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Vaccination against Infectious Bronchitis Virus: A Continuous Challenge

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Highlights

- Infectious bronchitis virus infection is a significant problem in the poultry industry.
- Current vaccines are either live-attenuated or killed and are used extensively.
- Novel vaccines are needed to combat emerging and variant IBV serotypes.
- Other factors that can influence the outcome of vaccination, including application method, vaccine combinations, chicken genetics, and immune responses, must be taken into account when developing vaccines.
1. Infectious Bronchitis Virus

The avian infectious bronchitis virus (IBV) is a highly infectious pathogen of commercial poultry with a predilection for the upper respiratory tract, but can also infect and cause disease in the kidneys and reproductive tract (Jackwood and de Wit, 2013). IBV is a gammancoronavirus with a single-stranded, positive sense, ~27 kb RNA genome that encodes many nonstructural proteins involved in replication, three major structural proteins (spike (S), envelope (E), and membrane (M)) involved in virion formation, and a protein involved in genome packaging (nucleocapsid (N)) (Cavanagh, 2007). Of these proteins, S is often regarded as the most significant due to its role in host cell binding and neutralizing epitope presentation, and has been the most researched (reviewed in (Wickramasinghe et al., 2014)), though the E, M, and N proteins are critical for virion production. The S glycoprotein is cleaved into two subunits, S1 and S2, which form the club-like head and transmembrane anchor of the protein, respectively. The S1 subunit of IBV contains the receptor binding domain (RBD) responsible for virus attachment to host cells (Promkuntod et al., 2014), and is the major inducer of neutralizing antibodies (Kant et al., 1992; Koch et al., 1990). Besides being the anchor, the ectodomain of S2 aids in virion attachment to host cells (Promkuntod et al., 2013) and has been shown to contain antigenic epitopes (Toro et al., 2014b).

IBV is worldwide in distribution and exists as many different serotypes. Serotypes are defined by specific circulating antibodies developed against epitopes on the S protein after exposure, and are traditionally determined by hemagglutination inhibition (HI) or virus neutralization (VN) assays (Jackwood and de Wit, 2013). Epitopes responsible for induction of neutralizing antibodies correlate closely with three hypervariable sequence regions (HVRs) in
the S1 portion of the spike protein gene (Cavanagh et al., 1988). Sequence of the HVRs can generally be correlated to serotype where viruses with >95% sequence similarity are considered the same type (Cavanagh, 2001) and viruses with <85% similarity are considered separate serotypes (Cavanagh, 2005), though this is not a hard-and-fast rule. The process of molecular evolution in IBV is responsible for the many different serotypes of the virus and is thought to be a combination of genetic shift through recombination events (Kusters et al., 1990) and genetic drift through the accumulation of genetic mutations due to the natural mechanism of virus replication that employs a low-fidelity RNA dependent RNA polymerase (Hanada et al., 2004). Emergence of new IBV serotypes is through selection acting on these molecular changes. Selection pressures can come from many different sources such as the use of vaccines in commercial poultry (Jackwood et al., 2012). Though the exact mechanisms for viral evolution and the emergence of new serotypes are not fully understood, we do know that dozens of individual serotypes and possibly hundreds of variants of those types exist worldwide making the study of molecular evolution in IBV extremely important.

IBV infection causes the disease known as infectious bronchitis (IB) in chickens. IB is characterized by conjunctivitis, tracheitis, and ciliostasis (loss of ciliary activity) in the upper respiratory tract in addition to drops in egg production in layer/breeder type birds. Some strains of IBV can also cause nephritis and urate deposition in the kidneys. Flocks that break with IB usually demonstrate 100% morbidity, but mortality is low unless complicated by nephritis or a secondary bacterial pathogen. Indeed, the predisposition to a secondary bacterial infection is the major economic concern of IB for the broiler industry. Airsacculitis caused by a secondary bacterial pathogen after infection with IBV will lead to condemnation at processing, and can cause the loss of an entire bird. Losses associated with an IB outbreak, including lost
performance, mortality, and condemnation can be upwards of $65,000 per week for an operation producing 1 million 7 pound broilers per week. If line speed at the processing plant is slowed, the costs associated bring the total loss to ~$450,000 per week, which is unsustainable for even short periods of time (personal communication, Dr. Don Ritter). In layer/breeder chickens, drops in egg production alone can cause significant economic losses. For these reasons, nearly every commercial chicken produced is vaccinated against IBV in an effort to prevent the disease.

