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REVIEW ARTICLE

A Review of the Advanced Immunogens for the Protection of Poultry Flocks Against Infectious Laryngotracheitis Virus

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Infectious laryngotracheitis virus is a member of a family of herpes viruses causing infection in avian species. It is a deadly virus that causes infection in multiple species of avians causing respiratory and ocular infections. ILT virus causes high mortalities, morbidities and economic losses in commercial poultry flocks. Traditionally, ILT is managed by the vaccination through live attenuated viruses synthesized either through tissue culturing or passaging through chicken embryos. The viral vector vaccines are also now commonly used commercially. Recent research highlights novel immunogenic candidates, which include viral vectors, genome-attenuated viruses, subunit vaccines, and DNA particles. The results of these vaccines have been tested in *in vivo* environment and they are providing sufficient levels of immunity against ILT. Further research is needed to remove the constraints of these vaccines to be used commercially.

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INTRODUCTION

Infectious diseases are of great concern because of their lethality and economic losses (Saeed *et al.*, 2023; Saeed and Alkheraije, 2023). Viral diseases are considered the most serious issues because no known treatment exists today (Trovato *et al.*, 2020; Meganck and Baric, 2021). Infectious laryngotracheitis (ILT) is among the most prevalent and pathogenic diseases of poultry (Tamilmaran *et al.*, 2020), causing high economic losses in commercial poultry farms (Gowthaman *et al.*, 2020). ILT is caused by the ILT virus (ILTV) which is classified as *Gallid herpes virus 1* belonging to the *Iltovirus* genus of the *Alphaherpesevrinae* subfamily.

ILT is horizontally transmissible, having the trachea and conjunctiva as its primary predilection sites (Fakhri *et al.*, 2020; Yegoraw *et al.*, 2021). The major clinical signs of ILT include dyspnea, inflammatory exudates, conjunctivitis, coughing, polypnea, and other upper tract respiratory system pathologies (Fakhri *et al.*, 2020). These problems result in a loss of productive performance in terms of meat and egg production losses (Gowthaman *et al.*, 2020; Hassan and Abdul-Careem, 2020; Pajić *et al.*, 2022). It is highly contagious, and the morbidity rate ranges from 90 to 100% (Bayoumi *et al.*, 2020; Zorman Rojs *et al.*, 2021) while it is also lethal, causing up to 70% mortality in infected flocks (Gowthaman *et al.*, 2020). Moreover, it can go into latency in the peripheral nerves, and later, because of stress it may reactivate and cause re-infections (Thilakarathne *et al.*, 2020b).

The ILT was the first time discovered in 1925 by May and Tittsler (Wu *et al.*, 2022) and soon after the discovery of the virus its vaccine was also developed (Bagust and Johnson, 1995; Bublot, 2024). The vaccine for the ILT virus was the pioneer vaccine for the viral diseases of poultry (Zhang *et al.*, 2022). Tissue culturing was used to develop the attenuated live vaccines by passing them into cell cultures. In a study, live virus vaccine was administered through water, and it was proved effective in controlling the disease (Ahaduzzaman *et al.*, 2020). Similarly, chicken embryos were also used to produce similar types of vaccines for commercial poultry vaccination. These vaccines have gained popularity among poultry farmers and are being popularly used in commercial poultry farming (Thilakarathne *et al.*, 2020a; Palomino-Tapia *et al.*, 2023). These vaccines are economical and have provided sufficient immunity against routine infections (Maekawa *et al.*, 2021a). These vaccines are still popular against ILT infections, but several farmers reported the limitations of these vaccines (Ahaduzzaman *et al.*, 2020), and because of these issues, alternatives to these vaccines are being researched (Zeng *et al.*, 2024). Routinely used live attenuated vaccines need high levels of care and handling (Assen *et al.*, 2020). The live attenuated

virus vaccine is being used to vaccinate the birds; however, it may be source of infection to any immunocompromised birds (Ravikumar *et al.*, 2022; Ganapathy and Parthiban, 2024). These vaccine viruses can rarely revert to virulent form and can cause infection at any time (Barboza-Solis *et al.*, 2020; Gowen *et al.*, 2021). Because of these issues, scientists are investigating multiple alternatives to these vaccines.

