AVIAN LARYNGOTRACHEITIS

Andrés Felipe Ospina-Jiménez, Arlen Gómez-Ramírez, Gloria Ramirez-Nieto.
Microbiology and Epidemiology Research Group. FMVZ.
National University of Colombia.

INTRODUCTION

Avian Infectious Laryngotracheitis (ILT) is a viral respiratory disease caused by GaHV-1, mainly affecting Gallus gallus species (chickens and hens), although it has also been reported in pheasants, partridges and peacocks. The ILT virus is one of the agents involved in the Avian Respiratory Complex, causing respiratory difficulty, variable morbidity and mortality, low egg production and latent infections that negatively impact the poultry industry. The virus's ability to establish latent infections makes it difficult to control and perpetuates disease during the production cycle, affecting production, and it becomes a health challenge. For this reason, ILT is considered a significant disease in the avian species and is included in the World Organization for Animal Health (WOAH) 's health code for terrestrial animals. ILT control has been based on the implementation of biosafety measures and the application of attenuated vaccines; however, its implementation has safety and effectiveness issues since the active circulation of vaccine viruses in avian populations has been shown to increase the probability of virulence reversal and genetic recombination. Globally, it is accepted that most disease cases are related to viruses genetically related to vaccine strains that revert their virulence due to poor practices during their implementation and insufficient vaccine coverage.

ETIOLOGY

ILT's causing agent is Gallid Alphaherpesvirus 1 (GaHV-1), classified within the *Iltovirus* genus of the *Orthoherpesviridae* family. This family includes other relevant agents in avian medicine, such as Marek's Disease Virus and Turkey Herpesvirus.

GaHV-1 is an enveloped virus, susceptible to most common disinfectants used in poultry farms. It has a double-stranded DNA genome, which gives it a relatively low mutation rate. However, the virus presents a considerable genetic variation due to genetic recombination processes (4, 21).

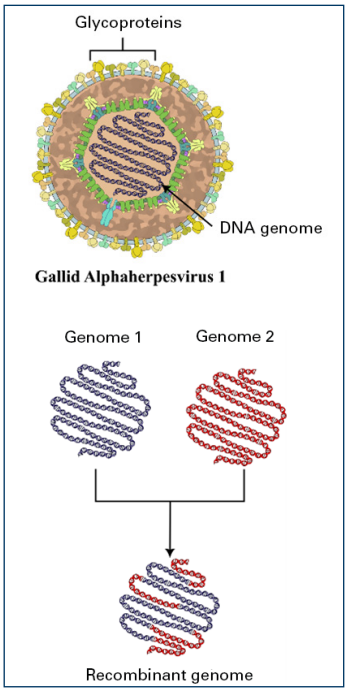

Figure 1. Schematic representation of Avian Infectious Laryngotracheitis virus and genetic recombination process.

Such processes occur when two or more viruses simultaneously infect the same cell, exchanging and mixing fragments of the genome, resulting in the appearance of viruses with intermixed genomic constitutions (Figure 1). This recombination process in GaHV-1 has been linked to the emergence of variants that cause disease outbreaks and reversion of vaccine viruses (13).

Although multiple glycoproteins (g) are recognized as antigenic determinants in GaHV-1 (gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL and gM)(9), differentiation of virus into serotypes has not been possible. All GaHV-1 are considered to be antigenically similar. Therefore, its differentiation has been based on genetic studies that allow its differentiation into vaccine and wild-type (WT) strains(16). These studies evaluate at least two genes amplified by the Polymerase Chain Reaction (PCR) technique and subsequently differentiated using the Restriction Fragment Polymorphisms (RFLP) pattern. According to the genes evaluated, between five and nine genotypes have been identified in GaHV-1 that can be summarized as WT field strains, vaccine strains produced in embryonated eggs (CEO), vaccine strains produced in cell culture (TCO), strains derived from CEO vaccines (CEO-derived) and strains derived from TCO vaccines (TCO-derived) (14).

Thanks to sequencing technologies, new analyses are being developed and implemented to study and characterize GaHV-1. These tools have led to the identification of two sublineages (European/American and Australian) and at least four viral clusters (WT, vaccine CEO, revertant CEO, and vaccine TCO) (4, 12, 21).