2. Vaccination

2.1. Vaccines

Live attenuated IBV vaccines are most commonly used in all sectors of the poultry industry. Live attenuated IBV vaccines are produced from pathogenic field isolates by serially passaging them in embryonated specific pathogen free (SPF) eggs. Attenuation is defined as the lack of clinical respiratory signs associated with disease in chicks after inoculation (Bijlenga, 1960). It can take up to 1-year to attenuate a field virus, but rapid attenuation can sometimes be achieved through a combination of heat-treatment and limited egg passage (Jackwood et al., 2010). Killed or inactivated IBV vaccines are also used in layer/breeder type chickens and are routinely produced by formaldehyde inactivation of live IBV. The Massachusetts (Mass) serotype IBV vaccine is used throughout the world as it was the first vaccine type produced and the only one available for many years. In many poultry producing countries, it is also the only licensed IBV vaccine serotype. As novel serotypes and variants have emerged, vaccines have continually been produced to combat regionally important serotypes, i.e. Arkansas (Ark), Connecticut (Conn), Delaware (Del), Georgia98 (GA98), Georgia 08 (GA08), and Georgia 13 (GA13) in the U.S., and 793/B, QX, and Q1 in Europe, Asia, and South America, to name a few (Jackwood, 2012). Recombinant IBV vaccines have been developed that express IBV antigens
alone (Johnson et al., 2003; Li et al., 2016; Toro et al., 2014a) or in combination with antigens from other pathogens (Yang et al., 2016; Yin et al., 2016), but have yet to show either the same protective level as live-attenuated vaccines, or ease of administration associated with mass hatchery application. Currently, there are no licensed recombinant IBV vaccines for use in the commercial poultry industry. Novel methods of vaccine production are discussed in more detail below.

2.2 Vaccination Timing

The timing of vaccination against IBV varies based on what type of bird is being vaccinated. Broilers are the shortest-lived type of chickens and are vaccinated the fewest number of times against IB. Typically, day-of-hatch chicks are mass vaccinated with 1-3 serotypes of live-attenuated IBV vaccine in the hatchery prior to placement on a farm. For many broilers, this is the only vaccination they receive for IBV. Longer-lived broilers (i.e. >49 days of age) may be field boosted in the broiler house between 14 and 18 days of age in an attempt to extend the duration of immunity. Broiler breeder and layer type chickens are much longer lived and receive multiple vaccinations against IB starting at 2 weeks of age with a live-attenuated vaccine in the pullet house, followed by live-attenuated vaccinations at 4 and 6 weeks of age. This schedule is fairly standard no matter company or breed. By 12 weeks of age, variations in vaccination begin to appear based on individual company, serotype of IBV challenge in the region, and cost of vaccination, but typically a live-attenuated and/or killed IBV vaccine will be given at this time. Once hens begin producing eggs (~20 weeks), almost all IBV vaccines given are killed, though some companies will occasionally give a live-attenuated vaccine if there is a challenge present in the region. The variability in timing and uniformity of live-attenuated and killed IBV vaccine application makes it difficult to effectively compare vaccination strategies to determine a “best-
case” field program that could work for every producer. It should also be noted that the vaccines used (both live and killed) are combined with Newcastle Disease Virus (NDV) vaccines, which adds another layer of complexity and variability.

