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Review Article

Field Evaluation of Vaccines against Respiratory Viral Disease in Chickens

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Abstract

In modern poultry industry, vaccination is the backbone for prevention of avian respiratory viruses parallel with strict hygienic measures such as good ventilation, proper stocking density, hygienic disposal of carcasses, and control of ammonia gas.

Vaccination regimen against these viruses vary from one to another as it was found that in Avian Influenza it could be prevented by only inactivated vaccines against subtype specific strain. On the other hand in case of Infectious Laryngotracchitis, only live vaccine is used and immune response produced mainly cellular immunity, while in case of Infectious Bronchitis it depends mainly on Protect-type phenomena taken in consideration the recommendation of using of different vaccinal strains start with classical parent one. In Newcastle disease, we depend on both live and inactivated vaccines parallel with serological monitoring using Haemagglutination Inhibition test for choose proper time of vaccination. Reo virus vaccine was used only in breeders with four times doses two live and two inactivated vaccinal doses. In case of swollen head syndrome; vaccinations occurs only in breeders with two doses one live for priming followed by second inactivated one together with good hygienic measures.

Field evaluation of viral respiratory vaccine is a field mirror for either vaccination success or failure which reflects on bird survival. Moreover vaccination success depend on different factors including type of vaccine used, route of application, age of bird, time of application, concomitant disease condition as well as type of production.

Viral respiratory diseases cause severe economic losses among poultry industry and strict vaccination regimen should be applied in order to prevent infection.

Keywords: *Vaccines; Respiratory Viral Disease; Chickens*

Introduction

Viral respiratory diseases affecting chicken respiratory system are characterized by variable respiratory signs, variable mortality and morbidity as well as affect egg production [1]. These viruses cause severe economic losses due to cost of eradication as well as loss of productivity [2].

Successful vaccination will be reflected on protection from morbidity and mortality against specific field virus and build immune foundation in newly hatched chicks [3]. On the other hand vaccination failure against these viruses cause severe economic losses and could not diagnosed easily as the main cause of this failure is complex and depend on different complicating factors [4].

Laboratory diagnosis can support field evaluation of this vaccine in order to evaluate immune response against each of them which will reflect on protection against challenge [5].

Poultry respiratory viral vaccines are typically characterized as live or inactivated. Live attenuated vaccines are relatively economical than inactivated one but Immunity from live vaccines is generally short-lived. Some exceptions to this exist for vaccines such as ILT [6].

Inactivated vaccines are generally whole antigen preparations combined with an adjuvant that are designed for subcutaneous or intramuscular injection. Inactivated vaccines generally consist of aqueous phase which contains the antigen, and the adjuvant phase which generally enhances the bird's response to this antigen [7].

This review aimed to give a sight on field evaluation and application of avian respiratory viral vaccines.

Most important avian respiratory viral diseases are Avian Influenza (AI), Newcastle disease virus (NDV), Infectious bronchitis virus (IB), Infectious Laryngotracheitis (ILT), Reo virus infection (Respiratory Enteric Orphan) and Swollen Head Syndrome in chickens "SHS" (turkey rhinotracheitis "TRT").

Avian Influenza (AI)

Avian influenza is caused by Myxovirus belonging to family Orthomyxoviridae. The disease in chicken varied according to affected strain which is the low pathogenic AI virus (LPAIV) or highly pathogenic AI virus (HPAIV). Infection with low pathogenic avian influenza virus resulting in no detectable signs, decrease in egg production and/or upper respiratory signs with excessive lacrimation are common [8]. Many of the lesions associated with LPAIV in the field have not been reproduced in SPF chicks [9] although they have been replicated in commercial chickens [10] and cause severe symptoms in association with secondary pathogen [11].

HPAIV H5N1 cause more severs clinical signs with high mortality. Mostly these signs are per acute and the neurological symptoms occur in individuals that survive the first few days of infection [12,13].

Vaccination against AI virus depends mainly on subtype specific related strains and so the most reliable way is to use vaccines contains virus subtype specific to particular hemagglutinin subtype at risk (autogenous vaccine) as it is not applicable in the field to vaccinate against all 16 haemagglutinin subtypes of Avian Influenza [14].

Field evaluation of vaccines against avian influenza

The main objectives of vaccination for avian influenza are: 1) to reduce the production losses caused by the disease. 2) To reduce the risk of spread of AIVs to animals and humans. 3) To reduce the shedding of the AIVs in the environment. 4) To create (by way of vaccine induced immunity) barriers between infected and free areas/compartments. 5) To help in the control and eradication of the disease [15].

