Review Article

Alternative approaches to live attenuated oral rotavirus vaccines

Makoye Mhozya Kanyema[1,2,3,4,5*] Junhong Wang[1,2,3,4] Gui-lian Yang[1,2,3,4]

Xin Cao[1,2,3,4] Chunfeng Wang[1,2,3,4]

Abstract

Oral attenuated rotavirus vaccine is safe and effective in developed countries but, however, it is less effective in low income countries such as African and Asian countries. Low vaccine effectiveness in low-income countries is arising from a variety of sources such as maternal antibodies, chronic enteropathy and other enteric diseases such as cholera, typhoid, and parasitic diseases, as well as the microbiota. There is increasingly evidence related to the risk of intussusception for the oral vaccine. The development of effective and safer rotavirus vaccines, particularly, non-living candidates, is critical. The aim of this review therefore is to explore some of the effective rotavirus vaccine alternatives to live attenuated oral virus vaccines. It primarily reveals non-living candidates such as inactive rotavirus vaccines, rotavirus-like particles, recombinant subunit proteins and non-living mucosal rotavirus vaccines. These approaches have been tested in a variety of animal models with great success in eliciting powerful immune responses. It is high time now for various vaccine manufacturers to look at these tested approaches, as they will provide hope to low-income countries in terms of producing vaccines that are both safe and effective.

Keywords: Attenuated vaccines, Inactivated vaccines, Rotavirus like particles, Recombinant subunit proteins, Non-living mucosal vaccines

[1]*College of Veterinary Medicine, Jilin Agricultural University, Changchun, China*
[2]*Jilin Provincial Key Laboratory of Animal Microecology and Healthy Breeding, Jilin Agricultural University, Changchun, China*
[3]*Jilin Provincial Engineering Research Center of Animal Probiotics, Jilin Agricultural University, Changchun, China*
[4]*Key Laboratory of Animal Production and Product Quality Safety of Ministry of Education, Jilin Agricultural University, Changchun, China*
[5]*Department of Agricultural Sciences Mwalimu Julius K.Nyerere University of Agriculture and Technology, Mara, Tanzania*
***Correspondence**: makoyemhozya86@gmail.com* (M.M. Kanyema)
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Introduction

Rotavirus (RV) gastroenteritis is a serious viral disease that affects children under the age of five and post-weaning neonates around the world. Dehydration is thought to be the cause of the deaths of 500,000 children per year. Low-income countries have the highest mortality and morbidity rates. Rotavirus replicates in mature enterocytes, cause the villi to be destroyed, resulting in malabsorption and diarrhoea. Poor nutritional absorption, prolonged dehydration and intussusception are all severe indications of RV infection (Azevedo *et al.,* 2013; El-Attar *et al.,* 2009; Lappalainen *et al.,* 2015).

Because of the reported risk of intussusception, Rotashield® was taken off the market shortly after the US Food Drug and Administration (FDA) authorized it. Rotateq® and Rotarix®, however, are currently available live attenuated RV vaccines on the market and have been approved in a number of countries. These oral RV vaccines are effective in developed countries but less effective in low income countries such as African and Asian countries. The interference of maternal antibodies, chronic enteropathy, other enteric diseases such as cholera, typhoid and parasitic diseases, as well as the microbiota, are believed to cause low vaccine efficacy in low-income countries. Moreover, research demonstrates that oral attenuated vaccination is strongly linked to an increased risk of intussusception (Arnold, 2018; Holmgren and Svennerholm, 2012; Li *et al.,* 2014, 2018; Sartorio *et al.,* 2020).

It has been documented that vaccine strains can be passed from the vaccinated to unvaccinated. It is even more risky to immune-compromised infants. Highly virulent strains may evolve if transmissible vaccine strains reassort among themselves or in combination with field strains. It's critical to develop effective and safer rotavirus vaccines, ideally non-living candidates, as an alternative to the existing oral attenuated rotavirus vaccines, which may pose public health hazards and, moreover, exert less effectivity in low-income areas (Bowen and Payne, 2012; Donato *et al.,* 2012; Kaneko *et al.,* 2017). The purpose of this review therefore is to discuss the suitability and superiority of alternative vaccine approaches to live attenuated, oral virus vaccines.

