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Review

Insights into recent advancements in human and animal rotavirus vaccines: Exploring new frontiers

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ABSTRACT

Rotavirus infections cause severe gastroenteritis and dehydration in young children and animals worldwide, leading to high rates of morbidity and mortality, predominantly in low- and middle-income countries. In the past decade, substantial progress has been made in the development and implementation of rotavirus vaccines, which have been essential in alleviating the global burden of this disease, not only in human being but also in livestock species like calves and piglets, where these infections can cause significant economic losses. By synthesizing the latest research and real-world evidence, this review article is designated to provide deep insights into the current state of rotavirus vaccine technology and its global implementation as well as the application of rotavirus vaccines in veterinary settings and their importance in controlling zoonotic transmission and maintaining food security.

INTRODUCTION

Rotaviruses (RV) are viral agents that lead to severe diarrheal disease and can be fatal in human and animals newborns (Ghonaim et al., 2023). Globally, an estimated 30 to 50 percent of pediatric hospitalizations for diarrhea are caused by rotavirus infections (Crawford et al., 2017). Rotavirus infections are also linked to more than 200,000 child fatalities every year in children under five, with the highest death rates occurring in Southern Asia and sub-Saharan African countries (Cohen et al., 2022; Crawford et al., 2017; Tate et al., 2016). Regarding animals, calf diarrhea is primarily caused by rotavirus worldwide with prevalence rate ranges from 7% to 94% worldwide (Ghonaim et al., 2023). The prevalence of porcine group A rotavirus (RVA) infections is highly variable, with a range of 3.3%–67.3%, and is often elevated at the farm level, in spite of the availability of vaccines (Vlasova et al., 2017). According to the infection chain of rotavirus infection, feco-oral route is considered the main transmission route, and risk factors include unsanitary surfaces, contaminated food or water, and inadequate hygiene (Ghonaim et al., 2023). In children, typical clinical signs of rotavirus infection include diarrhea, fever, nausea, and abdominal discomfort (Du et al., 2022). In calves, however, the disease usually does not present with fever unless there are secondary infections which lead to severe diarrhea followed by

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dehydration, and loss of appetite. Also, porcine rotavirus A is a major cause of enteritis in piglets during the weaning and post-weaning periods (Zhao et al., 2024). As of now, there is no available specific therapy for rotavirus infection, which is usually self-limiting, and typically resolves within 3–8 days (Bányai et al., 2018).

Vaccination is the most effective way to prevent rotavirus infection. Currently, six oral, live, attenuated rotavirus vaccines are available internationally for human use and four of them have passed the World Health Organization (WHO) prequalification. For veterinary applications, two bovine vaccines, two porcine rotaviruses vaccines, and three inactivated equine vaccines are available. The RV vaccines currently available for animal use rely on the immunization of pregnant animals (Carossino et al., 2024). Most of the vaccines commercially available are inactivated, while a small portion are live-attenuated ones. These vaccines are typically administered to pregnant animals in order to elicit strong maternal (passive) immunity, which will provide early protection to newborn animals against neonatal diarrhea (Kumar et al., 2022). Despite the introduction of rotavirus vaccines for infants in over 100 countries, the mortality rate associated with rotavirus remains high in low-income nations (Bonura et al., 2022; Omatola and Olaniran, 2022). To provide sufficient cross-protection, rotavirus monovalent and multivalent vaccines, which incorporate dominant and most circulating genotypes, have been introduced for domestic animals and also for humans. Considering the fact that genotypes of rotavirus continuously change in both humans and animals over time, ongoing surveillance of rotavirus becomes necessary in both populations. However, the current rotavirus vaccines have several disadvantages. For instance, they demonstrate poor efficacy in low-middle income countries, and there is a potential for the vaccine strain to revert to a virulent form. Moreover, the possibility of recombination between the vaccine strain and wild-type strains exists. This recombination raises concerns about the emergence of new, more pathogenic rotavirus strains (Granovskiy et al., 2024). The shortcomings of the existing vaccines highlight the need for newer approaches to rotavirus vaccine development (Granovskiy et al., 2024). Hence, this review aimed to address the advancements in vaccine implementation and to summarize the latest developments in the pipeline for rotavirus vaccine candidates.

ROTAVIRUS GENOME, CLASSIFICATION AND HOST RANGE

Rotaviruses are a group of viruses with segmented double-stranded RNA genome (16–21 kbp), belonging to the *Sedoreoviridae* (formerly *Reoviridae*) family (Ghonaim et al., 2024). The rotavirus particle is a non-enveloped, composed of three concentric protein layers, commonly referred to as a triple-layered particle (TLP), as show in Fig. 1. The wheel arrangement of the inner and outer capsid layer gives the virion a wheel-like appearance under electron microscopy, leading to the name "Rotavirus," derived from the Latin word "*rota*", meaning wheel, which is now the accepted genus name. The rotavirus genome has eleven segments, encoding six structural proteins and six nonstructural proteins. Structural proteins are designated as VP1, VP2, VP3 VP4, VP6 and VP7, with the nonstructural proteins are labeled as NSP1 to NSP6 (Chen et al., 2022). Rotaviruses are categorized into ten main groups (RVA–RVJ) based on VP6 serotypes, according to the International Committee on Taxonomy of Viruses website (http://ictv.globa/) (Ghonaim et al., 2023; Wu et al., 2024) as shown in Table 1. Among these groups, RVA is the most common and clinically significant species worldwide (Bányai et al., 2018; Suzuki, 2019).

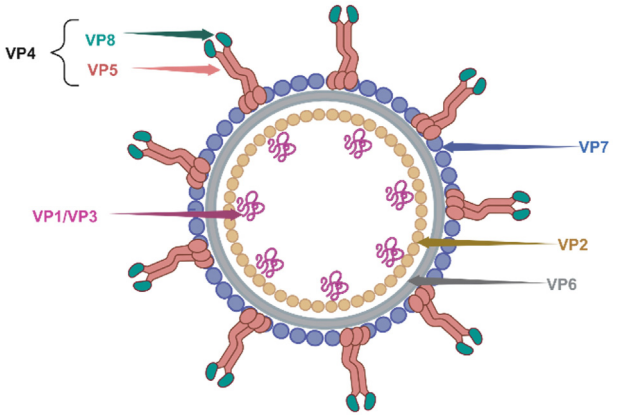

Fig. 1. Schematic diagram of the rotavirus particle. The infectious triple-layered particle (TLP) comprises an outer capsid composed of VP7 and VP4 spikes, a middle layer composed of VP6, and an inner layer composed of VP2 to which transcriptional enzymes VP1 and VP3 are anchored. Figure created with BioRender.com.

The outer capsid proteins VP4 and VP7 are crucial for rotavirus attachment and penetration, as well as for the induction of neutralizing antibodies (Ciarlet and Schödel, 2009; Sun Y. et al., 2024). RVAs are further classified into different G and P genotypes based on the *VP7* and *VP4* genes, respectively (Ghonaim et al., 2024). A more comprehensive classification system for RVAs has been established, using 11 gene segments, represented as Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, which details the characteristics of the *VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5* or *NSP6* coding gene, respectively (Matthijnssens et al., 2011a; Moutelíková et al., 2020). This allows for detailed analysis of the genetic relationships among RVAs from various host species. Human RVAs can be grouped into three main genotype constellations: Wa-like, DS-1-like and AU-1-related (Heiman et al., 2008; Matthijnssens and Van Ranst, 2012). Those human RVAs with a Wa-like backbone share a common ancestry with porcine RVAs (PoRVA), while those with a DS-1-like backbone are linked to bovine RVAs (BoRVA) (Ghosh and Kobayashi, 2011).

The most prevalent G and P genotypes for human RVA strains worldwide are G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] (Oliveira Matos et al., 2024). However, the specific G and P genotypes can vary among animal species (Bányai et al., 2012; Martella et al., 2010; Papp et al., 2013a). For PoRVA, the most common genotypes are G3, G4, G5, G9, and G11, often associated with P[6] and P[7]. G9P[23] is considered to be the most prevalent genotype (Ghosh and Kobayashi, 2014; Papp et al., 2013a; Yu et al., 2021). In BoRVA, the predominant genotypes are G6, G8, and G10, typically combined with P[1], P[5], and P[11] (Elkady et al., 2021; Papp et al., 2013a). The most prevalent RVA genotypes in felines are G3P[3] and G3P[9], whereas in canines, G3P[3] is the most common genotype (Ghosh and Kobayashi, 2014; Matthijnssens et al., 2011b).

