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Review

Vaccination against coronaviruses in domestic animals

Ian R. Tizard

Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77843, United States

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ABSTRACT

The current pandemic of COVID-19 has set off an urgent search for an effective vaccine. This search may well benefit from the experiences of the animal health profession in the development and use of coronavirus vaccines in domestic animal species. These animal vaccines will in no way protect humans against COVID-19 but knowledge of the difficulties encountered in vaccinating animals may help avoid or minimize similar problems arising in humans.

Diverse coronaviruses can infect the domestic species from dogs and cats, to cattle and pigs to poultry. Many of these infections are controlled by routine vaccination. Thus, canine coronavirus vaccines are protective in puppies but the disease itself is mild and self-limiting. Feline coronavirus infections may be mild or may result in a lethal immune-mediated disease – feline infectious peritonitis. As a result, vaccination of domestic cats must seek to generate- protective immunity without causing immune-mediated disease. Vaccines against bovine coronavirus are widely employed in cattle where they protect against enteric and respiratory disease in young calves. Two major livestock species suffer from economically significant and severe coronavirus diseases. Thus, pigs may be infected with six different coronaviruses, one of which, porcine epidemic diarrhea, has proven difficult to control despite the development of several innovative vaccines. Porcine epidemic diarrhea virus undergoes frequent genetic changes. Likewise, infectious bronchitis coronavirus causes an economically devastating disease of chickens. It too undergoes frequent genetic shifts and as a result, can only be controlled by extensive and repeated vaccination. Other issues that have been encountered in developing these animal vaccines include a relatively short duration of protective immunity, and a lack of effectiveness of inactivated vaccines. On the other hand, they have been relatively cheap to make and lend themselves to mass vaccination procedures.

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E-mail address: itizard@tamu.edu

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1. Introduction

The global pandemic of the disease COVID-19 caused by the SARS-CoV-2 coronavirus is an example of a "virgin soil" pandemic. Thus, a completely new virus has invaded a human population that totally lacks preexisting immunity. There are two possible outcomes of such a pandemic. If it sweeps unchallenged through susceptible populations, herd immunity will eventually develop in survivors and the severity of the outbreak will be reduced – but only after it has caused a huge number of deaths among the vulnerable. In addition, there may be waves of infection in different communities and countries depending upon the duration of protective immunity. The only long-term solution to this pandemic is therefore the development of an effective vaccine or vaccines. As a result, COVID-19 has triggered a worldwide effort to develop such vaccines for use in humans. Investigators have however, quite correctly, been reluctant to make definitive predictions regarding the efficacy of any such vaccines and there has been much speculation as to their potential effectiveness. Many commentators appear to be unaware that coronavirus vaccines have been widely employed in veterinary medicine for many years. They have been administered to both companion animals and to economically important livestock such as cattle, pigs and poultry. While it is important to emphasize that none of these animal vaccines will prevent COVID-19 in humans, the experience gained from the use of these vaccines and the problems associated with their use may be of benefit in developing and optimizing vaccines directed against SARS-CoV-2 in humans.

2. Coronaviruses

Coronaviruses are enveloped, single stranded, non-segmented, positive-sense RNA viruses belonging to the order Nidovirales [1]. They contain a large 25–30 kb genome, one of the largest genomes known among the RNA viruses. They are spherical viruses, 60–220 nm in diameter surrounded by a corona of multiple spikes 12–24 nm in length. They derive their name from this crown-like halo. Coronaviruses contain a linear, positive-stranded RNA molecule bound to a nucleocapsid (N) protein that encodes three major structural proteins, the spike (S) protein, the envelope (E) protein and the membrane (M) protein. The spike protein is responsible for viral attachment to receptors on target cells as well as membrane fusion. As a result, is also the immunodominant antigen responsible for stimulating the production of neutralizing antibodies. The M protein is the most abundant component of the envelope and contains epitopes that can be recognized by B cells. The E protein is required for viral assembly. Betacoronaviruses have an additional membrane protein the hemagglutinin esterase (HE), that forms short spikes on the viral surface. Coronaviruses also possess a variable number of accessory genes.

3. Canine coronavirus vaccines

Dogs harbor two unrelated coronaviruses. An alphacoronavirus (CCoV) that causes enteric disease and a betacoronavirus (CRCoV) that causes a mild self-limiting respiratory disease.