Inactivated rotavirus vaccine

The limited efficacy of attenuated RV immunizations in poor-resource African and Asian countries highlights the urgent need for new strategies to prevent morbidities and mortalities (Jiang *et al.,* 2008a; Wang *et al.,* 2010).

Such obstacles can be resolved using the inactivated vaccination approach. A number of animal models have demonstrated satisfactory immunogenicity with inactivated rotavirus vaccine (IRV) and when combined with adjuvants, strong immune responses have been induced (Hashim *et al.,* 2016). In mouse and rabbit models, IRVs were found to be effective against rotavirus challenge when correctly formulated and administered parenterally. The composition of the vaccine, the route of administration and the animal model used all had significant influence on the level of protection (Jiang *et al.,* 2008b; Lee, 2020).

According to studies, live triple-layered particles combined with adjuvant induced significant protection in mice via intramuscular (IM) injection. However, mice injected with formalin-inactivated triple-layered particles showed little protection and mice injected with double-layered particles showed even less protection. Gnotobiotic (Gn) piglets reacted positively when were orally supplied with purified formalin or heat inactivated candidates. Low immunogenicity was detected when given unpurified binary ethylenimine-inactivated candidates (Desselberger and Huppertz, 2011; Jiang *et al.,* 2008a; Yuan and Saif, 2002).

All of these suggest that IRV could be effective; an hypothesis that will need to be tested in clinical trials. There is, however, compelling evidence that an oral challenge resulted in a significant increase in antibody titers in vaccinated piglets, suggesting a novel promising opportunity for immunizing children first with an IRV and then with a live oral vaccine as a booster. This primary booster strategy will lessen the rare but serious occurrence of intussusception. This method will also prevent issues common with live oral rotavirus vaccines, such as the neutralization process in breast milk and interference from other microbiota in the gut of children in low-income countries, potentially improving vaccine efficacy and aiding in the achievement of the global immunization agenda (Wang *et al.,* 2010; Yuan and Saif, 2002).

The Center for Disease Control and Prevention (CDC) in the US is implementing an inactivated whole virus strategy. The strategy used the heat inactivated method to make the CDC-9 vaccine and the virion structure of the vaccine remained intact throughout the purifying process. When given IM to mice, the vaccine candidate provided significant protection together with aluminum hydroxide as an adjuvant. In mice, rats, rabbits and pigs, this candidate, which is also based on the G1P strain, has shown a heterotypic neutralizing antibody response. Mice showed systemic and mucosal immunity after being given an injection or a microneedle patch (Esona *et al.,* 2010; Jiang *et al.,* 2008b; Nakagomi and Nakagomi, 2009; Resch *et al.,* 2020; Velasquez *et al.,* 2015).

A bivalent inactivated polio vaccine (IPV)–IRV candidate is being investigated with this same strain (G1P) and it has demonstrated encouraging results without creating side effects from any of the two components. The inactivated G1P strain and Lanzhou lamb rotavirus (LLR) strain vaccines are two additional potential vaccines under development (Burke *et al.,* 2019; Lee, 2020).

The effectiveness of inactivated candidate vaccines is always influenced by the route of delivery. Both intramuscular and microneedle intradermal injection strategies have been shown to have a significant impact on immune protection. Some inactivated vaccines are currently undergoing early clinical trials, while manufacturers are being sought to assist with vaccine development and marketing (Carvalho and Gill, 2019; Kirkwood *et al.,* 2019; Y. Wang *et al.,* 2016).