In the last decade, recombinant vaccines have gained a lot of importance, and they have been researched for the control of ILT (Zeng *et al.*, 2024). Fig. 1 illustrates the structure of ILT virus showing its immunogenic proteins and other structures. The recombinant viruses have been tried and searched using the fowl poxvirus and herpesvirus of Turkey expressing the glycoproteins of the ILT virus (Chen *et al.*, 2020; Gaghan *et al.*, 2023). These vaccines have been reported to be used in some countries to replace classical attenuated membranes (Bhuiyan *et al.*, 2021b). This has encouraged researchers to find multiple other methods for producing vaccines for the infectious laryngotracheitis virus. These vaccines include classical live attenuated, viral vector-based, recombinant DNA, mRNA, subunit, and virus-like-particle types of vaccines (Mebatsion, 2021; Raji *et al.*, 2024). Despite problems reported with classical live attenuated vaccines, these vaccines are still in use. All the types of vaccines have their pros and cons which need to be observed. Previously Coppo *et al.* (2013) and Maricarmen and Guillermo (2019) have reviewed the advances in vaccine development but several research progresses have been done within the last 5 years. This review summarizes the latest research done in the last 5 years.

MATERIALS AND METHODS

The Scopus Document Search (https://www.scopus.com/search/form.uri#basic) was used as the basic search tool while NCBI-PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Google Scholar (Scholar.google.com) were used for refining the results. The keywords used were "Infectious Laryngotracheitis" AND "Vaccine". The inclusion criteria were time frame (2019-2024), "type of document ("Peer-reviewed research article"), and title with keywords. The filtered results were included in the construction of Table 1. The metadata with inclusion and exclusion criteria is displayed in Fig. 2.

Immunological properties of ILTV: The ILT virus has a great structural resemblance to other members of the subfamily *Alphaherpesvrinae* (Yang *et al.*, 2020). It is an enveloped virus with a nucleocapsid covered by a lipid membrane (Liu *et al.*, 2024). Its genome is comprised of double-stranded DNA inside the capsid (Bindari *et al.*, 2020). Several proteins are between the capsid and membrane which work as immunogens playing pivotal role in virulence of ILT virus which are named gB, gC, gD, etc(Sabir *et al.*, 2020; Elshafiee *et al.*, 2022). Some of the proteins are secreted by the host cells upon insertion of the ILT virus inside cells (Chen *et al.*, 2024).

Immune systems of vertebrates can be majorly categorized into two categories which are either humoral or cellular (Hira, 2022). The main humoral antibodies are produced by B cells matured in the bursa of the birds (Cheng *et al.*, 2023) while the cellular immunity is produced by the T cells matured in the thymus inside the chickens. Research states that removing bursa had no effects on the ILT virus immunization while removing thymus in experimentally induced birds resulted in reduced immunity (Beltrán *et al.*, 2019; Gopakumar *et al.*, 2024). It means that the T cells or cellular immune response has major roles. Although the B cell-mediated humoral immune response is the primary immune response in most viral diseases, the ILT virus has separate behavior because of several unknown mechanisms (Bela-Ong *et al.*, 2023). Understanding the behavior of immunity against ILT is the key to developing proper immunogens against ILT.

Recent advancements in immunization of ILTV: The development of vaccines via various routes is ongoing since the first trial of the vaccine (Palomino-Tapia *et al.*, 2023). Classical live attenuated chicken embryo or tissue vaccines are still being researched using various modern methods. Within 6 years (2019-2024), 17 peer-reviewed articles have been published (Scopus; Fig. 2) focusing importance of ILT in poultry. So, this review highlights all the modern methods searched for ILT vaccines in the 20220-2024 era. Following is a brief of these vaccines.

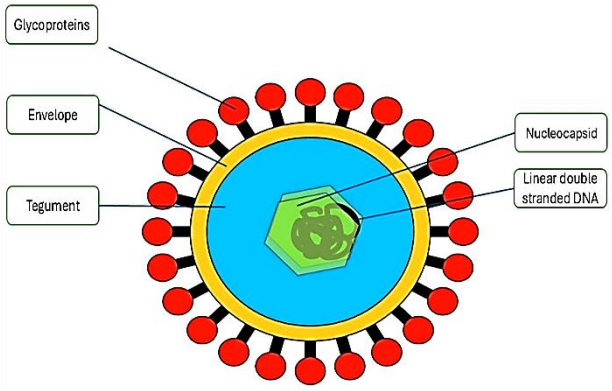

Fig. 1: Structure of ILT virus showing its immunogenic proteins and other structures.

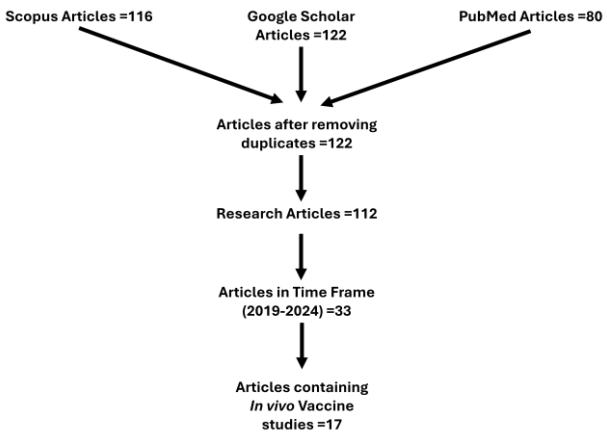

Fig. 2: The data obtained with specific keywords from search engines.