There are two different genotypes of CCoV, type I and type II. CCoV belongs to the same species as the cat (FCoV) and pig coronavirus (TGEV). Recombinant viruses between CCoV-1 and TGEV are widespread in dog populations. CCoV usually causes mild enteric disease in puppies with anorexia, fever and diarrhea.

Canine respiratory coronaviruses (CRCoV) were first identified in Europe in 2003 but are known to occur globally [2]. Serologic surveys across North America have indicated that as many as 50% of dogs may have been exposed to this virus [3]. Since CRCoV may show dual tropism for both the respiratory and digestive epithelium (like SARS-2) and can replicate in both sites, fecal-oral transmission may be possible [2].

3.1. Vaccines

The value of vaccination against CCoV is controversial. Although both inactivated and modified live vaccines against the group 1 virus are available, their use is not recommended because this virus usually only causes a mild, self- limiting or inapparent gastroenteritis with anorexia, fever and diarrhea. It usually affects puppies less than 6 weeks old and lasts for a few days. As a result, vaccination at the normal time of 8–12 weeks of age is too late to prevent the disease.

The vaccine appears to protect dogs from disease but not from infection. It is generally accepted that protection against CCoV is dependent on the presence of IgA in the intestine. Dogs vaccinated by the parenteral route do not mount an IgA response and so shed virus in their feces [4].

Immunity after natural exposure to enteric canine coronavirus does not provide complete protection against infection with the pantropic strain CB/05 [5]. Despite having high serum antibody levels as a result of prior exposure to enteric CCoV, challenged puppies still developed vomiting and diarrhea in addition to a profound lymphopenia. Viral RNA was detected in the thymus, spleen and lymph nodes of infected dogs. However viral shedding was of shorter duration and the clinical signs were milder than in puppies that lacked these antibodies.

Two types of vaccine have been developed against CCoV; inactivated and live-attenuated.

Vaccination of dogs with an inactivated CCoV vaccine reduced the level of viral shedding in the feces and was effective following experimental challenge in dogs. Thus 2/13 (15%) of vaccinated dogs developed mild diarrhea while 80% of control unvaccinated dogs developed diarrhea and 60% eventually developed severe watery or bloody diarrhea. The control dogs averaged 10.8 days of diarrhea compared to 1.4 days in vaccinated animals. 100% of the control animals shed virus compared to 38% of vaccinates [6]. A betapropiolactone-inactivated MF59-adjuvanted vaccine developed against a CCoV/TGEV recombinant, while safe, did not totally prevent the shedding of virus either [6]. It generated significant levels of virus neutralizing antibodies by 28 days. It prevented clinical disease in vaccinated puppies although the vaccinated dogs still shed low levels of virus in their feces. On the other hand, Pratelli et al demonstrated that a commercially available inactivated vaccine had a poor efficacy in reducing viral shedding in the feces after challenge with the field virus [4,7,8].

A modified live vaccine strain of CCoV (257/98-3c) has been tested in dogs when given by the intramuscular and oronasal routes [4]. Vaccine virus was not shed by the injected dogs but those that received the vaccine by the oronasal route shed it for a median of 6 days. The dogs were challenged with a virulent field strain of CCoV at 28 days post vaccination. None of the vaccinated dogs showed any clinical signs nor did they shed detectable virus.

A modified live vaccine was licensed in the USA in 1983 but was soon withdrawn due to a high rate of adverse reactions [9,10]. A high cell passage vaccine has been reported to generate sterilizing immunity in experimentally challenged dogs after receiving two doses of vaccine at 21-day intervals by the oronasal route. Dogs remained healthy and virus was not detected in their feces after oronasal challenge [11].

As of mid-2020, three monovalent coronavirus vaccines are licensed in the United states. Two are inactivated products given by the subcutaneous or intramuscular routes to dogs over six weeks of age. The duration of immunity has not been established. The other is a modified live product. The first dose is given by the subcutaneous or intramuscular routes to dogs over 6 weeks of age with a second dose 2–3 weeks later. Dogs under 12 weeks should be revaccinated every 2–3 weeks until they reach 12 weeks of age. Maternal antibodies will interfere with vaccination responses prior to that time. There are also a large number of multivalent licensed vaccines available that contain coronavirus combined with diverse other canine pathogens such as parvovirus, adenovirus, canine distemper and parainfluenza. These are all inactivated products given by injection.

