

# Infectious laryngotracheitis in broilers

By Felicity Kerr

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## INTRODUCTION

Infectious laryngotracheitis (ILT) is a highly infectious acute respiratory disease of fowl caused by infectious laryngotracheitis virus (ILTV). ILTV is classified as a member of the genus *Iltovirus* within the family *Herpesviridae*, subfamily *Alphaherpesvirinae*<sup>1</sup>. Taxonomically the virus is identified as *Gallid herpesvirus 1*<sup>1</sup>. The chicken is the primary natural host for the virus, however, pheasants, peafowl and turkeys are also susceptible<sup>2-4</sup>. Mild enzootic forms of infection are increasingly reported in developed poultry industries and manifest as mucoid tracheitis, sinusitis, swelling of infraorbital sinuses, haemorrhagic conjunctivitis, general unthriftiness and low mortality<sup>4</sup>. Severe epizootic forms of infection are characterised by signs of respiratory distress, gasping, expectoration of bloody mucus and high mortality<sup>4,7</sup>. Furthermore, infection with ILTV can predispose to other respiratory pathogens<sup>4</sup>. Whilst most characteristic clinical signs are seen in adult broilers (greater than 45 days), birds of almost any age (usually starting at 4 weeks of age) can become infected and develop disease<sup>8</sup>. Natural portals of entry for ILTV are through the upper respiratory and ocular routes, and to a lesser extent ingestion<sup>9,10</sup>. ILTV can be introduced to a flock through contact with respiratory exudates from affected birds or via mechanical transmission by fomites, or use of contaminated equipment and litter<sup>11</sup>. ILTV can also be introduced by carrier birds that have survived a previous outbreak of ILT (including backyard flocks) or through vaccinated birds. Transmission occurs more readily from acutely infected birds than through contact with clinically recovered carrier birds<sup>4</sup>. Following introduction of ILTV into a flock the appearance of clinical signs varies from 11 days to 10 weeks, though signs generally appear 6-12 days following natural exposure<sup>8,12</sup>. Currently there is no effective treatment for ILT, thus strategies for the control of the disease are largely based on preventing contact between the agent and the susceptible host through biosecurity, and/or vaccination strategies<sup>4,8</sup>. The currently available ILT vaccines include modified-live virus vaccines and vaccines based on recombinant DNA technology (not available in Australia). To generate modified-live virus vaccines, field strains of ILTV have been attenuated by sequential passages in cell culture (tissue-culture origin, TCO), embryonated chicken eggs (chicken-embryo origin, CEO) and via feather follicle inoculation of chickens<sup>12-17</sup>. ILT has the potential to cause severe economic losses through increased mortality, decreased production and export sanctions. Thus the poultry industry has placed major emphasis on decreasing exposure and eliminating ILT from broiler flocks.

## CURRENT PROBLEMS IN AUSTRALIA AND WORLDWIDE

ILT was first described in 1925, but some reports suggest it may have existed even earlier<sup>18-20</sup>. In 1934, Brandly and Bushnell devised a method for immunisation of chickens based on application of virulent virus into the cloaca<sup>21</sup>. ILT was subsequently the first major avian viral disease for which an effective vaccine was developed. To date ILT has been identified in most countries around the world and remains a serious disease wherever susceptible poultry populations occur in large numbers<sup>22</sup>. Severe epizootic forms of ILT were commonly reported in earlier years. However, recent times have seen the appearance of mild enzootic forms of ILT in the intensive poultry producing areas of Europe, Australia, New Zealand and the United States<sup>12,23-25</sup>. Such mild enzootic forms are associated with morbidity as low as 5% and very low mortality (0.1-2%)<sup>23-25,26</sup>. In developed countries ILT viruses tend to persist as endemic infections within backyard and exhibition chicken flocks<sup>4</sup>.

Despite improved biosecurity and vaccination programs in recent years, ILT continues to emerge in the field on a regular basis in the intensive poultry industry.

