Journal of Current Veterinary Research

ISSN: 2636-4026

Journal home page: http://www.jcvr.journals.ekb.eg

*Animal health & Zoonotic disease*

# Freezing, Heating and Some Factors Affecting Immune Response Induced by Inactivated Vaccines in Broiler Chickens

Diaa El Boraey[1*], Mousa A. Ayoub[1], Sabah I. Shaaban[1], Wael Elfeil[2], Mohamed A. Nossair[3], Said Ibrahim Fathalla[4], Ibrahim Said Abu-alya[4]

*(1)Department of Animal Hygiene and Zoonosis, Faculty of Veterinary Medicine, Damanhour University, Damanhour22511 Egypt.*

*(2)Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Suez Canal University, Egypt.*

*(3)Department of Animal Hygiene and Zoonosis, Faculty of veterinary medicine, Alexandria University, Egypt.*

*(4) Department of Physiology, Faculty of Veterinary Medicine, University of Sadat City, Egypt.*

**Corresponding author:* diaa.vet82@Gmail.com *Received: 15/11/2023* *Accepted: 26/11/2023*

## ABSTRACT

Poultry vaccines should have a specific storage temperature range to ensure their effectiveness. It is still unclear in Egypt, nevertheless, if these temperature ranges are rigidly specified as handling conditions or as recommendations. Large endemic and epidemic outbreaks of poultry illnesses frequently threaten Egypt's sizable conventional and exotic chicken industries. Although it is assumed in vaccine logistics that most of these safety measures are followed, handling vaccines within the specified temperature range is frequently not feasible. It is challenging to keep vaccines within a designated storage temperature range when they are being transported, according to earlier research. This is an issue not just in nations with weak cold chains but also in nations and areas with strong cold chains. Specifically, the chance of unintentionally being exposed to below-freezing conditions has been disregarded globally. This is a serious concern for vaccination consumers as well as the veterinarians who oversee vaccine usage. Because they cannot ensure the quality of vaccines whose storage temperature may have been beyond the prescribed range, veterinarians should advise consumers not to vaccinate birds with these vaccines. Additionally, the user is directly harmed in the uncommon case that a handling error impairs the quality of the vaccination.

**Keywords:** Consumers, Egypt, Handling, Storage temperature, Vaccines, and Veterinarians.

## INTRODUCTION

Poultry production is the fastest growing component of global meat production in developing and transitional countries (Assa, 2012). The demand for chicken meat has increased over time because of the increasing demand for quality food in the form of meat and eggs. They are a cheap source of quality animal protein. As a result, the Ministry of Agriculture has identified poultry production as key sector to deal with food security issues (LMP, 2015). More than

50,000 commercial producers operate in Egypt's vast poultry industry, which includes exotic broilers and layer chicken, native Baladi chicken, ducks (Muscovy, Peking, Sudani, and Mule types), ostriches, geese, quails, and turkey. Both conventional and contemporary hatcheries supply day-old birds. The current overall population of poultry is estimated to be one billion birds at any given time. Of these, 90% come from the commercial poultry industry, with the remaining 10% coming from home and small-scale poultry. These chicken farms are mostly located in Egypt's villages and near the cities (Ali et al., 2013).

Millions of low-income citizens depend on household poultry production for their livelihoods and employment, but the industry is characterized by intricate marketing chains and a wide range of challenges, including diseases with significant economic and public health implications. Over the past ten years, catastrophic financial losses have been brought on by major epidemic diseases of poultry, including extremely pathogenic poultry influenza (HPAI) H5N1 and Newcastle's disease (ND) (Marangon and Busani, 2007; Fasina et al., 2008).

Vaccines are commonly used to prevent endemic poultry illnesses, reduce their occurrence, and manage them. A vaccination needs to be pure, safe, powerful, and effective to maintain animal health (OIE, 2014). In 2014, 10.4 billion doses of vaccinations were used in Egypt; 96.43% of these doses were purchased abroad, while just 3.57% were made domestically (Central Laboratory for Evaluation of Veterinary Biologics -CLEVB, unpublished data).

The ideal chilled-chain temperatures for vaccine storage globally are +2ºC to +8ºC (35-46 F), since numerous vaccines are made from recombinant proteins, should be stored in liquid nitrogen (-196ºC) (Kumru et al., 2014). While the potency of vaccines generated plays a role in chicken vaccination programs, other factors that contribute to their performance include handling, maintaining the refrigeration chain throughout commercialization and transportation, and correctly administering vaccinations to target birds (Collett, 2013).

Poultry vaccine needs to be kept in a "cold chain" a range of safe handling and procedures on the vaccine from the time of manufacture through the transport processes until it is used in animals to retain its potency and immunogenicity. This cold chain goes beyond simply keeping the vaccine at the required temperature of +2º C to +8º C (Subramanyam, 1989; Cheyne, 1989; Chojnacky et al., 2010). Previous studies (Miller and Harris, 1994; Nelson et al., 2004) have confirmed that immunogenicity and responses to vaccines have been affected by a fewpractices, including incorrect dilutions and careless administration; these issues have also been linked to interference with maternally derived antibodies (Kim et al., 2010) and incorrect storage temperature.

The avian influenza H5N1 and ND outbreaks, along with other unreliable information from animal health professionals and farmers, have suggested severe vaccine failures despite the intensive vaccination of poultry, especially against H5N1HPAI and ND. Previous scientific evaluations have corroborated these reports (Kim et al., 2010; Arafa et al., 2012; Kilany et al., 2015).

## ***Overview of Vaccines and Poultry Vaccination programs***

### 1. ***Vaccine***

The Latin Variolae vaccinae, or cowpox, which Edward Jenner proved in 1798 could shield humans against smallpox, is where the name "vaccine" comes from. He coined the term in 1798 for the lengthy title of his investigation on the Variolae vaccine, now known as the "Cow Pox," in which he detailed how cowpox can ward off smallpox (Bax, 1999). Louis Pasteur suggested in 1881 that the phrases be expanded to include the newly discovered preventive vaccinations in remembrance of Jenner. These days, any biological preparation made from living things that either prevents disease or boosts protection against it is referred to as a "vaccine" (Poltkin and Plotkin, 2008). A vaccine is a natural product that offers protection against a specific infectious illness through active acquired immunity (Fiore et al., 2009).

### ***1.1. Vaccine nature***

According to definitions, a vaccine is "an immune-biological substance designed to produce specific protection against a given disease" or "an inactivated or attenuated pathogen or a component of a pathogen (nucleic acid, protein) that, when administered to the host, stimulates a protective response of the cells in the immune system (Melief et al., 2015). A vaccination is usually composed of weakened or destroyed versions of the pathogen, its toxins, or one of its surface proteins. Vaccines can be therapeutic (to treat an illness that has already developed, like cancer) or prophylactic (to stop or lessen the symptoms of a future infection by a natural or "wild" virus) (Ledford and Heidi, 2020). Certain vaccines provide fully sterilized immunity, which fully prevents infection (World Health Organization, 2013).