4. Feline coronavirus vaccines

Feline coronaviruses (FCoV) are highly contagious and endemic in multi-cat populations such as animal shelters and catteries in the United States [12]. They are classified into two serotypes based on the sequences of their spike proteins and their behavior [13]. Thus, the predominant serotype (serotype I) occurs in America and Europe. Serotype II in contrast is mainly found in Asia. It appears that serotype II has resulted from a homologous recombination between serotype I FCoV and CCoV [14]. As a result, about 10 kb of the FCoV genome has been replaced by an equivalent length of the canine genome including the S protein. Consequently, the two serotypes use different receptors for cell entry. Because of this recombination, serotype II viruses are cytopathic and grow to a high titer in feline cell culture. In contrast, serotype I viruses grow poorly. Both FCoV serotypes can be further separated into two pathotypes. One pathotype is called feline enteric coronavirus (FECV). The other pathotype is feline infectious peritonitis virus (FIPV). The vast majority of cat infections are caused by FECV and are either inapparent or cause a mild transient diarrhea. They primarily infect enterocytes and are transmitted by the oral-fecal route. They are controlled by both systemic IgG responses and mucosal IgA responses [15]. It is interesting to note that in addition to having their own coronaviruses, cats can be readily infected by several other coronaviruses including not only SARS-CoV-2 but also transmissible gastroenteritis of swine, canine coronavirus and even the human common cold coronavirus, 229E.

4.1. The disease

About 5–10% of FCoV-infected cats develop a highly lethal disease, feline infectious peritonitis. This is a fibrinous and granulomatous serositis affecting the body cavities. The disease clearly results in a switch in viral cell tropism from enterocytes to macrophages. This cell tropism is mediated by the spike protein. Thus, S protein sequence changes likely alter the target cells invaded by the virus [16,17]. Of the two distinct pathotypes of feline coronavirus, FECV prefers to replicate within intestinal epithelial cells, whereas FIPV prefers to replicate within macrophages. Macrophages also spread the virus throughout the body. The result of this infection depends on the nature of the immune response to the virus. Immunity to FCoV is predominantly cell mediated, and a type 1 response is protective. A cat that mounts a good type 1 response will become immune, regardless of the amount of antibodies it makes. Some cats, however, mount an antibody response to the viral spike proteins. In these animals, the antibodies and virus form immune-complexes that are more efficiently endocytosed by monocytes and macrophages. Virus-laden macrophages accumulate around the blood vessels of the omentum and serosa. Antibodies also generate immune-complexes that are deposited in the serosa, causing pleuritis or peritonitis, and in glomeruli, leading to glomerulonephritis. Cats with preexisting high levels of antibodies against FCoV develop effusive FIP rapidly on challenge. Administering antiserum to FCoV before challenge may also enhance the peritonitis. FIP tends to affect relatively young cats between 6 months and 3 years of age [17].

4.2. Vaccine

FIP is therefore an example of a virus disease similar to dengue or respiratory syncytial virus infection, in which serum antibodies, rather than being protective, increase the severity of the infection. This can be demonstrated experimentally by administering preformed IgG antibodies [18], or simply by vaccinating cats before experimental challenge [13]. Monoclonal antibodies to the spike protein of the virus have been shown to mediate this enhancement [18]. As a result, conventional vaccines have been uniformly unsuccessful in protecting cats. This may be relevant to some cases of COVID-19 where immune enhancement may play a role [19].

Since the results of stimulating a systemic IgG response by vaccination are totally unacceptable, an alternative strategy has been devised. This involves administering a temperature-sensitive vaccine by the intranasal route in an effort to stimulate a local IgA response that will prevent viral invasion and yet be both non-enhancing and protective.

A modified live intranasal vaccine is licensed in the USA for the prevention of FIP (Felocell® FIP, Zoetis). The vaccine contains a temperature-sensitive mutant of the FCoV strain DF2-FIPV that replicates only in the upper respiratory tract and as a result induces a local IgA response in the mucosa. Ideally this IgA is active in the oropharynx at the site where FCoV primarily enters the body. This local mucosal response thus prevents FCoV invasion without inducing high levels of serum antibodies. The vaccine is, however, only effective if administered prior to coronavirus exposure. In highly endemic situations where kittens are infected with FCoV at a young age, vaccination at 16 weeks of age may be too late to prevent infection. As a result of this age constraint, the "American Association of Feline Practitioners" does not recommend this vaccine [20]. Attempts to immunize cats against feline infectious peritonitis using an unattenuated field isolate of canine coronavirus have been unsuccessful [21].

