Chapter

Human and Veterinary Vaccines against Pathogenic *Escherichia coli*

Mariano Larzábal, Angel A. Cataldi and Daniel A. Vilte

Abstract

Pathogenic *Escherichia coli* constitute an important current problem of public health and animal production. Efforts have been made to fight the infections caused by these bacteria, and in this chapter, we present the progress made up to date in the vaccines generated for this purpose. Different vaccines have been tested against the pathotypes responsible for human diseases such as diarrhea and urinary infections. Also, the poultry market has deserved the effort of the researchers to obtain a product that fights the *E. coli* strains that cause diseases in them. Finally, advances are also presented for the zoonotic enterohemorrhagic *E. coli* (EHEC), which are a different problem due to their low importance as a disease factor in cattle, but they are a very important pathogen in humans. In several of these fields, authorized products have been developed and are currently being marketed.

Keywords: pathogenic *Escherichia coli*, vaccine, human, cattle, virulence factors

1. Introduction

This chapter deals with the current developments on human and veterinary vaccines against pathogenic *Escherichia coli* of following pathotypes: enterohemorrhagic *E. coli* (EHEC) and Shiga toxin producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), extraintestinal pathogenic *E. coli* (ExPEC), in particular uropathogenic *E. coli* (UPEC), and avian pathogenic *E. coli* (APEC). Other pathotypes were not considered because of a lesser development related to vaccines. In some cases, only vaccines tested in the target species (human, cattle, chicken, etc.) were considered due to the high abundance of publications where experimental vaccines were tested on rodent or on other animal models.

2. Vaccines against EHEC/STEC

2.1 Vaccines against EHEC/STEC for humans

Different factors make the development of a vaccine difficult to prevent EHEC/STEC infection and hemolytic uremic syndrome (HUS) in humans. The lack of knowledge about what type of immune response may confer protection, and the multiplicity of infection routes comprising bovine-derived food products, leafy green vegetables, pool or drinking water, person-to-person transmission [1], and the lack of reliable animal models complicate the advance in this field.

Szu and Ahmed developed polysaccharide conjugate vaccines composed of detoxified lipopolysaccharide (LPS) from *E. coli* O157, covalently linked to a carrier protein and a recombinant exoprotein of *Pseudomonas aeruginosa* (rEPA) that has been used for conjugation of polysaccharides and proteins [2]. Phase I and Phase II clinical studies were conducted in adults and in children ranging from 2 to 5 years old, respectively [3]. The *E. coli* O157 conjugate vaccines were safe for all ages, and a positive humoral IgG response with bactericidal activity was found in both age populations. However, there were certain limitations for using LPS-based vaccines. For example, LPS failed to induce a long-lasting humoral immune response especially in children, and STEC non-O157 serotypes were not covered. In one attempt to compensate for this shortcoming, the same group conjugated O-polysaccharide with the B subunit of Shiga toxin (Stx1) [2]. However, this formulation did not neutralize Shiga toxin (Stx2), the toxin type most frequently found in severe HUS cases.

The main virulence factor of STEC/EHEC is the Shiga toxin (Stx); in consequence, it is an optimal target to elicit neutralizing antibodies. Subsequently, various Stx-based vaccine approaches have been attempted. A vaccine consisting of a poly-N-acetylglucosamine (PNAG, a surface polysaccharide of STEC) conjugated to the B subunit of Stx1 was produced. The antibodies raised in rabbit neutralized Stx1 potently, but modestly Stx2. Passive transfer of antibodies indicates that anti-PNAG could confer protection, but the cross-reacting neutralization of Stx2 is limited [4].

To date, no vaccines have been approved for human use, exposing a void in both treatment and prevention of EHEC O157:H7 infections. Vaccine research and development efforts have oriented to cattle as the main reservoir.

2.2 Vaccines against EHEC for cattle

Up to date, different vaccine compositions have been tested to reduce the colonization of the bovine and the environmental dissemination of EHEC O157:H7. These vaccines have different immunogenic, adjuvants, inoculation pathways, number of doses, and of course differ in their development and evaluation level in experimental and natural conditions. In this occasion, we decided to consider the proposals whose capacity of protection was evaluated in cattle.

The key factor for achieving a protective immune response in the animal is the immunogen. Looking for the available literature, we can observe that there are several candidates, mainly colonization factors, which we can classify in: type III secretion system (T3SS) components, siderophore receptors and porin proteins, bacterins, whole-cell envelopes, flagellin, Shiga toxins toxoids, attenuated *Salmonella*, and combinations between more than one of these.