### ***1.2. Vaccine's mechanisms of action***

Vaccines function by inducing an immune system reaction against a virus or bacteria. This imbues the immune system with ‘memory'. The immunological memory function of the body enables it to ‘remember' a particular virus, therefore defending against it and averting possible illness. The vaccination exhibits the antigens characteristic of a certain virus.As a result, the immune system reacts similarly to how it would to the pathogen or toxin in its natural form. When the body is later infected with the active disease, it forms a defense and learns to recognize the antigens from the germs in the vaccination, enabling it to eliminate the pathogen quickly and effectively (Sharif and Ahmed, 2018).

## ***2. Poultry vaccine’s types***

It is possible to choose different vaccine strains for usage in various production systems and epidemiological contexts. It is important to be aware of the characteristics of various vaccines produced in various regions of the world to choose the right strain of vaccination for a certain manufacturing system or epidemiological area. As a result, the selection of vaccinations, particularly for the village system, is primarily focused on cost and transportability. Vaccines against bacterial and fungal infections are also available, however vaccinations against viral diseases are the most often used for poultry. In other regions of the world, vaccinations against other parasite illnesses, such coccidiosis, are also undergoing trials (Furuya et al., 2010).

There are a few solutions available for immunizing chickens, and they should be chosen based on the local disease conditions, farm biosecurity requirements, and degree of difficulty associated with each type of poultry business. A vaccination that has been destroyed or inactivated gives the flocks long-term protection (Hassan et al., 2017). Therefore, several immunizations and the inclusion of adjuvants to increase responsiveness are necessary for effective immunization with inactivated vaccines (Alexander and Shenne, 2003). Non-attenuated vaccinations are not the first option since they have a higher chance of causing illness in the vaccinated host, particularly in hens with weakened immune systems, as opposed to live attenuated vaccine strains (Ganguly et al., 2010). This is because insufficient attenuation leads to clinical illness and unfavorable vaccination responses, and reversion to virulence during viral replication in the host is the reason of the disease's extreme difficulty in managing (Furuya et al., 2010).

***2.1. Inactivated vaccines***

Most chicken vaccines were either live attenuated vaccines, which were frequently ineffective, or inactivated organisms combined with an oil-based adjuvant. Thus, the creation of innovative preventive and therapeutic veterinary vaccines has been aided by the discovery of antigen/gene delivery methods. Reverse vaccination has emerged as the most effective method recently. It predicts antigen localization using several bioinformatics algorithms and has been effectively used to immunize against a wide range of veterinary illnesses (Abdul Careem, 2015).

Inactivated vaccines are made up of viruses whose pathogenicity has been eliminated by chemical and physical techniques, but whose protein coat structure has been preserved and functions as an immunogen. Each chicken must be injected with an inactivated vaccination. Although inactivated vaccines are exceptionally effective at providing immunity without causing vaccination responses, they are not cheap and need a great deal of expertise when administered by non-veterinary professionals. Prior to injection, this kind of vaccination is combined with an adjuvant. Adjuvants such as oil or aluminum hydroxide are frequently utilized. Adjuvants increase vaccination stability in the chicken body, which boosts immunological response (Wambura and Kataga, 2011).

Since inactivated or dead viral vaccines are less immunogenic and do not encourage the body's natural synthesis of antigenic proteins that trigger the cell-mediated immune response, they are thought to be safer than traditional live attenuated vaccinations (Bosha and Nongo, 2012). These vaccines are rendered inactive by applying physical (heat and UV radiation) and chemical (formalin) techniques to render them pathogenicity-free, but the immunological protein coating architecture is retained.

***2.2. Live vaccines***

Live, non-attenuated, or attenuated vaccines can be used to provide vaccinations. Live attenuated vaccines are a particular kind of vaccination that stimulates the immune system by introducing attenuated virus strains whose virulence is lowered by a series of cell culture or embryo passage procedures. This form of vaccine elicits both cell- and antibody-mediated immune responses (Sanda, 2015).

Using a live attenuated vaccine strain has several benefits, including the ability to produce mucosal and systemic immunity and to be delivered by the natural route of infection (Butcher and Miles, 2017). It also helps with a lot of flocks because it is simple to administer and appropriate (Ganguly et al., 2010).

Live vaccinations are those that include the organism that infects the bird and then multiplies within the chicken's body, triggering the bird's immune system to fight the pathogen. The organism is only given a tiny quantity in the chicken's body to multiply. Increased organism identification by the chicken's immune system because of proliferation leads to an enhanced immune system (Wambura and Kataga, 2011). Live vaccinations against infectious bronchitis, infectious Bursal disease, and Newcastle disease are often utilized in poultry health care (Bell, 2000).

The most often recommended vaccinations for use in chicken farming are live vaccines. These vaccinations have been chosen for usage because of their many benefits. The most well-known benefits of live vaccinations are that they are affordable, simple to administer, and provide both individual birds and flocks with a reasonable level of immunity. However, there is a drawback: the birds may exhibit certain illness symptoms as a reaction to the immunization. The vaccination strain or the existence of concurrent infections with other diseases determine how severe this reaction will be (Jorge Dellagostin, 2017).

## 3. Scope of vaccination in poultry production

Protecting animals against infectious illnesses is the aim of this artificial stimulation of immunological responses (Bermudez and Stewart-Brown, 2003). The purpose of vaccines is to prevent or lessen the damage that can occur when organisms that cause illnesses show up on farms. The cost of vaccinations includes the risk of illness prevention. The expenses include vaccine cost, labor, time, tissue damage during vaccination administration, and losses from adverse responses to the vaccine (Wambura and Kataga, 2011).

**Table 1.** General features of chicken vaccinations, both inactivated and live:

| Live vaccines | Inactivated vaccine |
|---|---|
| -Reduced antigen amount to be used. The vaccination reaction depends on the bird's internal multiplication. | -Considerable antigen concentration to be used. No proliferation following administration. |
| -Heat and chemicals may easily destroy it. | -Stored easily. |
| -Drinking water and spray are very affordable, simple to use, and may be given to large numbers of people. | -Costly to create and implement since it is nearly usually done on an individual basis |
| -It is uncommon to adjuvate live vaccinations. | -It is necessary to adjuvate killed vaccines. |
| -Sensitive to the antibodies that are already present in birds (e.g., maternal immunity). | -Greater ability to provoke an immunological response despite the presence of antibodies. |
| -Booster vaccination is ineffective in immune birds. | -Additional immune response is commonly observed in immunological birds. |
| -Stimulate local immunity (gut or trachea). | -If administered as a booster, local immunity may be boosted again, but there is little to no secondary response. |
| -Risk of vaccination contamination, such as EDS. | -There is no risk of vaccination contamination. |
| -Tissue responses, often known as "vaccine reactions," can occur and are often apparent in a range of tissues. | -No microbe multiplication, so no tissue response other than that which is dependent on adjuvants. |
| -Restricted combinations as a result of simultaneous administration of many microorganisms interfering with one another (e.g. IB, ND and LT). | -Combinations, are not as common. |
| -Quick onset of protection. | -In general, immunity develops more slowly. |

Source: (Dana et al., 2000)

To immunize the birds against the pathogen in issue, non-pathogenic parts of the organism or closely related organisms that are almost identical to naturally acquired immunity are injected into the birds. Rather than preventing different illnesses from spreading throughout chicken flocks, this ensures that the effects of the infections will be less severe on clinical and production aspects (Saif and Barnes, 2003). To boost maternally produced antibodies (passive immunity) in the hatched progeny and shield the chickens from various infectious illnesses in their early years of life, proper immunization is also utilized to create extremely high antibody levels in hens (Furuya et al., 2018).