5. Bovine coronavirus vaccines

Bovine coronaviruses belong to the species betacoronavirus-1. The genome of betacoronavirus-1, in addition to encoding the S protein also encodes a short spike-like protein, the hemagglutinin esterase (HE). This acts as the major cell-binding ligand. Unlike other coronaviruses, BCoV uses N-acetyl-9-O-acetylneuraminic acid as its receptor for cell binding [22]. Phylogenetic analysis suggests that the endemic human coronavirus, HCoV-OC43 is derived

from BCoV and furthermore the transfer occurred around 1890 at a time when there was a pandemic of human respiratory disease [23].

5.1. Vaccines

In the United States, several successful bovine coronavirus vaccines, are currently licensed. Because the disease occurs in calves within a few days of birth it is essential that these vaccines work very rapidly indeed. For this reason, the vaccines are usually given to cows prior to calving so that their calves will be passively immunized by antibodies from the mother's colostrum. Alternatively, calves may receive an attenuated live intranasal vaccine at 1 day of age or slightly older. The intranasal vaccine induces an immediate innate response with interferon production that results in rapid onset of protection. Epidemiologic studies indicate that serum neutralizing and hemagglutinating antibody levels correlate with protection [24].

The intranasal vaccine is administered by syringe in a single dose between 3 and 4 days of age. These intranasal vaccines may also be administered to older calves when they enter a feedlot. Inactivated vaccines are also available for use in pregnant cows. There are also many multivalent inactivated vaccines available. These contain a mixture of other enteric pathogens such as rotaviruses, *E. coli* and Clostridia. Challenged calves show significant reductions in the severity and duration of coronavirus-mediated diarrhea.

BCoV expresses a viral hemagglutinin. As a result, Takamura and his colleagues investigated the use of a vaccine consisting of a solubilized cell extract of infected cells (BCV 66/H strain) mixed with an oil-based adjuvant. It was injected in two doses at 3-week intervals. The vaccine induced high hemagglutinating antibody titers in vaccinated cattle [25]. No adverse effects were noted. An aluminum hydroxide gel adjuvanted, formalin-inactivated BCoV is also licensed in Japan [26].

Welter adapted bovine coronavirus to growth in a diploid swine testicular cell line [25]. The virus replicated actively. After multiple passages in this line, the virus was sufficiently attenuated that it no longer caused disease in calves. It remained safe and effective even after five back-passages in calves. It provided protection against both winter dysentery and neonatal calf diarrhea [27].

6. Porcine coronavirus vaccines

Pig coronaviruses, as in other species, may cause respiratory or gastrointestinal diseases. Currently six coronaviruses are known to cause disease in pigs. Four of them are alphacoronaviruses, including transmissible gastroenteritis virus, (TGEV), porcine respiratory coronavirus (PRCoV), porcine epidemic diarrhea virus (PEDV) and swine acute diarrhea syndrome – coronavirus (SADS-CoV). One is a betacoronavirus, porcine hemagglutinating encephalomyelitis virus (PHEV). The sixth is porcine deltacoronavirus (PDCoV). TGEV, PRCV and PHEV have been recognized for many years. PEDV, PDCoV and SADS-CoV are emerging diseases. All three of these new viruses appear to have originated in China [28].

6.1. Porcine epidemic diarrhea

Porcine epidemic diarrhea virus (PEDV) is an alphacoronavirus. As with other coronaviruses, variations in its S gene and thus the epitopes on the spike protein have significant effects on its virulence and antigenicity. PEDV, as its name indicates, causes acute watery diarrhea, vomiting, anorexia, dehydration and death in piglets under two weeks of age.

