

Chapter 08

Use of Nanotechnology to Treat Infectious Bronchitis in Poultry

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ABSTRACT

Poultry industry has played a major role in managing food security and combating poverty for populations with food insecurities. For this purpose, it is important for the farmers to develop controls against diseases that can affect poultry production. One such control measure is vaccination. However, there are some diseases that can still lead to reduction in productivity despite vaccination. An example of one such disease is Infectious Bronchitis (IB). It is mainly a respiratory tract disease caused by the Infectious Bronchitis Virus (IBV) that can cause a decline in production of poultry birds irrespective of their status of vaccination. Hence, researchers observed that this was a disease that needed further control strategies to limit its impact. As it is a viral disease, use of antibiotics was ruled out. Another idea presented by researchers was use of innovative medicinal agents such nanoparticles to combat the disease. Since then several techniques and strategies have been outlined by researchers specifically for battling IB.

KEYWORDS

Infectious Bronchitis, Vaccination, Poultry production, Antibiotics, Nanoparticles

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INTRODUCTION

Poultry products are an integral part of our regular food consumption list and a tool for poverty alleviation when there is a lack of other suitable and nutritious food options (Hedman et al., 2020; Desta, 2021). Various crucial components like health, immunity, and production, capability of poultry birds are the main factors determining the future progress of poultry farming (Immunivt, 1998; Hafez and Attia, 2020). At the global level, new diseases of poultry birds are always being reported and become the main topic of concern in the poultry farming industry (De Boeck et al., 2015). Diseases like Infectious bronchitis (IB) have become a problem of common occurrence in poultry birds all over the world (Nkukwana, 2018; Yadav et al., 2019; Legnardi et al., 2020). Despite significant attention being paid to controlling infectious diseases for economic loss aversion, these ailments continuously begin to occur and reoccur (Ali et al., 2021). Infectious bronchitis develops due to the Avian Infectious Bronchitis Virus (IBV) infection. IBV belongs to the *Gammacoronavirus* genus and is a unique virus. IBV pioneered coronavirus when it was identified in 1993 by isolation from poultry. It was also assumed to be a crucial pathogenic virus of livestock (Schalk and Hawn, 1931). IBV belongs to the family of viruses that are enveloped and have non-segmented, single-stranded, positive-sense RNA (Wu et al., 2020).

Currently, IBV has become a pathogen of major concern for domestic poultry from an economic perspective as its infection leads to significant mortality and losses in production irrespective of the vaccination status of birds (Cavanagh, 2007). Many researchers reported global pandemics of IBV in chickens and other avian species that were marked by high rates of death, and disease, and with lowered production in terms of eggs and meat (Mansour et al., 2021; Parvin et al.,

2021). The use of conventional treatment options like antivirals against IBV leads to the development of resistance most of the time. Additionally, it can also produce side effects, and the re-emergence of the viral agent (Legnardi et al., 2020). Presently, vaccines of live attenuated nature are being mainly used for the control and prevention of IBV infections (Sultan et al., 2019; Toro, 2021). Despite the efforts to curb IBV, the higher rate of viral genetic diversity, composition and the occurrence of novel viral strains leads to a reduction in the efficacy of vaccination (Franzo et al., 2016; Sultan et al., 2019).

The utilization of antimicrobial agents as feed additives has led to a rise in the problem of drug residues. Drug residues consequently form the basis of antibiotic resistance in the pathogen. That is why the use of antibiotics at the sub-therapeutic level has been banned in Europe since January 2006 (Manyi-Loh et al., 2018). Hence, researchers must initiate the development of new strategies for controlling these diseases. To achieve this goal, attention is being diverted towards the development of antiviral herbs for useful combinations to get formulations with the least adverse effects on the health of birds and humans (Fuzimoto and Isidoro, 2020; Tagde et al., 2021). The effect of innovative and non-traditional medicine on the immune system and a reduction in the prevalence of unprecedented disasters can be very effective for defense against infectious diseases caused by viral agents (Hoang et al., 2020). It has been reported in previous research that some derivatives of plants and substances added to poultry feed including plant extract, prebiotics, probiotics, enzymes, and yeast, have an immunity-enhancing impact (Soccol et al., 2010; Gadde et al., 2017). The effects of these extracts and additives include improvement in metabolism, reduced physiological stress, prevention of cytokine expedition by macrophages, and antibacterial action, thus improving immune functionality (Al-Harthy, 2015; Patra et al., 2019). Mammal antibodies (Sastry et al., 2003) can be utilized for the purpose of diagnosis and therapy against the infection of various pathogens (Immunivt, 1998), however, the antibodies of mammals can be only obtained through the application of invasive extraction tactics. That is why avian eggs are considered the best alternate option to produce antibodies for the sake of diagnosis and therapy purposes against infection of pathogens (Pereira et al., 2019; El-Kafrawy et al., 2021).