## 4. Proofs of the Failure of Poultry Vaccines

When chickens receive a vaccination and either do not generate sufficient antibody titers or become prone to field disease outbreaks, this is known as vaccination failure. High-quality vaccinations and appropriate administration are necessary for the immunization program and vaccines to be applied effectively. Numerous reasons can lead to immunization failure in chickens that have received vaccinations, and each of these factors needs to be given equal weight (Mojtaba et al., 2012). Consequently, the most frequent causes of vaccination failure are listed below.

### 4.1. A vaccine as a cause vaccination failure

Even getting vaccinated causes worry. The chicken's capacity to mount an immunological response may be diminished by stress. Extremes in temperature and relative humidity, poor nutrition, parasitism, and other illnesses can all be sources of stress. When sick birds are vaccinated, it not only disrupts their immune system but also makes it more difficult for them to fight off the illness. Wait to vaccinate the birds till they are well. When the vaccination is given, the illness may already be incubating in the chickens. The birds get sick even when the medication is given correctly because it takes time for the manufacture of antibodies to start and reach protective levels. It is noteworthy to emphasize that antibodies IG, M, G, and A are first found 4 to 5 days after the initial exposure to a live virus-type vaccination. It takes several days for titers to reach levels that are protective (Brugh and, Siegel, 1978). The vaccines are produced at a processing facility where it may not be possible to preserve the titer of an antigen from a particular virus or bacterium. As a result, the inoculums may fail to trigger a protective immune response in birds. Birds with low immunity levels may have a low titer of antigen in their immunization vial. The immunogenicity of vaccinations is significantly impacted by virus concentration (Terfa et al., 2015).

#### 4.1.1. Vaccine expiration as a cause of vaccination failure

Vaccine failure and suboptimal immune response can occur when immunizations are administered beyond their expiration date. The survival of the vaccine's agents—virus and bacteria—as well as their multiplication in the birds determine how successful the shot is. Vaccines retain their effectiveness for a specific amount of time, but even if they are stored at the right temperature, their viability may be lost if they are past their expiration date. Furthermore, according to Alexander and Shenne (2003), the vaccine may become inactive if it encounters certain kinds of residual disinfectant on syringes and needles.

#### 4.1.2. Antigenic variations between field strains and currently available vaccines

Generally, dead vaccinations are less effective than modified live vaccines because to antigenic change between the vaccine and field strains (such as antigenic drift, antigenic shift). Accordingly, if the antibodies precisely attach to the antigenic strain on the viral surface, efficient antibody-mediated protection is produced. There is no strain-specific immunization in cell-mediated immunity, in contrast to antibody-mediated immunity. As such, it is imperative to separate the field strain that is currently in circulation and juxtapose it with the current vaccination strain (Nasser et al., 2010).

#### 4.1.3. Insufficient degree of protection

The ability of the vaccination strain to generate a robust and efficient immune response is contingent upon the antigenicity of the viral strain. For the first two weeks of life, maternal antibodies, often known as passive immunity, are derived from chicken eggs. Following a successful vaccination, most poultry vaccines normally take two to six weeks to achieve their maximal response, after which the amount of protection or immunity begins to progressively drop. As a result of neutralization, vaccinations against deadly infectious agents given to birds normally during these times of strong maternal antibody protection may not elicit an immunological response. Despite receiving vaccinations on a regular basis, birds might still get several poultry illnesses prior to developing an active immune response. To get a higher degree of protection, booster doses should be administered according to the suggested timetable (Bosha, 2012).

### ***4.2. The bird as a cause of vaccination failure***

The bird's past viral exposure status and passive immunity may have an impact on how the bird reacts to vaccination. There are two ways that passive immunity develops: first, it is brought about by hyperimmune sera; second, it is passed from the mother bird to her offspring through the yolk, shielding the young until they are between 14 and 30 days old. According to Abdullah et al. (2009), passive immunity, which is transferred from mother immunity to young chicks, might affect how they react to vaccinations.

#### ***4.2.1. Stress factors***

Stress is an indifferent reaction to environmental changes that places an undue burden on the birds' capacity for behavioral and physiological adaptation. Birds' bodies typically lack the resources necessary to protect them from illnesses and environmental changes. Stress is brought on by several predisposing conditions in poultry birds. Furuya et al. (2010) list a number of stressors that might cause this, such as inadequate ventilation, extreme heat or cold, high stocking density, insufficient spacing, poor sanitation, an excessive amount of litter, nutritional deficiencies, parasitism, fever, and more. Because poultry birds are very susceptible to adverse weather, health issues, and other management concerns, their immune responses are subsequently weakened. As a result, during disease outbreaks, hens become generally immune to immunizations and are vulnerable to infection. Furthermore, if live vaccinations are given to hens with weakened immune systems, it might result in massive epidemics. Therefore, stress puts chicken at risk for weakened immunity, failed vaccination campaigns, and financial losses from poor output (Marangon and Busani, 2007).

#### ***4.2.2. Disruption of the mother's antibodies***

Based on the incidence of each infectious illness, poultry are routinely vaccinated against it; also, the freshly hatched chicks receive passive immunity from the mother antibodies present in their blood. Some study indicates that during the first week of life, maternal immunity can shield the chicks from a variety of viral disorders, including the Newcastle disease virus and Gumboro (IBD) disease. These maternal antibodies may, nonetheless, react with the vaccination antigens to produce an antigen that neutralizes them. Thus, if a freshly hatched chicken receives a live vaccine within the first few weeks of its life, the vaccination's effect on immunity generation is mitigated by a decrease in antibody levels (Alexander and Shenne, 2003).

#### ***4.2.3. Co-existing and immunosuppressive diseases***

Due to a weakened immune system, immunosuppression raises the danger of infectious illnesses including mycotoxicosis, infectious Bursal disorders (Gumboro), infectious anemia, Marek's disease, and others in flocks of birds. Due to the stressors, the immune system was weakened, which might have an adverse influence on vaccination outcomes and efficacy (Alexander and Shenne, 2003).