6.2. Vaccines

When vaccinating neonatal piglets against a disease such as PED, there is insufficient time between birth and disease onset to permit an active immune response to occur in response to neonatal vaccination. As a result, it is necessary to rely on passive immunity. Infection of adult sows with an enteric virus triggers a local intestinal IgA response. During pregnancy, the IgA-producing B cells emigrate from the gut to other body surfaces including the mammary gland under the influence of the pregnancy hormones. As a result, the sow's colostrum and milk are also rich in specific IgA [29]. The presence of preexisting intestinal IgA may however block vaccine antigen absorption and prevent oral boosting by inactivated products. As a result, these booster vaccines are usually given parenterally.

While many different PEDV vaccines have been developed, most are considered to provide incomplete protection to naïve animals [30]. Because of the early onset of disease, most are designed for use in pregnant sows 2 to 4 weeks prior to farrowing although they are just as effective if given earlier in pregnancy [31]. The immunity conferred on the sows is transferred to their piglets via colostral immunoglobulins on suckling [32].

Multiple inactivated vaccines are available [33,34]. They are often combined with TGE and rotavirus vaccines in a single dose. Two inactivated PED vaccines are available in the United States. One is an adjuvanted inactivated whole virus vaccine containing both the S- and M-proteins, for pre-farrowing vaccination of pregnant gilts and sows. The other inactivated vaccine contains the S-protein only and is not adjuvanted. It is also given to sows prior to farrowing. Both will boost preexisting immunity but may not immunize naïve sows. For example, neutralizing antibody titers in sows milk and colostrum increased fivefold in vaccinated sows with preexisting immunity. This antibody response was not however seen in naïve sows [35].

Modified live attenuated PEDV vaccines that have been derived by multiple passages in Vero cell cultures have been widely used in Asia. They are usually given orally. For example, a trivalent, PEDV, TGEV and porcine rotavirus vaccine is used in China. They may reduce mortality, but the most highly attenuated vaccines do not appear to prevent virus shedding after challenge. In an effort to improve vaccine efficacy multiple different vaccination procedures may be used. Thus, both live and killed vaccines can be administered in series such as live-killed-killed or live-live-killed-killed [30]. Oral attenuated vaccines are available in South Korea and the Philippines for use in sows. This makes good sense since it is important to induce high antibody levels in colostrum and milk. The modified live-PED vaccines reduce mortality in piglets born to orally vaccinated sows but do not prevent infection or viral shedding.

Other vaccines that have been developed in efforts to control PED include viral vectored vaccines using swinepox virus or adenoviruses engineered to express the spike protein [36,37]. They also include subunit vaccines expressed in Baculovirus, yeast, or plant cells [38–40], however these are relatively expensive.

Plasmid-vectored DNA vaccines containing the genes for S, N or M proteins have also been developed [41,42]. For example, expression of the TGEV N gene in an attenuated *Salmonella typhimurium* vector is the basis of an oral vaccine in piglets. Piglets under 20 days of age received 1×10^{12} CFU and their immune responses evaluated. The vaccine induced IgG and IgA in addition to interleukin −4 and interferon-γ [43]. Wen et al [44] have used PEDV-loaded microspheres 700–900 μm in diameter and fed them to weaned piglets. The microspheres protected the antigens from destruction and induced high levels of both IgA and IgG in sera and saliva. These antibodies were neutralizing for PEDV in vitro. An alphavirus replicon RNA vaccine against PED has been provisionally licensed

by USDA. It is derived from a Venezuelan equine encephalitis replicon expressing the PEDV spike gene [45].

Multiple variant strains of PEDV continue to emerge among pig populations. The resulting antigenic variation has an adverse effect on the efficacy of currently available vaccines. As a result, this creates a constant demand for the development newer, more effective vaccines.

6.3. Transmissible gastroenteritis

Transmissible gastroenteritis (TGE) is an enteric disease of pigs caused by an alphacoronavirus related to PEDV. A respiratory variant of this virus, porcine respiratory coronavirus (PRCV), is associated with the pig respiratory disease complex. The PRCV variant is probably a deletion mutant of TGEV. It is not an important primary pathogen, but it contributes to the development of the porcine respiratory disease complex. Wild and domestic carnivores including mink, dogs and cats readily seroconvert to TGEV suggesting that they too are susceptible to this virus. As a result, virus excreted from TGEV-infected dogs remains infectious for pigs. TGEV is now considered to constitute a single virus species with feline and canine coronaviruses. Pigs inoculated with the PRCV variant do not develop disease (except perhaps a transient mild diarrhea) but respond by producing antibodies to TEGV [46].