The main role of field vaccination success against AI is matching of the vaccine and field strain (subtype relatedness) to provide optimal field protection including reduced shedding of the virus and protect against mortality when challenge with field virus [16].

Applications of inactivated whole AI virus vaccine for broiler chickens provide good protection against homologous (subtype specific) haemagglutinating virus, but poor protection against a heterologous [17].

Regarding the efficacy of inactivated oil – emulsion H9N2 avian influenza vaccines in Iran it was found that these vaccines hinder the rate of virus shedding into the environment [18].

Conventional H5N9 vaccine suppress shedding in specific – pathogen – free birds challenged with HPAI H5N1 (related subtype strains) and reduce (but not prevent) the amount of virus shedding into the environment [14].

The most frequent available and successful AI vaccine technology those inactivated whole AI virus using field outbreak strains (autogenous strain), this was prepared by reverse genetic generated AI vaccinal strain, followed by chemical inactivation and oil emulsification which is effective in preventing clinical signs and mortality when challenged with field virus [19].

Single vaccination dose of inactivated avian influenza vaccine (contain H5 antigen) was found to protect field challenge with highly pathogenic avian influenza H5N1 (HPAIH5N1) and hinder the transmissibility and spreading of the infectious field avian influenza virus [20].

Egyptian H5N1 strain was isolated, characterized by immunological, molecular levels and prepared inactivated vaccine from this isolates. Local H5N1 isolate vaccine showing 100% homology of both genes with previously published sequences of H5N1 isolates from Egypt and the Middle East. Also the prepared inactivated vaccine was highly immunogenic as it prevents mortality and reduce viral shedding after challenge with virulent virus [21].

Protective antibodies titer

should not be less than $10^{9}EID_{50}$ using Haemagglutination Inhibition (HI) test.

Newcastle disease (ND)

It is an acute highly contagious viral diseases caused by Paramyxovirus, only one serotype. Virus strains either mild strains (lentogenic), medium strength strains (mesogenic) which cause typical signs of respiratory distress or virulent strains (velogenic) and the strains used for production of live vaccines are mainly lentogenic.

Due to there is only one serotype of Newcastle disease and so prevention by vaccination usually protects the birds from the more serious consequences of the disease (mortalities, loss of production... etc.), but virus replication and shedding may still occurs even at a reduced level [22].

Field evaluation of vaccines against Newcastle disease virus

The interference between live Newcastle disease virus vaccine and live infectious bronchitis virus vaccine in broiler was studied and concluded that the interference induced by IB virus on the immune response against NDV only occurs when both vaccines are mixed together manually in farms. While if mixing in manufacturing laboratory (patent IB and NDV combined vaccine) no interference occurs and the immune response was similar to immune response of using NDV live vaccine alone. This phenomena could be explained due to rapid replication of IB virus than NDV and interfere on the receptors in respiratory tract while patent prepared vaccine IB and NDV concentration of NDV is higher than IB virus [23].

When studying the effect of field administration of garlic powder on humeral immune response of broilers against NDV vaccine (HB1), it was found a significant increase of total leukocyte 14 days after vaccination and the antibody titers on those receive garlic powder were higher than non-treated control group [24].

Field trails applied to study protection of Avinew vaccine in SPF chicken against challenge with two virulent genotypes (Goose Paramyxovirus (GPMV) and Rainbow challenge virus (RCV)) that infecting commercial and backyard poultry in South Africa. Results revealed that Avinew vaccine gave 100% protection from mortality against both challenge viruses, but not against infection and replication. The protective dose (HI titers) of Avinew vaccine against both GPMV and RCV was calculated at 10 (4.38) and 10 (4.43) respectively [22].

Trails were applied by administrating ginseng stem-and-leaf together with saponins (as immune elevator) orally in chickens in order to enhance the humeral immune response to inactivated ND and vaccines. Results revealed that these additives enhanced serum antibody response against ND with proved safety on chickens which maybe give a promising oral adjuvant to improve immunization in poultry [25].

Comparing the effect of *Lactobacillus casei* (*L. casei*) (as probiotics) and commercially mixed combination of fruit juice (as prebiotics) on mortality and antibody response after Newcastle live lentogenic vaccine application in fighting roosters revealed that fruit extract show that humoral immune response for ND live vaccine is higher than *L. casei* and both showed none or very low mortality [26].

