Research Article

Herpesvirus of Turkeys as a Vaccine Vector in Viral Diseases: Pros and Cons

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Abstract | Viral diseases of poultry have a devastating impact on the livestock industry leading to substantial economic loss in the worldwide economy. Recent advances in biotechnology have permitted the manipulation of viral genomes, thereby gaining a better understanding of the molecular insights of viral diseases. These have spurred the development of safer and efficient vector vaccines, which convey immunogenic genes of interest obtained from vitally important diseases. Herpesvirus of turkeys (HVT) has long been considered a versatile tool owing to its non-pathogenic characteristic, relatively higher transgenic capacity, and enabling the DIVA strategy. Numerous bivalent and multivalent HVT constructs have been generated by various methods and some of these constructs have been made commercially available so far. The efficacy of HVT-vectored vaccines has been assessed either individually or in combination with other vaccine formulations; hence, the optimal competence of these vaccines against viral diseases has been reported. Based on the current knowledge, this article outlines the benefits and drawbacks of HVT-vectored vaccines in combating health-threatening poultry infections and briefly pinpoints the future perspectives for improving the success of poultry vaccines.

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Introduction

The poultry sector has been exponentially growing owing to guard its profitability within the livestock industry throughout the world. Intensive poultry rearing systems enable an average consumer to afford cheap and high-quality protein sources. At this point, rigorous prevention and control strategies against pathogens are crucial to prevent the spread of diseases thereby sustaining long-term mass production. Vaccination is one of the main components of these strategies; therefore, a multitude of killed (KV) and live-attenuated (LAV) vaccines have been developed and introduced to the sector so far (Mottet and Tempio, 2017; Romanutti *et al.*, 2020).

With the advance of molecular biology techniques, vector vaccines have come forward with their superior features in the last decade. In general, a viral vector transduces the gene of interest effectively and stimulate the host immune system against targeted antigens (Ura *et al.*, 2014). Furthermore, recombinant vector vaccines do not revert their virulence; do not require rappels; have limited side effects; and offer differentiating vaccinated animals from naturally infected animals (DIVA) (Hein *et al.*, 2021). In recent years, turkey herpesvirus (HVT) has rapidly emerged as a promising viral vector for poultry. With the aid of genetic engineering technology, several bivalent or multivalent recombinant HVT vaccine (rHVT) formulations have been generated and approved by

authorities in various countries.

The *Mardivirus* genus consists of well-recognized avian herpesvirus species including *Anatid alphaherpesvirus 1*, *Gallid alphaherpesvirus 2*, *Gallid alphaherpesvirus 3* and *Meleagrid alphaherpesvirus 1*, the last of which is also known as turkey herpesvirus (HVT). HVT consists of low-virulent, non-oncogenic strains which have been broadly applied to elicit protection against Marek's Disease (MD). However, increased use of HVT in different formulations has given rise to novel variants with enhanced virulence; hence, multivalent vaccines including SB1, 301B/1 or CV1988 strains have been formulated for MD infections (Kim *et al.*, 2020). On the other hand, the concept of recombinant HVT vaccines was initially developed in the early '90s (Marshall *et al.*, 1993), and since then, they have been utilized as a viral carrier against some major poultry diseases in both bivalent and multivalent formulations.

Herpesvirus of Turkeys as a vaccine vector

Multiple approaches have been described to manipulate the herpesviral genome and they have been entitled under (1) bacterial artificial chromosome (BAC), (2) *en passant* mutagenesis, (3) homologous recombination (HR), (4) codon optimization and (5) CRISPR/Cas9 tool (Kamel and El-Sayed, 2019). BAC and HR techniques have achieved widespread application for generating recombinant vaccines, while CRISPR/Cas9 technology has had an increasing trend over the decade. Chronologically, HR based technique was initially described to construct an HVT vector and proved by expressing Newcastle disease (ND) fusion protein (Sondermeijer *et al.*, 1993). Then, the HVT genome turned into a plasmid construct via BAC technology, which also allowed gen manipulations such as insertion or deletion (Baigent *et al.*, 2006). However, these methods were quite laborious and demanding; therefore, targeted DNA nucleases (Bi *et al.*, 2014) had recently become a paradigm-shifting innovation that offered to insert a foreign gene into an exact position rapidly with higher specificity.