2.2.1 Vaccines based on T3SS components

The components of the T3SS were the first to be used as vaccines, because it was already known for the essential role that proteins such as intimin, Tir, EspA, and EspB play in the adhesion of EHEC O157:H7 to the host cell [5–7]. In 2004, Potter et al. [8] tested a vaccine composed by a protein supernatant of EHEC O157:H7 (containing various Esps and Tir) with the adjuvant VSA3, in animals that were later challenged with *E. coli* O157:H7, as well as in animals in a clinical trial. They observed significant increase in serum antibodies against proteins of T3SS and O157 lipopolysaccharide. There was also a decrease in the number of bacteria in feces, in the number of shedder animals, and in the duration of excretion in the vaccinated group. The clinical trial showed a reduced prevalence of EHEC O157:H7 in typical feedlot conditions when cattle were vaccinated. In 2005, Van Donkersgoed et al. [9]

published a field trial in nine feedlots using a vaccine similar to Potter et al. [8], and they did not observe a significant association between vaccination and pen prevalence of fecal *E. coli* O157:H7. Probably, the differences in the preparation of the secreted proteins, in this case with formalin, a different adjuvant and a different vaccination strategy, could cause the failure. Later, this same preparation, without formalin treatment and with VSA3 adjuvant, was standardized and analyzed in studies in commercial feedlots of beef cattle with a two-dose regimen. The authors evaluated the probability to detect the microorganism from terminal rectal mucosa as a measure of gut colonization [10] and other large-scale clinical trials on commercially fed cattle to test the efficacy of the regimen to reduce the environmental transmission of EHEC O157:H7 [11]. They concluded that the two-dose vaccine regimen was effective to reduce the probability for *E. coli* O157:H7 colonization of the terminal rectum of cattle at slaughter and reduces the probability for environmental transmission of the bacteria within commercial cattle feeding systems [12]. This evidence was accompanied by the generation of a commercial product known as Econiche(TM), which was developed by the Canadian company Bioniche Life Sciences. The vaccine was approved in Canada and the United Kingdom [13, 14] and had a pending conditional license in the U.S. [15], but in 2014, the Bioniche Animal Health business was purchased by Vèntoquinol SA [16], and the production of the vaccine was discontinued.

On the other hand, there were other groups that evaluated recombinant factor of the T3SS in various combinations. Van Diemen et al. [17] evaluated the carboxy-terminal 280 amino acids of intimin γ and β alone or combined with the portions of Efa-1 (EHEC factor for adherence). Immunized calves induced antigen-specific serum IgG and, in some cases, salivary IgA responses, but did not reduce the magnitude or duration of excretion of EHEC O26:H- (intimin β) or EHEC O157:H7 (intimin γ) after an experimental challenge. Similarly, immunization of calves with the truncated Efa-1 protein did not protect against intestinal colonization by EHEC O157:H7.

The vaccination of calves with recombinant EspA by intramuscular and intranasal routes induced high titers of antigen-specific IgG and salivary IgA, but these responses did not protect calves from intestinal colonization after a challenge with *E. coli* O157:H7 [18].

In 2010, McNeilly et al. [19] assessed whether three purified proteins, intimin (C-terminal 531 amino acids), EspA, and Tir, could reduce shedding of EHEC O157:H7. Furthermore, they evaluated if the inclusion of purified H7 flagellin to the vaccine could modify the vaccination efficacy. They used the intramuscular route and the rectal submucosal route and obtained a significant increased response in serum anti-EspA, anti-intimin, and anti-Tir IgG. When H7 flagellin was present, mucosal IgA and IgG anti-H7 was generated. After experimental infection with EHEC O157:H7, cattle showed that immunization with these purified antigens could significantly reduce the total levels of bacterial excretion and that the addition of H7 flagellin can improve this effect. More recently [20], this group optimized the formulation of this vaccine and concluded that the immunization with a combination of EspA, intimin, and H7 flagellin causes a significant reduction in shedding of EHEC O157:H7, more enough to impact on transmission between animals.

Vilte et al. [21] evaluated a vaccine composed by the C-terminal 280 amino acids of intimin γ and EspB. The intramuscular immunization elicited significantly high levels of serum IgG antibodies. Antigen-specific IgA and IgG were also induced in saliva, but only the IgA response was significant. Following experimental challenge with *E. coli* O157:H7, a significant reduction in bacterial shedding, was observed in vaccinated calves.

2.2.2 Vaccines based on siderophor receptors (SRP) and porins proteins

This proposal is based on reducing the ability of the bacterium to obtain iron from the environment to decrease the level of infection [22]. Thornton et al. [23] assessed the efficacy of an SRP-composed vaccine (Epitopix LLC) to reduce the prevalence and fecal excretion of EHEC O157:H7 in calves after an experimental infection. A significant response in serum anti-SRP antibody titers was detected, and they concluded that the vaccination tended to decrease the fecal prevalence and concentration of EHEC O157:H7. In other study [24], this group evaluated the vaccine to control the burden of *E. coli* O157:H7 in feedlot cattle in field conditions. Vaccination with SRP was associated with the reduction of fecal concentration of EHEC O157:H7 and suggested to reduce the burden of these bacteria on cattle. In a third assay, the vaccine was evaluated in feedlot cattle naturally shedding *E. coli* O157. There were two different inoculum volumes of vaccine, 2 and 3 ml. They concluded that SRP vaccine at the 3 ml dose reduced prevalence of *E. coli* O157. These results led to the commercial elaboration of a product known as *E. coli* bacterial extract vaccine with SRP® technology [25] and manufactured by Pfizer Animal Health (Now Zoetis Services LLC). It has conditional license of the U.S. Department of Agriculture.