Live attenuated vaccines: Attenuation of infectious agents to use as immunogens is the first method of vaccine development (Choy *et al*., 2022; Matić and Šantak, 2022; Zhou *et al*., 2022). Despite several developments and the introduction of novel methods in vaccine production (Lin *et al*., 2016), live attenuated vaccines are among the most

Table 1: A qualitative comparison of various vaccines used for protection against ILTV from 2019-2024 (Source: Scopus; Google Scholar, PubMed; n=17).

Type of Vaccine	licensed	Ease in administration	Level of protection	Production techniques	Chances of reversion	Recombinant virus production risk	Risk of latency
Live attenuated	Yes	Easy	Medium protection	Tissue cultured may be a bit difficult	High chances of reversion, especially in chicken embryo origin	Most often	High risks
Viral vectored	Yes	Easy	High	Easy	No risk of reversion	No risks of recombination	Low risk
Genetically attenuated	Yes	Easy	High	Easy	Conditional reversion may be present	Recombinant viruses have been observed	Latent phases have been observed
DNA	Only one	Unsure	Medium (limited results)	High technical skills required	Not present	No risks of recombination	Not determined
Subunit	No	difficult	Long-term studies not available	High technical skills required	Not present	No risks of recombination	Not determined
Virus-like particles	no	Data insufficient	Long-term studies not available	High technical skills required	Not present		Not determined

Table 2: Recent studies (2019-2024) compassing the ILT virus vaccination.

Type of vaccine	Strain/ srovar/ immunogen	Vaccine serotype/vector	Organism	Inoculation model	Experimental design	Parameters/mode of study	Results	References
LAT	CEO	Serva	Broiler	Eye drop	Factorial design (0-100%) flock vaccinated	Weight gain in	Weight reduction in 0, and 10% vaccinated birds compared to 20&100% flocks	(Assen *et al.*, 2023)
	CEO	-	White Leghorn	Eye drop	Randomized trial	Conjunctival lymphoid tissues and B & T cell immunity was compared	Expression of CD8 alpha and granzyme A gene expression reduces the viral proliferation	(Beltrán *et al.*, 2019)
LAT and Vector	CEO and TCO		White Leghorn		Randomized complete block	Comparative efficacy of vaccinated and unvaccinated Birds in the virulent challenge were evaluated	Recombinant vaccine has more immunogenic effects than the TCO and CEO vaccines	(El-Saied *et al.*, 2022)
GM	NHEJ-CRISPR/Cas9, Cre-LOX	--	LMH cell lines	-	Pairwise, comparisons	Immunogenicity tested in cell lines	The recombinant vaccines provided stuffiest response in cell lines	(Atasoy *et al.*, 2019)
VV	TCO	ILTV 1874C5	Broiler	*In ovo*	Randomized trials	Mortality, clinical signs and weight gain were tested	VV had positive results and provided long lasting immunity	(Maekawa *et al.*, 2019a)
VV, LAT	TCO (VV), CEO (LAT)	Innovax®-ILT	chicken	*In ovo*	Randomized	Mortality, clinical signs and weight gain were tested	Combination had the most pivotal results	(Maekawa *et al.*, 2019b)

popularly used vaccines against several infectious diseases including viral diseases (Gao *et al.*, 2019; Dong *et al*., 2024). Live attenuated vaccines are achieved by several passages of viruses in the non-host tissues (Imhof *et al.*, 2024). These passages make the pathogen less virulent. In the case of ILT viruses, Chicken embryo and tissue cultures are used to decrease the virulence of the virus (Thilakarathne *et al*., 2020a; Becerra *et al*., 2023; Palomino-Tapia *et al*., 2023). Vaccines of several types including A20, Serva, SA-2, and cover have been produced to protect commercial birds against ILT infection (Barboza-Solis *et al*., 2021; Elshafiee *et al*., 2022). These vaccines are administered through various routes to achieve high and durable immunity titers (Bhuiyan *et al*., 2021a; Wang *et al*., 2024b; Zeng *et al*., 2024). The most popular routes of administration include orally in water, spraying in the shed and ocular route (Gowthaman *et al*., 2020). The ocular routes are safe and provide maximum immunity (Conrady and Yeh, 2021; Wang and Zhang, 2023). Live attenuated vaccines, either of tissue culture or chicken embryo origin, provide high amount of immune response (Thilakarathne *et al*., 2020a; Perez-Contreras *et al*., 2021; Becerra *et al*., 2023). Because of this efficiency, ILT immunization is majorly achieved by live attenuated vaccines till today (Abdelaziz *et al*., 2024). They are economical and easy to be produced, and require less skill and labor than modern methods so, these make them suitable candidates to be used commercially (Shuja *et al*., 2022; Ganapathy and Parthiban, 2024; Tariq M *et al*., 2024).