The disruption of intergenic regions usually does not alter the virulence of herpesviruses, whereas virulence-associated gene indels do; therefore, the latter is considered to reduce virulence and insert an expression cassette simultaneously. At least eleven non-essential genes have been recognized in the HVT genome so far (Hall *et al.*, 2015) and *US2*, *US7* or *UL40* genes; *US3/US4*, *UL54/UL55*, *UL45/46* or *HVT065/HVT066*

intergenic regions have been used for foreign gene insertion. Foremost examples of the recombinant HVT vaccines are Newcastle disease (ND) vaccines. There were two types of conventional vaccines –LAV and KV– available against ND, and both had a plethora of drawbacks. It was quite challenging to provide optimized usage of the LAV vaccine, as it could cause iatrogenic infections, while KV required subcutaneous or intramuscular administration and rappels, also lack of engaging cell-mediated immunity (Dimitrov *et al.*, 2017). Considering these limitations, several HVT vector-based ND vaccines have been generated. Typically, fusion (*F*) gene expression cassettes have been constructed by using Rous sarcoma virus long terminal repeat, chicken β -actin or cytomegalovirus immediate-early promoters and inserted into *US10*, *UL45/UL46* or *US3/US4* positions (Bublot *et al.*, 1999; Dorsey *et al.*, 2002; Morgan *et al.*, 2019). These vaccines are proven to improve clinical signs of disease, elicit immune protection at least 70 weeks, and reduce virus shedding via excretes in chickens and turkeys (El-Khantour *et al.*, 2017; Palya *et al.*, 2014). On the other hand, although HVT-ND vaccines have been used extensively; recent studies have revealed the “immunologic gap” until the fourth week of age in maternal antibody-negative chickens because of delayed immunity (Palya *et al.*, 2014). This entails serious consequences in some geographic areas where the ND is endemic. Therefore, mass vaccination of 1-day-of age chicks with LAV is highly recommended to provide onset immunity in flocks.

HVT backbone has also been considered as an option to induce neutralizing antibodies via foreign gene expression in order to establish protection against infectious laryngotracheitis, which is another significant herpesviral disease of poultry. In this context, the first approach was to target glycoprotein B (gB) which is an essential gene of infectious laryngotracheitis virus (ILTV) functioning as a virus-cell fusion protein (Poulsen and Keeler, 1997). Hence, blockage of gB prevents cell entry of virus thereby blocking the cell binding in the first place. The second was to be expressed multiple proteins of ILTV instead, virulence-associated gI glycoprotein (involves in gE/gI complex) which takes a role in cell-to-cell spread of ILTV (Devlin *et al.*, 2006) along with essential glycoprotein gD, as it induces higher titres of neutralizing antibody and cell-mediated immune responses than gB alone (Ibrahim *et al.*, 2020; Kanabagatte Basavarajappa *et al.*, 2014). All

genes were inserted in intergenic regions of the HVT genome by the conventional HR method (Esaki *et al.*, 2013; Gimeno *et al.*, 2011). The gB expressing HVT was proven to provide adequate protection against virulent ILTV strain after subcutaneous injection to 1-day-old SPF chickens. Furthermore, a single *in ovo* inoculation on the eighteenth day of egg incubation protects 3 to 4 weeks old broiler chickens with 67% and 87% efficacies, respectively (Esaki *et al.*, 2013). Similarly, gI and gD expressing rHVT mitigated the symptoms of ILTV independently of administration routes in 57-day-old chickens and showed more efficacy than fowl poxvirus recombinants (Vagnozzi *et al.*, 2012), although this would be controversial (Johnson *et al.*, 2010). The most significant shortcoming of rHVT-ILTVs is the incompetency of abolishing viral replication in the trachea which may lead to the spread of ILTV through clinically healthy chickens (Barboza-Solis *et al.*, 2021; Johnson *et al.*, 2010; Vagnozzi *et al.*, 2012). To solve this, rHVT-ILTVs has been combined with traditional LAVs of ILTV or fowl poxvirus-vector vaccines (Guy and Byrd, 2016; Maekawa *et al.*, 2019).

Gumboro disease has been of considerable economic significance due to several reasons. First, it has a remarkable mortality rate arising from virulent infectious bursal disease virus (IBDV) strains in flocks, and the second is to the ability to cause immunosuppression, which results in a susceptibility to secondary infections and possible vaccine failures (Dey *et al.*, 2019; Giambrone *et al.*, 1976; Pejkovski *et al.*, 1979). The first generation of vaccines (LAVs) strictly depended on attenuating viral agents by serially passaging in the embryonated eggs or primary tissue cultures. In general, the level of neutralizing antibody titer correlates highly with the pathogenicity of vaccine strain; however, virulent strains can bring about bursal lesions and immunosuppression in laying hens (Thangavelu *et al.*, 1998). Therefore, most of the commercial LAV vaccines were derived from mild-intermediate strains and applied to hens on the verge of laying period to transfer maternal antibodies to chicks (Müller *et al.*, 2012). Molecular evolution mechanisms have constantly emerged that novel IBDV variants impaired the protection capacity of vaccines so that mild strains had become insufficient in immunizing chickens against these novel field strains (Rautenschlein *et al.*, 2005). Thus, various strategies were pursued to overcome this inefficiency problem. These included combining LAVs with