2.2.3 Vaccines based on bacterins and bacterial envelopes

To evaluate the protection conferred by a bacterin of EHEC O157:H7, van Diemen et al. [17] prepared a formalin-inactivated bacterin from EDL933nalR strain that was inoculated in a combined schedule by intramuscular (with Alu-Oil) and intranasal (mixed with cholera toxin B subunit) routes. It elicited significant IgG responses against intimin and LPS from *E. coli* O157:H7, but did not confer protection against intestinal colonization by EHEC O157:H7 after challenge.

In 2011, Sharma et al. [26] evaluated three heat-inactivated bacterins to reduce the fecal shedding of *E. coli* O157:H7. They used a *hha* + strain of *E. coli* O157:H7 and constructed a *hha* and *hha sepB* deletion mutants. These deletions enhance the expression and intracellular accumulation of T3SS proteins, respectively. There was a significant increase in IgG against LEE-encoded proteins in calves vaccinated with *hha* or *hha sepB* mutant bacterins compared to wild strain, and a reduction in the numbers of animals shedding EHEC O157:H7 and in the duration of the fecal shedding of bacteria in feces was also observed.

An alternative to bacterins was assayed by Vilte et al. [27] by means of empty envelopes of EHEC O157:H7 known as bacterial ghosts (BGs). These envelopes retain all surface components in a nondenatured form. Animals were vaccinated with BGs (without adjuvants) by subcutaneous route and elicited significant levels of specific IgG in serum. Following oral challenge with *E. coli* O157:H7, a significant reduction in both the duration and total bacterial shedding was observed in vaccinated calves.

2.2.4 Vaccines based on flagellin

In 2008, McNeilly et al. [28] assayed a systemic (intramuscular) and mucosal (intrarectal) immunization with purified H7 flagellin to evaluate its effects on the colonization of EHEC O157:H7 after a challenge. The vaccination induced high titers of anti-H7 IgG and IgA antibodies in both serum and nasal secretions by intramuscular injection, but the intrarectal route failed in generating any response against H7. With respect to colonization of EHEC O157:H7, they concluded that

immunization reduced colonization rates and delayed peak shedding, but did not affect total bacterial fecal shedding.

2.2.5 Vaccines based on attenuated Salmonella

In 2010, Khare et al. [29] assessed a live attenuated recombinant *Salmonella enterica* serovar Dublin *aroA* expressing intimin. The recombinant *Salmonella* was inoculated three times by oral route, but this did not produce a significant increase of intimin-specific IgA in serum and feces. Interestingly, they observed a transient clearance of *E. coli* O157:H7 in feces from vaccinated calves that subsequently reduced colonization and shedding of bacteria after an experimental challenge.

2.2.6 Vaccines based on Shiga toxins

An attractive target to research in cattle constitutes the Shiga toxins (Stx), the more important virulence factor for human health. In fact, Stx modulates cellular immune responses in cattle [30–32]. For that, in 2018, Schmidt et al. [33] evaluated the response, in a calf cohort, to immunization with recombinant Shiga toxoids genetically inactivated (rStx1MUT/rStx2MUT). Calves were passively (colostrum from immunized cows) and actively (intramuscularly) vaccinated, and this generated a significant difference in serum antibody titers compared with a control group. There was no EHEC O157:H7 challenge, but the natural presence of fecal STEC was monitored, and they observed less fecal positive (by PCR) samples from calves vaccinated than those from control animals. It is interesting because this investigation was not restricted to a determined serotype of EHEC.

In other study, Martorelli et al. [34] combined recombinant intimin and EspB with the B subunit of Stx2 fused to *Brucella* lumazine synthase (BLS-Stx2B) in order to evaluate whether the presence of Stx was able to improve the effect of the vaccine on fecal shedding of EHEC O157:H7 following an experimental inoculation. The immunization generates antibodies against Stx2B in serum and intestinal mucosa, but a superior level of protection compared with the use of intimin and EspB alone was not observed.

As was seen, there were and there are numerous efforts looking for a solution to reduce the contamination of cattle and its environment for EHEC O157:H7 and other dangerous serotypes too. Even two commercial products have been achieved, one of which has unfortunately been removed from the market. However, the fact that this pathogen does not constitute a direct problem for farmers, and because EHEC are not a cause of severe illness in cattle, makes our work more challenging. We have not only to find an adequate immunogen or formulation or doses that have a good response, but it must also be attractive enough for farmers to take it as a possible and desirable alternative to collaborate with one health perspective.

3. Vaccines against ETEC

ETEC is one of the leading bacteria that causes 200 million diarrheal cases and between 170,000 and 380,000 deaths annually in the world [35, 36]. Children under 5 years of age in developing countries are the most affected by ETEC infections and 42,000 deaths have been reported only in 2013 [37]. As well, ETEC infections are the main cause of diarrhea reported in persons who travel to Latin America, Africa, and Asia [38], where approximately 10 million traveler's diarrhea cases have been reported worldwide per year [39, 40].