CLINICAL SIGNS, INJURIES AND TRANSMISSION

GaHV-1 infection occurs through the virus in secretions of sick birds, vectors and fomites that reach susceptible birds' ocular and oral-nasal mucosa. Infection begins with its replication in the ocular mucosa and the epithelia of the upper respiratory tract and the oral-nasal cavity; then, it extends to the larynx and trachea glandular tissue, finally reaching the trigeminal nerve. Once there, a latent infection is set, allowing GaHV-1 to prevail, alternating between periods of inactivity, where there is no viral replication or disease, and periods of reactivation triggered when birds are stressed and suffer immunosuppression (Figure 2). During reactivation periods, GaHV-1 actively replicates, generating lesions in the respiratory tract, and is excreted in the secretions of sick birds. Such secretions are a source of infection in susceptible individuals. Thus, individuals with latent infections play an essential role in ILT's epidemiology. Since there is no evidence of vertical transmission of GaHV-1 or egg contamination during laying, it is acknowledged that its only form of dissemination is horizontal, as shown in Figure 2.

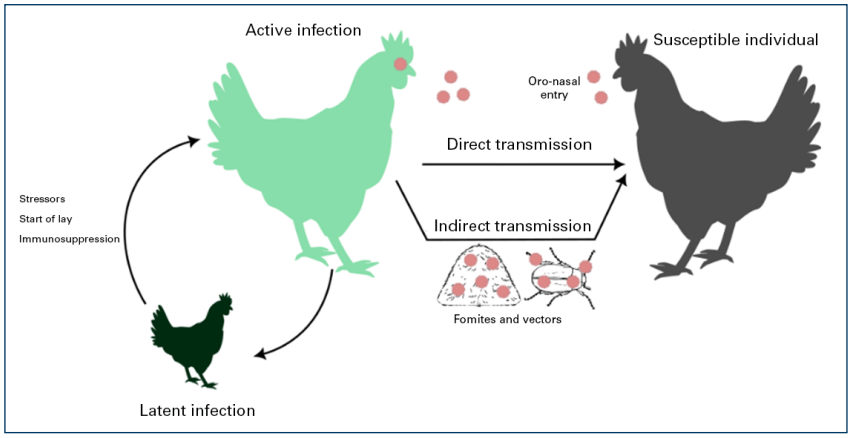

Figure 2. Transmission and circulation routes of GaHV-1 in avian populations.

GaHV-1 replication triggers a severe inflammatory reaction in the respiratory tract and ocular mucosa, causing edema, hyperemia, infiltration of immune cells and mucohemorrhagic exudates (Figures 3 and 4). These exudates obstruct the trachea and bronchi, causing respiratory sounds, dyspnea, and death by suffocation. Cyanotic birds can be observed during outbreaks, with nasal and eye secretions and panting. It is expected to identify spots of hemorrhagic expectorations on the floor and walls of sheds (Figure 5). Given that GaHV-1 induces damage to respiratory epithelium, its presence in farms can favour coinfections, resulting in severe disease and increased mortality. For this reason, GaHV-1 is to be considered a possible agent when there is suspicion of avian respiratory disease (5).

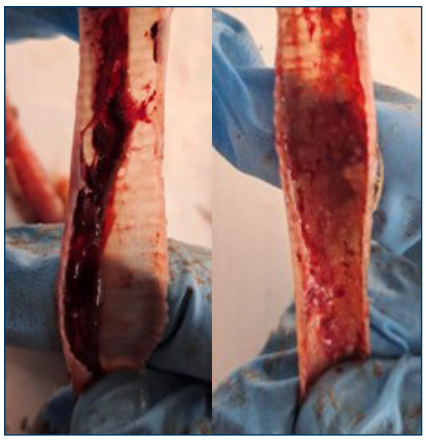

Figure 3. Presence of clots and mucohemorrhagic exudate in trachea lumen.