hyperimmune sera, which was defined as an “immune complex vaccine” (Whitfill *et al.*, 1995). The second-generation HVT-vector vaccines were constructed to respond urgent need for combating very virulent variants. VP2 gene was sought to make into expression cassette and inserted into intergenic regions or US7 locus of HVT backbone (Bublöt *et al.*, 1999; Darteil *et al.*, 1995). These vaccines were proven to present negligible lesions in bursa after s/c injection in 1-day-old chicks; protect SPF chickens against multiple IBDV strains with a high percentage (>95%) in both s/c and *in ovo* application and induce strong humoral and cell-mediated immune responses. Furthermore, rHVT-IBDV vaccines do not be affected by maternal immunity (Bublöt *et al.*, 2007; Gimeno *et al.*, 2016; Tsukamoto *et al.*, 2002). On the other hand, the efficacy of the US7-deleted rHVT-IBDV remained weak for MD despite providing full protection against IBDV (Darteil *et al.*, 1995). More recently, the UL45/UL46 intergenic region was selected to introduce VP2 expression cassette by CRISPR/Cas9 and Cre-Lox tools; however, *in vivo* stability and efficacy of vaccine candidate remained unknown (Tang *et al.*, 2018).

Avian Influenza (AI) virus is a constantly evolving virus owing to its unique eight-segmented RNA genome. Its low-fidelity RNA polymerase activity engenders multiple variations in each gene (antigenic drift), while genetic reassortment mechanism occurring amongst species assists novel recombinant strains (antigenic shift) (Suarez, 2000). For instance, H5 and H7 subtypes of low-pathogenic avian influenza (LPAI) are known to be capable of evolving to highly pathogenic avian influenza (HPAI), once they disseminate to the avian population (Sutton, 2018). Thus, an abundant number of subtypes and variants have been defined throughout the world. Viral surface glycoproteins neuraminidase (NA) and hemagglutinin (HA) have the ability to induce neutralizing antibody responses against AI virus (Crowe, 2019); however, the humoral immune response of the vaccinated animals triggers the evolutionary pressure on these proteins, thereby continuously assisting the emergence of novel vaccine escaping variants (Chang *et al.*, 2020; Lee *et al.*, 2004; Sitaras *et al.*, 2020). Whole particle KV vaccines have been utilized against several AI subtypes as a part of control measures. These vaccines fail to establish a protective immune response in the presence of maternal antibodies in chicks, or a potential impairment between vaccine and field strains (Fuchs *et al.*, 2009). Nonetheless, KV vaccines afforded to provide

sufficient immunization and reduce transmission between chickens (Swayne *et al.*, 2000). HVT vector rose to prominence as an alternative to KV and fowl poxvirus-vectored vaccines. The most common principle of these rHVT vaccines was to introduce the modified *Hx* gene cassette of HPAI viruses into the *UL45/UL46* intergenic region of HVT using various techniques such as BAC and/or CRISPR/Cas9 tools (Li *et al.*, 2011; Liu *et al.*, 2019). Several experimental evaluations of commercially licensed rHVT-H5 demonstrated that the vaccine significantly reduced the mortality (60–100%) and virus transmission rates depending on the challenged strain, also prevailed over maternal antibodies in chickens (Gardin *et al.*, 2016; Palya *et al.*, 2018; Steensels *et al.*, 2016). On the contrary, other studies pointed out the importance of booster vaccine implementation at 2–3 weeks of age in chickens, since maternal antibodies seemed to rarely interfere in vaccine induction (Kilany *et al.*, 2015; Rauw *et al.*, 2012). rHVT-Hx vaccines enable to set vaccinated animals apart from infected or KV immunized animals in case an *ad hoc* diagnostic method is applied.

The multi-pathogen vaccine concept has led to reap outstanding benefits, which are simply reducing the cost of vaccine production and transportation, also preventing infections by fewer vaccine implementation. In this regard, efforts have been directed towards inserting multiple antigen-expressing gene cassettes into HVT vectors using different techniques. A double recombinant vaccine architecture, rHVT-F-gI+gD, was constructed by placing corresponding gene cassettes into *US2* region by cosmid-based HR method. The *in vivo* challenge studies revealed that the rHVT-F-gI+gD induced excellent protection against NDV (strain GB Texas), ILTV (strain LT 96-3) and MDV (strain GA 5) when it was administered by either *in ovo* or subcutaneous route in 1-day-old chickens. Furthermore, vaccine response did not adversely affect when it was combined with LAVs against IBDV or MDV separately (Gergen *et al.*, 2019). Similarly, rHVT-F-VP2 was assembled using the same method and further tested on chickens. Results showed that this variation was also highly versatile and effective against MDV, IBV and MDV; maintained prolonged immunity and was capable of giving high immune protection ($\geq 90\%$) under field conditions (van Hulten *et al.*, 2021). A recent innovative study has brought three expression cassettes together into one rHVT construct which included *VP2* of IBDV in *UL45/*