Despite these advantages, there are still some issues being reported on commercial forms. In research and case studies, the reversion of live attenuated ILT vaccines into virulent forms is reported (Wong *et al.*, 2020; Nunberg *et al.*, 2024). The vaccine has been reported to revert into its virulent form in pre-exposed birds or birds with low immunity (Li *et al.*, 2016; Barboza-Solis *et al.*, 2021; Elshafiee *et al.*, 2022). It also has been reported that the live attenuated vaccine viruses made a new virus, sharing their genome with any other virus, leading to new pathogenic variants. Live attenuated vaccines developed though chicken embryos have been reported to be associated with outbreak while tissue culture produced vaccines viruses have been reported to have less risks of reversion and outbreak association. Similarly, the vaccines produced through tissue culturing, if revert into virulent form, have mild respiratory disorders compared to vaccines produced in chicken embryo passages (Maekawa *et al.*, 2021b). Studies are needed to understand the mechanisms of reversion and the appropriate tissue culture so that the live

attenuated vaccines may become successful for future use. Currently most of studies are using them as standard for comparisons to other vaccines, signifying their vitality in immunization strategies against ILT infection.

Vectored vaccines: These are almost as popular as the live attenuated vaccines for control of viral diseases of poultry. These vaccines are formulated by using a nonpathogenic virus as carrier for vaccine virus (Wang *et al*., 2024a). The first use of licensed viral vectored vaccine against ILT was published in 2006 using fowl pox virus expressing the gB antigen of ILT virus (García, 2017). Vectored vaccine for ILT vaccinations mainly herpes and fowl pox viruses act as carriers, but modern research is focusing on the use of ND virus as vector of IT virus (Bublot, 2024; Ganapathy and Parthiban, 2024). Most of the antigenic nucleic acids encoding the immunogenic glycoproteins B (gB), I(gI), g(D) etc. are used along with cytokines or interleukins in the carrier genome for the development of vectored vaccines (Jiang *et al*., 2024). In contrast to live attenuated vaccines the recombinant vaccines are not given orally but they are given subcutaneously or in wing web (El Boraey *et al*., 2024). The injection of vaccine inside egg is also recommended at commercial levels. Recombinant vaccines have less chances of reversion and are very much less pathogenic than the live attenuated vaccines. These vaccine ILT viruses don't go in latent phase and protect birds actively (Munuswamy *et al*., 2019; Schat and Skinner, 2022). However, it has been reported that although vaccine viruses may not go in latent phase, it may lead to naturally occurring wild-type viruses to go dormancy. It may result in later infections or infections in non-vaccinated birds (Table 2).

Genetically modified vaccines: In the current era, with the innovation of DNA recombinant technologies, attempts of genetically modified organisms are in a surge (Weiner, 2020). These attempts at making genetically modified organisms have been boosted by the introduction of CRISPR-CAS (Zhang *et al*., 2021a; Bhujbal *et al*., 2022). The viral vaccines with editing in the genome by inserting or deleting (knocking out) specific sequence lead to a virus with immunogenic and no-pathogenic variants (Wang *et al*., 2024c). Most of the time the genes responsible for virulence or replications are spliced out and the non-virulent, non-replicating virus is achieved to produce vaccine (Perkus and Paoletti, 2012; Kangethe *et al*., 2022). The spliced genome is replaced with some silent part or inactive analogue so that the main structure of genome remains maintained (Wong and Tremethick, 2024), and the virulence or replication-related part is maintained (Zhang *et al*., 2021b; Abushattal *et al*., 2022). Although these vaccines are safer and effective compared to the live attenuated vaccine, they have some constraints regarding their usage (Pandey *et al*., 2020b; Gupta and Pellett, 2023).