There have been several attempts to obtain a vaccine against ETEC. The greatest efforts have been focused on virulence factors such as fimbriae called colonization factor antigens (CFA) and colonization surface antigens (CS) and two enterotoxins, heat-labile (LT) and heat-stable (ST). These virulence factors are extremely important during the pathogenesis of ETEC. CFA promote the attachment to enterocytes in the small intestine and are critical for colonization. After the attachment, ETEC releases LT and/or ST enterotoxins that disrupt fluid and cause electrolyte homeostasis in small intestinal epithelial cells [41]. Therefore, a vaccine directed against CFA could prevent the adherence and intestinal colonization, avoiding the subsequent release of enterotoxins by ETEC. Although 23 immunologically distinct CFA adhesins have been identified, its high variation present in the different circulating strains worldwide has prevented the development of a protective vaccine [42–44]. Studies of killed whole-cell vaccines demonstrate the development of colonization factor antigen I (CFA/I) and LT IgA antibodies but only were protective against homologous strains [45, 46]. To date, isolated ETEC can be divided into 42 different clonal groups with a singular combination of colonization factors (CFs) and toxins [47]. Alternative approaches of CS targets have been evaluated. CFA/I fimbria, CS3, CS5, and CS6 are immunologically related to the more prevalent CFs covering a 50–80% of the clinical ETEC isolates. ACE527 and rCTB-CF are two whole-cell vaccines that include a wide repertory of CFs. Five CFA adhesins (CFA/I, CS2, CS3, CS5, and CS6), one CFA subunit (CS1), and the LT-B subunit compose the ACE527 vaccine, represented by three live attenuated ETEC strains [48, 49]. The orally inoculated ACE527 protects challenged adults with homologous strains [49, 50]; however, it had adverse effects on volunteers [51]. The rCTB-CF vaccine is composed by five formalin-killed ETEC strains, which presents CFA/I, CS1, CS2, CS3, CS4, and CS5 adhesins supplemented with recombinant B subunit of the cholera toxin (rCTB) [52, 53]. The immune response induced by rCTB-CF vaccine showed to reduce the risk of developing diarrhea in adult travelers [54], but presented little protection and some adverse effects in young children [55, 56]. Despite the improvements made to rCTB-CF and ACE527 [50, 51, 57], these vaccines fail to protect against some ETEC strains since they do not contain the heat-stable class a(STa) or LT-A antigens.

Neutralizing the effects of these enterotoxins is considered a highly effective approach for preventing ETEC diarrhea. However, the development of vaccines from toxoids has not presented satisfactory results either. Both LT and ST are potent toxins; therefore, no toxin can be used directly as a vaccine antigen. However, detoxified derivatives of LT including the B subunit (not toxic LT-B) have demonstrated immunological properties even as an adjuvant in many animal models [58–60]. The A subunit is also included in studies of ETEC LT (LT-A) vaccine. The purpose of this incorporation is to induce a mostly protective immune response [61, 62]. On the other hand, STa unlike LT is poorly immunogenic due to its small size.

Recent progress in toxoids antigens enhances the potential for developing an effective and safe subunit vaccine against ETEC diarrhea. A skin path vaccine containing LT toxin was applied to humans. Immunized adults developed strong IgG and IgA antibody responses to LT [63, 64], which reduced the incidence of moderate-to-severe diarrhea caused by ETEC in healthy adults traveling to Mexico or Guatemala [65]. A secondary study demonstrated that the LT patch provided protection against LT + ETEC diarrhea but provided no protection against STa + ETEC [66]. Therefore, the use of the LT patch alone cannot be considered a suitable approach for vaccinating against ETEC [67].

Subunit vaccine from a mutant LT toxin (mLT) has been proposed. Although it is safer than LT, up to now, mLT has not demonstrated a wide efficacy in the

protection against diarrhea caused by ETEC [66]. However, it has been explored mainly as a vaccine adjuvant. mLT demonstrated a higher protective efficacy of vaccine candidates for whole cell ETEC and a CFA + candidate adhesin subunit vaccine [68]. Therefore, its function as adjuvant favors a greater response of the candidate as well as allows the generation of anti-LT response.

Most of the ETEC strains isolated from patients with diarrhea are STa+ alone or LT+. The low immunogenicity and the high need to generate an immune response against STa led the researcher to develop mLT-STa fusions. Results of mouse immunization studies showed that LT-STaN12S toxoid fusion induces neutralizing anti-STa antibodies [69]. The high titer in mice presented against both toxoids makes it a promising antitoxin subunit vaccine.

Alternative adhesion tip of the CfaE and multiepitope fusion antigen (MEFA) were used as a conservative antigen for the development of a broadly protective ETEC antiadhesin vaccine [70]. Nonhuman primate immunized with CfaE showed protection against a CFA/I ETEC challenge [71]. However, the coadministration of CfaE and mLT did not protect against ETEC strains expressing Sta. MEFA is represented by epitopes from the seven most important CFA adhesins expressed by ETEC strains which was strongly immunogenic inducing high titers of antibodies specific to all adhesins [72]. This combination is an efficient means of developing a vaccine for antigenically heterogeneous pathogens like ETEC.