The rotavirus genetic diversity is influenced by interspecies transmission and genetic reassortment, which are important mechanisms in its evolutionary process (Bazzi et al., 2022; Doro et al., 2015). Humans can contract rotavirus strains from animals through direct transmission (Doro et al., 2015), or the reassortment of genome segments between human and animal rotaviruses (Banyai and Pitzer, 2016), can lead to antigenic shifts and/or increased virulence (Cui et al., 2022; Rojas et al., 2019).

Table 1
Rotavirus groups and susceptible species.

RV Group	Species
RVA	Humans, pigs, cows, goats, ostriches, chicken, dogs, and horses
RVB	Humans and some animal species (cattle, sheep, pigs, dogs, and rats)
RVC	Human, pigs, cattle, ferrets, and dogs
RVD, RVF and RVG	Birds
RVH	Humans and pigs
RVE	Pigs
RVI and RVJ	Dogs and bats

CURRENT ROTAVIRUS VACCINES

Vaccines for human use

Several key observations have led to the recognition that rotavirus infections are likely preventable by vaccination (Desselberger and Huppertz, 2011). These infections primarily occur within the first year of life, and reinfections after the age of 2–3 are typically not linked to gastroenteritis. Immunity gained from natural rotavirus infections is linked to the presence of rotavirus-specific antibodies, particularly IgA, in the serum and intestinal lumen (rotavirus-specific IgA) (Coulson et al., 1990, 1992; Coulson and Masendycz, 1990). The first licensed live-attenuated quadrivalent vaccine, RotaShield® (Wyeth Lederle Vaccines, Pearl River, NY, USA), received approval in 1998 after successful clinical trials (Irene et al., 2024; Joensuu et al., 1997; Kapikian et al., 1996; Pérez-Schael et al., 1997). This vaccine contained an attenuated simian rotavirus strain (G3P[7]) and human-animal reassortant strains with the human G1, G2, and G4 rotavirus *VP7* genes. Following licensure, the RotaShield® was quickly implemented as a universal childhood vaccination, administering over one million doses in the US. However, it was voluntarily discontinued by the manufacturer due to an observed heightened risk of intestinal intussusception in some vaccinated children (Wang et al., 2021).

Currently, six oral vaccines have been approved for use in humans (Fig. 2): Rotarix™ containing a single strain of the human rotavirus (G1P[8]) (GlaxoSmithKline, Belgium); RotaTeq™ containing five rotavirus strains (G1, G2, G3, G4, and P[8]) derived from both human and animal rotaviruses (Merck and Co, USA); Rotavac™ Containing the indigenous human rotavirus strain (G9P[11]) (Bharat Biotech International Ltd., India); Lanzhou lamb rotavirus vaccine (LLR) derived from a rotavirus strain isolated from lambs, specifically targeting the G10P[15] genotype (Lanzhou Institute of Biological Products, China); Rotavin-M1™ derived from a human rotavirus strain, specifically targeting the G1P[8] genotype (POLYVAC-Vietnam, Vietnam); and RotaSiil™ containing five human-bovine (UK) reassortant rotaviruses (G1, G2, G3, G4, G9) (Serum Institute of India Ltd., India).

Rotarix and RotaTeq were first licensed in 2006 and received WHO prequalification for worldwide use in 2009, while the Indian-produced Rotavac and RotaSiil were prequalified in 2018 (Burke et al., 2019). The above mentioned four vaccines that are prequalified by WHO are oral formulations, with a recommended two-dose series for Rotarix and a three-dose series for RotaTeq, Rotavac, and Rotasiil, to be given within the first six months of life (Burke et al., 2019). By 2021, 110 countries (56%) had implemented rotavirus vaccination, with 106 of those implementing it into their national childhood immunization programs (Cates et al., 2024). Additionally, there are two nationally licensed vaccines: Rotavin-M1 in Vietnam and the Lanzhou lamb rotavirus (LLR) vaccine in China. Both are oral, live-attenuated vaccines with monovalent compositions, specifically Rotavin-M1 (G1P[8]) and LLR (G10P[15]) (Wang et al., 2021).

During the past decade, an excess of seventy countries have initiated vaccination against rotavirus, indicating a significant worldwide effort to increase access to these vaccines and prevent hospitalizations and deaths from acute gastroenteritis (AGE) (Cates et al., 2024). In 2020, the World Health Organization's Strategic Advisory Group of Experts (SAGE) on Immunization recommended the global use of the four current WHO-prequalified Rotavirus vaccines (Cates et al., 2024). SAGE also advised for ongoing evaluation of Rotavac and Rotasiil effectiveness and safety. As of 2021, Rotarix was used in seventy-seven countries, RotaTeq was used in sixteen countries, and nine countries have introduced both Rotarix and Rotateq. Also, Rotavac was introduced in two countries; Rotasiil was introduced in three countries, while one country has introduced both Rotavac and Rotasiil. It should be noted that although the effectiveness of rotavirus vaccines is high, typically between 70% and 90% in developed nations (Desselberger, 2014), their efficacy is believed to be 20%–30% lower in low- and middle-income countries (Bonura

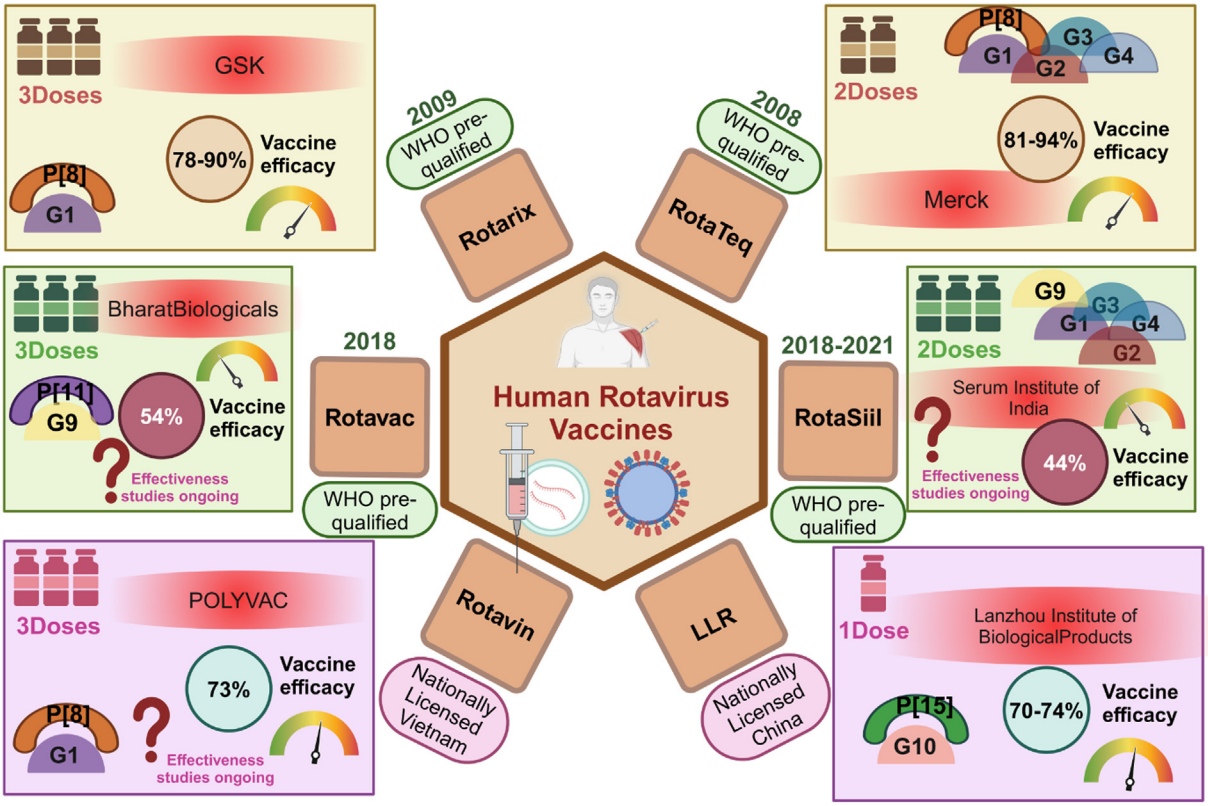

Fig. 2. The characteristics of currently available human Rotavirus vaccines. Figure created with BioRender.com.

Table 2
Characteristics of currently available human rotavirus vaccines.