Additionally, as the vaccine antigens are neutralized by an interaction with naturally occurring antibodies against infectious pathogens, the efficacy of the shot will be diminished if the stressed or sick birds receive the same vaccination. This results in a vaccination response in birds, which may exacerbate the illness and raise the incidence of morbidity and mortality. Consequently, it is crucial and strongly advised to ensure the health of birds prior to immunization (Bosha and Nongo, 2012).

#### *4.2.4. Genetic factors*

The kind of bird species determines the variation in response to vaccination. The individual variety of birds is attributed to the major histocompatibility complex (MHC) structures, which also influence how birds react to viral and bacterial antigens. Because birds lack some MHC structures that aid in the recognition of certain antigens, they may be more vulnerable to infection (Nelson et al., 2004).

## 5. *Managemental, practical, and technical errors as a cause of vaccination failure*

### *5.1. Insufficient cold chain storage and instability in vaccines*

Vaccines must be applied, transported, and kept in accordance with the manufacturer's instructions. Lack of storage equipment, improper storage temperature, insufficient refrigerators, mixing vaccines with other food items, and interactions between vaccines and disinfectants are common issues in developing nations that occur during vaccine storage and cause the vaccine to become inactive. Nelson et al. (2004) and Garg et al. (2017) stated that attempting to store or transport the vaccine without following cold chain protocols, handling the vaccine improperly, or preserving it properly would result in denatured antigen and vaccination failure. When a vaccination is exposed to direct sunlight, the antigens are destroyed, which lowers the vaccine's antigen concentration and efficacy. For optimal effectiveness, the vaccine should be positioned appropriately and shielded from direct sunlight (Bosha, 2012).

As time passes, the vaccine's efficacy diminishes, thus it must be at the right low temperature to stay stable and effective for extended periods of time. It is also important to store vaccines properly and maintain a cold chain temperature +2$^{o}$ C to +8 $^{o}$C or (35-46 F) (Evans and Pope, 1995). For underdeveloped nations, maintaining the cold chain throughout transit is a difficulty. Maintaining cold chain systems can be difficult for a variety of reasons, such as power outages, inadequate refrigeration, overchilling, etc. Furthermore, further cooling oil-based vaccinations causes adjuvant materials like aluminum salts, etc. to crystallize, which lowers the vaccines' efficacy. Thermostable vaccines are less expensive and more significant in situations when cold chain temperature is not maintained. They may be kept between 2 and 8 degrees Celsius (Siddique et al., 2016).

While freeze-dried vaccines should be maintained and stored at a low temperature in a refrigerator at 4$^{o}$C, even during vaccine transportation, thermostable vaccines have some tolerance to cold and hot environments. Avoiding freezing and thawing is necessary. The vaccinations must only be taken out of the freezer or refrigerator when needed on the farm. Live vaccinations in chicken flocks need to be administered within two hours of reconstitution. They lose their power quickly after being reconstituted. The reconstituted vaccinations must be administered as soon as possible. Any leftover vaccines should be thrown out after a maximum of six hours in the refrigerator. One potential solution to address challenges with cold chain and storage temperature is to employ vaccinations that are thermostable (Alders and Spradbrow, 2001). According to Ideris et al. (1987), the potency and vaccinal activity of thermostable vaccines may be sustained for a year at 2–8°C and for three months at up to 28°C when dried. The delivery of thermostable vaccinations can be done by ophthalmic, intranasal, parental (injection), and oral (drinking water and feed) routes (Tu et al., 1998; Wambura et al., 2000).

5.2. Incorrect administration route

Vaccinations against poultry can be given orally, subcutaneously, intramuscularly, by wing webs, drinking water, eye drops, or spray. A vaccine may not provide enough protection in chicken flocks if it is not administered according to the specified method of administration or at the proper immunization location. Furthermore, the widespread use of drinking water and aerosol spray vaccinations lacks dose consistency amongst individual birds during delivery, which prevents the development of effective immunity (Bosha, 2012).

***5.3. Inadequate dose***

Inaccurate vaccination administration, such as administering too little or too much, can lead to vaccine response and failure. The use of water containing antimicrobial agents, the high concentration of chlorine in water during vaccine manufacture, and incorrect dose calculations are some of the causes that lead to inappropriate vaccine formation. Furthermore, applying the vaccination to big flocks of birds more than what the manufacturer recommends may result in an insufficient dosage, which will lower the level of vaccine titers and cause the vaccine to become inactive (Bell, 2000).

***5.4. Incorrect vaccination formulation and diluent use***

Saline water should be used as a particular diluent for preparing and formulating poultry vaccinations, in accordance with the manufacturer's instructions. Certain diluents are designed specifically for a single vaccination (lyophilized vaccine diluents), and they contain preservatives that can significantly reduce the efficacy of the other vaccine. The efficacy of vaccines can also be reduced by improper dilution and a non-standard method of formulation that uses a single syringe of diluent. As a result, while mixing many vaccinations in one syringe, it is important to thoroughly consider the potential consequences of interaction (Bosha, 2012).

***5.5. Utilizing just one adjuvant***

Since using a single adjuvant does not meet all vaccination criteria, there are several disadvantages to this approach. One of the drawbacks of utilizing a single adjuvant is the induction of weak, insufficient, and transient immune responses (Shane, 2005).

***5.6. Sanitary procedures***

The bird's resistance to illness may be strengthened by the vaccination, but if proper care procedures are not followed, this resistance may be overcome. The efficacy of chicken vaccinations was lowered by poor sanitation in addition to other variables including stress, overcrowding, and concomitant illness. The challenge dosage might be too high, or illness could happen too fast if subsequent flocks aren't cleaned out and disinfected (Spackman et al., 2014).

***5.7. Failure induced by administering several vaccinations at the same time.***

Birds often receive poultry vaccinations for different kinds of illnesses at the same time. There is, however, a paucity of evidence discussing the safety and effectiveness of providing several vaccines. Various studies indicate that when given in combination, some vaccinations may cause a decreased antibody response and raise the risk of both morbidity and death (Spackman et al., 2014).

***5.8. Inaccurate vaccination schedule and timing***

It is often advised to vaccinate chickens during the colder hours of the day, particularly in the early morning and late afternoon or evening. To have a better response from the immunization, now is the most comfortable and ideal moment to obtain it. However, if the birds get the vaccination during the hottest part of the day (heat stress), the vaccination's effectiveness may be diminished in vaccinated flocks (Furuya et al., 2010). Taking the bird's age into account is crucial while administering the immunization. Certain antigenic receptors are age-specific because they mature from the time a chick hatches until it is quite old. The receptors for infectious bronchitis, infected bursal disease (IBD), and Newcastle disease begin to form in young birds, but the receptors for chicken pox and infectious bursa disease grow with age. Because vaccination failure results from a host's lack of receptors for the antigen, it is thus advised to vaccinate birds after these receptors have developed in their bodies (Furuya et al., 2010).