Inactivated vaccine alternatives are equally effective against RV infection in food animals like cattle and pigs as they are in humans. When IRV is given to

pregnant cows, it causes secretory antibodies to be produced, which provides passive protection to newborn calves. Colostrum, milk and colostrally ingested antibody in the gut of calves are most likely involved in eliciting this immune response (Dhama *et al.,* 2009; Papp *et al.,* 2013). Also, IRV is being used in pregnant and/or nursing sows to give passive immune protection to suckling piglets in some countries (Matías *et al.,* 2017; Saif and Fernandez, 1996).

Rotavirus Like Particles (RV-VLPs)

Because of their significant efficacy and safety, virus like-particles (VLPs) offer awesome vaccine antigens (Chen and Lai, 2013; Nooraei *et al.,* 2021). Recombinant VLPs are potent immune inducers against RV in small animals (Azevedo *et al.,* 2013; Parez *et al.,* 2006). In vitro and in vivo models have demonstrated the potency of DNA vaccines, rotavirus virus-like particles (RV-VLPs) and recombinant proteins in activating homologous and cross-protective immune responses. Conventional vaccines, on the other hand, are inferior to RV-VLPs as RV-VLPs are conformationally close to the parent virus and therefore can induce both cellular and humoral immune responses (Changotra and Vij, 2017; Parez, 2008). These promising findings provide a solid experimental foundation and research direction for the production of RV-VLPs in the future (Wang *et al.,* 2020; Ward and McNeal, 2010).

The viral proteins of RV-VLPs in combinations such as 2/6, 2/6/7, and 2/4/6/7 have been immunogenic in various animal models such as mice, rabbit and gnotobiotic piglets. Routes of administration such as parenteral, intranasal and intra-rectal were also significantly researched. Besides, 2/4/6/7-VLPs have had homologous immunological effect in rabbits and heterologous immunological activation in mice when administered parenterally (Azevedo *et al.,* 2013; Conner *et al.,* 1996; Desselberger and Huppertz, 2011; Istrate *et al.,* 2008; O'Neal *et al.,* 1997).

Clinical trials have demonstrated recombinant VLPs as powerful immunogens. The VLP conformation boosts the immune response by displaying a large number of antigenic epitopes on its surface, which more closely resemble the surface proteins of the natural virions. As they are void of nucleic acid, VLPs are safer as antigens in vaccine formulations than native virions (Calzas and Chevalier, 2019; Roldão *et al.,* 2010; Vieira *et al.,* 2005).

Furthermore, parenteral immunization has shown to improve the potency of VLP candidate vaccines in low-income countries over oral attenuated vaccines. RECOMBIVAX HB® was the early recombinant VLP vaccine approved in 1986 and consisted of a single recombinant HBs-Ag protein. This vaccine is a great example of the VLP recombinant vaccine with substantial immune protection in humans. Human papillomavirus and hepatitis E virus VLP vaccines were also approved about two decades later (Roldão *et al.,* 2010). But the scenario is different with rotavirus in which recombinant double-layered VLPs still remain developed only in insect and mammalian cells (Crisci, 2013; Li *et al.,* 2014).

Recombinant Subunit Proteins

Recombinant subunit vaccines are non-replicating protein vaccines and when given parenterally bypass the intestine, potentially improving efficacy and safety(Wen *et al.,* 2014; Xue *et al.,* 2016).

The outer capsid protein of rotavirus, VP4, is usually cleaved into VP5* and VP8* fragments when it comes into contact with intestinal proteases. VP8*, which is present at the distal end of the VP4 protein, facilitates viral penetration by binding to cells. VP8* contains neutralizing epitopes, making it a useful target for vaccine development. It is a potent immunogen expressed in various culture systems. It has portrayed potential immunogenicity in mice, guinea and gnotobiotic pig (Li *et al.,* 2018; Ramesh *et al.,* 2019; Salgado *et al.,* 2018; Velasquez and Jiang, 2019; Zárate *et al.,* 2000).