Trade name	Target antigen & original strains (genotype)	Vaccine type	Year of WHO pre-qualification	Form	Vaccine efficacy[a]	Post-licensure vaccine effectiveness[a] (VE)
Rotarix	One rotavirus strain of G1P[8]	Live attenuated	2009	Liquid	LMC: 90% MMC: 78% HMC: 54%	LMC: 83% MMC: 67% HMC: 58%
RotaTeq	Four reassortant rotaviruses express one of the outer capsid, VP7, proteins (serotypes G1, G2, G3, or G4) from the human rotavirus parent strain and the attachment protein VP4 (type P7) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the attachment protein VP4, (type P1A), from the human rotavirus parent strain and the outer capsid protein VP7 (serotype G6) from the bovine rotavirus parent strain.	Live, oral pentavalent vaccine that contains five rotavirus strains	2008	Liquid	LMC: 94% MMC: 81% HMC: 44	LMC: 85% HMC: 45%
Rotavac	Containing a G9P[11] human strain isolated from an Indian child	Live attenuated, monovalent vaccine	2018	Liquid (frozen) and nonfrozen liquid (Rotavac 5D)	India: 54%	VE studies are ongoing
Rotasiil	Five human-bovine (UK) reassortant rotaviruses (G1, G2, G3, G4, G9)		2018 (lyophilized) 2021 (liquid)	Lyophilised and liquid forms available	India & Niger: 44%	VE studies are ongoing
Rotavin-M1	The vaccine contains a G1P[8] human rotavirus strain.	Single, attenuated human rotavirus strain	N/A	Liquid (frozen) and nonfrozen liquid (Rotavin)	None published; IgA Seroconversion: 73%	VE studies are ongoing
Lanzhou lamb Rotavirus vaccine	It contains a G10P[12] lamb rotavirus strain.	Live, attenuated oral rotavirus vaccine	N/A	Liquid	Any severity: 57% Severe RVGE: 70% Inpatient RVGe: 74%	35%–73%

Notes: LMC = low-mortality countries; MMC = medium-mortality countries; HMC = high mortality countries.
[a] Against severe rotavirus gastroenteritis, per protocol analysis.

et al., 2022; Omatola and Olaniran, 2022) (Table 2). The causes of these variations are not yet fully understood, but they may be related to factors such as malnutrition, zinc deficiency, avitaminoses, gut microbiota, co-infections with RVs and other enteric pathogens, immaturity/functional reduction of the infant's immune system, environmental enteropathy, passive transfer of maternal antibodies, and genetic factors (Armah et al., 2010; Desselberger, 2014; Glass et al., 2014; Lopman et al., 2012; Patton, 2012).

Vaccines for animal use

The primary strategy to reduce the burden on rotavirus infection is vaccination (Geletu et al., 2021; Sun M. et al., 2024). Vaccines are available to prevent rotavirus infections in cattle, horses, and swine, as rotavirus-induced diarrhea can significantly impact these livestock (Barrandeguy et al., 1998; Zhang and Cui, 2023). Most commercial vaccines are inactivated, with some live-attenuated options. They are typically administered to pregnant animals to boost maternal immunity, which is then transferred to newborns, offering early protection against neonatal diarrhea during their most vulnerable period. The passive immunity conferred from vaccinating the pregnant females helps safeguard the young livestock against the devastating economic effects of rotaviral diarrheal outbreaks (Barrandeguy et al., 1998; Saif and Fernandez, 1996). First-milking colostrum is vital, as it provides essential nutrients and passively transferred maternal antibodies that protect newborn calves from infections during their initial weeks and months. Newborns are born with low antibody levels, and they rely on colostrum to acquire these protective antibodies (Geletu et al., 2021). Therefore, efforts to prevent diarrhea through dam vaccination are ineffective unless newborns receive colostrum, ideally within the first two to 4 h after birth. Colostrum is rich in antibodies, immune cells (such as neutrophils, macrophages, and T and B cells), complement proteins, lactoferrin, insulin-like growth factor-1, transforming growth factor, interferon, and essential nutrients (Geletu et al., 2021).

Vaccines against bovine RVA

Bovine RVA is a major cause of neonatal diarrhea in calves. Among the various G and P genotypes, only G6, G8, and G10 combined with P[1], P[5], and P[11] are considered epidemiologically significant (Geletu et al., 2021). The G6 genotype is the most common, representing about 40%–78% of circulating BoRVA strains, followed by G10 at 21% and G8 at 3%, mainly in Africa. For P-types, P[5] is the most prevalent at 37%–50%, followed by P[11] at 15%–35%, and P[1] at 2%. The dominant strain combinations include G6P[5], G6P[11], and G10P[11] (Geletu et al., 2021).

Commercial vaccines for BoRVA consist of both live-attenuated and inactivated types, often combined with other agents for calf diarrhea, as shown in Table 3. Most vaccines have similar antigenic profiles, including the G6 and G10 genotypes. For bovines, two types of vaccines are available. One is a bivalent vaccine based on G10P[11] and G6P[1], while the other is a monovalent vaccine containing G6P[5] (Fritzen et al., 2019). These inactivated vaccines are recommended for injection in pregnant cows during late gestation to boost maternal immunity and provide partial protection to newborn calves. However, instances of vaccine failure have occurred, often due to differences in antigenic properties between the vaccine and field strains, which can reduce efficacy. The only available live-attenuated vaccine is Calf-Guard® from Zoetis Animal Health, which includes attenuated strains of both BoRVA and bovine coronavirus (Carossino et al., 2024). It is administered orally to newborn calves immediately after birth, not to pregnant cows.

Vaccinating pregnant cows with inactivated vaccines has shown at least partial efficacy in the field, resulting in reduced duration and severity of diarrhea in calves and decreased viral shedding in feces.

Table 3
Characteristics of currently available Veterinary rotavirus vaccines.

Trade name	Manufacturer	Composition	Vaccine type
Bovine rotavirus vaccines			
ScourGuard® 4K	Zoetis animal health	Bovine RVA (G6 and G10) Bovine coronavirus Entero-toxigenic strains of *Escherichia coli* (*E. coli*) containing K99	Inactivated vaccine
ScourGuard® 4 KC	Zoetis animal health	Bovine RVA (G6 and G10) Bovine coronavirus Entero-toxigenic strains of *E. coli* containing K99 *Clostridium perfringens* type C	Inactivated vaccine
Fencovis®	Boehringer Ingelheim	Bovine RVA (G6 and G10) bovine coronavirus Entero-toxigenic strains of *E. coli* containing K99	Inactivated vaccine
Bovilis® Rotavec®	Merck	Bovine rotavirus strain UK-Compton serotype G6 P5 Bovine coronavirus strain Mebus *E. coli* strain CN7985 serotype O101:K99:F41	Inactivated vaccine
Bovilis® Guardian®	Merck	Bovine group A serotype G6 and G10 rotaviruses Bovine Coronaviruses types 1 and 3 *E. coli* pilus type K99 *Clostridium perfringens* types C & D	Inactivated vaccine
KOLIBIN RC Neo	Bioveta	Bovine rotavirus, serotype G6P1, strain TM-91 *E. coli*, serotype O9:K35 *E. coli*, serotype O8:K35 (fimbrial adhesin F5) *E. coli,* serotype O101:K30 (fimbrial adhesin F5) Bovine coronavirus, strain C-197	Inactivated vaccine
Rotagal	Pharmagal	Bovine rotavirus, serotype G6P1, strain TM-91 Bovine coronavirus, strain C-197 *E. coli,* serotype O9:K35 (fimbrial adhesin F5)	Inactivated vaccine
Calf-Guard®	Zoetis animal health	Attenuated strains of bovine RVA Attenuated strains of bovine coronavirus	Live-attenuated vaccine
Porcine rotavirus vaccines			
ProSystem® Rota	Merck animal health	Attenuated porcine RVA strains G5P[7] OSU G7P[7] A2 G4P[6] Gottfried	Live-attenuated vaccine
ProSystem® RCE	Merck animal health	Attenuated porcine RVA strains G5P[7] OSU G7P[7] A2 G4P[6] Gottfried Different *E. coli* pilus antigens *Clostridium perfringens* type C	Live-attenuated vaccine
Equine rotavirus vaccines			
Duvaxyn® R	Zoetis animal health	Cell-culture-adapted H2 (G3P[12]) strain of equine RVA inactivated vaccine (RVA/Horse-tc/GBR/H-2/G3P[12]/1976)	Inactivated vaccine
Rotamix Equin	Biochemiq	Bovine RVA NCDV-Lincoln G6P[1] strain and H2 strain of equine RVA (RVA/Cow-tc/USA/NCDV-Lincoln/G6P[1]/1967), the simian RVA SA11 G3P[2] strain (RVA/Simian-tc/ZAF/SA11/G3P[2]/1958), and the *E. coli* antigen	Inactivated vaccine
N/A	Nisseiken, Tokyo, Japan	RVA HO-5 (G3BP[12]) strain (RVA/Horse-tc/JPN/HO-5/G3P[12]/1982)	Inactivated vaccine

N/A = not available.

Future efforts to enhance vaccine efficacy will focus on incorporating more contemporary strains and adopting advanced vaccine design techniques (Maier et al., 2022).