***5.9. Absence of booster or repeated dose.***

An antigen dosage called a booster dose is given after the first vaccination dose in order to promote effective immunity. Hence, depending on the vaccination type, administering multiple doses over a set length of time is crucial. Additionally, booster doses are required to maintain the highest level of protection against an antigen, but earlier vaccination is required to introduce the vaccine's antigen into the birds' bodies. Nevertheless, the lack of a booster dosage leads to low antibody titers, which ultimately causes the vaccination to fail (Furuya et al., 2010).

***5.10. Inadequate biosecurity measures***

In poultry farms, biosecurity is a mandatory, all-encompassing procedure that guards against biological threats, both deliberate and inadvertent. In rural locations, biosecurity measures can be developed and used to small-scale agriculture systems. Periodic risk assessment is ideal for biosecurity, and local conditions may demand adjustments to the precautions in place. The farm, the movement of animals on and off the property, and general management procedures are associated with several risk factors for infection. As a result, the absence of comprehensive biosecurity plans leaves chickens prone to various disease outbreaks. Due to the vaccine strain switching to a pathogenic one, this weakens the chicken's immune system and increases the likelihood of vaccination failure (Butcher and Yegani, 2009). As a result, many real-world factors influence immunization and lead to vaccine failure.

**Table 2:** An overview of the elements that reduce the effectiveness of vaccines.

| Elements | Impacts |
|---|---|
| Maternal immunity | Interact with the vaccine's antigens earlier |
| Coexisting illness and immunosuppressive medications | The immune system of birds is unable to provide a strong enough defense against the pathogenic pathogens. |
| Incompatibility between the vaccination and the field virus (field strain pathotype) | Absence of sufficient antibody similarity between the vaccination strain and the circulating viral strain (field virus) to provide complete protection |
| The vaccine's immunogenicity and antigen content | If there is insufficient antigen to trigger an immune response, then no sufficient immune response is elicited. |
| The state of the hens' health | When the bird's immune system fails to provide a strong enough defense |
| Coverage of vaccinations (the total number of immunized birds) | Protecting Many birds is necessary to limit the transmission |
| Bird species or breed | For the best Response, the target species should be considered during preparation. |
| How long immunity lasts | Birds with reduced immunity are more vulnerable to illness and infection because their immunity is weakened. |
| Number of dosages | Over time, booster doses should be administered to increase immunity. |
| Administration technique quality | The right tools should be used to provide a complete and ideal dosage. |
| The rate at which vaccination results in the best possible amount of protection | Any encounter prior to the establishment of enough immunity might result in illness. |

Source: (Butcher and Yegani, 2009).

## ***6. The Main Prevention Techniques for Immunization Failure***

Vaccines for poultry are often employed in a variety of production methods. Before putting the control choices into practice, a cost-benefit analysis should be carried out to determine the best possible strategy while considering various scenarios. Accordingly, the following factors should be considered when performing a cost-benefit analysis: the type of birds, the production system, the density of birds, the antigenicity and virulence of the virus in question, the availability of veterinary facilities, and the effect on trade (Ganguly et al., 2010; Spackman et al., 2014). The following approaches are thought to be the most effective way to manage poultry vaccination failure.

### ***6.1. The vaccines' appropriate formulation***

Since vaccine preparation and composition might vary, it is always advised to heed the advice given by the manufacturer of the vaccination. For example, if the chemicals (sanitizers) used to clean the drinkers are combined with the vaccine during production, the vaccine's vital components may be lost. Furthermore, Nwanta et al. (2005) state that consideration should be given to the timing, duration of effectiveness, and kind of diluent while preparing vaccines.

***6.2. Considering maternal immunity***

The most of poultry farms routinely vaccinate their animals, particularly parent stocks, in response to different bird outbreaks and common infectious diseases. As a result, through antibodies carried in eggs, freshly born day-old chickens automatically have passive immunity from their parent stock in their blood. Nevertheless, vaccinations, particularly live ones, will be neutralized if they are given within the first two weeks when the mother's antibodies are at their highest. To obtain the optimum amount of immunization from the vaccine, vaccination should be administered to newly hatched chickens depending on the antibody titration level against the recommended vaccinations, according to various study findings and poultry manuals. For example, immunization against Gumboro disease (IBD) should occur at least 11 days after hatching, whereas vaccination against Newcastle disease should occur when the chicken is 7 days old (CEVA, 2005).

***6.3. Keeping the cold chain temperature and proper storing***

Vaccines for poultry should be carried and stored according to the manufacturer's recommended temperature since variations in temperature directly affect the vaccine's effectiveness. Additionally, the vaccine needs to be stored apart from other things like food, chemicals, and pathology samples. Because of this, some live vaccinations, such as those for Marek's disease, can quickly become inactive if handled or stored incorrectly (Alexander and Shenne, 2003). To preserve the vaccine's viability, the appropriate cold chain temperature needs to be maintained. The cold chain temperature is affected by several issues, including overchilling, a lack of energy sources, a lack of cooling systems (such as refrigerators), and a lack of ice boxes for delivering vaccines. Similarly, further cooling oil-based vaccinations causes the adjuvants (aluminum salts) to crystallize, which lowers the vaccines' efficacy. While freeze-dried vaccines should be transported using ice blocks or a cooling system to maintain the recommended temperatures, thermostatic vaccines are designed to withstand temperature fluctuations in both hot and cold environments (Alexander and Shenne, 2003).

Additionally, if freezing affects the quality of a vaccine, it must be avoided during handling and storage. In vaccine logistics, it is presumed that most of these precautions are observed; however, it is often impossible to handle vaccines within the stated temperature range. Previous studies have shown that it is difficult to maintain a specified storage temperature range during vaccine transport (Matthias et al., 2007; Kartoglu and Milstien, 2014; Das et al., 2019; Falcón et al., 2020).

***6.4. Do not utilize vaccines that have been expired.***

The most of chicken vaccines manufactured globally have varying table shelf lives, which are explicated in detail in the manufacturer's leaflets. Certain vaccines, such as live lyophilized vaccines, have a long shelf life (1-2 years), whereas others, such as oil-based vaccinations, have a shorter shelf life (3-6 months). Before applying, the vaccine vial should be thoroughly examined, and its expiration date determined. It is important to refrain from using vaccinations that have expired and to dispose of them appropriately or replace them with fresh ones directly from the manufacturer (Nelson et al., 2004).

***6.5. Adjuvant and stabilizers should be utilized.***

An adjuvant is a supplement that is given to a vaccination to improve the immune system's reaction to the target antigen and boost the vaccine's bioavailability. This in turn aids in lowering the necessary antigenic dosage to generate effective immunity and raising the immunogenicity of vaccinations to offer long-term protection. Adjuvants may have an impact on triggering a mucosal immune response, which contributes to the vaccine's increased safety. For example, the microbial barriers in the gastrointestinal tract present a significant challenge for oral vaccination, and adjuvants are needed to preserve the antigen to overcome these obstacles and stimulate the immune system. For vaccinations against particular and more sensitive flocks of chicken populations, an adjuvant platform that combines many adjuvants is particularly favorable and promising in the case of inadequate vaccines. As opposed to single adjuvants, combined adjuvants work in concert to activate and stimulate many kinds of immune cells, including lymphocytes, macrophages, and dendritic cells (Shane, 2005).