UL46 intergenic region, both *gI* and *gD* genes of ILTV in *HVT065/HVT066* intergenic regions, and *H9* gene of AI in *US2* gene using CRISPR/Cas9 tool (Tang *et al.*, 2020). Taken together, multi-pathogenic rHVT vectors appear to be more advantageous than using a combination of multiple bivalent rHVT vectored vaccines, as the repeated application results in neutralization of virus (Dunn *et al.*, 2019)

Conclusion and Recommendations

Achieving the balance between safety and efficacy is essential to developing an efficient recombinant vaccine. The use of HVT-vectored vaccines in poultry has been in an increasing trend due to their versatility and effectiveness. rHVT vaccines allow *in ovo* or at hatch application options, do not be eliminated by the maternal antibodies of a targeted disease, and most importantly, maintain durable immunity with a single dose. In addition, rHVT enables distinguishing infected from vaccinated animals.

On the other hand, the titer of neutralizing antibody required for the complete protection against targeted disease may delay up to four weeks; therefore, a booster vaccination is needed in most cases. Furthermore, HVT-vectored vaccines must be transported in liquid nitrogen because of the cell-associated nature of HVT. This *natura* also assists to evade maternal antibody thereby increasing the vaccine efficiency.

Overall, rHVT vaccines have delivered a great performance in the induction of systemic immunity. However, local immunity protection is relatively lower which is crucial for combating ILTV or NDV (Romanutti *et al.*, 2020). To address these limitations of rHVT-vectored vaccines, multiple approaches can be implemented to augment vaccine efficacy. For example, deletion of viral immune-mediated genes from the HVT backbone; insertion of immunomodulatory and/or fusion protein gene cassettes to improve cell-mediated immune response; testing various viral or cell promoters for increasing the expression level; combining vector with other immunogens such as new generation vaccine adjuvants should be comprehensively evaluated.

Novelty Statement

HVT vectors are recognized as a reliable tool to develop rational multivalent vaccine design. Thus, many

HVT-vectored vaccine candidates have been commercialized and made available in the field against the major viral diseases of poultry. It is conceivable that the elucidation of virus-host relationships and the perspectives provided by novel vaccine technologies will lead to the development of safer and more effective HVT-vectored vaccines in future.

Conflict of interest

The author has declared no conflict of interest.

