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Research paper

Spread of the newly emerging infectious laryngotracheitis viruses in Australia

Rebecca Agnew-Crumpton [a,1], Paola K. Vaz [b,1], Joanne M. Devlin [b], Denise O'Rourke [a], Hayley Patricia Blacker-Smith [a], Barbara Konsak-Ilievski [a], Carol A. Hartley [b], Amir H. Noormohammadi [a,*]

[a] Asia Pacific Centre for Animal Health, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Werribee, Victoria 3030, Australia
[b] Asia Pacific Centre for Animal Health, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria 3052, Australia

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ABSTRACT

Infectious laryngotracheitis (ILT) is a significant viral disease of chickens in many countries around the globe. In this report the status of ILT in Australia has been used as a model to evaluate the evolution of the ILT viruses (ILTVs). Due to its geographical isolation, Australia harbored a distinct lineage of ILT viruses (ILTV) up to 2007. However examination of the ILT viruses (ILTV) involved in outbreaks between 2007 and 2009 has revealed that many of the outbreaks were caused by two new viral genotypes, class 8 and class 9. These two recombinant viruses were found to emerge as a result of recombination between previously existing live vaccine strains (SA2 and A20), and another live vaccine strain (Serva) introduced into the country in 2007. The new recombinant ILTVs were also shown to possess significantly higher virulence and replication capacity compared with a previously predominant ILTV, class 2. In the current study, examination of a large number of ILTVs isolated from outbreaks between 2009 and 2015 revealed the emergence of yet another recombinant virus (class 10) that appears to have become a predominant genotype in New South Wales. In Victoria however, the recombinant class 9 gradually became the predominant virus, replacing class 2. Therefore, there was an unusual pattern in geographical spread of the newly emerged viruses in different states of the country. These results suggest that ILTV is fast evolving towards a greater transmissibility and therefore greater capacity to spread into ILTV-free areas.

1. Introduction

Evolution of pathogens is driven largely by their interaction with host species and can lead to diversification of ecological and epidemiological patterns (Burdon et al., 2013). Pathogens affecting food animals are particularly under an evolutionary pressure, especially with the rise in food-animal populations and globalisation of the animal food market which facilitates transmission of the pathogens (Engering et al., 2013). Commercial poultry constitutes the largest population amongst the food producing animals and its global production is rapidly expanding. Most if not all of this expansion is due to scale-up of the intensive farming practices which triggers the emergence, spread and persistence of pathogens with novel traits. Infectious laryngotracheitis (ILT) is a highly contagious respiratory disease of poultry and has been reported in most countries around the world. The disease continues to cause significant economic losses to the intensive chicken industry in many developed countries including Australia, where ILT outbreaks continue to occur despite preventative and biosecurity measures which are in place. Up until 2007, five different genotypes of ILTV were present in Australia (Kirkpatrick et al., 2006). After 2007 however, 4 additional genotypes were reported in Australia (Blacker et al., 2011). Of these genotypes, two (class 8 and class 9) were recombinant viruses emerged as a result of recombination between a previously existing Australian vaccine strain (class 1) and a vaccine (class 7) introduced into the country in 2007 (Blacker et al., 2011; Lee et al., 2012). A recent study by Lee et al. (2015) revealed that compared to the historical class 2 ILTV, the newly emerged recombinant class 9 grew to a significantly higher titre in cell culture and embryonated eggs, induced greater tracheal pathology and weight loss, and more readily and consistently transmitted to naïve birds. These results in combination indicated an improved fitness of the recombinant virus over the previously predominant field strains (Lee et al., 2015). These findings are likely to account for displacement of wild-type viruses by vaccine-like strains after the introduction of live vaccines in a number of countries including

Abbreviations: ILT, infectious laryngotracheitis; ILTV, infectious laryngotracheitis virus; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

* Corresponding author at: Asia Pacific Centre for Animal Health, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria 3052, Australia.
E-mail address: Amirh@unimelb.edu.au (A.H. Noormohammadi).

[1] The first two authors contributed equally to this manuscript.

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Northern Ireland (Graham et al., 2000), Taiwan (Chang et al., 1997) and the USA (Garcia and Riblet, 2001).

In this study, the contemporary status of ILT in Australia has been used to demonstrate that recombinant ILTVs with greater transmissibility and pathogenicity are fast spreading in the susceptible host populations, replacing somewhat less virulent historical strains. Results have implications for the evolution of herpesviruses affecting production animals globally.

2. Material and methods

2.1. Field submissions

A total of 384 field submissions made during 2009–2015 and confirmed positive for the presence of ILTV using thymidine kinase (TK) PCR (see below) were used in this study (Supplementary Table 1). Submissions included fresh tracheal/laryngeal tissues, or swabs taken from affected tracheal/laryngeal or conjunctival tissues presented to the Asia Pacific Centre for Animal Health laboratories, the University of Melbourne. The majority of submissions (360) were made from the two most affected states of Australia, Victoria (VIC) (219) and New South Wales (NSW) (141) and only a small number of submissions from other states including Queensland (QLD) (19), South Australia (SA) (3), Western Australia (WA) (1) and Tasmania (1). Most specimens were from commercial poultry flocks although a small number (10) were also from small backyard flocks. From those submitted from commercial flocks, the majority belonged to broiler flocks but a small number (50) were also from layer flocks. Based on the history provided by the submitters, the age of the birds from which specimens submitted ranged from 18 days old to 46 weeks old. Clinical signs and lesions (recorded in our laboratory or provided by the submitter) ranged from none to severe respiratory signs/lesions. Vaccination history was provided only for some of the submissions but at least 103 submissions were from flocks with known vaccination history and at least 33 were from unvaccinated flocks. The vaccines used in these flocks were one or combination of the three commercially available Australian vaccines, SA2, A20 (Zoetis Pty Ltd., Baulkham Hills, NSW, Australia), and Nobilis ILT (MSD Animal Health, Bendigo, VIC, Australia).