However, the most improved candidate vaccine currently available contains a truncated VP8. It has the P2 epitope of tetanus toxin bound to it. The recombinant fusion protein P2-VP8-P[8] induced immune tolerance and robustness in clinical trials that included adults in the United States and toddlers and infants in South Africa. To homologous P[8] rotavirus strains, this vaccine elicited strong neutralizing antibody responses but only moderate induction with heterologous strains P[4] and P[6] (Agarwal *et al.,* 2020; Wen *et al.,* 2014; Xue *et al.,* 2016).

In one of the clinical trials, infants were challenged with Rotarix one month after receiving their third dose of the improved candidate vaccine. According to the results, vaccinated infants shed significantly less rotavirus than placebo recipients, implying its potential mucosal protection. However, a trivalent vaccine formulation comprising VP8 subunits from P[4], P[6], and P[8] strains was engineered to optimize responses in order to control the most dominant rotavirus strains around the world (Burke *et al.,* 2019; Groome *et al.,* 2020; Wang *et al.,* 2020).

In addition, one study used the gnotobiotic pig model to determine the immune protection of a novel P24-VP8* nanoparticle vaccine. Intramuscular immunization of three subsequent doses of P24-VP8* (200 g/dose) with Al(OH)3 adjuvant (600 g) provided substantial protection against the challenge of virulent Wa strain rotavirus. On post-challenge day (PCD) 1–7, there was a considerable reduction in the mean duration of diarrhea, virus shedding in feces and lower fecal cumulative consistency scores when comparing immunized pigs to mock immunized controls.

From post-inoculation day 21 to PCD 7, the vaccine induced robust VP8*-specific serum IgG and Wa-specific virus-neutralizing antibody responses in gnotobiotic pigs. But it did not trigger mucosal immune response or a strong effector T cell response, which is consistent with the vaccination route, adjuvant used, and the non-replicating vaccine's nature (Ramesh *et al.,* 2019).

The RV VP6 protein is a highly conserved and potent immunological capsid protein present in the virion's intermediate layer. While VP6 does not stimulate neutralizing antibodies, it has been shown to stimulate heterotypic cross-protective cellular immunity (Afchangi *et al.,* 2019; Lopez-Guerrero *et al.,*

2018). In mice, it provides active protection against viral shedding. While these findings are promising, it is yet to be established if cross-protective, non-neutralizing VP6 antigen induces immune response against RV in humans (Blazevic *et al.*, 2011; Jalilvand *et al.*, 2015; Ward and McNeal, 2010).

In addition, a bivalent vaccine including the VP6 subunit as well as norovirus virus-like particles is being developed. In mouse models, this candidate has shown a positive immune response (Burke *et al.*, 2019; Li *et al.*, 2014).

Non-living mucosal rotavirus vaccines

Despite the excellent efficiency of attenuated oral vaccines with significant antigenic epitope delivery at the mucosa, their manufacturing entails numerous processes that repeatedly fail to create vaccines that are safe and durable. As a result, there is still a strong case for developing nonliving whole cell or subcomponent mucosal vaccines (Heyse *et al.*, 2008; Minor, 2015; Sartorio *et al.*, 2020).

Secretory IgA has a unique ability to withstand protease enzymatic degradation, which distinguishes mucosal immunity from systemic immunity. Pathogen neutralization in the gut relies heavily on the resiliency of secretory IgA. One great feature of the mucosal vaccination is the ability to induce secretory IgA and serum IgG which is contrary to the parenteral vaccination that exerts no development of secretory IgA in the mucosa. In other words, parenteral vaccination does not induce mucosal immunity but it induces systemic immunity (Neutra and Kozlowski, 2006; Owen *et al.*, 2013).