In areas of intensive production and large concentrations of poultry, such as the United States, Europe, China, Southeast Asia and Australia, ILT is usually controlled in layer flocks by the use of modified-live virus vaccines<sup>4</sup>. Despite vaccination, mild to moderate outbreaks of ILT are not uncommon in commercial layer flocks worldwide<sup>27</sup>. In intensive broiler production, the short growth cycle and high level of on-site quarantine can reduce the need for prophylactic vaccination. However, in recent years, sporadic outbreaks of ILT in broiler flocks have been identified as an emerging problem in several countries, including Australia<sup>28-29</sup>. A growing body of evidence supports the notion that most field outbreaks are caused by viruses indistinguishable from chicken-embryo-origin (CEO) vaccine strains, leading to the term ‘vaccinal laryngotracheitis’ being used to describe outbreaks in broilers<sup>30</sup>. This is certainly the case for many overseas disease outbreaks, however, it differs to what is currently occurring in Australia. Live CEO vaccines are used to inoculate breeders and layers and may serve as a reservoir of virus capable of causing broiler outbreaks<sup>31-32</sup>. Such outbreaks are likely a result of lack of uniform flock immunity and the transmission of a vaccine strain from vaccinated to unvaccinated birds<sup>33-34</sup>. Furthermore, it is possible that some live attenuated strains of ILTV may occasionally revert to parental-type virulence, causing sporadic ILT outbreaks<sup>35</sup>.

Interestingly, most of the recent ILT outbreaks in Australia have not been caused by vaccine strains<sup>27</sup>. In 2007 and 2008, a series of ILTV outbreaks were reported in commercial layer and broiler flocks, predominantly in Victoria<sup>36</sup>. The ILTV isolates from these recent outbreaks were identified using polymerase chain reaction (PCR) and restriction fragment length polymorphism analyses (RFLP) and found to be mostly the same ILTV genotypes as those identified in a previous study<sup>27</sup>. An additional, novel ILTV genotype was discovered in several flocks from a single broiler company that was distinct from all recent and historical isolates in Australia<sup>27</sup>. The Australian vaccine strains SA2 and A20 were not found responsible for any of the outbreaks investigated in this study<sup>27</sup>. Thus further investigation into the involvement of environmental factors such as air/wind and insects may be necessary to fully understand the route of transmission of ILTV in the recent Australian outbreaks. Poor biosecurity has also emerged as a predominant factor in starting broiler ILT outbreaks<sup>4</sup>. The source of the virus may be latent infections in backyard flocks or alternatively, CEO vaccines used in long-lived birds or broilers<sup>31,35</sup>.

#### AVAILABILITY OF VACCINES

Vaccination has proven to be a satisfactory method for developing resistance in susceptible chicken populations. However, given that vaccination can give rise to latently infected carrier birds, it is recommended for use only in geographic areas where disease is endemic. Alternatively vaccination can be used in the face of an outbreak to effectively limit virus spread and shorten the duration of disease. The recent outbreak of ILT in broiler flocks has created the need for mass vaccination of broilers against ILT. In Australia the sudden increase in vaccine demand during the 2007/8 ILT disease outbreaks led to a shortage of the key commercially available vaccines (SA2 and A20, Fort Dodge)<sup>36</sup>, which complicated control of these outbreaks. Thus rapid availability of effective vaccines and mass application to large flocks are key components for managing and limiting disease outbreaks.

#### PROPERTIES OF CURRENT AUSTRALIAN VACCINES

The commercially available vaccines used to immunise chickens against ILT in Australia include the live attenuated vaccines SA2 and A20 (Fort Dodge, Australia, Pty. Ltd.) and more recently the Nobilis ILT vaccine (Intervet Pty. Ltd.)<sup>36</sup>. The vaccine strain SA2 originated from an Australian field isolate that was attenuated through sequential passages in chicken embryo<sup>37</sup>. Strain A20 originated from the SA2 strain through further passages in chicken embryonic cell culture as a means of reducing residual virulence<sup>38</sup>. The Nobilis vaccine has only recently been introduced into the Australian market and consists of a live attenuated Serva strain of ILTV. The A20 vaccine is considered relatively safe and can be used in very young chickens<sup>36</sup>. Thus the A20 vaccine is suitable for the prophylactic vaccination of birds of all ages, as well as in the advent of a disease outbreak. The SA2 and Nobilis ILT vaccines on the other hand, are less attenuated and are only recommended for use in adult birds (eg. minimise the spread and duration of disease during outbreaks) or as a booster vaccine following A20 administration<sup>36</sup>.