A stabilizer is a material that is given to a vaccine to help it stay stable and last longer while other vaccines are being prepared and administered. Stabilizers come in a variety of forms and are sold to prepare different kinds of vaccines. For instance, the best stabilizers for live oral vaccines are Vac-Safe (Intervet) and Vital Blue, which are used for infectious bronchitis, Newcastle disease, and Gumboro disease (Nelson et al., 2004).

***6.6. Reducing stress and using antibiotics***

Stress may be controlled by determining its possible origins and causes, which can be divided into two categories: predictable stress, which is associated with problem-solving and management, and unpredictable stress, which is associated with temperature fluctuations and varying rates of disease outbreaks. The development of a technique for recognizing and measuring stress is necessary to manage vaccination failure brought on by unanticipated stress. For this reason, to improve living quality and boost immune response sources, the feeding, housing, microbial flora, and breeding systems should all be altered to the appropriate standards. Second, administering various treatments to reduce the degree of immune suppression in birds caused by non-specific or unknown variables to improve their quality of life and boost their immunological responses. These methods shouldn't be viewed as the only ways to avoid stress and its negative effects, though. In addition, after determining the cause of the stress, remedial actions including decreasing bird density, boosting feeder and drinker counts, enhancing ventilation, and other measures should be implemented (Bermudez and Stewart-Brown, 2003). Antimicrobials are used in chicken production both before and after vaccinations. They are administered to reduce and avoid the formation of a high risk of secondary bacterial illnesses caused by opportunistic microorganisms during stressful times. When choosing antimicrobials, consider their range of action, potential against pathogenic opportunistic microorganisms, and compatibility with the immune system.

***6.7. Vitamin and mineral administration***

Immunological-compromised birds should be given vitamins and minerals that promote the immunological response by acting on the immune cell and quickly enhance the generation of antibodies; this will improve the birds' immunity more quickly. In order to stimulate the immune system and mitigate some of the negative effects of stress, vitamins A, E, C, and B are added to chicken feed. In addition, taking vitamins before and throughout a known stressful situation is crucial and need to be continued until the stressful event is over. Vitamins are involved in various stress reactions and are either directly depleted during the synthesis of glucocorticosteroids (Vitamin C) or indirectly depleted by increasing the number in most intermediate metabolic reactions (Vitamin B). This should be administered 24 hours prior to vaccination (Alexander and Shenne, 2003).

### *6.8. Appropriate immunization schedule and proper biosecurity*

Depending on the illness's frequency in the region, a suitable vaccination program should be in place for chicken flocks to reduce disease outbreaks and the accompanying financial losses. It is preferable to vaccinate the birds before the sickness manifests itself so that they can acquire the necessary level of antibody titration. For example, vaccinations against avian influenza and infectious bronchitis should be administered prior to the winter season because bird infections often peak around this time. Furthermore, maintaining a high standard of biosecurity is essential to preventing and controlling poultry outbreaks and should be used in conjunction with vaccination programs. Furthermore, Butcher and Yegani (2009) recommend adhering to stringent stamping-out (all in, all-out) guidelines to prevent cross-contamination among flocks.

### *6.9. Preparing flocks for immunization*

Depending on the route of delivery, the age of the birds, the vaccination technique, and the type of vaccine, flocks of birds need to be appropriately prepared for vaccination. To further enhance the effectiveness of the vaccine, preventative measures including restricting food and liquids for two to three hours before immunization should be rigorously adhered to. For example, spray vaccination is being used to mass-vaccinate older chicks post-hatch in cabinets using the aerosol approach. Old chicks are applied to individual chickens through the eye on eye drop day. The most popular method of administering vaccines is orally. The vaccinations should be prepared and provided in clean equipment (drinkers), and they should be given to the flock after they have fasted for a few days. This will assist the flock finish the immunization quickly up to two hours. To do this, there must be enough drinkers, and the birds must be moved often and well monitored to ensure they consume an adequate amount of the vaccine (Nelson et al., 2004).

### *6.10. Adhere according to the instructions provided by the manufacturer.*

When administering vaccinations, one should follow the manufacturer's instructions for preparation, usage, storage, and administration method. These instructions should be followed for all vaccine types, including inactivated, live attenuated, DNA, and recombinant vaccines. Therefore, to reduce the possibility of vaccine-related side effects, such as local tissue response (in the case of activated or dead vaccinations) and vaccine reaction (in the case of live attenuated vaccines), immunization should always be administered by a trained or certified veterinarian. Furthermore, the most of manufacturers recommended that poultry vaccinations be applied after determining the appropriate antibody titration level, in order to prevent stressful environments like as transportation, crowding, and hot weather. For example, early or late in the day are thought to be the best times to get vaccinated, particularly in the summer (Nwanta et al., 2005).

## CONCLUSION AND RECOMMENDATIONS

In emerging and transitional nations, the fastest-growing segment of the world meat output is poultry production. Poultry producers and the industry are at risk from infectious illnesses that cause significant financial losses. One of the most crucial and economical methods for preventing disease in poultry, minimizing losses, and lowering the use for antibiotics in chicken products is vaccination. However, there are issues with poultry vaccinations that need to be addressed in order to prevent vaccine failure. These issues include the vaccine's instability, improper handling, storage, and transportation, as well as administration, all of which call for novel approaches to vaccine development. Therefore, to address these issues, it is best to adhere to the manufacturer's requirements for vaccines, since they are crucial in preventing failure. In addition, flock health and the quantity of titers obtained from mothers should be considered while giving the vaccination. Thus, in light of the aforementioned finding, the ensuing suggestions were sent Veterinarians employed at various field clinics must adhere to the vaccination makers' recommendations regarding administration, storage, timing, and expiration dates. It is vital to keep an eye on the health of the chickens prior to vaccination. It's crucial to use the appropriate vaccination techniques, the right dose, and the right timing. It is important to educate chicken owners on the proper use of vaccinations in preventing diseases in their birds. More research is required to determine the best timing to provide the vaccination to a flock of immunized parents. It is necessary to do a thorough analysis of the various commercially available vaccinations. Veterinarian advice should be sought before administering any vaccines to chicken producers. Furthermore, research on the relative effectiveness of vaccinations from reputable sources is required.

Handling and storage of vaccines must be done without freezing them as freezing lowers their quality. Although it is assumed in vaccine logistics that the most of these safety measures are followed, handling vaccines within the specified temperature range is frequently not feasible. It is challenging to keep vaccines within a designated storage temperature range.

## REFERENCES


Abdul Careem, M.F. (2015). Main challenges in poultry farming: Vaccination failure. Zaragoza, Spain: Grupo Asis.

Abdullah, U., Adamu, S., Ahmed, A. (2009). Investigating on some causes of poultry vaccination Nigerian Journal of Experimental and Applied Biology 14(1): 47-50.