Among the different useful applications including a number of nanoparticles which are used to enhance the interaction between the virus and molecules, and also helps the researchers to build a electroanalytical biosensing analyzer which is portable and which helps in the detection of the virus effectively (Neethirajan, 2017; Power et al., 2018) and the production of nano-based vaccine against viruses. Usually, there are first-generation vaccines which are developed by killing the organisms or inactivation of living organisms or by live attenuation of organisms while on the other hand second and third-generation vaccines are prepared using subunits of either RNA or DNA (Saha et al., 2013; Scallan et al., 2013). In comparison to conventionally developed vaccines, subunit-based vaccines have more advantages including low cost and high proficiency of vaccine to develop immune response against the pathogens (Brisse et al., 2020). The cons of subunit-based vaccines include poor or low immunogenicity, adverse effects, and in in-vivo conditions they are unstable in addition to multiple boosters. So in comparison to conventional vaccines, nanotechnology is the best way to deal with the shortcomings of the vaccines and helps to provide better immune response and produce vaccines that work along with adjuvant and protect the host from the pathogens (Yan et al., 2020). Genomic assortment along with the massive outbreaks of such infectious diseases can lead to an epidemic which results in the spread of adverse side effects globally which not only affects poultry industry but also disturbs human health (Shao et al., 2017; Blagodatski et al., 2021).

Nanotechnology vs Infectious Bronchitis

The word nano originated from the Latin word "nanus" meaning a small object, lesser, dwarf, minute unit that is around 1 nm or 10^{-9} m (Youssef et al., 2019). Nanotechnology is one of the innovative skills that have an unmatched potential of being utilized with regards to a socio-economic prospective in the poultry industry all across the globe (Abd El-Ghany et al., 2021). Nanotechnology has progressed the field of biomedical sciences, with a huge number of NPs of various kinds and capabilities in terms of diagnostics and therapy against infectious viral ailments (Ramos et al., 2017; Krishnan et al., 2021). Investigation has been done to check the antiviral effects of nanocomposites of G-Ag against IBV and Feline CoV (Chen et al., 2016a; Wu et al., 2020). Li et al. (Li et al., 2018) found help in preparing a vaccine on the basis of BIV-flagellin self-assembled protein nanoparticles (SAPNs) which are used against IBV by using spike protein as an adjuvant with the flagellin. Chicken infected with IBV were administered with nano-vaccine which we mentioned above and the results show the enhanced antibody action which confirms the role of the vaccine giving protective immunity. Chandrasekar et al., shows that another adjuvant-based nano-carriers of Quil-A and chitosan (QAC) were developed having the size of less than 100 nm (Chandrasekar et al., 2020).

Other than that some procedures are also done to make encapsulation with plasmid DNA (pQACN) vaccine and coding nucleocapsid which were then given through the intranasal route. The results show increased immunogenic and protective outcomes in both humoral and cellular immunity against IBV infections. Furthermore, it is observed that the rate of viral load decreases and the severity of symptoms also reduces. Polymeric carbonized nano-gels (CNGs) are considered as effective therapeutic agents against IBV. CNGs work by prohibiting the S1 and S2 glycoproteins to interact with cells of the host as the infection proceeds, CNGs are vulnerable to viruses as they are very much absorbent on the virus. In the process of development of CNGs, a very high temperature is required to make these nano-gels, due to the high temperature formation the CNGs exhibit a positive charge. This positive charge helps CNGs to neutralize the charge present on the IBV which reduces the level of pathogenicity of virus (Liu et al., 2020). Chou et al. (Chou et al., 2021) proposed that if amalgamation of the CNGs using pyrolysis method is done with lysine hydrochloride, this may prohibit the virus against IBV. CNGs were administered at a very low concentration of 30ug/ml in IBV-infected chicken embryos and

the efficacy was determined as it shows an inhibitory effect >98%. Many uses of full NP to detect and trace virus has been demonstrated as the NP was based on magnetic and gold quantum dots (QDs) (Kang et al., 2021). Ahmed et al. (Ahmed et al., 2018) suggested a new technique in which he links anti-IBV Abs with QDs and produced an immune-link of chiral-QDs. This immune-link is referred to as a chiro-immuno-sensor for IBV collected from chicken's blood samples.