Additionally, oral passive immunotherapy using anti-RVA IgY (IgY DNT) has been evaluated on dairy farms to help reduce bovine rotavirus infections in calves during their first two weeks of life, effectively supporting existing vaccination practices. More recently, Immunotherapy with anti-RVA IgY protected monkeys against severe rotavirus-induced enteritis, as manifested by histopathological results (Bentes et al., 2024).

Vaccines against porcine RVA

Porcine rotavirus A is a major cause of enteritis in piglets during the weaning and post-weaning periods. The predominant G-types in pigs include G5 (71.43%), G4 (8.19%), G3 (3.57%), G9 (2.31%), G11 (1.89%), G10 (1.26%), and G1 (1.05%), while P-types are dominated by P[7] at 77.22% and P[6] at 12.07% (Ghonaim et al., 2024; Jiang et al., 2024). Although G5P[7] was historically the most common genotype, a recent analysis of PoRVA strains in the U.S. from 2004 to 2012 revealed that G9P[13] emerged as the most prevalent genotype, accounting for 60.9%. This was followed by G9P[7] and G4P[13] at 8.7%, and G11P[13] and G11P[7] at 4.3%. Notably, no G5 strains were detected, indicating spatio-temporal variations and the re-emergence of genotypes in PoRVA epidemiology (Vlasova et al., 2017).

Currently, two vaccines are certified by the United States Department of Agriculture (USDA) to prevent RVA in swine: ProSystem® Rota and ProSystem® RCE (Merck Animal Health) (Kumar et al., 2022), as shown in Table 3. ProSystem® RCE is administered to pregnant sows to boost maternal immunity, while ProSystem® Rota is given to piglets, with the initial dose orally and the second dose intramuscularly, typically seven to ten days before weaning (Hoblet et al., 1986; Vlasova et al., 2017). The ProSystem® Rota vaccine is also frequently provided to gilts to help them acclimate before joining the sow herd. However, there is limited field data on the efficacy of these vaccines. Despite their availability, they do not provide complete protection, and PoRVA infections continue to affect swine producers. Additionally, concerns have been raised about the potential for live-attenuated vaccines to increase the genetic diversity of PoRVA, possibly leading to new variant emergence, similar to trends observed with human RVA vaccines. This highlights the urgent need for alternative vaccines that are safer and more effective than current live-attenuated options.

Recently, vaccine manufacturers like Merck Animal Health and Harrisvaccines have been exploring new technologies for PoRVA vaccine development. They have implemented particle RNA technology to create prescription vaccines tailored to specific VP7 sequences from circulating RVA strains (Sequivity®) and developed a vaccine targeting porcine rotavirus group C (RVC) using SirraVaxSM RNA Particle Technology

from Harrisvaccines. These innovations aim to address the challenges of isolating and propagating RVC for traditional vaccine development. However, enhancing existing vaccine platforms to elicit more effective protective responses against porcine RVA remains a critical need in the industry (Carossino et al., 2024).

Vaccines against equine RVA

Equine rotavirus A has a widespread global distribution, with repeated seropositivity observed in adult horses (Conner and Darlington, 1980). In horses, G-types (G3, G5, G6, G8, G10, G13, and G14) and P-types (P[1], P[3], P[7], P[11], P[12], and P[18]) have been detected. The G3P[12] and G14P[12] genotypes are widespread in horse populations globally (Carossino et al., 2018; Garaicoechea et al., 2011; Matthijnssens et al., 2012, 2015; Nemoto et al., 2011). The other genotypes such as G3P[3] (Garaicoechea et al., 2011), G5P[7] (Ciarlet et al., 2001; Hoshino et al., 1983), G8P[1] (Iša et al., 1996), G10P[11] (Imagawa et al., 1993; Iša et al., 1996), and G13P[18] (Browning et al., 1991) are less commonly reported. There are three types of inactivated vaccines for equine RVA worldwide, as shown in Table 3.

There are three types of inactivated equine rotavirus vaccines with varying formulations, but all contain G3 genotype (Bailey et al., 2013). One inactivated vaccine based on the cell-culture-adapted H2 (G3P[12]) strain of equine RVA, is produced by Zoetis Animal Health (Kalamazoo, MI, USA), and is used in several countries. A similar inactivated vaccine, produced by Biochemiq (Buenos Aires, Argentina), has been available in Argentina since 1996 and features the H2 strain of equine RVA, along with the bovine RVA NCDV-Lincoln G6P[1] strain, the simian RVA SA11 G3P[2] strain, and the *Escherichia coli* (*E. coli*) antigen (Bailey et al., 2013; Barrandeguy et al., 1998; Garaicoechea et al., 2011; Papp et al., 2013b; Powell et al., 1997). Additionally, another inactivated vaccine containing the equine RVA strain HO-5 (G3BP[12]) strain was approved in Japan in 2001 (IMAGAWA et al., 2005). Equine rotavirus vaccines are typically given to pregnant mares intramuscularly during the 8th, 9th, and 10th months of gestation. This ensures that the foal receives appropriate colostral immunity, which helps prevent rotaviral diarrhea (Bailey et al., 2013; Barrandeguy et al., 1998; Powell et al., 1997; Sheoran et al., 2000). However, vaccinating foals directly is deemed ineffective because rotavirus infections typically occur very early in life, before colostral immunity diminishes. These vaccines induce neutralizing antibodies in the mother, which are then passed to the foal, reducing the incidence and severity of diarrhea, as well as the levels and duration of viral shedding. Nonetheless, the vaccines do not provide complete protection, and foals can still contract the virus (Bailey et al., 2013; Barrandeguy et al., 1998; Powell et al., 1997). The limited efficacy of the vaccines is likely due to several factors, including the type of inactivated vaccines used, the immunization methods, the adjuvants in the formulations, the challenges of neutralizing antibodies effectively reaching the foal's intestinal lumen, and the narrow strain composition (only G3P[12]) (Carossino et al., 2018; Hardy et al., 1991; Nemoto et al., 2012; Papp et al., 2013b). Current studies have shown that the antibody response generated by the G3P[12] vaccine strains only provides limited neutralization of the G14P[12] equine strains, which are one of the most common genotypes found in horse populations globally (Carossino et al., 2018; Nemoto et al., 2018).

Limitations identified in both laboratory studies and by field practitioners regarding vaccine efficacy further highlights the need to explore modern vector platforms to enhance equine rotavirus A vaccines. Potential emergence of equine rotavirus B should be additionally considered for future incorporation in vaccine formulations to provide comprehensive protection. In light of these limitations and emerging threats, there is a critical need to explore and develop innovative vaccine technologies that can provide more effective and broad-spectrum protection against the various genotypes and strains of equine rotaviruses, including both RVA and potentially RVB (Carossino et al., 2024). Adopting modern vector platforms and expanding the antigenic composition of the vaccines may be essential to improving the overall efficacy and utility of equine rotavirus vaccines going forward.

VACCINES IN DEVELOPMENT

To successfully prevent and manage infections caused by various genotypes of rotaviruses, it is essential to develop an effective and broad-spectrum rotavirus vaccine that can elicit strong cross-protective immunity (Luo et al., 2024a). Both rotavirus infections and vaccinations provide protection against heterotypic rotavirus infections. The major protective antigens of rotavirus are VP4, VP7, and VP6. VP4 and VP7 are capable of inducing neutralizing antibodies, whereas it is known that antibodies produced to target VP6 are of both IgA and IgG isotypes, which help mediate protection through intracellular neutralization during transcytosis (Caddy et al., 2020). As an alternative to existing live-attenuated vaccines, several non-replicating parenteral rotavirus vaccines are currently being developed. Different methods have been employed to create these non-replicating vaccines, including the use of inactivated rotavirus particles, protein subunits, or virus-like particles (VLPs) that mimic the structure of live viruses (Kurokawa et al., 2021), as mentioned in Table 4.

Clinical trials have shown that oral rotavirus vaccines of the live-attenuated type can achieve up to 97% protection against severe gastroenteritis caused by rotaviruses. This can lead to a marked reduction in global disease burden (Wang et al., 2021). However, clinical trials have demonstrated reduced efficacy and effectiveness of these vaccines in low- and middle-income countries (LMICs) that exhibit higher mortality related to diarrheal diseases. Reasons underlying different real-world effectiveness in middle-income countries as compared to high-income countries remain unclear (Bergman et al., 2021; Cates et al., 2022; Lee, 2021; Varghese et al., 2022). Additionally, post-marketing studies have demonstrated the risk of intussusception related to vaccination in both middle-income and high-income countries (Clark et al., 2019; Yen et al., 2016). This has led to a rising interest in the development of non-replicating parenteral vaccines, which may have the potential to overcome the drawbacks of the current rotavirus vaccines (Cárcamo-Calvo et al., 2021; Hausdorff et al., 2022; Song, 2021). Due to these observed limitations, non-replicating vaccines including subunit or inactivated vaccines should be necessarily investigated and developed.