References

- Baigent, S.J., Petherbridge, L.J., Smith, L.P., Zhao, Y., Chesters, P.M., and Nair, V.K., 2006. Herpesvirus of turkey reconstituted from bacterial artificial chromosome clones induces protection against Marek's disease. *J. Gen. Virol.*, 87(4): 769–776. <https://doi.org/10.1099/vir.0.81498-0>
- Barboza-Solis, C., Najimudeen, S.M., Perez-Contreras, A., Ali, A., Joseph, T., King, R., Ravi, M., Peters, D., Fonseca, K., Gagnon, C.A., van der Meer, F., and Abdul-Careem, M.F., 2021. Evaluation of recombinant herpesvirus of Turkey laryngotracheitis (rHVT-LT) vaccine against genotype VI Canadian wild-type infectious laryngotracheitis virus (ILTV) Infection. *Vaccines*, 9(12): 1425. <https://doi.org/10.3390/vaccines9121425>
- Bi, Y., Sun, L., Gao, D., Ding, C., Li, Z., Li, Y., Cun, W., and Li, Q., 2014. High efficiency targeted editing of large viral genomes by RNA-guided nucleases. *PLoS Pathog.*, 10(5): e1004090. <https://doi.org/10.1371/journal.ppat.1004090>
- Bublot, M., Laplace, E., and Audonnet, J.C., 1999. Non-essential loci in the BamHI-I and -F fragments of the HVT FC126 genome. *Acta Virol.*, 43: 181–185.
- Bublot, M., Pritchard, N., Le Gros, F.X., and Goutebroze, S., 2007. Use of a vectored vaccine against infectious bursal disease of chickens in the face of high-titred maternally derived antibody. *J. Comp. Pathol.*, 137: 81–84. <https://doi.org/10.1016/j.jcpa.2007.04.017>
- Chang, P., Sealy, J.E., Sadeyen, J.R., Bhat, S., Lukosaityte, D., Sun, Y., and Iqbal, M., 2020. Immune escape adaptive mutations in the H7N9 avian influenza hemagglutinin protein increase virus replication fitness and decrease pandemic potential. *J. Virol.*, 94(19): 1–15. <https://doi.org/10.1128/JVI.00216-20>
- Crowe, J.E., 2019. Antibody determinants of influenza immunity. *J. Infect. Dis.*, 219: S21–S29. <https://doi.org/10.1093/infdis/jiz010>
- Darteil, R., Bublot, M., Laplace, E., Bouquet, J.F., Audonnet, J.C., and Riviè, M., 1995. Herpesvirus of Turkey recombinant viruses expressing infectious bursal disease virus (IBDV) VP2 immunogen induce protection against an IBDV virulent challenge in chickens. *Virology*, 211(2): 481–490. <https://doi.org/10.1006/viro.1995.1430>
- Devlin, J.M., Browning, G.F., and Gilkerson, J.R., 2006. A glycoprotein I- and glycoprotein E-deficient mutant of infectious laryngotracheitis virus exhibits impaired cell-to-cell spread in cultured cells. *Arch. Virol.*, 151(7): 1281–1289. <https://doi.org/10.1007/s00705-005-0721-8>
- Dey, S., Pathak, D., Ramamurthy, N., Maity, H.K., and Chellappa, M.M., 2019. Infectious bursal disease virus in chickens: Prevalence, impact, and management strategies. *Vet. Med. Res. Rep.*, 10: 85–97. <https://doi.org/10.2147/VMRR.S185159>
- Dimitrov, K.M., Afonso, C.L., Yu, Q., and Miller, P.J., 2017. Newcastle disease vaccines. A solved problem or a continuous challenge? *Vet. Microbiol.*, 206: 126–136. <https://doi.org/10.1016/j.vetmic.2016.12.019>
- Dorsey, K.M., Saitoh, S., Okuda, T., and Kubomura, M., 2002. WO2003064595 recombinant herpesvirus of Turkeys and use thereof, Available at: <https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2003064595> (accessed 16 Jan 2022).
- Dunn, J.R., Dimitrov, K.M., Miller, P.J., Garcia, M., Turner-Alston, K., Brown, A., and Hartman, A., 2019. Evaluation of protective efficacy when combining Turkey herpesvirus-vector vaccines. *Avian Dis.*, 63(1): 75–83. <https://doi.org/10.1637/11979-092818-Reg.1>
- El-Khantour, A., Darkaoui, S., Tatár-Kis, T., Mató, T., Essalah-Bennani, A., Cazaban, C., and Palya, V., 2017. Immunity elicited by a Turkey herpesvirus-vectored newcastle disease vaccine in Turkey against challenge with a recent genotype iv newcastle disease virus field strain. *Avian Dis.*, 61(3): 378–386. <https://doi.org/10.1637/11547-120216-ResNoteR>