6.4. Vaccines

In the United States, both modified live and inactivated TGEV vaccines are licensed by USDA [47]. Unlike PEDV, the prevalence of TGE is declining, as is the market for TGE vaccines. The appearance of the PRCV variant appears to have reduced the prevalence of the parent TGEV strain and perhaps implies some degree of cross-protection. The modified live TGEV vaccines are administered orally to pregnant sows in order to induce passive immunity in their offspring. They may also be given orally to nursing or weaned piglets to induce active immunity. The inactivated vaccines are given to nursing or weaned piglets by intramuscular injection. They do not induce a strong protective response against acute disease but are useful in controlling low-level enzootic infections.

Unfortunately, the modified live TGEV vaccines do not stimulate a strong secretory IgA response since they do not replicate sufficiently within enterocytes. As a result, they are not always protective for the sow as well. Likewise killed vaccines administered parenterally do not stimulate a strong IgA response either.

The purified spike proteins of TGEV may act as effective vaccine antigens provided, they can be delivered in such a way as to induce a mucosal IgA response [36]. An oral recombinant corn-based vaccine has been shown to work well in pigs [40,48].

7. Avian infectious bronchitis vaccines

Avian infectious bronchitis is arguably the most important disease threat for commercial poultry producers worldwide. The combination of high morbidity, and loss of growth performance accompanied by secondary bacterial infections can lead to unsustainable losses in poultry production. It can only be controlled by mass vaccination, but the continuing emergence of new viral variants ensures that vaccine development is an ongoing process [49].

7.1. The virus

In 1937, infectious bronchitis virus (IBV) was the first coronavirus to be discovered. [50]. IBV is a gammacoronavirus with a 27 kb genome that affects chickens. Related coronaviruses occur in turkeys and pheasants [51]. It resembles the alphacoronaviruses in that it possesses the same number of major structural proteins, a large spike (S) glycoprotein, a smaller membrane glycoprotein (M), some envelope (E) glycoproteins and a nucleocapsid (N) protein. The spike protein contains two subunits, S1 and S2 that form the large head and the transmembrane components of the molecule respectively. The S1 subunit contains the receptor-binding domain and hence is responsible for cell binding [52]. The amino-terminal subunit of the S1 subunit is the immunodominant and protective antigen [53]. S1 forms the major epitope against which neutralizing antibodies are directed but the S2 subunit also plays an important role. Thus, administration of a recombinant Newcastle Disease vectored vaccine expressing the S2 gene of IBV resulted in a significant reduction in viral load compared to unvaccinated chickens [54]. This phenomenon may be exploited in order to produce cross-protection between virus strains that differ in their S1 subunits.

Like PEDV, IBV is characterized by the continuous emergence of new, and different viral serotypes. This diversity results from recombination events leading to antigenic shifts [55] as well as antigenic drift as a result of the use of a low-fidelity RNA-dependent RNA polymerase [56]. Because of this antigenic drift, minor changes (<5%) in the amino acid sequence of the S1 protein in circulating viruses may have significant effects on vaccine effectiveness and cross-protection. In general, different serotyped of IBV do not cross-protect. The continuous emergence of new genotypes and the lack of cross-protection between most of them means that IBV vaccines must also continue to change in order to minimize their impact and chicken flocks may have to be repeatedly vaccinated. A single application of a vaccine containing a single serotype is usually insufficient to provide reliable and consistent protection.

7.2. The disease

As its name indicates, avian infectious bronchitis virus causes a severe upper respiratory tract infection in chickens. It colonizes the nose and trachea and eventually moves to the lungs and air sacs where it can cause pneumonia. However, IBV can also replicate in the kidneys where it causes nephritis, in the oviduct, and along most of the alimentary tract. The intestinal infection generally has no clinically obvious effect. The virus persists in the oviduct and as a result, is re-excreted at the onset of egg laying [57].

7.3. Vaccines

Almost all commercial chicken flocks are vaccinated against IBV. As a result, both inactivated and live attenuated IBV vaccines are widely available. The original attenuated IBV vaccines used virus strains attenuated by passage in embryonated eggs. Subsequently the inactivated products were introduced to be used as boosters in older, egg-laying chickens.