#### ADMINISTRATION OF VACCINES

The first successful immunisation against ILT was achieved by application of virulent virus into the cloaca<sup>21</sup>. Since this time effective vaccination has been achieved via inoculation of infraorbital sinuses, intranasal instillation, feather follicles, eye-drop, intratracheal, *in ovo*, spray and orally through drinking water<sup>39-41</sup>. However, many of these routes of inoculation are impractical in large scale poultry production due to time and labour constraints.

ILT vaccine administration via drinking water or spray is desirable for rapid, mass application, however, several problems have been associated with these routes of inoculation. Administration of ILT vaccine by drinking water was found to result in a high proportion of chickens that failed to develop protective immunity<sup>42</sup>. Successful vaccination via this route requires vaccine virus coming in contact with susceptible nasal epithelium. This occurs via aspiration of virus through external nares or choanae, which reportedly occurs infrequently in chickens vaccinated by drinking water<sup>42</sup>. Successful vaccination by drinking water is also dependent on the proximity of the birds to the water source<sup>4</sup>. If application of ILT vaccines by spray is performed incorrectly adverse reactions may occur as a result of insufficient attenuation of vaccine virus, deep penetration of the respiratory tract due to small droplet size or excessive dose<sup>37,43</sup>. In addition, single dose vaccination administered by spray often fails to induce adequate immunity<sup>44</sup>. Vaccine application by eye-drop route has been shown to provide more uniform protection following a single dose application compared with spray and drinking water routes<sup>4</sup>.

#### CURRENT VACCINATION PROTOCOLS

Chickens can be successfully vaccinated as early as 1 day of age, however, chickens less than 2 weeks of age do not respond as well as older birds<sup>13,45-46</sup> and are more likely to encounter severe adverse reactions to vaccination. Vaccination of chickens over 2 weeks of age with modified-live virus or field exposure confers complete protection against challenge by 6-8 days<sup>40</sup>. Significant flock immunity is generally observed for 15-20 weeks following vaccination<sup>13</sup>. Layer flocks are typically vaccinated twice before the commencement of egg production. Vaccines are typically administered via eye-drop at 7 weeks age and eye-drop, spray or drinking water at 15 weeks of age<sup>4</sup>. For intensive broiler production, the short growth cycle, all-in all-out production and a high level of biosecurity can reduce the need for prophylactic vaccination. However, vaccination of broiler flocks may be necessary when the flocks are in close proximity of ILT outbreaks or when disease has previously occurred on the farm. Under such circumstances, broilers are generally vaccinated at 10-21 days with vaccine administered by drinking water. A recombinant fowl pox virus-vectored vaccine is commercially available in the United States for immunisation of chickens against ILT<sup>47</sup>. It is typically

used in multi-age layer flocks and is administered via wing-web inoculation to chickens that are at least 8 weeks old and at least 4 weeks prior to the onset of egg production.

#### IDEAL VACCINE

There are many effective ILT vaccines currently in existence, however, no one vaccine could be considered ideal for all situations. An ideal vaccine needs to be safe, and not cause disease or death in itself. Thus many of the modified-live virus vaccines fall short due to their tendency to revert to virulence. Secondly the vaccine must be able to produce protective immunity in a high proportion of vaccinates, which is generally the case for the current vaccines if administered appropriately. Thirdly, the vaccine should generate long-lived immunological memory so that 'booster' vaccinations are not required. Many of the current vaccines require a booster vaccination and provide only short term immunity (up to 20 weeks)<sup>13</sup>. It is also desirable for one vaccine to be cross-protective for all virus genotypes. ILTV strains are antigenically homogenous, thus a single ILT vaccine produces cross-protective immunity for all ILTV strains<sup>4</sup>. Lastly there are many practical considerations that need to be addressed. The vaccine needs to be cost-effective, biologically stable, easily stored and transportable, easily administered to large populations and associated with few side-effects. Thus it is not hard to see why the currently available vaccines fall short of the ideal.