Aldres, R.G. and Spradbrow, P.B. (2001). SADC Planning workshop on Newcastle disease control in village chickens. In: Proceedings of an international workshop, Maputo, and Mozambique.

Alexander, D. J. and, Shenne, D. (2003). Newcastle disease. Diseases of poultry 11(1): 64-87.

Ali A., Ankers P., DeHaan N., Saad A., Hussein S., Lubroth J. &Jobre Y. (2013). Mapping influenza A (H5N1) virus transmission pathways and critical control points in Egypt. FAO Animal Production and Health Working Paper 11 (http://www.fao.org/docrep/017/i3272e/ i3272e.pdf accessed on 31 January 2016).

Ali A., Ankers P., DeHaan N., Saad A., Hussein S., Lubroth J. &Jobre Y. (2013). Mapping influenza A (H5N1) virus transmission pathways and critical control points in Egypt. FAO Animal Production and Health Working Paper 11 (http://www.fao.org/docrep/017/i3272e/ i3272e.pdf accessed on 31 January 2016).

Arafa A., Suarez D., Kholosy S., Hassan M., Nasef S., Selim A., Dauphin G., Kim M., Yilma J. & Swayne D. (2012). Evolution of highly pathogenic avian influenza H5N1 viruses in Egypt indicating progressive adaptation. A*rch Virol*, 157 (10), 1931-1947.

Assa, M. (2012).Poultry production and rural poverty among small scale farmers in Mzimba District of Malawi. Livestock Res for Rural Dev.24, 177-82.

Bax, D. (1999). "Edward Jenner's Inquiry; a bicentenary analysis" Vaccine. 17 (4): 301–307.

Bell, J. G. (2000). A comparison of the different vaccines available for the control of Newcastle Disease in village chickens. In: SADC planning workshop on Newcastle disease.

Bermudez, A. J. and Stewart-Brown, B. (2003). Disease prevention and diagnosis. In disease of poultry (Y.M. Saif, ed.), 11th Ed. Iowa State University Press, Ames, 17-54.

Bosha, J. (2012). Common breaches in poultry vaccine handling and administration in Makurdi Metropolis: A recurrent phenomenon in the tropics. Vom Journal of Veterinary Science 9(1): 11-16.

Bosha, J. and, Nongo, N. (2012). Investigating vaccine handling and administration in Makurdi metropolis, Benue state, Nigeria. Paper presented at Proceedings of 37th conference of Nigerian society of animal production (NSAP), University of Agriculture Makurdi, Bennue state, Nigeria.

Brugh, M. and, Siegel, H. S. (1978). Inactivated Newcastle disease vaccines: Influence of virus concentration on the primary immune response. Poultry Science, (57): 892-896.

Butcher and Miles (2017). Vaccine Failure in Poultry: Factors to Consider. University of Florida Institute of Food and Agricultural Sciences, http://edis.ifas.ufl.edu/vm174, Accessed on September 17th, 2019.

Butcher, G. D. and Yegani, M. (2009). Investigating Vaccination Failure in Poultry Flocks.

CEVA, (2005). Factors affecting vaccination. Vaccines and Vaccination in Poultry Production: Santé Animal, 81-98.

Cheyne J. (1989). Vaccine delivery management. *Review of Infectious Diseases*, 11 (Suppl 3), S617-S622.

Chojnacky M., Miller W. & Strouse G. (2010). Thermal analysis of refrigeration systems used for vaccine storage Report on pharmaceutical grade refrigerator and household refrigerator/freezer. NISTIR 7753 (http:// www.nist.gov/pml/div685/grp01/upload/NISTIR7753-Thermal-Analysis-of-a-Dual-zone-Refrigerator-and-Pharmaceutical-Refrigerator-for-Vaccine-Storage.pdf ).

Collett S.R. (2013). Principles of disease prevention, diagnosis, and control. *In* Diseases of poultry (David E. Swayne, ed.), 13th Ed. Iowa State University Press, Ames. Kumru O.S., Joshi S.B., Smith

Collett S.R. (2013). Principles of disease prevention, diagnosis, and control. *In* Diseases of poultry (David E. Swayne, ed.), 13th Ed. Iowa State University Press, Ames.

Dana, S., Rathore, B. and, Kaul, P. (2000). Morbidity and mortality pattern in desi chicken reared by the santal tribe of West Bengal. Indian Journal of Animal Research, 34(1): 49-51.

Das MK, Arora NK, Mathew T, Vyas B, Sindhu M and Yadav A. (2019). Temperature integrity and exposure to freezing temperature during vaccine transfer under the universal immunization program in Three States of India. Indian Journal of Public Health, 63: 139-142.

Evans, M. and Pope, M. (1995). Vaccine handling and storage in general practice. Health Trends, 27: 124-126.

Falcón VC, Porras YVV, Altamirano CMG and Kartoglu U. A. (2020). vaccine cold chain temperature monitoring study in the United Mexican States. Vaccine, 38: 5202-5211.

Fasina F.O., Sirdar M.M. & Bisschop S.P. (2008). The financial cost implications of the highly pathogenic notifiable avian influenza H5N1 in Nigeria. *Onderstepoort J Vet Res*, 75 (1), 39-46.

Fiore, A. E., Bridges, C. B. and Cox, N. J. (2009). "Seasonal influenza vaccines". Vaccines for Pandemic Influenza. Curr. Top. Microbial. Immunol. Current Topics in Microbiology and Immunology. Vol. 333.

Furuya, Y., Regner, M., Lobigs, M., Koskinen, A. and Müllbacher, A. (2010). Effect of inactivation method on the cross-protective immunity induced by whole 'killed' influenza A viruses and commercial vaccine preparations. Journal of General Virology 91(6): 1450-1460.

Ganguly, S., Paul, I. And Mukhopadhayay, S. (2010). Different types of vaccines and vaccination. The most accepted trend to control and eradicate infections. Indian Pet Journal 5: 34-37.

Garg, R. Babiuk, L. and Gerdts, V. (2017). A novel combination adjuvant platform for human and animal vaccines. Vaccine, 35(35): 4486-4489.

Hassan, A. M., Garba, and M. S. and Durodola, O.S. (2017). Causes of Vaccination Failures among Poultry-Journal of Agric & Agric Tech 3(1): 104-110.

Ideris, A.; Ibrahim, A.L.; Spradbrow, P.B. and Hung, H.C. (1987). Development of food pellet Newcastle disease Vaccine. In: Copland JW, editor. Newcastle disease in poultry. A new food pellet vaccine, Canberra, ACIAR., 20-23.

Jorge, S. andDellagostin, O.A. (2017). The development of veterinary vaccines: a review of traditional methods and modern biotechnology approaches. Biotechnology Research and Innovation, 1(1): 6-13.

Kartoglu U. and Milstien J. (2014). Tools and approaches to ensure quality of vaccines throughout the cold chain. Expert Review of Vaccines, 13: 843-854.