Novel antigens, such as the glycoprotein EtpA and the outer membrane adhesin EaeH, have been identified by genome sequencing [73]. Antibodies against EtpA demonstrated a significant reduction in the colonization of mice by the challenge ETEC strain (H10407) [74]. The identification of new antigens could be the way to incorporate epitopes that allow a greater range of protection against the different ETEC strains. These new epitopes, incorporated into the candidate vaccines that contain the most conserved and representative virulence factors of ETEC, could enhance the protection against diarrhea caused by ETEC.

ETEC is the most common cause of *E. coli* diarrhea in farm animals, and in the first four days of calves, life can be responsible for severe diarrhea with high mortality [75]. The strains are characterized by the surface adhesins fimbriae being F5, F7, and F17, more frequently involved in diarrhea in calves [76–79]. In addition, CS31 adhesin is prevalent on isolates from calves with *E. coli* septicemia [80, 81]. In regards to toxins, STa is the only toxin associated with disease in neonatal calves infected with ETEC [82], rarely LT are identified [76, 83]. Killed ETEC possessing F5-fimbriae or purified F5 fimbriae are contained in the commercial vaccines for calves. These vaccines do not contain F17, CS31, or STa; however, the impact of their absence is unknown. The maternal vaccination with these vaccines protects the neonatal ETEC infections by passive colostral and lactogenic immunity [84, 85]. Once the lactation stage is over, the cattle being more resistant [86]. In this way, vaccination dams are an effective strategy to prevent ETEC diarrhea in neonates calves [87, 88].

4. Vaccines against ExPEC

ExPEC causes a vast majority of urinary tract infections (UTIs), mostly in women with highly common recurrent episodes. ExPEC pathotypes causing UTI are called uropathogenic *E. coli* (UPEC). A recent review of Nesta and Pizza describes progresses in UPEC vaccines [89]. Most of the vaccines are aimed to stimulate the mucosal immune system. Initial attempts to the development of vaccines against ExPEC infections have been unsuccessful [90, 91]. The immunogen in these vaccine

was single-purified virulence factors such as hemolysin [92], pilin, or the O-specific polysaccharide LPS, conjugated to either *Pseudomonas aeruginosa* endotoxin A (TA) or cholera toxin (CT) as carrier proteins [93, 94]. Because of high heterogeneity of O-specific polysaccharide, the design of a polysaccharide vaccine able to prevent ExPEC infections has been extremely challenging [95]. The O18-polysaccharide conjugated to either cholera toxin or to *P. aeruginosa* exoprotein A (EPA) was safe and able to induce antibodies with opsonophagocytic killing activity (OPK) in human volunteers. IgG purified from immunized individuals were protective in mice in an *E. coli* O18 challenge sepsis model [93]. However, a further test with a 12-valent O-antigen showed difficulties of cross protection.

Three vaccines against UTI reached market status in different countries. Vaccines based on whole or lysed fractions of inactivated *E. coli* have been evaluated in human clinical trials and have been so far the most effective in inducing some degree of protection in patients with recurrent urinary tract infections. The sublingual vaccine Uromune, an inactivated whole preparation of *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Enterococcus faecalis*, evaluated as prophylactic treatment in a multicenter retrospective observational study, demonstrated a certain degree of clinical benefit in terms of reduced recurrence rate in women suffering recurrent UTI [96].

The Solco Urovac vaccine, a vaginal suppository polymicrobial vaccine consisting of 10 inactivated uropathogenic bacteria, including six *E. coli* serotypes, *Proteus mirabilis*, *Morganella morganii*, *K. pneumoniae*, and *E. faecalis* strains, showed a minimal efficacy in Phase I and two Phase II trials in women suffering of recurrent UTIs [97–99]. However, in two additional clinical studies, the vaginal mucosal vaccine given for a 14-week period increased the time to reinfection in UTI susceptible women, representing a valuable alternative to the antibiotic-based prophylactic regimens [98, 100].

One of the first vaccine tested was based on *E. coli* extract was presented by Frey et al. [101]. This development lead to Uro-Vaxom, a commercial vaccine that was assessed in larger clinical trials a few years later [102] leading to the recommendation of Uro-Vaxom for prophylactic treatment of patients with recurrent urinary tract infections. OM-89/Uro-Vaxom vaccine demonstrated modest protection in women [103]. However, in a more recent trial on 451 female subjects, the lyophilized lysate of 18 *E. coli* strains, OM-89/Uro-Vaxom, manufactured using a modified lytic process, based on alkaline chemical lysis and autolysis, failed to show a preventive effect on recurrent uncomplicated UTIs [104].