Newer oral vaccines

A leading candidate in the rotavirus vaccine pipeline is the RV3-BB vaccine, developed by PT BioFarma in Indonesia (Lee, 2021). This three-dose oral vaccine is intended for neonates shortly after birth and is based on a naturally attenuated G3P[6] strain that can replicate in the presence of maternal antibodies. This early administration may provide timely protection against rotavirus disease. Ongoing studies are focused on optimizing its production.

In another research effort, scientists have developed a live attenuated trivalent PoRVA vaccine using dominant Korean strains G8P[7], G9P[23], and G5P[7]. This vaccine has proven safe and effective in protecting piglets from diarrhea and enhancing RVA-specific antibody levels (Park et al., 2019).

Additionally, researchers created a dissolvable oral polymeric film containing a live attenuated tetravalent rhesus-human reassortant rotavirus vaccine (RRV-TV) and antacid ($CaCO_3$) (Hensley et al., 2021). They found that two doses of this vaccine were highly immunogenic and provided strong protection against virus shedding and diarrhea in a gnotobiotic pig model upon challenge with a high dose of a virulent human RV (G1).

An oral hexavalent live human-bovine reassortant rotavirus vaccine (RV6) was developed by Wuhan Institute of Biological Products Co., Ltd (WIBP). RV6 is based on the bovine RV strain UK (G6P [5]), reassorted with VP7 gene from human G1, G2, G3, G4, G8, and G9 strains. It has

Table 4
Summary of promising rotavirus vaccine candidates in development.

Vaccine candidate	Technology used	Advantage
VP7 based vaccine candidates	Genetic engineering approaches and messenger RNA (mRNA) vaccines	• Can provoke IgG production and a protective immune response and preserve neutralizing Abs • The mRNA vaccine approach offers a short development cycle, ease of industrial production, and inducingbetter humoral and cellular immunity
VP6 based vaccine candidates	VLPs technology, DNA vaccines, subunit vaccine containing recombinant VP6 protein, and self-assembled structures	• This protein is highly conserved structural protein, containing highly immunogenic epitopes. • Induce protective immune responses with the absence of neutralizing antibodies. • As VP6 tubes/VLPs are more immunogenic than other VP6 preparations and in combination with other structural proteins can induce significant cellular immunity, marked by high levels of IFN-γ production, as well as mucosal IgG and IgA antibodies, and elevated cross-reactive serum IgG against various rotavirus strains
VP4 based vaccine candidates	Recombinant subunit VP8* protein vaccine, nanoparticle-based chimeric VP8* antigen-NoV, and mRNA-based vaccine candidates	• Enhance prominent neutralizing both homotypic and heterotypic Ab response. • The mRNA vaccine candidates demonstrated strong humoral immunity, primed effector T cells, and significant reduction in symptoms.
RV3-BB vaccine developed by PT BioFarma in Indonesia	Based on a naturally attenuated G3P[6] strain that can replicate in the presence of maternal antibodies.	• This three-dose oral vaccine is intended for neonates shortly after birth. • This early administration may provide timely protection against rotavirus disease
PoRVA vaccine	A live attenuated trivalent vaccine using dominant Korean strains G8P[7], G9P[23], and G5P[7].	• This vaccine has proven safe and effective in protecting piglets from diarrhea and enhancing RVA-specific antibody levels
RRV-TV	A dissolvable oral polymeric film containing a live attenuated tetravalent rhesus-human reassortant rotavirus vaccine and antacid ($CaCO_3$)	• They found that two doses of this vaccine were highly immunogenic and provided strong protection against virus shedding and diarrhea in a gnotobiotic pig model upon challenge with a high dose of a virulent human RV (G1).
RV6 developed by Wuhan Institute of Biological Products Co., Ltd (WIBP)	Oral hexavalent live human-bovine reassortant rotavirus vaccine, based on the bovine RV strain UK (G6P [5]), reassorted with VP7 gene from human G1, G2, G3, G4, G8, and G9 strains	• Has high efficacy by providing comprehensive coverage against both major and rare prevalent epidemic strains in China and is safe for use in infants.
CDC-9 vaccine candidate by the Chinese centers for disease control and prevention	A monovalent, heat-inactivated whole virus vaccine based on the G1P[8] strain	• Can elicit strong serum neutralizing antibody responses either homotypic or heterotypic, as well as reduce fecal virus shedding after oral challenges in various animal models. • Has been successfully delivered via a novel-coated skin microneedle patch.

finished a randomized, placebo-controlled phase III clinical trial in four provinces of China in 2021 (Wu et al., 2022; Zou et al., 2023).

Promising VP7 based vaccine candidates

The VP7 glycoprotein antigen can provoke IgG production and a protective immune response (Zhao et al., 2015). Studies have shown that VP7 can preserve a neutralizing immunity when expressed in the potato genome, as mice injected with the transformed potato tubers successfully generated VP7 specific serum IgG and mucosal IgA (Wu et al., 2003). Researchers revealed that the use of modified VP6 as a vector to construct three epitopes carrying chimeric proteins originating from VP7 has been shown to produce antibodies that recognize VP6 and VP7 of RVA, and protect mammalian cells from SA11 and Wa infection *in vitro* (Zhao et al., 2015). A recombinant plasmid, pPI-2.EGFP.VP7.S, was constructed based on the dominant antigen genes of porcine epidemic diarrhea virus (PEDV) *S* gene and PoRV *VP7* gene as vaccine and evaluated in mice. This vaccine candidate exhibited a good immunogenicity, eliciting both humoral and cellular responses against PoRV and PEDV (Yin et al., 2019). In addition to these genetic engineering approaches, messenger RNA (mRNA) vaccines have emerged as the third generation of nucleic acid vaccines, following traditional inactivated and live-attenuated vaccines, as well as newer subunit and viral vector vaccines. mRNA vaccines function by delivering mRNA that encodes one or more target proteins into host cells particularly in cytoplasm, allowing the cells to express the antigenic proteins and present them to the immune system and activating antibodies production (Lu et al., 2024). The mRNA vaccine approach offers a short development cycle, ease of industrial production, straightforward production process, the ability to respond quickly to new variants, and the potential to induce better humoral and cellular immunity. The VP7-mRNA vaccine produced a powerful antiviral immune response, demonstrating the activation and differentiation of antiviral $CD8^+$ T cells and the secretion of interferon-gamma (IF-γ) to mediate cellular immune responses. Additionally, the vaccine was able to activate signaling pathways which are mediated through chemokines and also the overall immune response (Lu et al., 2024).

Promising VP6 based vaccine candidates

Among the proteins of rotavirus, VP6 was the first antigen utilized for the classification of rotavirus strains. This protein is highly conserved structural protein, containing highly immunogenic epitopes and capable of self-assembling into multiple forms, including trimeric, spherical, tubular, and sheet structures. The ability of structural proteins from various viruses to produce immunogenic virus-like particles (VLPs) has been well-documented and can be used to develop vaccines that neutralize the pathogens of interest (Mohsen et al., 2017; Plummer and Manchester, 2011). The benefit of VLPs technology method originates from its ability to mimic the natural structure and antigenic properties of the rotavirus, thereby eliciting a vigorous and effective immune response against the virus.

Various vaccine modalities targeting the rotavirus VP6 antigen, including DNA vaccines (Afchangi et al., 2019; Jalilvand et al., 2015; Lopez-Guerrero et al., 2018), subunit vaccines containing recombinant VP6 protein (Afchangi et al., 2018, 2019; Feng et al., 2017; Jalilvand et al., 2015; Lappalainen et al., 2014; Malik et al., 2023; Malm et al., 2019; Tamminen et al., 2021; Vega et al., 2012, 2013; Zhou et al., 2010), and self-assembled structures (Afchangi et al., 2019; Blazevic et al., 2011, 2016; Heinimäki et al., 2020; Li et al., 2019b; Malm et al., 2016, 2017, 2019; Tamminen et al., 2020; Wu et al., 2021), have been reported to trigger an immune response and/or protection in animal models. Studies involving immunization of animals have demonstrated presence of VP6

protective immune responses with the absence of neutralizing antibodies. One study found that the VP6 protein from the BoRVA C-486 strain (P[1]G6), expressed in insect cells and used to immunize cows, was able to reduce diarrhea and virus shedding in a colostrum-deprived calf model, compared to unvaccinated calves (Gonzalez et al., 2010). Neutralizing epitopes are known to present only on the RV VP4 and VP7 proteins. Consequently, it appears that neutralizing antibodies targeting these proteins may not be required for protection against RV infection, as evidenced by the protection observed in animals that received VP6 preparations missing VP4 and VP7 proteins (Afchangi et al., 2019; Jalilvand et al., 2015). Furthermore, several reports have indicated that non-neutralizing antibodies, such as IgA and IgG, can clear RV infection through intracellular inhibition mechanisms, such as transcytosis (Aiyegbo et al., 2013; Burns et al., 1996; Caddy et al., 2020; Feng et al., 2002). It's interesting to note that the strongest immunogens among the many VP6 preparations have been found to be self-assembled VP6 structures, including VP6 tubes and VLPs. These self-assembled VP6 structures demonstrate adjuvant and immunological carrier qualities in addition to eliciting a more robust immune response and increased levels of immunogenicity and protection (Blazevic et al., 2011).