Antigen presentation and lymphocyte priming (Figure 1) are the two features of mucosal DCs found in mucosal associated lymphoid tissue (MALT). Antigen presentation work may also be done by epithelial microfold cells (M cells). The potential for developing new vaccines by targeting DCs with an antigen delivery system is growing tremendously (Kaul and Ogra, 1998; Kavanagh *et al.*, 2010; Wang *et al.*, 2014). Lactic acid bacteria (LAB) produce a significant amount of lactic acid as a byproduct of glucose metabolism. LABs are gram positive, non-pathogenic bacteria termed as 'generally recognized as safe' (GRAS). They have been utilized in the food fermentation industry for decades because of their non-pathogenic properties. In the field of recombinant vaccine technology, LAB has recently received a lot of attention as a mucosal delivery vehicle. *Lactococcus lactis*, *Lactobacillus plantarum* and *Lactobacillus casei* are some of the most commonly utilized LAB as recombinant vectors. They have had a lot of success delivering non-replicating antigens to the mucosa of the intestine (Leblanc *et al.*, 2013; Takahashi *et al.*, 2019; M. Wang *et al.*, 2016).

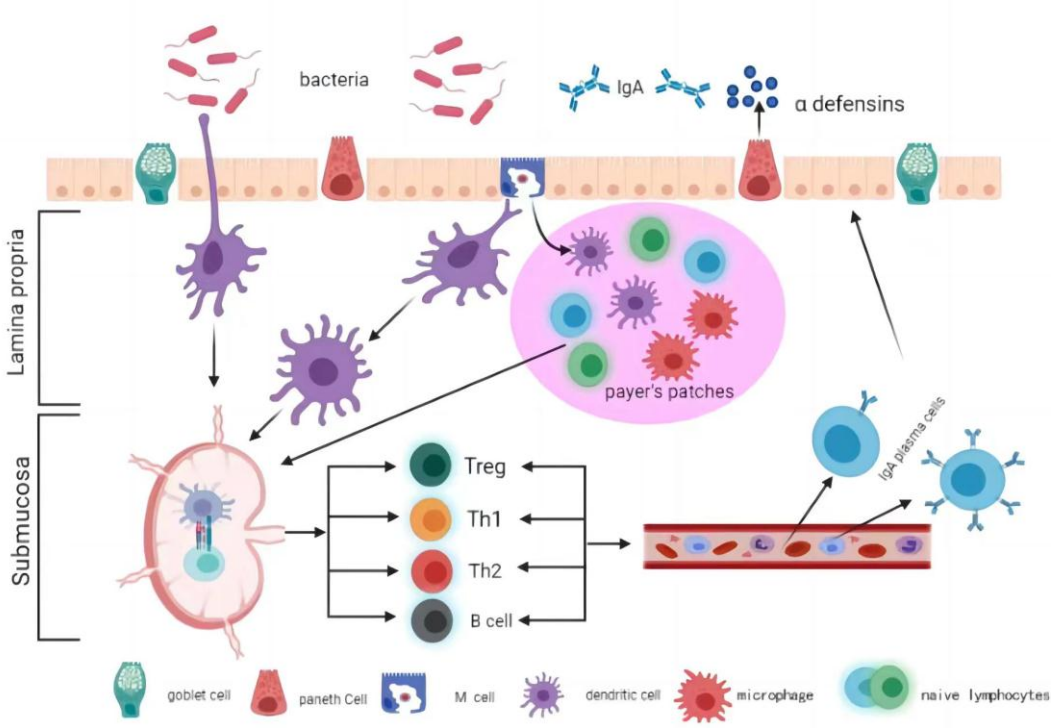


Figure 1 The mucosal immunity of the intestine. Dendritic cells catch microbial antigens through extending dendrites across the tight junctions of the epithelial cells or receive antigens from M-cells and use them to induce lymphocyte differentiation in the induction sites such as payer's patches and mesenteric lymph nodes. However, the M-cell may present the antigen to a dendritic cell or a macrophage in the payer's patches, which subsequently initiates lymphocyte priming. It is worthwhile to note that final lymphocyte differentiation and maturation frequently happen in the mesenteric lymph nodes. In this instance, initiated lymphocytes from the payer's patches migrate to mesenteric lymph nodes for the purpose. T and B cells mature in the mesenteric lymph nodes before being discharged into the circulatory system to affect tissue immunological responses. When delivered to the lamina propria, however, the IgA-secreting plasma cells become completely differentiated and functional. The lamina propria is also populated by effector T cells from the circulation, which carry out cellular-mediated immunity.
Treg-Regulatory T Lymphocyte, Th1 and Th2-Helper T lymphocyte 1 and 2 respectively, IgA-Immunoglobulin A