2.3 Vaccination Programs

It is well known that a single serotype of IBV vaccine does not provide adequate protection from a heterologous challenge. Therefore, producers give more than 1 vaccine serotype to increase the breadth of protection in their flocks. This “multi-monovalent” strategy requires at least 2 serotypes of IBV vaccine, but more can be used if the challenge in a poultry producing area is highly diverse. The logic behind this strategy is that vaccination with multiple serotypes in combination will induce neutralizing antibodies against each of the vaccine types individually and protect chickens from those serotypes through sterilizing immunity, which will also protect the cilia in the trachea (preventing ciliostasis) and prevent secondary pathogen infections. This strategy has historically been used throughout the world because it has shown to be effective in practice and principle (Jackwood and de Wit, 2013). For this strategy to remain effective however, the pathogenic serotypes of IBV circulating in the field must be known so that the proper serotype vaccines can be given. If a novel serotype is introduced or a variant emerges, the “multi-monovalent” strategy may not provide protection against that virus. Indeed, emergence of novel or variant serotypes are observed in the face of vaccination (Lee and Jackwood, 2001; Wang et al., 1998; Yu et al., 2001). The continual evolution of IBV and constant need to update the vaccine repertoire is also a drawback to this vaccination strategy.

A second strategy used with multiple serotype vaccination is applying only two serotypes of IBV vaccine that provide a level of broad cross-protection against heterologous virus types (Cook, 1999). This strategy has been termed “protectotype”. Protectotype vaccination is not
based on specific neutralizing antibodies providing sterilizing immunity, though certainly neutralizing antibodies are developed against the two vaccine types. Protectotype vaccination centers on reducing IBV infection enough to protect the cilia in the trachea. Protection from ciliostasis will then reduce the incidence of secondary bacterial infections, which contributes to most of the losses associated with the disease (Jackwood et al., 2015). The Mass and 793/B group IBV serotypes have been the most widely used vaccine types in the protectotype strategy, and have been shown to give the largest breadth of cross-protection, especially when the Mass serotype was given at day-of-hatch and the 793/B serotype was given 14 days later (Cook, 1999; Terregino et al., 2008). The reason for the effectiveness of this vaccination strategy is currently not fully understood and is probably related to many factors including vaccine titer, level and timing of vaccine replication in the chicken, and actual neutralizing titer. As stated previously, Mass is licensed for use nearly everywhere in the world while 793/B type vaccines are not, making this strategy impossible in many poultry producing regions. Therefore, other vaccine combinations have been tried (Bru et al., 2016; Gelb et al., 1989; Jackwood et al., 2015), but to date, none have shown the same breadth of protection as the Mass and 793/B combination (de Wit et al., 2015). There is also some controversy regarding the protectotype vaccination strategy, especially when a 793/B type vaccine is being considered for introduction into a country that does not have any 793/B type pathogenic field viruses. Research has also shown that vaccinating with a single vaccine multiple times can significantly reduce viral loads in chickens after heterologous challenge (Toro et al., 2015), possibly alleviating the need for introduction of a novel vaccine type.

3. Vaccine Application

3.1. Spray Vaccination
Infectious bronchitis virus vaccines have routinely been mass applied by coarse spray in the hatchery on the day chicks are pulled from the hatching. IBV vaccines are mixed in a large reservoir and placed at the site of vaccination in the hatchery. Most spray vaccination systems use syringes to draw the vaccine out of the reservoir and expel it onto the chicks by forcing it through small spray nozzles that aerosolize the vaccine. The process of spray vaccination is a standard practice in modern hatcheries, where thousands to hundreds of thousands of chicks are vaccinated every day. There is a common misconception that spray application is designed to create droplets that contain vaccine that chicks can inhale. The thought has been that coarse spray was used because smaller droplets would be inhaled too deeply in the respiratory tract of the chicks, which in turn would cause the chicks to react more harshly to the vaccine. Measuring droplet size created by a spray nozzle shows that there is always a range of sizes, but that even the minimum droplet created is larger than a chick could inhale (Purswell et al., 2008). In actuality, spray vaccination recreates individual oculo-nasal inoculation on a large scale.