As VP6 tubes/VLPs are more immunogenic than other VP6 preparations, they are the preferred choice for VP6-based RV vaccine candidates. Despite the difficulties in efficiently generating VLPs at a large-scale using insect cell expression systems, establishing IC-BV expression systems has been prioritized for the production of VP6 tubes and VLPs. The initial studies in 1987 demonstrated the feasibility of this approach (Estes et al., 1987), where the expression of VP6 proteins with the ability to form tubular structures was achieved through the use of a recombinant baculovirus containing the *VP6* gene of RV strain SA11. These VP6 tubes and VLPs made from insect cells kept their original oligomeric structure and antigenic determinants (Estes et al., 1987), and several studies have since shown their immunogenicity. For instance, combining norovirus GII-4 VLPs with RV VP6 tubes to immunize BALB/c mice resulted in a strong systemic antibody response that was cross-reactive and capable of blocking both norovirus and rotavirus (Blazevic et al., 2011). This suggests that both antigens in the combined mixture show acceptable immunogenicity, without one dominating over the other. Building on this, the same team of scientists created a trivalent combination vaccination that included RV VP6 tubes and norovirus GII-4 and GI-3 VLPs (Tamminen et al., 2013). Mice immunized with this trivalent vaccine showed that VP6 tubes may elicit strong, enduring, and highly specific IgG responses with high avidity against several strains of RV (Tamminen et al., 2013). Furthermore, the same research group also evaluated the immunogenicity of the VP6 tubes and double-layered (dl) 2/6-VLPs, which are produced by co-expressing VP2 and VP6 (Lappalainen et al., 2013). Their findings indicated that these VP6-based vaccine formulations induced a balanced Th1/Th2-type immune response. This response includes significant cellular immunity, marked by high levels of IFN-γ production, as well as mucosal IgG and IgA antibodies, and elevated cross-reactive serum IgG against various rotavirus strains (Lappalainen et al., 2013). Notably, the mucosal VP6-specific antibodies detected in the immunized animals' intestines, particularly against the VP6 tubes, may act as primary defense against infection by different rotavirus strains, both *in vitro* and *in vivo* (Lappalainen et al., 2013, 2014; Tamminen et al., 2013). Additional research has shown that serum VP6-specific IgA titers correspond with a minimum of 65% protection against RV infection, independent of the mode of administration (intramuscular or intranasal) (Lappalainen et al., 2015). These findings imply that VP6-specific IgA and mucosal immunity play a major part in blocking RV replication and also in offering heterotypic protection against infection. It's also important to emphasize that injecting VP6 tubes intradermally or intranasally into mice can produce a variety of $CD4^+$ T cell subsets and stimulate the production of antiviral cytokines IFN-γ and IL-4, as well as the pro-inflammatory cytokine IL-17 (Heinimäki et al., 2018). This diverse cellular immune response indicates that immunization with VP6 tubes may protect against rotavirus infection through mechanisms beyond the intracellular inhibition provided by VP6-specific IgA and IgG. A single dose of VP6 tubes elicited the highest IgG titers and provided protection against rotavirus infection comparable to that achieved with two doses of either dl2/6-VLPs or trimers, according to research comparing the immunogenicity of various RV VP6 assemblies, such as dl2/6-VLPs, VP6 tubes, and trimers (Pastor et al., 2014). This indicates that RV VP6 tubes are superior for formulation of vaccines (Pastor et al., 2014).

Apart from the baculovirus expression strategy, several RV VP6 assemblies have also been produced using the bacteria *E. coli*, including tubes, VLPs, and trimers (Bugli et al., 2014; Li et al., 2014, 2019a, 2019b). The morphology of the *E.coli*-derived VP6 structures has been verified using techniques such as transmission electron microscopy (TEM) and atomic force microscopy (AFM) (Bugli et al., 2014; Jiménez-Zaragoza et al., 2018; Li et al., 2014, 2019a, 2019b; Venkataraman et al., 2006). It's interesting to note that *E. coli*-derived double-layered particles (DLP2/6), which consist of the VP2 and VP6 proteins, were found to be substantially more effective against RV infection than VP6-VLPs or trimers (Li et al., 2014). In addition to baculovirus and *E. coli* expression methods, other host systems have also been demonstrated to be able to create RV VP6 structures that self-assemble, including mammalian cells (da Silva Junior et al., 2012) and herpes simplex virus type 1 (HSV-1)-based vectors (Palacios et al., 2015), and plant cells (Zhou et al., 2010). These self-assembled VP6 structures can induce immune responses that provide protection against RV infection (Malm et al., 2019; Palacios et al., 2015). Most recently, researchers expressed VP2, VP6, and VP7 VLPs successfully using baculovirus expression system based on PoRV G5 genotype and found that immunization of mice with these VLPs induced both humoral and cellular immune responses, including the production of neutralizing antibodies, as well as significantly lowered levels of RNA copy numbers (Yan et al., 2024).

To date, RV-VLPs have been generated by co-expressing various structural proteins, including VP6, VP7, VP4, and/or VP2, via baculovirus expression systems. The immunogenicity of these RV-VLPs has been extensively evaluated in mice (Crawford et al., 1994; Peralta et al., 2009). Generally, among the different RV VP6 preparation strategies, the VP6 tubes and it seems that VLPs provides the best potential as a platform for non-living RV vaccines. This is attributed to their solid immunogenicity and the ability to maintain the conserved epitopes of the VP6 protein, which is a key antigen for eliciting protective immune responses against RV.

Promising VP4 based vaccine candidates

The rotavirus VP4 protein is a non-glycosylated protein with functions related to viral adhesion, penetration, and immune response (Zeng et al., 2022). VP4 (88 kDa) is further cleaved by trypsin into two subunits: VP8*(28 kDa, amino acids [aa] 1–247) and VP5* (60 kDa, 248–776 aa) (Estes et al., 1981). This cleavage event facilitates viral entry into host cells (Kaljot et al., 1988), and both VP8* and VP5* can trigger the generation of neutralizing antibodies (Nair et al., 2017). To be more precise, VP4's distal VP8* heads interact with glycan receptors of rotavirus, which initiates a host-specific infection process (Xia et al., 2019). Hence, the VP8* protein is vital in determining the antigenic types of rotaviruses and is regarded as an ideal vaccine target for combating rotavirus infection.

Studies have shown that the *E. coli*-expressed bovine rotavirus VP8* protein can elicit neutralizing antibodies in seronegative mice and rabbits. Furthermore, it markedly enhanced antibody responses in the colostrum and milk of cows that were previously exposed to rotavirus, under normal conditions (Lee et al., 1995). Researchers have also explored the use of truncated recombinant subunit VP8* protein vaccine candidates. These vaccines contain amino acid residues 64 (or 65)–223 with specificity for the P[8], P[4], or P[6] rotavirus strains, expressed in *E. coli*. Each of these ΔVP8* proteins produced significant amounts of homotypic neutralizing antibodies and varying degrees of heterotypic

