium acetate 3M and washed in ethanol 70% and finally eluted in 50 µl of TE buffer (10 mM Tris-HCl, 5 mM EDTA, pH 8.0).

Amplification and Sequencing of ICP4 and TK Genes

In order to assess the presence of ILTV in tracheal samples, a 222 pb fragment corresponding to the ICP4 gene was amplified using PCR primers described by Creelan et al, 2006 (ICP4f 5'-CTCTTCTCCTTTCCTCAT-3' and ICP4rev 5'-GTTACTGACTGAACCGACCC-3'). A 649 pb fragment corresponding to the Thymidine Kinase gene (TK) was amplified as previously described (Han & Kim, 2001) using the primers TKIPf 5'-CTTAGCGGAACCTATGCAAG-3' and TKIPrev 5'-TAGCGTCTGGTTCGATTGAAG-3'. Amplicons sequences were compared to those of field and vaccinal viruses using BLAST and ClustalX (MEGA version 5.0) (Tamura et al, 2011). All samples were tested for the beta-actin gene amplification, which was used as an internal control.

RESULTS

We amplified ILTV DNA sequences (ICP4 and TK genes) from 80% (n=8) of farms housing laying hens and none from broiler farms. The viral nucleotide sequences from the Ecuadorian samples had two unique single nucleotide polymorphisms (SNPs) and two unique insertions when compared with ILTV sequences in the GenBank (Figure 1). The TK sequences were identical to other

TK sequences in the GenBank. These results suggest that ILTV was one of the causes of respiratory sickness observed in Ecuadorian laying hens in 2011. Differences in nucleotide sequences of amplicons suggested that the viruses causing the 2011 outbreak did not correspond to any previously sequenced ILTV.

DISCUSSION

Our study provides evidence of ILTV infection in Ecuador; this is the first report of the disease in this country. The analysis of nucleotide sequences obtained in this research suggested that the outbreak was not caused by vaccinal strain or any other previously described ILTV. We speculate that the virus may have entered Ecuador from neighboring countries where the disease has been reported previously (Alvarado et al, 2013).

Our results suggest that PCR amplification and amplicon sequencing is a simple and inexpensive method to detect and characterize ILTVs. Additionally, nucleotide sequences provide a portable record which may allow monitoring of viral strains causing disease in different parts of the world.

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