ILT has three forms depending on the signs' severity and mortality. The hyperacute form is considered the most serious and shows outbreaks of severe respiratory distress in a significant number of birds, which is accompanied by a mortality close to 70%. The subacute form is characterized by high morbidity and mortality, close to 30%. When the signs are mild and mortality does not exceed 2%, it is referred to as a moderate or chronic presentation (10, 17).

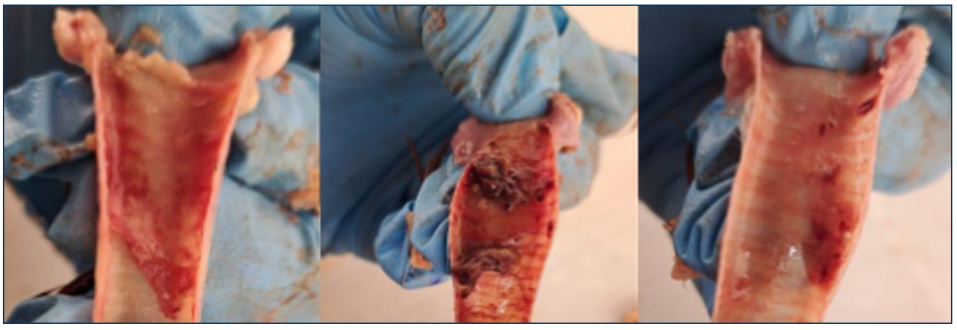

Figure 4. Different degrees of tracheal mucosa hyperemia and injury in birds with Avian Infectious Laryngotracheitis.

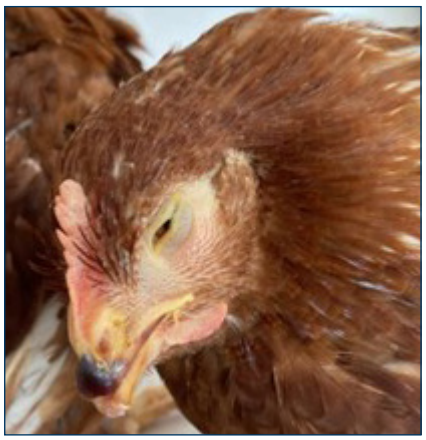

Figure 5. Facial swelling and edema in a bird infected with GaHV-1.

Table 1. Tests and samples available for ILT diagnosis in commercial birds			
Test Type	**Technique**	**Sample**	**Remarks**
Serological	Enzyme-Linked Immunoassay (ELISA)	Serum	Mainly used in determining vaccine coverage.
	Seroneutralization		
	Antigen capture ELISA	Exudates and secretions	Hard and rarely used technique.
	Immunofluorescence	Swabs and tracheal tissues or exudates	Hard techniques and results can be delayed.
Virological	Viral isolation	Swabs and tracheal or laryngeal tissues and exudates	Slow, expensive and hard process that requires confirmation with other techniques.
Molecular	PCR conventional or real-time	Swabs and tracheal or laryngeal tissues and exudates	Allows the detection of specific viral genes (e.g. TK and gC) but does not differentiate between vaccine viruses and WT.
	PCR coupled to RFLP (PCR-RFLP)		Allows detection of specific genes of virus and its genetic differentiation.

DIAGNOSIS

Because the clinical signs of ILT can vary and resemble those of other diseases, other agents, such as the Infectious Bronchitis Virus (IBV), Newcastle disease (NDV), and Influenza A virus (IAV), must be considered during respiratory problems care. Additionally, the rules and regulations of each country must be considered regarding the care of suspected cases of ILT.

Following the provisions of the WOAH (2023), ILT diagnosis can be made through serological tests that detect the bird's response to viral antigens or antibodies against the virus, viral isolation, and molecular biology tests that lead to the detection of GaHV-1 and/or parts of its genome. Table 1 summarizes the foremost diagnostic techniques recommended by the WOAH and some observations that should be considered when establishing the diagnosis of the disease.