Other vaccines reached clinical trial status. The development of ExPEC4V, a novel tetravalent bioconjugate vaccine developed by Glaxo Smith Kline against extraintestinal pathogenic *E. coli*, started by an epidemiological screening of the prevalent *E. coli* serotypes causing infection in women in Switzerland, Germany, and the USA. The authors selected the O antigens from LPS from the prevalent serotypes. It was evaluated for safety, immunogenicity, and clinical efficacy in placebo-controlled phase Ib trial [105]. By glycoengineering, the O antigens were conjugated in *E. coli*. The vaccine was well tolerated and elicited a robust antibody response in patients suffering from recurrent UTIs. Data indicated a reduced incidence of UTIs after vaccination, especially for higher bacterial loads. Clinical trial was performed in a population of healthy women with a history of recurrent UTI allowed for an additional, preliminary assessment of the candidate's clinical efficacy. In a multicenter Phase Ib clinical trial, 92 healthy adult women with a history of recurrent UTI received a single injection of either intramuscular ExPEC4V or placebo. The authors concluded that the tetravalent *E. coli* bioconjugate vaccine candidate was well tolerated and elicited functional antibody responses against all vaccine serotypes [106].

Mobley et al. investigated four defined antigens (IreA, Hma, IutA, and FyuA) associated with iron uptake, as an immunogen to prevent UTI [107]. The adjuvant used was cholera toxin. They tested the formulation in mice and observed antigen-specific IgG response. High antibody titers correlate with low colony forming units (CFUs) of UPEC following transurethral challenge of vaccinated mice. In addition, sera from women with and without histories of UTI have been tested for antibody levels to vaccine antigens. They indicated that iron uptake components are a suitable target for vaccination against UTI. Later, it was observed that the iron receptor FyuA is present in 77% and it is highly conserved among UPEC isolates [108]. FyuA immunization of mice reduced the colonization of UPEC in bladder and kidney. Adhesins and bacterial appendages as flagella have a long history as immunogenic single antigens component of experimental vaccines against UTI. FliC (or pilin) and FimH (from type 1 fimbriae) were administered to mice as a fusion or mixed and elicited higher levels of serum and mucosal. Different combinations and adjuvants elicited good protection against UPEC [109].

5. Vaccines against APEC

APEC that belongs to the ExPEC pathotype is a major causative agent of colibacillosis, aerosacculitis, polyserositis, septicaemia, and other diseases in chickens, turkeys, and other avian species. It is responsible for significant loss for the poultry industry. Main APEC serogroups associated with disease are O1, O2, and O78.

An ideal vaccine for poultry has to be able to induce cross protection against various APEC serogroups capable of causing disease. To be deliverable via a massive immunization method such as administering the antigens in drinking water or feed, *in ovo* and spray, in order to immunize thousands of broiler chickens, must be used. And, the vaccine has to be administered at a young age so that the birds develop a protective immune response by the age of 21 days when they are most vulnerable to APEC infection [110].

Inactivated bacterin vaccines or autovaccines of APEC are frequently used in the field, but their protective efficacy was not demonstrated. Landman and van Eck studied the protection conferred in laying hens against *E. coli* peritonitis syndrome (EPS) disease. Vaccines were formulated either as aqueous suspension or as water-in-oil induced protection against homologous challenge, while protection against heterologous challenge was inconclusive. However, other study [111] indicated no protection against a challenge with homologous or heterologous strain, in spite of a raise of IgY titer in vaccinated animals.

A recombinant *Salmonella enterica* serovar *Typhimurium* strains expressing the heterologous O polysaccharide of *E. coli* O1 and O2 was used to immunize chickens and elicited production of serum IgG and mucosal sIgA antibodies against the LPS of APEC O1 and O2. The immune response induced resulted protective against a lethal dose of both APEC serogroup strains [112]. An attenuated *Salmonella* (*Δlon*, *ΔcpxR*, and *ΔasdA16*) delivery system containing the genes encoding P-fimbriae (*papA* and *papG*), aerobactin receptor (*iutA*), and CS31A surface antigen (*clpG*) of APEC was constructed, and its potential as a vaccine candidate against APEC infection in chickens was evaluated. It induced an immune response and an effective protection against colibacillosis caused by APEC [113].

Mixed recombinant APEC surface proteins EtsC (a type I secretion system protein), the porins OmpA and OmpT, and TraT of APEC were used as antigens to immunize chickens seeking for a broad protection against several serotypes of APEC. The experimental vaccine elicited specific IgY and the induction of diverse cytokines in spleen and resulted in a reduction of lesion scores in different organs and a reduction of bacterial loads in blood and organs [114].

A commercial vaccine (Gall N tect CBL) against avian colibacillosis for layer hens is produced and marketed in Japan since 2012. It consists of a live attenuated O78 APEC with a *Δcrp* deletion. A big trial in layer hens [115, 116] demonstrated that it prevents avian colibacillosis infection and improves productivity. Live attenuated APEC strains were used as experimental vaccines for various research groups in colibacillosis fields. Strains deleted in *aroA* [117], *carAB* [118], and *galE* [119] were tested. Another commercial vaccine, based in subunit components, is Nobilis (MSD) composed by F11-and FT-antigens of APEC in a water-in-oil emulsion. No trials have reported by the company, but Gregersen et al. in 2010 [120] observed that in a controlled trial the vaccine application did not affect the overall mortality rate between the vaccinated and control flocks, but mortality due to *E. coli* infections made up only 8.2% in vaccinated birds compared with 24.6% in unvaccinated birds. Also, differences in average first week mortality, average weight at 38 days, and food conversion rate among vaccinated and control birds, respectively, were not found.