Insertion of mutation in the genome may result in various viral abnormalities which may interfere with the cellular process of infected chicken cells (Gul *et al.*, 2022). These may result in the development of recombinant vaccines (Hellmich *et al.*, 2020; Kim *et al.*, 2023). The genetically modified vaccines also have spliced virulence or replication genes which make their reversion difficult, however, lower immune responses are also observed in these vaccines. The immune response is immediately produced but is of a lower level and has a short duration. Currently researchers are focusing on selection of those parts that ensure that the virus will not revert into virulent type along with strong and long-lasting immunity (Triggle *et al.*, 2021). Some modified vaccines have also been produced and licensed e.g. Barboza-Solis *et al*. (2020) have stated that the pathogenic protein gG deleted vaccine of ILTV has been licensed having proven more effective and less pathogenic effects than the commercial live attenuated vaccines (Gopakumar *et al*., 2024).

DNA vaccines: DNA vaccines are based on nucleic acids and consist only immunogenic part of nuclear material. These DNA produce immunogenic proteins providing efficacy (Lin *et al*., 2016; Qin *et al*., 2021; Ruan *et al*., 2023). Although DNA vaccines are being tried for decades, but the first licensed DNA vaccine has been licensed in 2017 by United states Department of Agriculture for use in poultry against Avian influenza (Guyonnet and Peters, 2020). After this, the research is focusing the use of DNA vaccines against many pathogens of poultry species (Mustafa *et al*., 2024). These vaccines are proving themselves effective to control the poultry disease. Recently a few studies have been conducted to evaluate the DNA vaccines against ILT virus (Ahaduzzaman *et al*., 2020; Barboza-Solis *et al*., 2020; Gamal and Soliman, 2023). DNA vaccines are proving themselves effective in controlling ILT signs and symptoms (Jogi *et al*., 2024). Further work is needed to reduce the risks of vaccine failure and DNA insertion failure.

Subunit vaccines: Subunit vaccines focus insertion of only specific immunogens so that the vaccinated animal may not suffer overburden and immunity to diseases may be achieved (Lopez *et al*., 2023; Barbosa *et al*., 2024). These are only immunogenic proteins containing no part of nuclear material, so there is no risk of transmission or reversion as the pathogen (Pandey *et al*., 2020a; Citarasu *et al*., 2023). Although not many studies have been presented recently but the subunit vaccines have been tried against infectious laryngotracheitis (Chen *et al*., 2011) in the past. They have been proven effective so subunit vaccines can be a good candidate to be searched as vaccine candidates for ILT vaccination (Table 2).

Virus-Like Particles: Virus-like particles are synthetic analogs to wildtype viruses, they are synthetically made to elicit an immune response without any risk of reversion or failure (Pankrac, 2020). These virus-like particles cannot replicate but represent viruses like immunogens inducing immune responses. Attempts have been made to synthesize the virus-like proteins using the immunogenic proteins gB of ILT viruses and interferon gamma against ILT viruses.

Limitations and Prospects: ILT vaccination is being tried to control the ILT in the commercial poultry to avoid the losses in the flocks (García and Zavala, 2019). Live attenuated, vectors, recombinant, subunit, DNA based etc. types of vaccines are being searched (Cid and Bolívar, 2021; Gupta and Pellett, 2023). Commercially available vaccines against ILT include the live attenuated (tissue

culture or chicken embryo based) vaccines, vectored vaccines and recombinant vaccines (Becerra *et al.*, 2023) while the subunit, DNA and virus like proteins are being tried. The route of administration in practice includes spray, oral in water, ocular, wing web, subcutaneous, in ovo etc. (Wolfrum, 2020). All this research focuses on the new particles, but no study is focusing on use of delivery vehicles like nanoparticles for effective and long lasting immunity. Multiple research projects have been reported presenting that the use of adjuvants and delivery vehicles for vaccines in poultry increases the effectiveness of vaccines (Nooraei *et al.*, 2023; Abdelaziz *et al.*, 2024). Work on delivery systems for ILT viruses is needed. Further the reasons and gene involved in reversion must be sorted out to limit these issues in future vaccine regimes. Research is reporting the efficacy of vaccines, but no research is being made on the improving the availability of vaccines and moreover the estimating the factors associated with the possible adverse effects of modern types of vaccines is lacking. The DNA and virus-like particles may have some genetic shifts and to avoid future risks long-term study are needed. Despite some shortcomings, the ongoing research is being carried out at a good pace which is paving to counter all the hurdles.

Conclusions: ILT vaccination is necessary to avoid the economic losses. Despite the Live attenuated and vectored vaccines are still being used, but because of viral reversion and vaccines failure the research is needed to synthesize novel vaccines. Recent studies have been conducted to estimate the potential of traditional vaccines and new methods of vaccination. Encouraging results of novel methods encouraging scientists to investigate the future aspects to achieve sustainable protection against ILT infection in poultry.

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