Although serological and viral isolation tests continue to be valuable tools in ILT diagnosis, currently, diagnosis of GaHV-1 is made through the PCR-RFLP test. PCR-RFLP serves to classify viruses into WT or vaccine strains (CEO - chicken embryo origin and TCO - tissue culture origin) through the detection of differences in specific genes that influence recognition by restriction enzymes (Figure 6), which induce cuts in particular regions of genes studied, resulting in obtaining genomic fragments of different sizes depending on the sequence of each virus. Multiple genes and restriction enzymes have been used in the classification of GaHV-1; however, WOAH recommends amplification of TK, UL15, UL47, Gg, OFR-BTK and/or ICP4 genes and their processing by four enzymes (17).

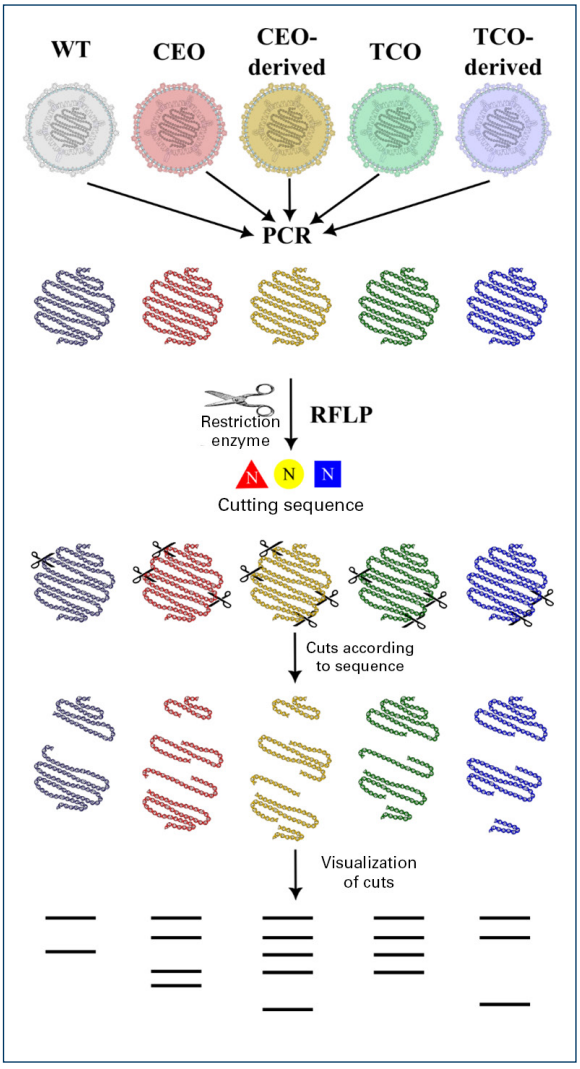

Figure 6. Schematic representation of the PCR-RFLP technique for the diagnosis and genotyping of GaHV-1.

IMPACT OF VACCINES ON THE GENETIC VARIABILITY AND EPIDEMIOLOGY OF GAHV-1

ILT was first described around 1920 in Canada as an emerging disease in chickens. Ten years later, it was identified in Australia and Europe and was later reported in South America and Asia (6, 19).

During the first half of the 20th Century, ILT cases were exclusively related to the circulation of WT viruses. However, this panorama changed after introducing vaccines against the disease in 1958 (2). Such vaccines held attenuated replicating viruses that displaced the WT strains, establishing themselves widely in avian populations, where they have undergone recombination and virulence reversal processes (13). Such processes have occurred more frequently in CEO vaccine viruses after continuous passage in birds with poor or no immunity because the attenuation of CEO viruses is less than that of TCO viruses (11). Therefore, since the implementation of vaccines against ILT, most disease outbreaks have been caused by viruses genetically related to vaccine strains.

In North America and Australia, the importance of CEO vaccine viruses has been demonstrated as they are mainly responsible for ILT outbreaks in both commercial and backyard birds with and without vaccination. Studies conducted in North America show a high diversity in GaHV-1, with nine different genotypes described based on PCR-RFLP (I-IX) (15). Of these, the predominant one has been genotype V, which corresponds to CEO vaccine viruses that reverted (CEO-derived) and have been maintained in avian populations, causing outbreaks mainly in backyard poultry (1,4,15).