6. Conclusion

A high interest in the development of vaccines against pathogenic *E. coli* occurred in recent years. This interest is related both to pathotypes affecting human and animal health. Few vaccines have been licensed and reached market and public health status. There is an intrinsic difficulty in directing the immune response to a bacterial species that is commonly part of the animal microbiota. The state of the art consists in identifying antigenic components that are exclusive of pathogenic subtypes.

In spite of these difficulties, science has gained a relevant knowledge of virulence, pathogenicity, genomics, and epidemiology of pathogenic *E. coli,* and with no doubt this will benefit vaccinology concerning pathogenic *E. coli*.

Conflict of interest

The authors declare no conflict of interest.

Author details

Mariano Larzábal[1], Angel A. Cataldi[1*] and Daniel A. Vilte[2]

1 Institute of Biotechnology, IABIMO-CONICET, Center of Veterinary and Agronomical Research, National Institution of Agricultural Technology, Argentina

2 Institute of Pathobiology, IPVet-CONICET, Center of Veterinary and Agronomical Research, National Institution of Agricultural Technology, Argentina

*Address all correspondence to: cataldi.angeladrian@inta.gob.ar

References

[1] Rivas M, Chinen I, Miliwebsky E, Masana M. Risk factors for Shiga toxin-producing *Escherichia coli*-associated human diseases. Microbiology Spectrum. 2014;**2**. DOI: 10.1128/microbiolspec.EHEC-0002-2013

[2] Szu SC, Ahmed A. Clinical studies of *Escherichia coli* O157:H7 conjugate vaccines in adults and young children. Microbiology Spectrum. 2014;**2**. DOI: 10.1128/microbiolspec.EHEC-0016-2013

[3] Ahmed A, Li J, Shiloach Y, Robbins JB, Szu SC. Safety and Immunogenicity of *Escherichia coli* O157 O-specific polysaccharide conjugate vaccine in 2-5-year-old children. The Journal of Infectious Diseases. 2006;**193**:515-521. DOI: 10.1086/499821

[4] Lu X, Skurnik D, Pozzi C, Roux D, Cywes-Bentley C, Ritchie JM, et al. A poly-N-acetylglucosamine-Shiga toxin broad-spectrum conjugate vaccine for Shiga toxin-producing *Escherichia coli*. MBio. 2014;**5**:e00974-e00914. DOI: 10.1128/mBio.00974-14

[5] DeVinney R, Stein M, Reinscheid D, Abe A, Ruschkowski S, Finlay BB. Enterohemorrhagic *Escherichia coli* O157:H7 produces Tir, which is translocated to the host cell membrane but is not tyrosine phosphorylated. Infection and Immunity. 1999;**67**:2389-2398

[6] Tzipori S, Gunzer F, Donnenberg MS, de Montigny L, Kaper JB, Donohue-Rolfe A. The role of the eaeA gene in diarrhea and neurological complications in a gnotobiotic piglet model of enterohemorrhagic *Escherichia coli* infection. Infection and Immunity. 1995;**63**:3621-3627

[7] Dean-Nystrom EA, Bosworth BT, Moon HW, O'Brien AD. *Escherichia coli* O157:H7 requires intimin for enteropathogenicity in calves. Infection and Immunity. 1998;**66**:4560-4563

[8] Potter AA, Klashinsky S, Li Y, Frey E, Townsend H, Rogan D, et al. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. Vaccine. 2004;**22**:362-369. DOI: 10.1016/j.vaccine.2003.08.007

[9] Van Donkersgoed J, Hancock D, Rogan D, Potter AA. *Escherichia coli* O157:H7 vaccine field trial in 9 feedlots in Alberta and Saskatchewan. The Canadian Veterinary Journal. 2005;**46**:724-728

[10] Smith DR, Moxley RA, Peterson RE, Klopfenstein TJ, Erickson GE, Bretschneider G, et al. A two-dose regimen of a vaccine against type III secreted proteins reduced *Escherichia coli* in beef cattle in commercial feedlots. Foodborne Pathogens and Disease. 2009;**6**:155-161

[11] Smith D, Moxley R, Peterson R, Klopfenstein T, Erickson G, Clowser S. A two-dose regimen of a vaccine against *Escherichia coli* O157:H7 type III secreted proteins reduced environmental transmission of the agent in a large-scale commercial beef feedlot clinical trial. Foodborne Pathogens and Disease. 2008;**5**:589-598. DOI: 10.1089/fpd.2008.0080

[12] Smith DR, Moxley RA, Klopfenstein TJ, Erickson GE. A randomized longitudinal trial to test the effect of regional vaccination within a cattle feedyard on *Escherichia coli* O157:H7 rectal colonization, fecal shedding, and hide contamination. Foodborne Pathogens and Disease. 2009;**6**:885-892. DOI: 10.1089/fpd.2009.0299

[13] *E. coli* Vaccine Gets Full CFIA License | Canadian Cattlemen n.d. https://www.canadiancattlemen.ca/

daily/e-coli-vaccine-gets-full-cfia-license [Accessed: October 8, 2018]