neutralizing antibodies in guinea pigs that were hyperimmunized intramuscularly (Wen et al., 2012). Additionally, a truncated VP4 protein (amino acids 26–476), containing the VP8 and VP5, was expressed in a soluble form in *E. coli*. In animal models, this truncated VP4 protein showed high protective efficacy, significantly reducing diarrhea severity and viral shedding when combined with an aluminum adjuvant. It also produced high titers of neutralizing antibodies (Li et al., 2018). Furthermore, a chimeric norovirus (NoV) S60 nanoparticle displaying 60 copies of the rotavirus neutralizing VP8* antigen has been constructed. This nanoparticle-based vaccine elicited a strong IgG response in mice towards the displayed VP8* antigens and exhibited high neutralizing activities against rotavirus infection in cell culture. Another approach involves constructing a norovirus S particle-VP8* vaccine, where the murine rotavirus (EDIM strain) VP8* antigen was displayed on the S particle. Immunizing mice with this vaccine candidate resulted in the generation of high titers of anti-EDIM VP8* IgG antibodies (Xia et al., 2019). In another study, BALB/c mice were orally immunized with a recombinant *L. lactis* strain that expressed the VP4 protein. This led to the production of specific anti-VP4 secretory IgA and IgG antibodies in the feces, as well as in ophthalmic secretions, vaginal secretions, and in serum which were experimentally able to neutralize porcine rotavirus infection (Li et al., 2010). Additionally, a bacterially- expressed subunit vaccine candidate containing P2 universal $CD4^+$ T cell epitope (aa 830–844) fused to a P[8] ΔVP8* antigen induced high levels of both homotypic and heterotypic neutralizing antibodies in guinea pigs and significantly delayed the onset and shortened the duration of diarrhea when challenged with the virulent human rotavirus Wa strain (Wen et al., 2014). Furthermore, two tandem recombinant VP8* proteins based on an *E. coli* expression system resulted in the generation of high titers of homotypic and heterotypic neutralizing antibodies against human rotaviruses bearing various G and P type combinations, including G1-G4, G8, G9 and G12 with P[8], P[4] or P[6] (Wen et al., 2015). The VP8-1 (aa 26–231) antigen's immunogenicity was enhanced by the intramolecular adjuvant of the nontoxic B component of cholera toxin (CTB). When compared to the VP8-1-CTB construct in mice, the CTB-VP8-1 fusion protein produced greater titers of neutralizing antibodies and had greater protective effectiveness (Xue et al., 2016). Another trivalent vaccine candidate [formed of joined monomeric VP8 domain of RV spike protein (VP4), fused dimeric protrusion (P) proteins of astrovirus (AstV) and hepatitis E virus (HEV)] was able to elicit significantly higher immune responses to the three viral antigens compared to a mixture of the three individual free proteins (mixed vaccine) when administered to mice (Xia et al., 2016).

A randomized, double-blind, placebo-controlled trial involving infants and toddlers in South Africa has been performed to evaluate the safety and immunogenicity of the P2-VP8-P[8] subunit rotavirus vaccine, where researchers found that significant immune responses were observed in infants receiving the P2-VP8-P[8] vaccine, with 81% and 98% demonstrating serum IgA and IgG seroresponses in the 30 μg group, respectively, compared to only 20% and 9% in the placebo group (Groome et al., 2017). Later on, the same research team revealed that the trivalent P2-VP8 subunit rotavirus vaccine elicited significantly higher anti-P2-VP8 IgG seroresponses in infants across all vaccine groups (99%–100%) compared to the placebo group (10%–29%). While IgA seroresponses were modest (20%–34%), the vaccine demonstrated a favorable safety profile, with no serious adverse events in adults and a low incidence of serious events in toddlers and infants (Groome et al., 2020). However, Program for Appropriate Technology in Health (PATH) announced that results from the phase 3 study indicate that the vaccine candidate is safe and well tolerated by infants, but unfortunately, the trivalent P2-VP8 provided inferior protection against severe rotavirus gastroenteritis (SRVGE) compared to the licensed oral rotavirus vaccine ROTARIX® (Data now shown). In a different investigation, the Vi capsular polysaccharide (Vi) of *Salmonella Typhi* and the RV ΔVP8* antigen were covalently conjugated to create a bivalent vaccine known as Vi-ΔVP8*, which was able to elicit specific immune responses in inoculated mice against both the Vi and the RV ΔVP8*antigens (Park et al., 2021).

Researchers have also developed trivalent P24-VP8* nanoparticles displaying the RV VP8* (aa 65–223) of the P[8], P[4], and P[6] genotypes. The nanoparticles were able to spontaneously self-assemble and elicited a robust IgG antibody response specific to the three homologous VP8* antigenic types when tested in mice. Moreover, these nanoparticles demonstrated the ability to neutralize the replication of all three distinct rotavirus P types in cell culture experiments (Xia et al., 2021). Furthermore, an oral multi-vaccine based on a *Lactobacillus* vector platform, containing the VP4 antigens of transmissible gastroenteritis virus (TGEV), PEDV, and PoRVA, was developed. This vaccine was effective in eliciting both mucosal and systemic humoral immune responses that were targeted against multiple viruses known to cause diarrhea in piglets. Furthermore, the vaccine was able to protect piglets from becoming infected by these viral pathogens (Guo et al., 2022). The VP4* proteins (aa 26–476) of P[4], P[6], and P[8] genotype rotaviruses were expressed in *E.coli*. When the protein antigens were formulated with an aluminum-based adjuvant, they induced high levels of neutralizing antibodies in both rabbits and guinea pigs. However, a bivalent VP4*-based vaccine (comprising P[8] + P[6]-VP4*) was also capable of stimulating elevated neutralizing antibodies levels against different rotavirus genotypes which were similar to those levels induced by more complex trivalent formulations (Luo, 2022). Another approach involved inserting the RVA $VP8^*_{26\text{-}231}$ antigen (Ren et al., 2022), within the major immunodominant region (MIR; amino acids 76 to 82) of the histo-blood group antigens (HBGAs) to construct chimeric HBc-VP8-VLPs (cVLPVP8*). Immunization of mice with this vaccine candidate resulted in higher IgA and IgG levels, and also higher IgG1/IgG2a ratio, compared to vaccination with VP8* alone (Latifi et al., 2023).

In another study, researchers developed mRNA-based vaccine candidates, a design with a single P2 (T cell universal epitope) antigen fused to VP8*, as well as a construct incorporating a lumazine synthase (LS) component linked to the P2 and VP8* antigens. When evaluated in guinea pigs, the LS-P2-VP8* mRNA vaccine was found to induce superior humoral immune responses compared to the P2-VP8* mRNA vaccine, both when administered as a monovalent and trivalent formulation. These mRNA vaccine candidates demonstrated strong humoral immunity, primed effector T cells, and significant reduction in diarrhea duration and viral shedding. These promising results provide proof of concept for further development of mRNA vaccines against rotavirus infections (Hensley et al., 2024).

Additionally, a bivalent subunit PoRV vaccine was developed using truncated versions (VP4*) of the VP4 proteins from P[7] and P[23] genotypes. Combining two distinct VP4* antigens, VP4*[7] and VP4*[23], for mouse immunization elicited more robust neutralizing antibody and cellular immune responses compared to administering each antigen individually (Tang et al., 2024). Furthermore, when BALB/c mice were immunized with the $VP4_{26\text{-}476aa}$ (VP4*) and $NSP4_{86\text{-}175aa}$ (NSP4*) antigens in combination via the subcutaneous (SC) route demonstrating that the NSP4* component had strong adjuvant activity, up-regulating the VP4*-specific secretory IgA level, antibody responses, and IFN-γ secretion (Li et al., 2024). In one study, five VP4 (aa 26–476) proteins from diverse P[8] lineages of human rotavirus were expressed in *E. coli* including the Wa-VP4 protein. This Wa-VP4 protein was able to induce significantly elevated levels of neutralizing antibodies homologous and heterologous rotaviruses, compared to the other VP4 proteins from different P[8] lineages when tested in rabbits, cynomolgus monkeys, pigs and mice (Luo et al., 2024b). More recently, researchers developed a universal recombinant rotavirus antigen (URRA) derived from a diverse range of rotavirus strains. The URRA antigen alone was only able to elicit anti-URRA antibody titers after two immunizations in a mouse model. However, when the URRA antigen was paired with plant virus-based spherical particles (SPs), it triggered a strong immune response after just one immunization (Granovskiy et al., 2024). Moreover, a multivalent vaccine candidate has been developed that includes nine different VP8*

proteins, (P[4], P[6], P[8]LI, P[8]LIII, P[8]LIV, P[9], P[11], P[14], and P[25]), which are all expressed in *E. coli*. Antibody responses were elicited in mice against six of the nine used immunogens with higher antibody titers significantly noticed against the VP8*P[8]LI genotype compared to mice immunized solely with the P[8]LI antigen (Cárcamo-Calvo et al., 2024). Most recently, researchers mentioned that nanoparticles utilizing the SpyCatcher-SpyTag system can effectively display truncated VP8-1 proteins, significantly enhancing their immunogenicity. The bivalent presentation of VP8-1 from simian and lamb rotaviruses not only improved immune responses to homotypic antigens but also generated strong heterotypic immunity, providing effective protection against diverse rotavirus strains in an adult mouse model, suggesting a promising approach for developing broad-spectrum rotavirus vaccines (Luo et al., 2024a). These studies highlight the ongoing efforts to develop more broadly protective rotavirus vaccine candidates by targeting a diverse array of VP4 and VP8* antigens, either as monovalent or multivalent formulations, and incorporating various expression systems and adjuvant strategies to enhance the vaccines' immunogenicity and cross-reactivity.