Circulation of this genotype and vaccine viruses, such as the European Serva strain, have contributed to the emergence of recombinant variants. Similarly, in Australia, multiple classes of GaHV-1 have been described (class 1-9) (3). In that country, many outbreaks have been caused by CEO-derived viruses associated with SA2, A20 and Serva vaccine strains (Blacker et al., 2011). According to genetic analyses, the introduction of Serva vaccine strains in Australia contributed to the emergence of highly virulence recombinant viruses and the emergence of class 7, 8 and 9 viruses (3, 12, 20). Despite the above, in North America and Australia, the detection of other genotypes corresponding to WT viruses as causes of ILT outbreaks is still reported, as is the circulation of vaccine viruses (1, 4).

Evidence of the association of WT and CEO-derived viruses in ILT outbreaks has been found in other parts of the world, with viruses of this type reported as the most relevant in ILT cases in Asia, Latin America, and Europe.

ILT PREVENTION AND CONTROL

Given that birds infected and vaccinated with CEO and TCO viruses will have latent infections that can be reactivated, the primary biosecurity measure on farms is based on avoiding contact with these birds with susceptible individuals. This is achieved by implementing good livestock practices that guarantee shed isolation and the absence of cross-contamination by fomites between groups. Likewise, when there are ILT outbreaks, it is necessary to decontaminate sheds since GaHV-1 can persist in bedding material and surfaces where biofilms exist (8, 18).

Given the high impact of ILT on the poultry industry, three types of vaccines for the prevention of ILT are currently commercially available (Figure 4). For its use and/or implementation, it is essential to review and adhere to the regulations of each country.

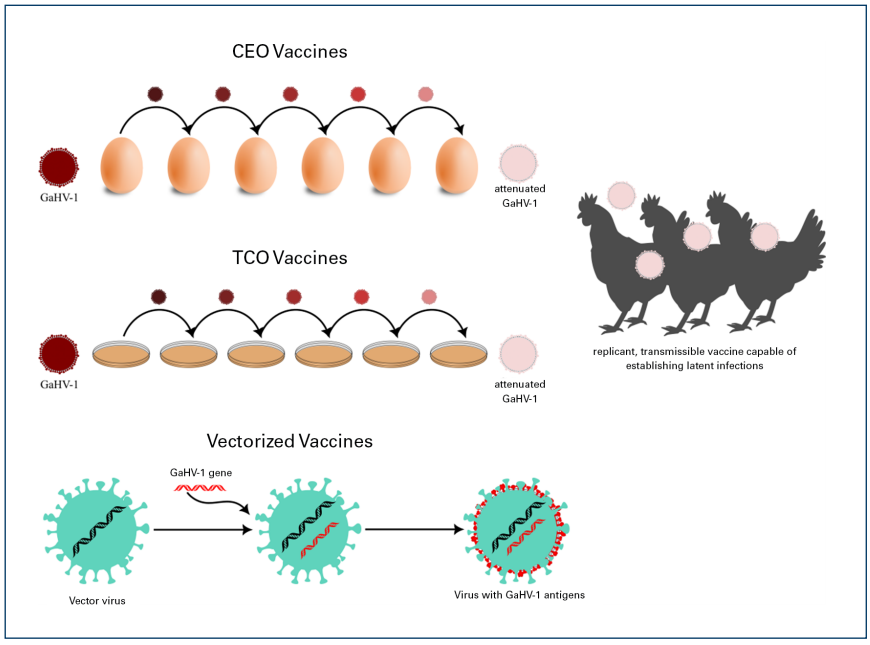

Figure 7. Principles and types of commercially available vaccines against ILT.

The most widely used vaccines are those with live attenuated CEO and TCO viruses. Both vaccines are produced through the serial passage of live viruses and either in chicken embryos (CEO) or in cultured tissues (TCO) (Figure 7). These biologicals have demonstrated adequate efficacy in inducing good immunity in birds. The main advantage of CEO vaccines is that they can be applied as a spray or in drinking water, facilitating mass immunization. However, the application of these vaccines is critical since, due to their residual virulence and failures in vaccine coverage, they favour the reversion of attenuated viruses. For this reason, CEO vaccines have been questioned, even being banned in some countries. TCO vaccines are safer and have lower reversals than CEO vaccines. However, its application is mainly done through the eye, which makes its mass application difficult. To avoid safety problems with OCT vaccines, it must be guaranteed that immunization is carried out by the route recommended by the manufacturer and achieves good vaccine coverage.