Comparing two live lentogenic vaccines (HB1 and Lasota) In order to evaluate the efficiency of different techniques (drinking water, ocular rout and spray) of Newcastle disease live vaccines administration on broiler chicks results revealed that ocular rout is the most efficient technique as it induced the highest antibody titer (log(2)6.6) and 93.3% protection from challenge followed by drinking water method while the lowest was spray technique as it induced antibody titer of log(2)5.9 and only 53% of chicks survived challenge. Moreover, when studying the economic point of view for all used live vaccines, it was found that ocular method for application of HB1 and Lasota vaccines at 1-and 21-day-old chicks gave the highest revenue followed by drinking water method [27].

Protective titer for NDV

- Broiler: not less than 104,5 - 10^5 EID50.
- Layers and Breeders: not less than 10^7 - 10^8EID50.

Infectious Bronchitis (IB)

It is common highly contagious viral disease caused by Corona virus has several serotypes, only chickens are susceptible. There are two forms of the disease either classical form or variant (nephritis nephrosis syndrome) form.

The classical form in chicks characterized by respiratory signs (gasping, coughing, sneezing, tracheal rales and nasal discharge) with variable mortality, in post mortem examination found cheesy exudates in the bifurcation of the bronchi. In chickens greater than 6 weeks of age and adult birds the disease may pass unnoticed and does not cause mortality [28].

In layers egg production will dramatically decreased, deformed eggs with wrinkled shells will often be laid. The severity of the production declines may vary with the period of laying and with the causative virus strain [29].

Variant strain infection characterized by wet dropping, increase water intake and mortality, in post-mortem examination nephrosis with urolithiasis [1].

In case of IB virus it is well known that protection varies according relatedness between vaccines used (protect-type) and virulence of IB infectious strain taken in consideration types of strain either variant or classical [30].

Field evaluation of vaccines against Infectious Bronchitis virus

Both live and inactivated virus vaccines are used in immunization against IB virus. Live vaccine (H120) is used in broiler chickens for initial vaccination while in breeder and layers pullets it was used for priming mainly and those live vaccines are variable in their pathogenicity according to attenuating procedures [31].

Based on the fact that vaccine protects against the same serotype of virulent virus (protect type) it was found that field application of IB virus H120 live attenuated vaccine was able to protect broiler against clinical signs when challenged with field virulent strain of the same serotype [30].

Many field trails support the routine use of inactivated IB vaccines either by intramuscular or subcutaneous rout of injection in layers, and breeders. These vaccines induce serum antibody and provides protection to internal tissues, kidney and reproductive tract in layers and breeders. Also uses of these vaccines reduce the incidence of virus present in the respiratory tract of challenged broiler chickens, so it limits the transmission to other susceptible birds (Ladman., *et al*. 2002).

Using of live attenuated IB vaccine together with concomitant infection with low pathogenic avian influenza virus, revealed that it exacerbates the severity of H9N2 LPAIV clinical signs in infected birds and increases replication and shedding of infecting virus. So it is recommended not to use avian respiratory viral vaccines during concomitant respiratory infection or respiratory distressed birds [32].

Studding immune responses of broiler chickens according to different field vaccination routs (spray – eye drop – drinking water) against IB field viruses infection results revealed that eye – drop method induce the highest antibody titers compared to other routes [33].

Vaccination of one day old broiler Ross with H120 vaccine using spray route resulting in severe post vaccinal reaction. So it is recommended to use spray route carefully in farms and preferable in non-respiratory distressed birds [34].

Infectious Laryngotracheitis (ILT or LT)

It is an acute viral disease of chickens, pheasants, and peafowl caused by herpes virus. Most of outbreaks in chickens occur in mature or nearly mature chickens and the disease characterized clinically by marked dyspnea, coughing, gasping, and expectoration of bloody exudate with high morbidity and considerable mortality rate [35].

The principal mediator of ILT resistance is the local cell – mediated immune response in the trachea which induced by live vaccine application (Davison., *et al.* 2006).

Field evaluation of vaccines against ILT virus disease

Commercially, there are two types of live attenuated ILT virus vaccines either chicken embryo – origin or a tissue culture – origin. It is recommended in field application to start with tissue culture vaccine for priming parallel with suitable disinfectant effective against ILT virus during regular farm disinfection. It was found that involvement of modified – live ILT vaccine viruses in field outbreaks give possible evidence of possible reversion of vaccinal virus of embryo origin to virulence, in spite of the virulence of all vaccinal viruses was low compared with field isolates [36].