Inactivated vaccines

The Chinese Centers for Disease Control and Prevention (CDC) is developing a monovalent, heat-inactivated whole virus vaccine based on the G1P[8] strain CDC-9 human rotavirus. This strain is notable for its ability to grow to high titers and maintain stability in its infectious, TLP form. An inactivated vaccine using this strain is appealing because it retains all relevant surface neutralizing epitopes (Wang et al., 2021). Extensive preclinical testing has shown that the CDC-9 vaccine candidate can elicit strong serum neutralizing antibody responses either homotypic or heterotypic, as well as reduce fecal virus shedding after oral challenges in various animal models. Notably, it has been successfully delivered via a novel-coated skin microneedle patch.

Two commercial partners, Serum Institute of India and Zhifei Lvzhu Biopharmaceutical, have begun Good Manufacturing Process production and regulatory agreements processes in preparation for phase one clinical trials (Wang et al., 2021). Additionally, Bharat Biotech is working on an inactivated version of their Rotavac vaccine for parenteral administration, though details on its progress are limited. The CDC-9 vaccine candidate has also been assessed alongside an inactivated polio vaccine (IPV-IRV), with no interference observed. This combined vaccine is currently being tested with a microneedle patch, and early-phase clinical trials are underway.

Furthermore, gamma(γ)-irradiation has been used to inactivate whole RV for the development of γ-irradiated RV (γ-RV) vaccine candidate. Immunization of mice with this γ-RV vaccine produced robust rotavirus-specific neutralizing antibody responses without the use of an adjuvant (Shahrudin et al., 2018). Lastly, parenteral administration of an inactivated rotavirus vaccine (IRV) to mice, either through an adjuvant-free dissolving polymer microneedle (MN) patch or an alum-adjuvanted intramuscular (IM) injection, was found to induce the expression of the gut-homing receptor LPAM-1 on both T and B cells in the spleen and mesenteric lymph nodes of the immunized animals (Resch et al., 2018). Fig. 3 shows the disadvantages of currently available Rotavirus vaccines in comparison with the advantages of the new vaccine candidates.

FUTURE VACCINE CHALLENGES AND PROSPECTS

While significant advancements have been made in rotavirus vaccine research and implementation, there remain several key challenges that need to be addressed to further improve the global impact of these vaccines.

One critical challenge is expanding the breadth of strain coverage provided by current rotavirus vaccines. The licensed oral vaccines target a limited number of the most common rotavirus genotypes, leaving populations vulnerable to emerging or less prevalent strains. Developing

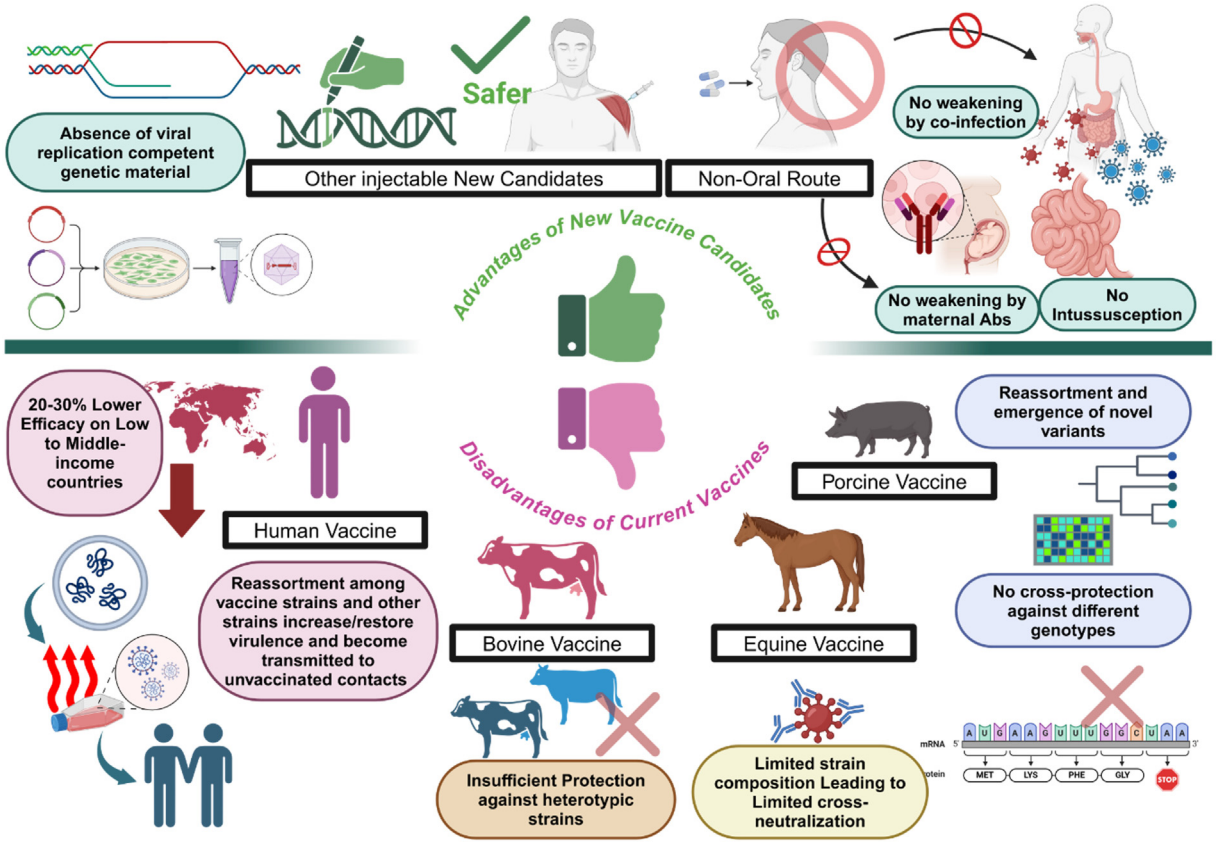

Fig. 3. Disadvantages of currently available rotavirus vaccines versus advantages of new vaccine candidates. Figure created with BioRender.com.

vaccines with broader serotype inclusion or cross-protective antigens could help address this issue and ensure more comprehensive protection.

Improving thermostability of rotavirus vaccines is another important priority. Many current formulations require a cold chain for storage and transport, which can be logistically challenging and limit access, especially in resource-limited regions. Advancing heat-stable vaccine technologies would facilitate more reliable and equitable vaccine delivery.

For veterinary applications, a key future challenge is harmonizing rotavirus vaccination strategies across various livestock and companion animal species. While there is increasing focus on rotavirus vaccines for animals, the specific vaccine strains, immunization schedules, and implementation programs vary significantly depending on the host species and production systems. Establishing more standardized approaches could optimize the effectiveness of these vaccines.

Moreover, increasing global access and uptake of rotavirus vaccines, particularly in low- and middle-income countries, remains a significant challenge. Factors such as vaccine cost, healthcare infrastructure, and community acceptance can affect vaccination coverage. Innovative financing mechanisms, targeted delivery programs, and robust communication campaigns will be essential to ensure that these life-saving interventions reach the populations most in need.

Finally, continued research into next-generation rotavirus vaccine technologies, such as mucosal or needle-free formulations, could enhance ease of administration, immunogenicity, and overall effectiveness. Addressing these multifaceted challenges is vital to fully realize the potential of rotavirus vaccines and reduce the substantial global burden of this prevalent and debilitating disease in both human and animal populations.

The recent introduction of a plasmid-based reverse genetics system for rotavirus marks a crucial advancement with the potential to significantly speed up rotavirus research. This innovative technology is anticipated to enhance existing platforms and support the development of new vaccine candidates (Desselberger, 2020).

CONCLUSIONS

Rotavirus infections are a significant threat to the health of both human and animal populations worldwide. Effective vaccines have greatly alleviated this burden, especially in public health, where licensed oral live-attenuated vaccines have successfully reduced severe disease and healthcare costs. Newer candidates, such as the pentavalent RV5 and monovalent RV1, promise broader protection against diverse strains. Additionally, there is increasing focus on veterinary vaccines, which are crucial for protecting livestock and ensuring food security due to the severe economic losses caused by rotavirus infections.

Despite these advancements, challenges remain, including the need to expand strain coverage, improve thermostability, and ensure equitable access, particularly in resource-limited regions. Continued innovation in vaccine technologies and delivery methods is essential for maximizing the impact of rotavirus vaccines on global health. Overall, the progress in rotavirus vaccine development is a notable success in infectious disease prevention, with the potential to further safeguard both human and animal health as research and deployment efforts continue.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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