2.2. PCR and restriction fragment length polymorphism (RFLP)

DNA was extracted directly from swabs taken from infected trachea using a method described previously (Kirkpatrick et al., 2006). Extracted DNA was used in PCR and RFLP procedures targeting the genes for TK, ICP4 and ICP18.5, and the genomic regions between OFRB-TK, according to the procedures described previously (Kirkpatrick et al., 2006) with modifications described in a subsequent report (Blacker et al., 2011).

2.3. High throughput sequencing, and genome assembly and analysis

The class 10 ILTV was isolated and propagated initially in chorioallantoic membrane of 10-day-old chicken embryos, and then propagated in LMH cells, viral DNA extracted from purified nucleocapsids and sequenced using Illumina high-throughput sequencing technology as previously described (Vaz et al., 2015). The genome was assembled using the software package Geneious V6.1.7 (Kearse et al., 2012) using the class 9 genome sequence of ILTV (GenBank accession JN804827) as the reference sequence.

The genome sequence was aligned with the genome from other Australian ILTVs available in the GenBank database including class 1 (commercial vaccine strains SA2 and A20, JN596962 and JN596963, respectively), class 2 (V1-99, JX646898), class 7 (Serva vaccine strain, HQ630064), class 8 (JN804826) and class 9 (JN804827) viruses. Alignments were performed using the Multiple Alignment with Fast Fourier Transformation (MAFFT) version 7 plugin in Geneious (Katoh and Standley, 2013) using class 10 as the reference sequence. Sequences were trimmed to the reference and large gapped regions were removed. The terminal repeat region (which is identical to the internal repeat region) was excluded from the alignments.

Analysis of the genome for evidence of historical recombination events between the isolates was performed using SplitsTree 4 (Huson, 1998) and RDP4 , as previously described (Vaz et al., 2015). Short tandem repeat regions were identified in the aligned genomes using the Phobos plugin in Geneious V6.1.8 and removed prior to analysis. Statistical analyses of the recombination networks were performed using the Phi test as implemented by SplitsTree4 (Bruen et al., 2006).

3. Results

3.1. ILTV class 9 is the predominant ILTV in Australia

The number of ILT positive specimens and the distribution of the ILTV classes identified between 2009 and 2015 in Australia are summarized in Supplementary Table 1 and Fig. 1. The majority of the submissions were made from two most affected states, NSW and VIC. Except for 2011, the predominant class of ILTV in Australia since 2009 appeared to be class 9. In contrast, class 8 which was first detected in 2008 (Blacker et al., 2011), and class 2 became less frequent after 2009. Class 7 (Serva strain, Nobilis ILT) was also found in a number of submissions every year although the largest number of class 7 positive samples occurred in 2011 which included 8 separate submissions of class 7 from NSW.

A small number of cases were submitted (and the ILTV strains involved fully typed) from other states including QLD, SA and WA. These included one submission of class 1, four of class 2, one of class 3 and four of class 7 from QLD in 2009, one submission of class 3 from WA and three submissions of class 7 from QLD in 2010, four submissions of class 7 from QLD in 2011, and one of class 3 from SA in 2013.

3.2. Replacement of the historical genotype 2 by the recombinant genotype 9 in Victoria

The number of ILT positive specimens and the distribution of the classes identified between 2009 and 2015 in the state of VIC are summarized in supplementary Table 2 and Fig. 2. When examined using PCR-RFLP (Blacker et al., 2011; Kirkpatrick et al., 2006), ILTV class 2 was reported to be the predominant ILTV class detected in VIC until 2009. Since then however, class 2 was very rarely detected and instead class 9 constituted the largest number of ILTVs detected in VIC. A relatively small number of ILTV class 1, and small number of other classes (2, 3, 7 and 8), and a single case of class 5 were also detected during the course of this study in VIC.

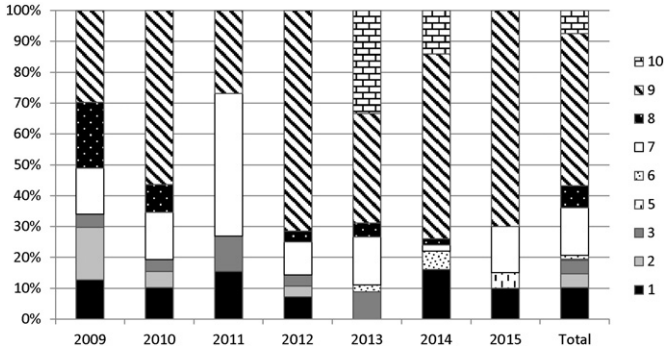

Fig. 1. Percentage of ILTV classes detected in Australia between 2009 and 2015.