It is recommended to administrate ILT live vaccine only in areas where the disease is endemic, since vaccination can result in the occurrence of long-term "carrier" birds due to the virus' ability to enter a latent state in the sensory ganglia. This was proved by comparative trail on two ILT vaccines (chicken embryo and tissue culture origin),which revealed that when both vaccines passaged in SPF (specific pathogen free) chicken the vaccine of chicken embryo origin increased in virulence while the tissue culture - origin is not. After 10 serial passages the chicken embryo vaccine gain virulence comparable to highly virulent strains [37].

Comparing the protection induced by live attenuated ILT chicken embryo vaccine and recombinant viral vector vaccines against infectious laryngotracheitis in broiler chickens revealed that chicken embryo origin vaccine provided optimal protection, while the viral vector vaccines applied in-ovo and subcutaneously provided partial protection and reducing to some degree clinical signs together with challenge virus replication in the trachea. On the other hand in terms of safety the recombinant vaccines found to be safer than embryo origin vaccines [38].

Route of administration of live ILT virus vaccine is of great value as it was found that use of drinking water cause vaccination failure in high proportion of chickens that fail to develop protective immunity. It is recommended to implement two vaccinations doses for developing of optimum protection against challenge, regardless the rout (eye drop, drinking water, or spray) and vaccine source. However it was found that vaccine applied by eye drop route provide more uniform protection compared with spray, drinking water routes [39].

Reo virus infection

Avian Reo virus infection is viral disease infecting broiler breeder chickens between 6 and 10 weeks of age. It is responsible for several pathological entities. Susceptibility of chickens to infection decreased with advancing age, the virus is carried by eggs, airway or digestive tract. The main route of transmission is via ingestion of water and feed [40].

In case of Reo virus; The immunogenic protein is Sigma C protein which is the most variable protein in the virus and it induces the production of neutralizing antibodies (immunogenic part) (Vasserman., *et al*. 2004).

Field evaluation of vaccine against Reo virus

Presence of maternal immunity in broilers does not preclude the successful protective immunization with attenuated live Reo virus vaccine in field at 1-day-old of age. This means that this vaccine could apply safely in early days of age in broiler breeders [41].

Comparative field study on safety, protectivity and antibody response of seven avian Reovirus live vaccines in SPF chickens revealed that all seven commercial live vaccines provide protection against virulent field virus challenge, but the protection is correlated with the remaining virulence of the virus and relatedness to immunogenic part (Sigma C protein) between field virus and those examined live Reo virus vaccines [42].

Recombinant Reovirus vaccine sigma C protein produced in plants demonstrated that it has the potential for large – scale successful vaccination against Avian Reo Virus in commercial poultry production in the term of safety and protection of challenge [43].

Gallimune 201 IBD – REO (commercial inactivated combined vaccine) effectiveness against IBD and avian Reo virosis Flu, was proved to be effective for successful active immunization of laying hens (breeder) against infectious bursal disease and avian reovirosis flu and protecting breeders from challenge with field virus [44].

It was found that some Reo viruses cause immunosuppression by produce atrophy of lymphoid organs and replicate in blood monocytes, this give rise for the need for proper Booster revaccination with inactivated combined vaccine (IBDV + NDV + IBV + Reo virus) together with supplementation with prebiotic or probiotic (immune stimulant) for breeders during egg production period, was a useful tool to keep the hens antibody titers in high levels resulted in producing chicks with high maternal antibody titers and minimizing the number of unprotected chicks [45].

Immunogenicity of a DNA vaccine of avian Reo virus to eliciting antibody production in six – day – old SPF chickens which were orally vaccinated with this vaccine then boasted 2- weeks interval revealed that antibody was generated 2 weeks after immunization, which was significantly higher than control groups beside proved protection against subsequent challenge [46].

Swollen Head Syndrome (SHS)

Swollen-head syndrome is a disease seen in broiler chickens 4 - 6 weeks of age caused by pneumovirus associated with complicating agents such as bacterial complications (*E. coli* and *Mycoplasma gallisepticum*) or viral complications (adeno virus, reovirus, NDV and IB). Together with bad hygienic measures as the pneumovirus it self-did not play a causal role in SHS in commercial poultry flocks [47].