The potential of LAB to influence gut immunological activity, the ease with which it may be genetically modified and its ability to survive in the harsh environment of the gut epithelium are all characteristics that make it a promising recombinant vector (Abdo *et al.,* 2019; Medina and Guzmán, 2001; Seegers, 2002) .

Dendritic cell activation for innate and adaptive immunity is affected differently by different LAB species and strains. They also have different effects on the ability to maintain immunological homeostasis by balancing the responses of T-cell subsets (Th1 and Th2). Careful selection of LAB species and strains for recombinant vectors is crucial for optimal antigen delivery at the mucosa that generates controlled inflammation to prevent immunological disorders (Medina and Guzmán, 2001; Owen *et al.,* 2013; Seegers, 2002; Wells, 2011).

Recombinant lactobacillus-based vaccines could fill a gap in the market for non-replicating subunit vaccines that provide the benefits of probiotics while still being safe for use in neonates and infants. Few studies have tested candidate rotavirus vaccines based on lactobacillus as vectors in animal models, some listed in Table 1. In one experiment, mice were given a standard oral vaccine with *L. casei*, which induced mucosal antibodies and neutralized serum antibodies against the porcine rotavirus major protective antigen (PA) VP4 (Afchangi *et al.,* 2021; Israr *et al.,* 2018; Pellissery and Nair, 2013).

Table 1 Candidate rotavirus vaccines based on lactobacillus vectors

Pathogen	Lactobacillus species	Antigen(s) Released	Animal modal	Result(s)	Reference
Porcine RV	*L. casei*	VP4	Mice	Serum IgG, mucosal IgA, neutralization	(Qiao *et al.,* 2009)
Porcine RV	*L. acidophilus*	VP7	Mice	Mucosal IgA	(Hu and Wang, 2008)
Porcine RV	*L.casei*	VP4	Mice	Serum IgG, mucosal IgA, neutralization	(Yin *et al.,* 2016)

RV-rotavirus, L-lactobacillus, VP-viral protein, Ig-immunoglobin

Since this vector successfully induced an important and specific antiVP4 IgA response (VP4), it is suggested that, *L. casei* expressing recombinant VP4 could be used in the oral vaccination of pigs against porcine rotavirus infections (Qiao *et al.,* 2009).

Current research on bacterial spores as vectors has revealed the ability of *Bacillus subtilis* spores to deliver mucosal antigens and demonstrating immunogenicity (Amuguni and Tzipori, 2012; Batista *et al.,* 2014; Rosales-Mendoza and Angulo, 2015). Despite their poor immunogenicity and limited residence time in the gastrointestinal tract of the host after oral vaccination, the spores of *B. subtilis* are desirable for use in vaccine delivery due to their greater tolerance to adverse conditions for longer periods of time, heat resistance, probiotic effects, low production cost and GRAS status (da Silva *et al.,* 2014).

In one study, *Bacillus subtilis*-based rotavirus VP6 vaccines were found to be successful in inducing protective immunity in adult mice against rotavirus challenge. When cholera toxin (CT) and mutant Escherichia coli heat-labile toxin (mLT) was applied to the *B. subtilis* spore-based vaccines, protective immunity was elicited but not when spores were given without adjuvants. This is in line with previous research suggesting that rotavirus VP6 antigen-based vaccines need an adjuvant to achieve high levels of immunogenicity (Lee *et al.,* 2010).