Inactivated vaccines may be used alone or in combination with modified live vaccines in layer/breeder flocks to induce maternal immunity and thus protect newly hatched chicks. As in other diseases, the inactivated vaccines induce a relatively weak antibody-mediated immune response and thus require multiple doses and the use of adjuvants. These in turn increase handling costs and may cause significant injection site lesions in vaccinated birds. As with other vaccines, live attenuated vaccines tend to generate better protection than inactivated vaccines. On the other hand, live vaccines also carry with them the risk of reversion to virulence [56–59].

Live attenuated vaccines are usually given to chicks at one day of age. The initial response is protective against homologous strains. However, immunity begins to wane by about 9 weeks, especially if highly attenuated live vaccines have been used. Currently most of these live-attenuated vaccines contain the

Massachusetts strain of the virus either alone or in combination with others. Modified live IBV vaccines containing the common strains are usually administered in the drinking water or by coarse spray and given at day 1 or within the first week. (Coarse spray vaccines are delivered either in an enclosed cabinet or by manual spraying of the flock. While some of the droplets are inhaled, most gets on the plumage. The birds then ingest the vaccine when grooming their feathers). Some short-lived broilers (chickens used for meat) receive only this single dose. For longer-lived birds, a second dose may be given 2–3 weeks later. Long-lived birds used for breeding and egg production receive multiple vaccine doses at 2, 4 and 6 weeks. Revaccination after that depends upon the local threat assessment. In practice it is usual to vaccinate long-lived egg producing birds with two or three doses of live attenuated vaccines and then maintain their immunity with repeated doses of the inactivated vaccines [58].

The great diversity among the attenuated strains employed as IBV vaccines depends in large part on their geographic location. For example, in North America the major vaccine strains are M41 (Massachusetts), Arkansas and Connecticut. In Europe strains 4/91 and D274 predominate. These may be ineffective in other countries or locations. The QX strain is the predominant circulating strain in China [59]. This strain has also caused outbreaks in Africa, the Middle East, Europe and Asia. These modified live vaccines induce a potent protective response but reversion to virulence, recombination, or mutation, are ever-present risks (see Table 1).

Efforts are ongoing to reliably and consistently attenuate these strains. For example, Cavanagh et al, [60] have demonstrated that the four small IBV proteins are not required for viral replication. Viruses with these genes deleted are less aggressive than wild type viruses. Likewise exchanging the S protein genes between strains also reduces pathogenicity.

Experimental recombinant vaccines against IBV using fowlpox, adenovirus and multiple other viruses as vectors have been investigated. An experimental fowlpox vectored vaccine expressing not only the S1 protein of IBV but also the chicken IFN-γ gene gave greatly improved results. [61]. Likewise, a fowlpox vectored S1 vaccine expressing the chicken IL-8 gene was also very effective [62]. Adenovirus recombinants were highly protective against both homologous and heterologous challenge [63,64]. Li et al have generated an IBV recombinant vaccine in duck enteritis virus that appears to be protective [65]. Falchieri and colleagues have developed a stable recombinant vaccine in a subtype A avian metapneumovirus to incorporate IBV QX genes [66]. Eyedrop inoculation of this recombinant vaccine in one-day old chicks provided protection against virulent QX challenge 3 weeks later. However preexisting immunity to the vector virus, especially passive immunity from vaccinated hens reduces their efficacy. Likewise, inappropriate folding and other posttranslational changes reduce their effectiveness These recombinant vaccines may be enhanced to protect against multiple serotypes [67].

Subunit based vaccines have been developed using selected sequences from epitopes within the S1 and N genes. Multiepitope IBV vaccines have been shown to induce both humoral and cell mediated responses [68]. Thus, the genes for the three major IBV peptides, one S and two N genes were incorporated into a plasmid fused to the 3′ terminal of the glutathione S transferase gene and expressed in *E. coli*. The purified fusion proteins were recognized by anti IBV serum. They induced 80% protection on challenge.

Several different plasmid DNA vaccines for IBV have also been evaluated. They include one using the S1 gene of the Arkansas strain. It is administered *in ovo* around 18–19 days incubation. This vaccine alone provided about 80% protection, but this could be significantly enhanced by boosting with a live attenuated vaccine at two weeks of age [69]. An intramuscular liposome-encapsulated DNA vaccine containing the genes for the S1, S2 and N regions also generated a protective response of 80% [70]. Good results have also been obtained by using a DNA vaccine encoding N or S1 genes together with either GM-CSF or IL-2 genes. The vaccine containing the genes encoding S1 performed better than those encoding the N protein [71,72]. Similar positive results were obtained by Tarpey et al [73] who expressed the IBV and IL-2 genes in turkey herpesvirus and administered the vaccine *in ovo*.