Many trails proved that *E. coli* is one of the main complicating agents with swollen head syndrome infection in broiler chickens and suggested that hygienic measures should be implemented together with antibiotic treatment to eliminate E.coli – induced SHS in broilers in Dakahlia [48].

In laying hens it was found that challenge virus could induce a drop in egg production accompanied by malformation of egg shells [49].

Vaccination against SHS with live attenuated vaccine stimulate both systemic and local immunity in respiratory tract of chicken and successful vaccination start by proper priming by live vaccine followed by inactivated one parallel with good hygienic measures (Cook., *et al*. 2001).

Field evaluation of vaccines against SHS

Live attenuated vaccine is now successfully attenuated on cell culture and was proved to be commercially available for field priming of breeders against field virus infection [50].

Inspite of humoral antibody response is poor following primary live vaccination; birds may still be protected from challenge via cell mediated immunity in the respiratory tract [51].

To produce complete protection in breeding flocks against virulent field challenge, it was found that SHS inactivated vaccine should be applied at 16 - 20 weeks of life prior to production, preferable to be primed with live swollen head syndrome vaccine. There is evidence that live infectious bronchitis vaccine can interfere with the replication of avian metapneumovirus live vaccines in chickens and so it is recommended to separate between both vaccines field application with 2-3 days [52-54].

Conclusion and Recommendations

Viral respiratory diseases is one of the main problems in poultry industry as it is incriminated in many serious conditions either alone or together with complicating factors, including bacterial complications (such as *Mycoplasma* and *E. coli*) or management factors (such as high ammonia concentration in the farm, high stocking density and bad ventilation).

In order to prevent and control this viral respiratory disease, it is recommended to use proper vaccination program parallel with good hygienic measures, in order to prevents this diseases completely.

Vaccination programs varies from virus to another as it is subtype specific in avian influenza, using only inactivated vaccine, while in ILT virus only live vaccine is used, depend mainly on cell mediated immunity. Infectious Bronchitis protection depend on protect-type phenomena, while in NDV the faster to develop and maintain proper immunity, the better protection against challenge. Reo virus immunity developed mainly against Sigma C protein (immunogenic part) while prevention of swollen head syndrome depend mainly on prevention of complicating factors (hygienic, bacterial and viral complication) beside vaccination.

Recommendation for proposal for proper vaccination program varies from virus to another and place to another.

Each virus has its own vaccination program as in avian influenza vaccine used only inactivated and in early age (8 - 10 days) in broiler, while in layer two inactivated vaccinal doses extra were recommended.

In NDV start with live vaccine at 5 - 7 days of age (parallel with inactivated vaccine in endemic area) then repeated every 7 - 10 days in broiler, while in layers two extra doses with inactivated vaccine also used (45 and 100 days of age). In respiratory distressed birds it is recommended to use NDV vaccine either clone strain or of enteric origin as emergency vaccination.

Protect-type phenomena was a guide in IB vaccination and so it is recommended in broiler to use two live vaccine doses one classical at 1 - 7 days and other variant at 14 days of age parallel with one dose of inactivated vaccine. In layer and breeders extra additional dose of inactivated vaccine 2 - 3 weeks prior egg production.

In ILT virus infection, it is recommended in broiler to be used only in endemic area or in Baladi production one dose at 35 - 40 days of age preferable tissue culture origin, while in layer and breeders two vaccinal doses start with tissue culture vaccine at 35 - 40 days of age and second dose with embryo origin vaccine at 85 - 90 days of age taken in consideration it is only live vaccine.

In case of Reo virus vaccine, it is used only in breeder start with two doses of live vaccine; one in drinking water in 1st two weeks of live and second injectable at 35 - 40 days of life, followed by two inactivated doses one at 70 days of life and the last 2 - 3 weeks before egg production.

In swollen head syndrome, it is recommended to prime with live vaccine (recommended chicken not turkey origin vaccine) then use inactivated one prior to egg production.

Correction of managemental procedure is of great value not only for prevent viral respiratory diseases but also for all poultry diseases as it prevent occurrence or disease progress as well as prevent mortality and morbidity in susceptible flocks.

It is recommended also to diagnose the condition from all views start from field diagnosis parallel with laboratory diagnosis and use of a suitable medication for complicating microorganism and main cause.

Finally it is of great value to do not jump or anticipate the final diagnosis of the main cause until studding it well from all arms and aspects.

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