In addition, a nanostructure was made for the limit which was self-assembled, used for the detection and targeting the virus while using the EID (egg infection dose) 47.91/50ml was very efficient in the process of examination of targeting virus (Ahmed et al., 2017). Virus-like particles (VLPs) have been studied widely and used as a transportation tool for many compounds especially medicines, proteins (peptides), RNA/DNA, antibiotics, and vaccines. They work as an adjuvant or antigen nano-carriers to help immune cells to exhibit humoral immune response and protection against viruses (Nasrollahzadeh et al., 2020). Surface protein S used to bind with the receptor which triggers the body to respond against it and shows an immune response (Kato et al., 2019). Chen et al. (Chen et al., 2016b) have demonstrated a technique for the usage of CoV VLPs-based S protein in which the incubation of 100-nm gold np was done with optimal concentration of viral proteins. The finding of the study was the impulsive production of proteins with the installment of virus-like nanostructure assembly with fundamental particles having viral antigen coating. In addition, the results of VLPs from this study was come to the conclusion that validate the successful production of synthetic VLPs (sVLPs) from NP (Liu et al., 2013; Wang et al., 2017).

Encapsulation of the IB live attenuated vaccine using chitosan nanoparticles has been demonstrated to show brilliant results in enhancing the antibodies responses and cell mediated responses. This specially includes increased level of IgA at mucosa and enhanced the expression of genes (interferon gamma related genes) especially at the sites where virus replicates primarily (Lopes et al., 2018). Saponins were used as immunostimulant in this study which was further recognized in several studies later. Berezin et al. (Berezin et al., 2013) determined the noticeable improvement in antibody (IgG, IgM and IgA) and cell mediated (IFN-gamma and IL2) immune responses. Single dose of intranasal immunization using saponins and delivery system was used against influenza. After that, Yu et al. (Yu et al., 2015) demonstrated the use of saponins (oral ginseng stem-leaf saponins) to increase the response of vaccine in chicken especially with immunosuppression. Hence both the chitosan and saponins are used for dual purpose including as an adjuvant or as an immunomodulator to enhance the immune responses (Greenland and Letvin, 2007).

AgNps and *P. betle*

Attributed to their astonishing antimicrobial properties, AgNps drew the attention of the researchers. These nanoparticles are usually present in less than 100 nanometers in size, and contain a large surface area available for the interaction with viruses (Karna et al., 2023). The special properties possessed by the AgNPs makes them unique against many viruses, especially those against viruses which cause bronchitis. These nanoparticles are very effective against some common bronchitis viruses for example respiratory syncytial virus and influenza virus (Nefedova et al., 2021). Several studies have demonstrated that the use of AgNPs at the early stages of infection can inhibit the infection while interacting with the viral envelope (Saadh et al., 2021; Saadh, 2022; Saadh, 2023a). The mechanism of action of AgNPs is to disrupt the viral envelope in response to which virus is not able to enter in the host cells and start replication itself (Saadh et al., 2021). Furthermore, another unique property of AgNPs is that they do not induce viral resistance so used as an effective solution for long term use against viruses (Saadh, 2022; Saadh, 2023a). For centuries *P. betle* leaves have been used in cultural practices and as a traditional medicine. The leaves of *P. betle* are contains a rich amount of several bioactive compounds polyphenols including acetyl eugenol, transisoeugenol, chavicol, chavibetol acetate, chavibetol, and allyl pyrocatechol diacetate (Saadh, 2023b). These polyphenols have been used for the production of silver nanoparticles (AgNPs) which is one of the potential uses of these polyphenols which gathered the attention of various industries (Saadh, 2023b). These polyphenols present on the *P. betle* leaves act as reducing and capping agents when present on the surface of silver nanoparticles. This property of these polyphenols inhibits the aggregation of nanoparticles which in turn reduces the size of AgNPs giving several benefits including increasing the stability of nanoparticles and improving their performance (Saadh, 2023b). AgNPs perform their action through the disruption of viral envelope while attaching with the viral genome. After disruption, the virus is no longer capable of causing infection in the host cell and cannot replicate. The contact of viruses with AgNPs is enhanced due to the small size and increased surface area of these nanoparticles (Saadh, 2023b).

Conclusion

Since time of its introduction chicken has become an integral part of food in terms of culture and nutrition. With introduction of new farming techniques poultry overshadowed other meat producing industries by many levels. However, this progress came with its own problems. Soon it was found that various diseases affected poultry flocks leading to high mortality rates, inefficient FCR and low production. One of such diseases is Infectious Bronchitis. It is a viral respiratory tract infection caused by Infectious Bronchitis Virus (IBV). IB is one of the viral diseases that severely affect production and viability of poultry flocks and effect the economical horizon of agricultural market. This disease reportedly affects the production of birds irrespective of the vaccination status. Such dire straits demanded development of a new weapon for defense against IB. Such a weapon was presented by researchers in form of nanotechnology. Nanoparticles of silver and other material when given alone or integrated into vaccine, were proved to

be supremely effective. Hence this technology has emerged as a beacon of hope for poultry farmers that can help them control IB and limit its effects at the same time.

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