V vectorized vaccines have been developed as safer alternatives to the CEO and TCO vaccines. They use other modified viruses expressing GaHV-1 antigens (gB and UL32 / gD and gI) (Figure 7). In these vaccines, agents such as NDV, Avian Pox, and other Herpesviruses such as Turkey Herpesvirus or Marek's Disease Virus have been used to carry the GaHV-1 gene. The safety of these vaccines lies in using only a part of GaHV-1, so the vaccine does not take the ILT agent as such in its composition (Figure 7) (10). This type of vaccine is given parenterally or in ovo. It has the disadvantage that it induces a neutralizing immune response less than that achieved with live attenuated vaccines (7).

PHOTOGRAPHS

The authors express their appreciation to veterinary doctors Alberto Paredes and Jesús Peña for their shared photographs.

REFERENCES

1. Barboza-Solis, C., Contreras, A. P., Palomino-Tapia, V. A., Joseph, T., King, R., Ravi, M., Peters, D., Fonseca, K., Gagnon, C. A., van der Meer, F., & Abdul-Careem, M. F. (2020). Genotyping of Infectious Laryngotracheitis Virus (ILTV) Isolates from Western Canadian Provinces of Alberta and British Columbia Based on Partial Open Reading Frame (ORF) a and b. Animals, 10(9), 1634. https://doi.org/10.3390/ANI10091634
2. Benton, W. J., Cover, M. S., & Greene, L. M. (1958). The Clinical and Serological Response of Chickens to Certain Laryngotracheitis Viruses. Avian Diseases, 2(4), 383. https://doi.org/10.2307/1587478
3. Blacker, H. P., Kirkpatrick, N. C., Rubite, A., O'Rourke, D., & Noormohammadi, A. H. (2011). Epidemiology of recent outbreaks of infectious laryngotracheitis in poultry in Australia. Australian Veterinary Journal, 89(3), 89–94. https://doi.org/10.1111/J.1751-0813.2010.00665.X
4. Contreras, A. P., van der Meer, F., Checkley, S., Joseph, T., King, R., Ravi, M., Peters, D., Fonseca, K., Gagnon, C. A., Provost, C., Ojkic, D., & Abdul-Careem, M. F. (2020). Analysis of Whole-Genome Sequences of Infectious laryngotracheitis Virus Isolates from Poultry Flocks in Canada: Evidence of Recombination. Viruses, 12(11), 1302. https://doi.org/10.3390/V12111302
5. Couto, R. M., Braga, J. F. V., Gomes, S. Y. M., Resende, M., Martins, N. R. S., & Ecco, R. (2016). Natural concurrent infections associated with infectious laryngotracheitis in layer chickens. Journal of Applied Poultry Research, 25(1), 113–128. https://doi.org/10.3382/JAPR/PFV075
6. Cover, M. S. (1996). The early history of infectious laryngotracheitis. Avian Diseases, 40(3), 494–500. https://doi.org/10.2307/1592256
7. García, M., & Zavala, G. (2019). Commercial Vaccines and Vaccination Strategies Against Infectious Laryngotracheitis: What We Have Learned and Knowledge Gaps That Remain. Avian Diseases, 63(2), 325–334. https://doi.org/10.1637/11967-090218-REVIEW.1
8. Giambrone, J. J., Fagbohun, O., & Macklin, K. S. (2008). Management Practices to Reduce Infectious Laryngotracheitis Virus in Poultry Litter. Journal of Applied Poultry Research, 17(1), 64–68. https://doi.org/10.3382/JAPR.2007-00017
9. Goraya, M. U., Ali, L., & Younis, I. (2017). Innate Immune Responses Against Avian Respiratory Viruses. Hosts and Viruses, 4(5). https://doi.org/10.17582/JOURNAL.HV/2017/4.5.78.87