Relatively few laboratory studies have been performed to evaluate the efficiency of spray vaccination. Based on electron microscopy, individual IBV vaccine viruses do not show any evidence of structural damage after spray (no loss of spike proteins or fracturing of virion membrane), but titer was lost presumably from the mechanical force applied to the vaccine during the administration process (Roh et al., 2015). This indicates that many viruses make it through the spray application process intact, but that some are inactivated. Other studies showed that a significant drop in titer occurs when comparing what comes out of the spray nozzle to what gets to the level of the chicks, indicating that virus is being lost to the environment during the spray process as well (Jordan, 2016). This is especially true when smaller application volumes (7 ml) are used, which has been standard practice in the United States for many years.
Small application volumes are applied through smaller flow rate nozzles, promoting smaller droplet formation upon aerosolization (Jordan, 2016). This makes the droplets more susceptible to air movement where they are literally blown away from the chicks. Increasing the application volume to 14 or 21 ml through larger spray nozzles increases the amount of vaccine reaching the chicks, which results in better vaccine coverage.

The spray application equipment currently used in the poultry industry has not changed in nearly 40 years. Out of a need for innovation, and, for some, as a result of the research discussed above, new vaccine application technologies have been developed. One of the main points of failure on the spray apparatus is the plastic syringe used to draw in and expel vaccine. Single use syringes that are used over and over countless times, can become dislodged, broken, or stuck in the machine and they are directly responsible for vaccine destruction through the shearing forces applied to the vaccine during the application process. Because of this, the new spray technologies have focused on removing the syringes and utilizing another mechanism to expel vaccine. One technology uses a volumetric pump, wherein the vaccine is drawn in to and expelled from a piston-like pump. The mode of action for this system is similar to the syringe, but the problems with mechanical failure are alleviated as these pumps are fully enclosed and made of stronger material. The effect of pressures and forces on the vaccine in this type of system still needs to be evaluated. Another novel spray technology recently introduced has removed the syringe/pump mechanism altogether. In this system, the vaccine reservoir is pressurized and a switch at the nozzle activates to allow vaccine to be dispersed. In this way, vaccine is dispensed at the recommended pressure for the nozzle and mechanical failures and shearing forces from the syringe are removed, thereby maintaining the IBV vaccine titer throughout the entire system.

3.2. Gel Vaccination
Gel diluents, the newest technology in IBV vaccine application, were not originally designed for IBV vaccines, but has been adapted for this purpose. Gel diluents were initially introduced into the poultry market as a delivery method for coccidiosis vaccines. In this system, the coccidia vaccine is mixed into a thick gel diluent, and applied onto the chicks in streams through a gel applicator bar. Because of the viscosity of the gel, it forms large droplets on the chicks which remain stably intact until they are preened or shaken off by the chick. It can be imagined that this application method may increase the uptake of coccidia oocysts that must be ingested. However, this application system is counterintuitive for IBV vaccines, when considering the target route of vaccination is oculo-nasal. But, recent laboratory research and commercial field trials have shown that it can be as effective as spray vaccination for IBV (personal communication). More extensive research needs to be performed to fully evaluate gel vaccination as a viable alternative to spray for IBV vaccines.

4. Evaluation of Protection after Vaccination

Infectious bronchitis virus vaccine efficacy tests for licensure in the U.S. are required to evaluate protection from pathogenic homologous challenge by virus re-isolation in embryonated SPF chicken eggs (USDA, 1999). But, in other countries, vaccine efficacy can also be evaluated based on protection from ciliostasis (Pharmacopoeia, 2016). Clinical respiratory signs in the birds and histopathology of the trachea have also been used as measures of protection after challenge. As molecular diagnostic and research tools have progressed, virus detection by quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) both after vaccination and after challenge have become more popular. These methods are discussed below.