- Esaki, M., Noland, L., Eddins, T., Godoy, A., Saeki, S., Saitoh, S., Yasuda, A., and Dorsey, K.M., 2013. Safety and efficacy of a Turkey herpesvirus vector laryngotracheitis vaccine for chickens. *Avian Dis.*, 57(2): 192–198. <https://doi.org/10.1637/10383-092412-Reg.1>
- Fuchs, W., Römer-Oberdörfer, A., Veits, J., and Mettenleiter, T.C., 2009. Novel avian influenza virus vaccines. *OIE Rev. Sci. Tech.*, 28(1): 319–332. <https://doi.org/10.20506/rst.28.1.1862>
- Gardin, Y., Palya, V., Dorsey, K.M., El-Attrache, J., Bonfante, F., De Wit, S., Kapczynski, D., Kilany, W.H., Rauw, F., Steensels, M., and Soejoedono, R.D., 2016. Experimental and field results regarding immunity induced by a recombinant Turkey herpesvirus H5 vector vaccine against H5N1 and other H5 highly pathogenic avian influenza virus challenges. *Avian Dis.*, 60(1): 232–237. <https://doi.org/10.1637/11144-050815-ResNote>
- Gergen, L., Cook, S., Ledesma, B., Cress, W., Higuchi, D., Counts, D., Cruz-Coy, J., Crouch, C., Davis, P., Tarpey, I., and Morsey, M., 2019. A double recombinant herpes virus of turkeys for the protection of chickens against Newcastle, infectious laryngotracheitis and Marek's diseases. *Avian Pathol.*, 48(1): 45–56. <https://doi.org/10.1080/03079457.2018.1546376>
- Giambrone, J.J., Eidson, C.S., Page, R.K., Fletcher, O.J., Barger, B.O., and Kleven, S.H., 1976. Effect of infectious bursal agent on the response of chickens to Newcastle disease and Marek's disease vaccination. *Avian Dis.*, 20(3): 534–544. <https://doi.org/10.2307/1589386>
- Gimeno, I.M., Cortes, A.L., Faiz, N., Villalobos, T., Badillo, H., and Barbosa, T., 2016. Efficacy of various HVT vaccines (Conventional and Recombinant) against marek's disease in broiler chickens: Effect of dose and age of vaccination. *Avian Dis.*, 60(3): 662–668. <https://doi.org/10.1637/11415-040116-Reg.1>
- Gimeno, Isabel M., Cortes, A.L., Guy, J.S., Turpin, E., and Williams, C., 2011. Replication of recombinant herpesvirus of turkey expressing genes of infectious laryngotracheitis virus in specific pathogen free and broiler chickens following in ovo and subcutaneous vaccination. *Avian Pathol.*, 40(4): 395–403. <https://doi.org/10.1080/03079457.2011.588196>
- Guy, J., and Byrd, M., 2016. Protective immunity against laryngotracheitis (LT) is enhanced by combined in ovo vaccination with herpesvirus of turkeys-vectored LT vaccine and fowl poxvirus-vectored LT vaccine (HVT-LT + FPV-LT). In: 65th Western Poultry Disease Conference, (April 24-27, 2016), Vancouver, BC, Canada.
- Hall, R.N., Meers, J., Fowler, E.V., and Mahony, T.J., 2015. Identification of non-essential loci within the Meleagrid herpesvirus 1 genome. *Virol. J.*, 12(1): 130. <https://doi.org/10.1186/s12985-015-0362-9>
- Hein, R., Koopman, R., García, M., Armour, N., Dunn, J.R., Barbosa, T., and Martinez, A., 2021. Review of poultry recombinant vector vaccines. *Avian Dis.*, 65(3): 438–452. <https://doi.org/10.1637/0005-2086-65.3.438>
- Ibrahim, M.J., Ali, S.A., Abd-elrahman, K.A., and Almofti, Y.A., 2020. Vaccinomic approach for multi epitopes vaccine from glycoprotein D of virulent strains of avian infectious laryngotracheitis virus. *J. Appl. Environ. Microbiol.*, 8(1): 8–24.
- Johnson, D.I., Vagnozzi, A., Dorea, F., Riblet, S.M., Mundt, A., Zavala, G., and García, M., 2010. Protection against infectious laryngotracheitis by in ovo vaccination with commercially available viral vector recombinant vaccines. *Avian Dis. Dig.*, 5(4): e22–e23. <https://doi.org/10.1637/9552-940110-DIGEST.1>
- Kamel, M., and El-Sayed, A., 2019. Utilization of herpesviridae as recombinant viral vectors in vaccine development against animal pathogens. *Virus Res.*, 270(July): 197648. <https://doi.org/10.1016/j.virusres.2019.197648>
- Kanabagatte Basavarajappa, M., Kumar, S., Khattar, S.K., Gebreluul, G.T., Paldurai, A., and Samal, S.K., 2014. A recombinant Newcastle disease virus (NDV) expressing infectious laryngotracheitis virus (ILTV) surface glycoprotein D protects against highly virulent ILTV and NDV challenges in chickens. *Vaccine*, 32(28): 3555–3563. <https://doi.org/10.1016/j.vaccine.2014.04.068>
- Kilany, W.H., Hassan, M.K., Safwat, M., Mohammed, S., Selim, A., VonDobschuetz, S., Dauphin, G., Lubroth, J., and Jobre, Y., 2015. Comparison of the effectiveness of rHVT-H5, inactivated H5 and rHVT-H5 with inactivated H5 prime/boost vaccination regimes in commercial broiler chickens carrying MDAs against HPAI H5N1 clade 2.2.1 virus. *Avian Pathol.*, 44(5): 333–341. <https://doi.org/10.1080/03079457.2015.1080000>