CEO vaccines can be readily administered on mass via eye-drop, water, or spray and as such can be used practically and economically in disease outbreaks<sup>48</sup>. However, CEO vaccines have the potential to increase their virulence with bird-to-bird passage<sup>35</sup>. Furthermore, broilers vaccinated with CEO vaccine serve as carriers and can re-excrete the virus under stressful conditions, thereby spreading virus to other susceptible poultry<sup>35,49</sup>. TCO vaccines lack the tendency to revert to virulence following bird passages, which is a major advantage over the CEO vaccines<sup>35</sup>. However, TCO vaccines can only be given by eye-drop, making their administration labor intensive and cost-prohibitive in many countries<sup>49</sup>. All the vaccines currently available in Australia are CEO vaccines.

Given the limitations of attenuated virus strains in terms of residual pathogenicity and reversion to virulence, current vaccine development has turned to recombinant vaccines and the development of novel vaccine strains that lack virulence genes. Glycoprotein G (gG) is a virulence factor in ILTV<sup>50</sup>. Recently a gG-deficient ILTV administered via eye-drop or drinking water was found to be comparable to currently available commercial ILT vaccines in terms of safety and efficacy<sup>50</sup>. In 2002 a recombinant fowl pox virus-vectored ILT vaccine was licensed by the USDA for use in chickens by injection via the wing web<sup>51</sup>. This vaccine does not utilise live virus and thus avoids the risk of reversion to virulence. In addition the vaccine was found to be safe and effective following *in ovo* administration in combination with Marek's and IBDV vaccines<sup>51</sup>. *In ovo* vaccination has advantages related to mass vaccination at a single location, improving the uniformity of vaccination and reducing labour and time expenditure compared to field applications<sup>51</sup>. Thus recombinant DNA vaccines have potential for use in large-scale poultry vaccination programs, with the distinct advantage of preventing the perpetuation of ILTV in surrounding flocks.

#### BIOSECURITY AND CONTROL

Development and implementation of an effective biosecurity plan is essential in preventing the introduction of diseases into a poultry flock<sup>52</sup>. Site quarantine and hygiene are central in preventing the movement of potentially contaminated personnel, equipment, feed and birds. The essentials of a biosecurity plan include purchasing birds from a reliable, disease-free source and avoid mixing of birds from different sources. Restricting barn access to authorised personnel and insisting upon the use of personal protective equipment (coveralls and boots) is also important. Viricidal boot-dips

should be placed at entry points to all barns and changed daily. Vehicle traffic should be restricted to specific areas on the farm. Measures should also be in place to control dogs, cats, rodents and wild birds from accessing barns<sup>4</sup>. Dead carcasses should be removed and disposed of accordingly. Employees of the farm should not have flocks of poultry or companion birds of their own, nor should they visit other poultry farms. Finally a thorough cleanout and disinfection should be conducted between flocks. ILTV infectivity is readily inactivated outside the chicken host by disinfectants and warm temperatures<sup>4</sup>. Thus adequate cleanup between successive flocks can prevent carryover of ILTV. Current recommendations are that all potentially contaminated carcasses, feathers, feed, water and litter remain within the poultry house which is then heated to 38 °C for 100 hours<sup>4</sup>. All buildings and equipment should be washed and sprayed with disinfectants such as phenolics, sodium hypochlorite, iodophors or quarternary ammonium compounds<sup>4</sup>.

#### CONCLUSION

ILT remains an important disease of intensively farmed chickens worldwide. A better understanding of the disease pathogenesis may facilitate the introduction of further biosecurity measures in an attempt to prevent ILT outbreaks. Recombinant DNA vaccines represent a realistic means of controlling and preventing disease outbreaks without posing a threat to unvaccinated flocks. In the advent of an ILT outbreak, the most effective approach is a co-ordinated effort between government and industry. This involves rapid diagnosis, institution of a vaccination program, increasing biosecurity, use of geographic information system technology, cleaning and disinfection, and communication between stakeholders<sup>4,30,53</sup>. This coordinated management approach is not unique to ILT outbreaks. The same strategy can be applied to the control of various other poultry diseases which ensures the industry is effectively prepared in the advent of any poultry health issue.

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