4.1. Virus Isolation
Infectious bronchitis virus isolation in embryonated SPF chicken eggs post challenge has always been the gold standard for determining protection from an IBV challenge (Jackwood and de Wit, 2013) and is still performed routinely. A negative virus isolation test indicates that a chicken is free of virus and that the vaccine provided sterilizing immunity, which is the highest form of protection. This test is costly and time consuming however, and SPF eggs are not available in all regions of the world. This test is also more suited for vaccination strategies designed around induction of neutralizing antibodies, i.e. monovalent or “multi-monovalent” vaccinations. Another drawback of this test is that it does not differentiate between IBV vaccine or pathogenic challenge virus. In a case where a vaccine may persist, a positive result by virus isolation may actually be the vaccine. In this situation, further analysis would need to be done to determine the true outcome of the efficacy test.

4.2. Ciliostasis

Protection from ciliostasis is an alternative test that is routinely used by countries outside the USA for evaluating the efficacy of an IBV vaccine. The ciliostasis test can be used to evaluate the cross-protective ability of combinations of vaccines in the protectotype vaccination strategy, and it can be used when homologous vaccine/challenge experiments are performed since induction of sterilizing immunity also protects the cilia. The ciliostasis test is a measure of functionality of the cilia in the trachea, and is designed to measure the level of ciliary activity. It has been shown that increased ciliary activity (lack of ciliostasis) correlates to increased protection from a secondary bacterial challenge since the respiratory route of infection is common for opportunistic bacteria (Jackwood et al., 2015). Performing the ciliostasis test requires special training and it is somewhat laborious, since several people are needed to “read” the tracheal rings to evaluate the cilia. The test can also be somewhat subjective. Since the
ciliostasis test is a purely physiological assay, no information on the induction of neutralizing antibodies or amount of virus in the chicken post-challenge can be obtained.

4.3. Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR)

As molecular technologies have advanced and become more economical, qRT-PCR has become a common detection method for IBV in chickens after challenge. qRT-PCR assays have been developed to generally detect all IBV types (Callison et al., 2006), or to specifically detect individual serotype viruses (Roh et al., 2014). The generic qRT-PCR assay for IBV targets a portion of the viral RNA genome that is highly conserved between different IBV types, making it suitable for detecting IBV but not discerning genotype. Genotype specific qRT-PCR assays target highly diverse HVR sequences that are correlated with serotype. qRT-PCR assays are less expensive than virus isolation, once the upfront cost of equipment has been absorbed, and reagents for these assays are readily available from a number of sources. qRT-PCR is also as sensitive as virus isolation and, in some cases, more sensitive (Roh et al., 2014) and results can be achieved in a single day by a single person. This sensitivity must be considered when interpreting data obtained from qRT-PCR however; a sample positive by qRT-PCR, but with a low number of viral RNA copies, may be negative by virus isolation creating a false positive qRT-PCR result. Published serotype specific qRT-PCR assays are highly specific for the target serotype, and a negative assay for one serotype will not provide any information on the presence or absence of other serotypes. It should also be noted that qRT-PCR does not differentiate between infectious and noninfectious virus; the assay only measures the amount of viral RNA in the sample being analyzed.

4.4. Correlation of Vaccine Efficacy Evaluation Methods
Depending on evaluation method used to determine vaccine efficacy, different conclusions could be reached. Jackwood et al. (2015) showed that, when evaluating protection, clinical signs, ciliostasis, virus detection by qRT-PCR, and histopathology correlated very well to each other in chickens that were either clearly protected or clearly not protected. In chickens that could be considered marginally protected (mild clinical signs, positive for virus detection by qRT-PCR but negative for ciliostasis) however, the evaluation methods did not all agree. This is significant considering that many laboratories only perform one method to evaluate protection. The experimental design of the vaccine/challenge experiment can also greatly affect the outcome. This has been extensively reviewed (de Wit and Cook, 2014), and the type of bird used (SPF vs broiler), maternal antibody state, age at vaccination, vaccine application method, timing of challenge, titer of challenge virus, and method used to evaluate protection can all influence the outcome of the experiment.