0/03079457.2015.1053840

- Kim, T., Volkening, J.D., and Spatz, S.J., 2020. Comparative molecular characterization of three gallid alphaherpesvirus Type 3 Strains 301B/1, HPRS24, and SB-1. *Avian Dis.*, 64(2): 174. <https://doi.org/10.1637/0005-2086-64.2.174>
- Lee, C.W., Senne, D.A., and Suarez, D.L., 2004. Effect of vaccine use in the evolution of Mexican lineage H5N2 avian influenza virus. *J. Virol.*, 78(15): 8372–8381. <https://doi.org/10.1128/JVI.78.15.8372-8381.2004>
- Li, Y., Reddy, K., Reid, S.M., Cox, W.J., Brown, I.H., Britton, P., Nair, V., and Iqbal, M., 2011. Recombinant herpesvirus of turkeys as a vector-based vaccine against highly pathogenic H7N1 avian influenza and Marek's disease. *Vaccine*, 29(46): 8257–8266. <https://doi.org/10.1016/j.vaccine.2011.08.115>
- Liu, L., Wang, T., Wang, M., Tong, Q., Sun, Y., Pu, J., Sun, H., and Liu, J., 2019. Recombinant turkey herpesvirus expressing H9 hemagglutinin providing protection against H9N2 avian influenza. *Virology*, 529: 7–15. <https://doi.org/10.1016/j.virol.2019.01.004>
- Maekawa, D., Riblet, S.M., Newman, L., Koopman, R., Barbosa, T., and García, M., 2019. Evaluation of vaccination against infectious laryngotracheitis (ILT) with recombinant herpesvirus of turkey (rHVT-LT) and chicken embryo origin (CEO) vaccines applied alone or in combination. *Avian Pathol.*, 48(6): 573–581. <https://doi.org/10.1080/03079457.2019.1644449>
- Marshall, D.R., Reilly, J.D., Liu, X., and Silva, R.F., 1993. Selection of marek's disease virus recombinants expressing the *Escherichia coli* gpt gene. *Virology*, 195(2): 638–648. <https://doi.org/10.1006/viro.1993.1415>
- Morgan, R.W., Gelb, J., Schreurs, C.S., Lütticken, D., Rosenberger, J.K., and Sondermeijer, P.J., 2019. Protection of chickens from Newcastle and Marek's diseases with a recombinant herpesvirus of Turkeys vaccine expressing the Newcastle disease virus fusion protein. *Avian Dis.*, 36(4): 858–870. <https://doi.org/10.2307/1591544>
- Mottet, A., and Tempio, G., 2017. Global poultry production: Current state and future outlook and challenges. *World's Poult. Sci. J.*, 73(2): 245–256. <https://doi.org/10.1017/S0043933917000071>
- Müller, H., Mundt, E., Eterradossi, N., and Islam, M.R., 2012. Current status of vaccines against infectious bursal disease. *Avian Pathol.*, 41(2): 133–139. <https://doi.org/10.1080/03079457.2012.661403>
- Palya, V., Tatár-Kis, T., Kovács, E.W., Kiss, I., Homonnay, Z., Gardin, Y., Kertész, K., and Dán, Á., 2018. Efficacy of a recombinant Turkey herpesvirus AI (H5) Vaccine in preventing transmission of heterologous highly pathogenic H5N8 Clade 2.3.4.4b challenge virus in commercial broilers and layer pullets. *J. Immunol. Res.*, 2018: 3143189. <https://doi.org/10.1155/2018/3143189>
- Palya, V., Tatár-Kis, T., Mató, T., Felföldi, B., Kovács, E., and Gardin, Y., 2014. Onset and long-term duration of immunity provided by a single vaccination with a turkey herpesvirus vector ND vaccine in commercial layers. *Vet. Immunol. Immunopathol.*, 158(1–2): 105–115. <https://doi.org/10.1016/j.vetimm.2013.11.008>
- Pejkovski, C., Davelaar, F.G., and Kouwenhoven, B., 1979. Immunosuppressive effect of infectious bursal disease virus on vaccination against infectious bronchitis. *Avian Pathol.*, 8(1): 95–106. <https://doi.org/10.1080/03079457908418330>
- Poulsen, D.J., and Keeler, C.L., 1997. Characterization of the assembly and processing of infectious laryngotracheitis virus glycoprotein B.J. *Gen. Virol.*, 78(11): 2945–2951. <https://doi.org/10.1099/0022-1317-78-11-2945>
- Rautenschlein, S., Kraemer, C., Vanmarcke, J., and Montiel, E., 2005. Protective efficacy of intermediate and intermediate plus infectious bursal disease virus (IBDV) vaccines against very virulent IBDV in commercial broilers. *Avian Dis.*, 49(2): 231–237. <https://doi.org/10.1637/7310-112204R>
- Rauw, F., Palya, V., Gardin, Y., Tatar-Kis, T., Dorsey, K.M., Lambrecht, B., and Van Den Berg, T., 2012. Efficacy of rHVT-AI vector vaccine in broilers with passive immunity against challenge with two antigenically divergent Egyptian clade 2.2.1 HPAI H5N1 strains. *Avian Dis.*, 56(4): 913–922. <https://doi.org/10.1637/10172-041012-Reg.1>
- Romanutti, C., Keller, L., and Adriana, F., 2020. Current status of virus-vectored vaccines against pathogens that affect poultry. *Vaccine*, 38(45): 6990–7001. <https://doi.org/10.1016/j.vaccine.2020.09.013>
- Sitaras, I., Spackman, E., de Jong, M.C.M., and