Kilany W.H., Khalifa M.K., Safwat M., Mohammed S., Selim A., VonDobschuetz S., Dauphin G., Lubroth J. &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 challenge. *Avian Pathol*, 44 (5), 333-341.

Kim J.K., Kayali G., Walker D., Forrest H.L., Ellebedy A.H., Griffin Y.S., Rubrum A., Bahgat M.M., Kutkat M.A., Ali M.A., Aldridge J.R., Negovetich N.J., Krauss S., Webby R.J. & Webster R.G. (2010). Puzzling inefficiency of H5N1 influenza vaccines in Egyptian poultry. *Proc Natl Acad Sci USA*, 107 (24), 11044-11049.

Kumru O.S., Joshi S.B., Smith D.E., Middaugh C.R., Prusik T. &Volkin D.B. (2014). Vaccine instability in the cold chain: mechanisms, analysis and formulation strategies. *Biologicals*, 42 (5), 237-259.

Ledford, and Heidi, (2020). "What the immune response to the coronavirus says about the prospects for a vaccine". Nature. 585 (7823): 20–21. Bibcode: 2020 Natur.585.20 L.

LMP (Livestock Master Plan), (2015). Road maps for Growth and Transformation. Poultry Development Roadmap 2015/16-2019/20, 48 – 62.

Marangon S. &Busani L. (2007). The use of vaccination in poultry production. *Rev Sci Tech OIE*, 26 (1), 265-274.

Marangon, S. and Busani, L. (2007). The use of vaccination in poultry production. Revue Scientifique ET Technique-Office International des Epizooties 26(1): 265-274.

Matthias DM, Robertson J, Garrison MM, Newland S and Nelson C. (2007). Freezing temperatures in the vaccine cold chain: a systematic literature review. Vaccine, 25: 3980-3986.

Melief, C. J., van Hall, T., Arens, R., Ossendorp, F. and van der Burg, S.H.

(2015). "Therapeutic cancer vaccines". The Journal of Clinical Investigation. 125 (9): 3401–3412.

Middaugh D.E., C.R., Prusik T. &Volkin D.B. (2014). Vaccine instability in the cold chain: mechanisms, analysis and formulation strategies. *Biologicals*, 42 (5), 237-259.

Miller N.C. & Harris M.F. (1994). Are childhood immunization programmes in Australia at risk? Investigation of the cold chain in the Northern Territory. *Bull World Health Organ*, 72 (3), 401-408.

Mojtaba, Y., Gary, D., Butcher, Amir, Nilipour, Richard, D., Miles, and Ali, R. (2012). The culprits of vaccination failures. World Poultry - Elsevier Volume 18, No 8. '02.

Nasser, M., Lohr, J. E., Mebratu, G. Y., Zessin, K. H., Baumann, M. P. O. and Ademe, Z. (2010). Oral Newcastle disease vaccination trials in Ethiopia. Avian Pathology, 29: 27– 34.

Nelson C.M., Wibisono H., Purwanto H., Mansyur I., Moniaga V. &Widjaya A. (2004). Hepatitis B vaccine freezing in the Indonesian cold chain: evidence and solutions. *Bull World Health Organ*, 82 (2), 99-105.

Nelson, C. M., Wibisono, H., Purwanto, H., Mansyur, I. and Moniaga, V. (2004). Hepatitis B vaccine freezing in the Indonesian cold chain: evidence and solutions. Bulletin of the World Health Organization, 82(2): 99-105.

Nwanta, J., Umoh, J., Abdu, P., Ajogi, I. and Egege, S, (2005). Comparison of the Cost of unvaccinated and oral vaccinated local chickens with a Malaysian Thermostable Newcastle Disease Vaccine (NDV4HR) In Kaduna State, Nigeria. Bulletin of Animal Health and Production in Africa 53(3): 203- 210.

Ontario Ministry of Health and Long-Term Care (OIE) (2014). Vaccine storage and handling guidelines. GSIN: 7540-19600E May 2013. Queen's Printer for Ontario, 2013.(http://www.health.gov.on.ca/en/pro/programs/publichealth/oph_standards/docs/reference/vaccine%20_storage_handling_guidelines_en.pdf accessed on 1 October 2023).

Plotkin, S.L. and Plotkin, S.A. (2008). A short history of vaccination. In Vaccines 5th edition, S Plotkin, W Orenstein and P Offit, Eds, Saunders Elsevier, China.

Ramirez, L. A., Arango, T. and Boyer, J. (2013). Therapeutic and prophylactic DNA vaccines for HIV-1. Expert Opinion on Biological Therapy.13:563-573.

Saif, Y. M. and Barnes, H. J, (2003). Diseases of poultry. 11th Edition. Ames, Iowa: Iowa State Press.

Sanda, M, (2015). Effects of Vitamin-Mineral Supplement on the Immune Response of Broilers to Newcastle Disease Vaccination. International Journal of Agricultural and Veterinary Sciences (IJAVS) 1(1): 10-13.

Shane, S. M, (2005). Handbook on Poultry Diseases. (2nd Edn.): American Soybean Association, Singapore.

Sharif, A. and Ahmad, T. (2018). Preventing Vaccine Failure in Poultry Flocks. Immunization-Vaccine Adjuvant Delivery System and Strategies: Intech Open.

Siddique, F.; Iqbal, A. and Manzoor, M. (2016). Thermostable vaccine: A New Horizon in poultry Industry: A review. Scholar's advances Animal Veterinary Research, 3: 42-46.

Spackman, E., Pantin, J. and wood, M. J, (2014). Practical aspects of vaccination of poultry against avian influenza virus. The Veterinary Journal 202(3): 408-415.

Subramanyam K. (1989). Vaccine distribution: an operations research study. *Rev Infect Dis*, 11 (Suppl 3), S623-S628.

Terfa, Z., Garikipati, S., Dessie, T. P., Wigley, J. M. and Bettridge, (2015). Farmers' willingness to pay for a village poultry vaccine service in Ethiopia:

Prospect for enhancing rural livelihoods. Food Security 7(4): 905-917.

Tu, T.D.; Phuc, K.V.; Dinh, N.T.K.; Quoc, D.N. and Spradbrow, P.B. (1998). Vietnamese trial with a thermostable Newcastle disease vaccine (strain 1-2) in experimental and village chicken. Preventive Veterinary Medicine, 34: 205-214.

Wambura, P. N. and Kataga, S, (2011). Putative protective antibody response following oral vaccination of multi age free ranging helmeted guinea fowls (Numidia meleagris) with Newcastle disease virus strain I-2 coated on oiled rice. Tropical animal health and production 43(1): 99-102.

Wambura, P.N.; Kapaga, A.M. and Hyera, J.M. (2000). Experimental trial with thermostable Newcastle disease virus (strain) in commercial and village chickens in Tanzania. Preventive Veterinary Medicine. 43: 75-83.

World Health Organization, (2013). Global vaccine action plan, 2011–2020. WHO https://www.who.int/immunization/globalvaccine_action_plan/GVAP_doc_2011_2020/en/.