5. Novel IBV Vaccines

Much research has gone into developing novel, more broadly effective IBV vaccines over the past 20 years, but there has been relatively little technological advancement in the development or production of IBV vaccines that have come to market (Bande et al., 2015). Live-attenuated vaccines are still the predominant vaccine type, with killed or inactivated vaccines being the only alternative. There are two main limiting factors that have hindered the development of novel IBV vaccine technologies. The first limiting factor is cost. Commercial poultry companies operate on very small profit margins, making any additional cost per bird significant. Current live-attenuated and killed IBV vaccines cost on the order of a few cents per dose. Standard recombinant vaccines against other poultry pathogens are much more expensive relative to live-attenuated or killed IBV vaccines, but still frequently used because of their
efficaciousness and ease of application. If a novel IBV vaccine could match those specifications, cost would not as big of an impediment. The second limiting factor is application method. Broiler chickens are vaccinated in-ovo at transfer from the setter to the hatcher or on day of hatch by a mass application method (spray or gel). Layer/breeder chickens are also vaccinated multiple times with live IBV vaccines by spray. Any vaccine that cannot be mass applied (and be efficacious) is not compatible with the large-scale production practices of the poultry industry.

5.1. Recombinant Vaccines

Recombinant vaccines are extensively used in the poultry industry and are most commonly based on herpesvirus of turkeys (HVT) or fowlpox virus vector backbones. Genes encoding antigenic proteins from NDV (Morgan et al., 1992), infectious bursal disease virus (IBDV) (Darteil et al., 1995), and infectious laryngotracheitis virus (ILTV) (Vagnozzi et al., 2012) have been inserted into these viral hosts and used effectively to induce an appropriate immune response in chickens. Attempts have been made to develop a recombinant IBV vaccine using these viral backbones expressing the S1 portion of the spike gene (Johnson et al., 2003; Toro et al., 2014a) with differing levels of protection from homologous challenge. To date however, none of these candidates have come to market. Currently, these IBV vaccine candidates don’t meet the application requirement set out above. For these vaccines to be effective, chicks must be vaccinated by intramuscular or subcutaneous injection or by individual oculo-nasal routes, and often multiple vaccinations are needed. This is not feasible in a commercial setting, especially for broiler chickens. Other recombinant systems where the S1 or the S2 portion of the IBV spike gene has been incorporated into other viral backbones, such as NDV, duck enteritis virus, and avian metapneumovirus, have also been evaluated (Falchieri et al., 2013; Li et al., 2016; Toro et al., 2014b). These recombinant IBV vaccine candidates, especially those using the
NDV and metapneumovirus virus backbones, show promise as these viruses have a respiratory tropism and may be able to induce a more robust immune response in the head associated lymphoid tissues. Though the initial studies did show some protection from homologous challenge, even when given at one day of age, further evaluation is needed.

5.2. Subunit, Peptide, and DNA Vaccines

Subunit, peptide, and DNA vaccines are all based on the concept of presenting the host immune system with an antigen that will then stimulate the immune system to develop antibodies against the disease agent. Subunit vaccines are typically an antigenic protein from a pathogen, peptide vaccines are short segments of amino acids derived from an antigenic protein from a pathogen, and non-plasmid based nucleic acids and plasmid based DNA vaccines encode an antigenic protein that is expressed inside the hosts’ cells. Some of these vaccine types have been examined (Fang et al., 2013; Guo et al., 2010; Yang et al., 2009) and have shown some success at achieving partial protection from pathogenic IBV challenge. The immune response generated to these vaccines can be enhanced by the addition of immune factors (such as cytokines) or adjuvants, but for these vaccines to be efficacious, they must be injected intramuscularly, multiple times, which is not feasible in large-scale poultry production. These types of vaccines are also costly to produce on a large scale.