Another study found that porcine rotavirus VP8* could successfully show up on the surface of the recombinant *B. subtilis* Bacterial Vaginosis (BV) with biological activation and its spores could trigger both secretory and serum immune responses in mice. The virus VP8* protein is linear and conservative and it can cause neutralizing antibodies. It is associated with cell adhesion and effective cell entrance and it consists of most of the epitopes of VP4. The VP8* protein has been identified as a potential subunit vaccine candidate (Li *et al.,* 2019).

Bacillus subtilis-based vaccines are a novel approach to rotavirus immunization that may address issues with live, orally delivered rotavirus vaccines, especially in developing countries. They may also be safer because they do not contain infectious virions (Batista *et al.,* 2014).

As the vaccines are non-replicating and delivered parenterally, biological interference from maternal antibodies and other viral intestinal illnesses is avoided (Oh *et al.,* 2020; Zhang *et al.,* 2020).

Adjuvants for Rotavirus subunit vaccines

Vaccine adjuvants are substances that are co-administered with a vaccine in order to improve the immunogenicity of the vaccine antigens. Adjuvants can help increase the efficacy of rotavirus vaccines more so in low-resource and developing countries, where vaccine take-up and immunogenicity are low (Bastola *et al.,* 2017; Shah *et al.,* 2017).

Vaccine adjuvants such as aluminum salts and oil emulsions have been extensively tested for use with the parenteral inactivated polio vaccine (IPV) in order to minimize vaccine dose and therefore vaccine cost. But with live, oral rotavirus vaccines, few adjuvant candidates have been tested (Chauhan *et al.,* 2017; Mount *et al.,* 2013; Tissera *et al.,* 2017).

Live attenuated vaccines can contain several antigens that also function as adjuvants, whereas non-living rotavirus vaccines cannot. Since these components cannot work optimally, substantial adjuvants are needed to elicit adequate responses (Montomoli *et al.,* 2011; Vetter *et al.,* 2018).

Alum, cholera toxin, LT(R192G), LT(R72), LT(K63), chimeric A1 subunit of cholera toxin (CTA1)-DD, Resiquimod, poly[di(carboxylatophenoxy) phosphazene] (PCPP) and CpG ODN are all potent adjuvants that have shown immunogenicity to

rotavirus subunit vaccines in diverse experimental animals (Istrate *et al.,* 2008; Johansen *et al.,* 2003).

In addition, cholera toxin (CT) and mutant *Escherichia coli* heat-labile toxin (mLT) have also demonstrated immunological adjuvants with RV virus-like particle immunizations(Cimica and Galarza, 2017; Freytag and Clements, 2005; Lightowlers, 2005).

However, bacteria toxins are not suggested as adjuvants for human usage; instead, adjuvants like QS-21 and ISCOMs can be utilized successfully with rotavirus VLPs (Aguilar and Rodríguez, 2007; da Hora *et al.,* 2011).

The ionotropic adjuvant PCPP was successfully used in mice with the rotavirus VP6 protein for the first time. It has recently been studied in mice with rotavirus VLPs. PCPP has also been used as an adjuvant with human viral strains such as HSV, HIV, HBV, and influenza. A single dose of influenza virus with PCPP adjuvant induced adequate immune responses contrary to three doses with the use of QS-21 as an adjuvant (Carvalho and Gill, 2019; El-Attar *et al.,* 2009).

Concluding remarks

Approaches alternative to live attenuated rotavirus vaccines have been investigated in different animal models with great success of inducing strong immune response. It is high time now for different manufacturers to pay attention to these approaches as this will give hope to low-income settings for producing vaccines that are safe and effective. It is better for the ongoing new generation vaccine manufacturers and those who are ready to initiate pre-clinical and clinical trials to continue with strong determination as fast as possible to saving lives in low-income settings. Moreover, the non-living vaccines that are promising and are at advanced stages of clinical trials like P2-VP8-P[8] have to finalize their scientific logistics and get access to the market.

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