- Parris, D.J., 2020. Selection and antigenic characterization of immune-escape mutants of H7N2 low pathogenic avian influenza virus using homologous polyclonal sera. *Virus Res.*, 290: 198188. <https://doi.org/10.1016/j.virusres.2020.198188>
- Sondermeijer, P.J.A., Claessens, J.A.J., Jenniskens, P.E., Adrian Mockett, A.P., Thijssen, R.A.J., Willemse, M.J., and Morgan, R.W., 1993. Avian herpesvirus as a live viral vector for the expression of heterologous antigens. *Vaccine*, 11(3): 349–358. [https://doi.org/10.1016/0264-410X\(93\)90198-7](https://doi.org/10.1016/0264-410X(93)90198-7)
- Steensels, M., Rauw, F., Van Den Berg, T., Marché, S., Gardin, Y., Palya, V., and Lambrecht, B., 2016. Protection afforded by a recombinant Turkey herpesvirus-H5 vaccine against the 2014 european highly pathogenic H5N8 avian influenza strain. *Avian Dis.*, 60(1): 202–209. <https://doi.org/10.1637/11126-050615-Reg.1>
- Suarez, D.L., 2000. Evolution of avian influenza viruses. *Vet. Microbiol.*, 74(1–2): 15–27. [https://doi.org/10.1016/S0378-1135\(00\)00161-9](https://doi.org/10.1016/S0378-1135(00)00161-9)
- Sutton, T.C., 2018. The pandemic threat of emerging H5 and H7 avian influenza viruses. *Viruses*, 10(9): 1–21. <https://doi.org/10.3390/v10090461>
- Swayne, D.E., Perdue, M.L., Beck, J.R., Garcia, M., and Suarez, D.L., 2000. Vaccines protect chickens against H5 highly pathogenic avian influenza in the face of genetic changes in field viruses over multiple years. *Vet. Microbiol.*, 74(1–2): 165–172. [https://doi.org/10.1016/S0378-1135\(00\)00176-0](https://doi.org/10.1016/S0378-1135(00)00176-0)
- Tang, N., Zhang, Y., Pedrera, M., Chang, P., Baigent, S., Moffat, K., Shen, Z., Nair, V., and Yao, Y., 2018. A simple and rapid approach to develop recombinant avian herpesvirus vectored vaccines using CRISPR/Cas9 system. *Vaccine*, 36(5): 716–722. <https://doi.org/10.1016/j.vaccine.2017.12.025>
- Tang, N., Zhang, Y., Sadigh, Y., Moffat, K., Shen, Z., Nair, V., and Yao, Y., 2020. Generation of a triple insert live avian herpesvirus vectored vaccine using crispr/cas9-based gene editing. *Vaccines*, 8(1): 97. <https://doi.org/10.3390/vaccines8010097>
- Thangavelu, A., Dhinakar Raj, G., Elankumaran, S., Murali Manohar, B., Koteeswaran, A., and Venugopalan, A.T., 1998. Pathogenicity and immunosuppressive properties of infectious bursal disease virus field isolates and commercial vaccines in India. *Trop. Anim. Health Prod.*, 30(3): 167–176. <https://doi.org/10.1023/A:1005059619825>
- Tsukamoto, K., Saito, S., Saeki, S., Sato, T., Tanimura, N., Isobe, T., Mase, M., Imada, T., Yuasa, N., and Yamaguchi, S., 2002. Complete, long-lasting protection against lethal infectious bursal disease virus challenge by a single vaccination with an avian herpesvirus vector expressing VP2 antigens. *J. Virol.*, 76(11): 5637–5645. <https://doi.org/10.1128/JVI.76.11.5637-5645.2002>
- Ura, T., Okuda, K., and Shimada, M., 2014. Developments in viral vector-based vaccines. *Vaccines*, 2(3): 624–641. <https://doi.org/10.3390/vaccines2030624>
- Vagnozzi, A., Zavala, G., Riblet, S. M., Mundt, A., and García, M., 2012. Protection induced by commercially available live-attenuated and recombinant viral vector vaccines against infectious laryngotracheitis virus in broiler chickens. *Avian Pathol.*, 41(1): 21–31. <https://doi.org/10.1080/03079457.2011.631983>
- van Hulten, M. C. W., Cruz-Coy, J., Gergen, L., Pouwels, H., ten Dam, G.B., Verstegen, I., de Groof, A., Morsey, M., and Tarpey, I., 2021. Efficacy of a turkey herpesvirus double construct vaccine (HVT-ND-IBD) against challenge with different strains of Newcastle disease, infectious bursal disease and Marek's disease viruses. *Avian Pathol.*, 50(1): 18–30. <https://doi.org/10.1080/03079457.2020.1828567>
- Whitfill, C.E., Haddad, E.E., Ricks, C.A., Skeeles, J.K., Newberry, L.A., Beasley, J.N., Andrews, P.D., Thoma, J.A., and Wakenell, P.S., 1995. Determination of optimum formulation of a novel infectious bursal disease virus (IBDV) vaccine constructed by mixing bursal disease antibody with IBDV. *Avian Dis.*, 39(4): 687–699. <https://doi.org/10.2307/1592404>