As of mid 2020 there were 57 vaccines containing IBV licensed by USDA for use in chickens. Of these 19 contained IBV alone. The remainder were multivalent vaccines.

The correlates of protection against IBV are not clearly understood. Serum antibody levels do not correlate well with protection. For example, birds that received vaccine by eye drops generated a strong IgA primary response whereas the memory response was dominated by IgG antibodies [74]. Adoptive transfer of $CD8^+$ T cells from immune to susceptible chicks has been shown to transfer

Table 1
Summary of the Coronavirus vaccines currently licensed in North America.

Virus species	Vaccine type	Route of administration	Indications	Other vaccines added
Canine coronavirus	Inactivated	Subcutaneous intramuscular	Dogs over 6 weeks of age	Adenovirus Distemper Parvovirus Leptospirosis Lyme disease
Feline coronavirus	Modified live	Intranasal	Cats over 16 weeks of age	None
Bovine coronavirus	Modified live	Oral, Intranasal	Neonatal calves	Rotavirus
	Inactivated	Subcutaneous Intramuscular	Healthy Pregnant cattle	Clostridia E. coli
Porcine Epidemic diarrhea virus	Inactivated	Intramuscular	Healthy pregnant sows	None
Transmissible gastroenteritis virus	Inactivated	Intramuscular	Healthy pregnant sows	None
	Modified live	Oral Intramuscular	Healthy pregnant sows	
Infectious bronchitis virus	Inactivated	Subcutaneous Intramuscula	Chickens over 12 weeks	Newcastle disease
	Modified live Arkansas Massachusetts Connecticut Georgia Delaware	Aerosol Coarse spray Intranasal Intraocular Drinking water	Chickens 1 day of age	Infectious bursal disease Reovirus

protection [75]. While birds may be protected against clinical disease, there are often a significant fraction that are not [60].

The ability to control or prevent infectious bronchitis outbreaks is however, rendered very difficult by the continuous emergence of new IBV genotypes, serotypes and variants as a result of mutation and recombination. Small differences in the amino acid sequence of the S protein epitope can change the viral serotype and the effectiveness of a vaccine. Over 50 serotypes and hundreds of variants have been identified and more continue to emerge. These variants arise as a result of sequence changes in a hypervariable region of the viral spike (S) glycoprotein. There are a very large number of different serotypes recognized and cross-protection between them is often poor. Thus, as variants appear and disappear, they necessitate the continual development of new vaccines.

8. Discussion

None of these existing domestic animal vaccines are likely to be in any way protective against SARS-1, MERS or SARS-2. Nor do most of the domestic animal diseases closely resemble the acute, lethal pneumonic diseases of animals. The human vaccines will inevitably have to be developed independently. It is clear from the veterinary experience however that vaccines do work against coronaviruses. Both inactivated and live attenuated vaccines are effective in domestic species and will produce protective immunity Table 1. This immunity may be mediated by IgA against superficial enteric or respiratory disease or by IgG against viremic disease. It is also clear that for many of these coronaviruses a T cell-mediated response is required for significant protection. On the other hand, evidence from COVID-19 cases suggests that immunological mechanisms may contribute to the disease pathogenesis – the so-called cytokine storm, in some patients and it will be important to ensure that vaccines do not contribute to this.

As pointed out above, many coronaviruses cause severe disease in neonatal animals. As a consequence, provided that they can protect during the vulnerable neonatal period, these vaccines are not required to confer long-lasting protection. Thus, the duration of immunity against these viruses may be relatively short.

The situation in livestock species is somewhat different from that in companion animals. Livestock producers need to minimize disease losses while at the same time avoiding the expenses incurred by unnecessary vaccination. These expenses may be considerable in large, intensive livestock operations. In the poultry industry it is now normal to measure the level of flock immunity with antibody tests and then only revaccinate when it becomes apparent that immunity has waned significantly. Similar procedures are being adopted in the swine industry. It is possible that monitoring for protection of this type may be required to keep COVID-19 under control in human populations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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