5.3. Recombinant IBV Generated by Reverse Genetics

The most promising, though not commercially available, novel vaccine candidate is a recombinant IBV generated by a reverse genetics system that can be engineered in the laboratory to fit the needs of the poultry industry. With these vaccines, the genome of an IBV vaccine virus is cloned in the laboratory and can be manipulated by researchers through homologous recombination, PCR, or cloning of other genes of interest directly into the genome (Britton et al.,
2005; Casais et al., 2001). These reverse engineered genomes are then transfected into an appropriate cell culture system that will replicate the genome, produce the viral proteins necessary for assembly and, ultimately, yield a fully functional IBV. The majority of the research with recombinant IBV has been performed to better understand the function of the viral proteins expressed in the IBV genome, and to determine how they interact with the host cell (Casais et al., 2005; Casais et al., 2003; Cavanagh et al., 2007), but research into vaccine development is also ongoing. Results show that removing the spike gene in a recombinant IBV and replacing it with the spike gene from a different serotype of IBV changes the virus to that new serotype. The newly created recombinant IBV can then be used to induce a protective immune response against the pathogenic virus containing that spike (Armesto et al., 2011; Hodgson et al., 2004). The advantage of this system is that an attenuated live vaccine to a newly emerged variant IBV can be created relatively quickly by recombinant techniques compared to the time it takes to attenuate the virus by passage in embryonated eggs. Recombinant IBVs have also been investigated as a delivery mechanism for other antigens, extending their possible use as a vaccine (Bentley et al., 2013).

Recombinant IBVs have several advantages to other possible IBV vaccine candidates. First, recombinant IBVs are live viruses and capable of infection, replication, and generation of an immune response in the same way that a traditional IBV vaccine would. Since all proteins in the virus are present, generation of a response to immunogenic proteins other than spike can be achieved. Second, as we learn more about the function of all of the proteins encoded by the IBV genome, we can develop recombinant IBVs that have been strategically modified to possibly alter pathogenicity (Cavanagh et al., 2007), rather than rely on continual chicken embryo adaptation. Third, the ability to swap only spike protein genes and produce a new serotype
vaccine in a constant background of accessory proteins would be a significant advancement in
the general predictability of IBV vaccines. The genomes of current live-attenuated vaccines vary
in many locations outside of the spike gene, making predictions of the behavior of the new
vaccines challenging. Fourth, and perhaps most important, recombinant IBVs fit in the
application and cost model for poultry vaccines. Since they are live viruses, they can be applied
by traditional methods, or even in-ovo in some cases (Cavanagh et al., 2007). The initial cost for
creating the recombinant virus is significantly higher than the traditional embryo passaging
method, but this is a one-time cost. Once the recombinant IBV is produced it can be propagated
and maintained in embryonated eggs just like any other IBV vaccine. The impact of the initial
production cost can be spread out over the long-term use of the vaccine and thereby only
minimally affect the cost per dose.

There is still at least one major hurdle that recombinant IBV vaccines must clear: licensing. Currently, each new recombinant IBV created for vaccine purposes would have to be licensed as a new virus, which could take two years or more. The hope would be that licensing agencies would adopt a platform approach similar to avian influenza recombinant vaccines that allows for expedited approval for use. Public perception must also be considered when considering the use of recombinant IBVs, since they are genetically modified organisms (GMOs). Currently, GMOs are not in favor with the general public and that sentiment may affect their use.

6. Conclusion

Even with extensive vaccination, outbreaks of IB in commercial poultry remain a
significant problem. New serotypes and variants are continually emerging, forcing poultry
producers and animal health companies to continually evaluate their vaccination plans and
produce new vaccines. Research into and field data on IBV vaccine combinations shows that some combinations can achieve cross-protection against heterologous challenges, allowing producers to keep their vaccine programs simple while still maintaining bird health. Research into novel vaccine technologies is continually broadening our understanding of IBV and will lead to better control strategies, while focusing on application methods will ensure that vaccination failures do not occur because of poor technique. Furthering our understanding of chicken genetics and how the genetic background affects the development of immunity may also shed light onto new research focus areas for vaccine development or application methods that will increase immune stimulation and induce better protection. Ultimately, it will take advances in all areas of IBV control, research, and development to create a strategy that is fully protective and cost effective, while maintaining mass application viability.

7. Conflicts of Interest

The author has no conflicts of interest to disclose.

Conflict of Interest Statement:

I have no conflict of interests regarding the preparation of this manuscript.
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