10. Gowthaman, V., Kumar, S., Koul, M., Dave, U., Murthy, T. R. G. K., Munuswamy, P., Tiwari, R., Karthik, K., Dhama, K., Michalak, I., & Joshi, S. K. (2020). Infectious laryngotracheitis: Etiology, epidemiology, pathobiology, and advances in diagnosis and control – a comprehensive review. Veterinary Quarterly, 40(1), 140–161. https://doi.org/10.1080/01652176.2020.1759845

11. Guy, J. S., Barnes, H. J., & Smith, L. (1991). Increased virulence of modified-live infectious laryngotracheitis vaccine virus following bird-to-bird passage. Avian Dis., 35(2), 348–355.

12. Lee, S. W., Devlin, J. M., Markham, J. F., Noormohammadi, A. H., Browning, G. F., Ficorilli, N. P., Hartley, C. A., & Markham, P. F. (2013). Phylogenetic and Molecular Epidemiological Studies Reveal Evidence of Multiple Past Recombination Events between Infectious Laryngotracheitis Viruses. PLOS ONE, 8(2), e55121. https://doi.org/10.1371/JOURNAL.PONE.0055121

13. Lee, S. W., Markham, P. F., Coppo, M. J. C., Legione, A. R., Markham, J. F., Noormohammadi, A. H., Browning, G. F., Ficorilli, N., Hartley, C. A., & Devlin, J. M. (2012). Attenuated vaccines can recombine to form virulent field viruses. Science, 337(6091), 188. https://doi.org/10.1126/SCIENCE.1217134/SUPPL_FILE/LEE.SM.PDF

14. Menendez, K. R., García, M., Spatz, S., & Tablante, N. L. (2014). Molecular epidemiology of infectious laryngotracheitis: a review. Avian Pathology, 43(2), 108–117. https://doi.org/10.1080/03079457.2014.886004

15. Oldoni, I., & García, M. (2007). Characterization of infectious laryngotracheitis virus isolates from the US by polymerase chain reaction and restriction fragment length polymorphism of multiple genome regions. Avian Pathology, 36(2), 167–176. https://doi.org/10.1080/03079450701216654

16. Oldoni, I., Rodríguez-Avila, A., Riblet, S., & García, M. (2008). Characterization of Infectious Laryngotracheitis Virus (ILTV) Isolates from Commercial Poultry by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP). Avian Diseases, 52(1), 59–63. https://doi.org/10.1637/8054-070607-REG

17. OMSA. (2023). Laringotraqueítis Infecciosa Aviar. In Manual Terrestre de la OIE. OMSA.

18. Ou, S., Giambrone, J. J., & Macklin, K. S. (2011). Infectious laryngotracheitis vaccine virus detection in water lines and effectiveness of sanitizers for inactivating the virus. Journal of Applied Poultry Research, 20(2), 223–230. https://doi.org/10.3382/JAPR.2010-00300

19. Parra, S. H. S., Nuñez, L. F. N., & Ferreira, A. J. P. (2016). Epidemiology of Avian Infectious Laryngotracheitis with Special Focus to South America: an update. Brazilian Journal of Poultry Science, 18(4), 551–562. https://doi.org/10.1590/1806-9061-2016-0224

20. Sabir, A. J., Olaogun, O. M., O'Rourke, D., Fakhri, O., Coppo, M. J. C., Devlin, J. M., Konsak-Ilievski, B., & Noormohammadi, A. H. (2020). Full genomic characterisation of an emerging infectious laryngotracheitis virus class 7b from Australia linked to a vaccine strain revealed its identity. Infection, Genetics and Evolution, 78, 104067. https://doi.org/10.1016/J.MEEGID.2019.104067

21. Wu, M., Zhang, Z., Su, X., Lu, H., Li, X., Yuan, C., Liu, Q., Teng, Q., Geri, L., & Li, Z. (2022). Biological Characteristics of Infectious Laryngotracheitis Viruses Isolated in China. Viruses, 14(6). https://doi.org/10.3390/V14061200