[14] UK Approves *E. coli* O157:H7 Vaccine for Cattle | Food Safety News n.d. https://www.foodsafetynews.com/2012/08/uk-approves-e-coli-o157h7-vaccine-for-cattle/ [Accessed: October 8, 2018]

[15] Econiche(TM) Vaccine Efficacy Challenge Study Summarized in The Canadian Journal of Veterinary Research n.d. https://www.prnewswire.com/news-releases/econichetm-vaccine-efficacy-challenge-study-summarized-in-the-canadian-journal-of-veterinary-research-121343543.html [Accessed: October 8, 2018]

[16] Vétoquinol to Acquire the Animal Health Business of Bioniche Life Sciences inc. | Vetoquinol n.d. https://www.vetoquinol.com/eng/content/vetoquinol-acquisition-animal-health-business-bioniche-life-sciences [Accessed: October 8, 2018]

[17] van Diemen P, Dziva F, Abu-Median A, Wallis T, van den Bosch H, Dougan G, et al. Subunit vaccines based on intimin and Efa-1 polypeptides induce humoral immunity in cattle but do not protect against intestinal colonisation by enterohaemorrhagic *Escherichia coli* O157:H7 or O26:H. Veterinary Immunology and Immunopathology. 2007;**116**:47-58. DOI: 10.1016/j.vetimm.2006.12.009

[18] Dziva F, Vlisidou I, Crepin VF, Wallis TS, Frankel G, Stevens MP. Vaccination of calves with EspA, a key colonisation factor of *Escherichia coli* O157:H7, induces antigen-specific humoral responses but does not confer protection against intestinal colonisation. Veterinary Microbiology. 2007;**123**:254-261. DOI: 10.1016/j.vetmic.2007.02.016

[19] McNeilly TN, Mitchell MC, Rosser T, McAteer S, Low JC, Smith DGE, et al. Immunization of cattle with a combination of purified intimin-531, EspA and Tir significantly reduces shedding of *Escherichia coli* O157:H7 following oral challenge. Vaccine. 2010;**28**:1422-1428. DOI: 10.1016/j.vaccine.2009.10.076

[20] McNeilly TN, Mitchell MC, Corbishley A, Nath M, Simmonds H, McAteer SP, et al. Optimizing the protection of cattle against *Escherichia coli* O157:H7 colonization through immunization with different combinations of H7 flagellin, Tir, intimin-531 or EspA. PLoS One. 2015;**10**:e0128391

[21] Vilte DA, Larzábal M, Garbaccio S, Gammella M, Rabinovitz BC, Elizondo AM, et al. Reduced faecal shedding of *Escherichia coli* O157:H7 in cattle following systemic vaccination with γ-intimin C280 and EspB proteins. Vaccine. 2011;**29**:3962-3968. DOI: 10.1016/j.vaccine.2011.03.079

[22] Emery D, Straub D, Huisinga R, Carlson B. Active immunization using a siderophore receptor protein. 6,027,736; 2000

[23] Thornton A, Thomson D, Loneragan G, Fox J, Burkhardt D, Emery D, et al. Effects of a siderophore receptor and porin proteins-based vaccination on fecal shedding of *Escherichia coli* O157:H7 in experimentally inoculated cattle. Journal of Food Protection. 2009;**72**:866-869

[24] Thomson DU, Loneragan GH, Thornton AB, Lechtenberg KF, Emery DA, Burkhardt DT, et al. Use of a siderophore receptor and porin proteins-based vaccine to control the burden of *Escherichia coli* O157:H7 in feedlot cattle. Foodborne Pathogens and Disease. 2009;**6**:871-877. DOI: 10.1089/fpd.2009.0290

[25] *E. coli* Bacterial Extract vaccine with SRP® technology. n.d. https://

www.zoetisus.com/_locale-assets/mcm-portal-assets/services/documents/srpecoli/srp_ecoli_conditional_license_study_tech_bulletin_final.pdf

[26] Sharma VK, Dean-Nystrom EA, Casey TA. Evaluation of *hha* and *hha sepB* mutant strains of *Escherichia coli* O157:H7 as bacterins for reducing *E. coli* O157:H7 shedding in cattle. Vaccine. 2011;**29**:5078-5086. DOI: 10.1016/j.vaccine.2011.04.073

[27] Vilte DA, Larzábal M, Mayr UB, Garbaccio S, Gammella M, Rabinovitz BC, et al. A systemic vaccine based on *Escherichia coli* O157:H7 bacterial ghosts (BGs) reduces the excretion of *E. coli* O157:H7 in calves. Veterinary Immunology and Immunopathology. 2012;**146**:169-176. DOI: 10.1016/j.vetimm.2012.03.002

[28] McNeilly TN, Naylor SW, Mahajan A, Mitchell MC, McAteer S, Deane D, et al. *Escherichia coli* O157:H7 colonization in cattle following systemic and mucosal immunization with purified H7 flagellin. Infection and Immunity. 2008;**76**:2594-2602. DOI: 10.1128/IAI.01452-07

[29] Khare S, Alali W, Zhang S, Hunter D, Pugh R, Fang FC, et al. Vaccination with attenuated *Salmonella enterica* Dublin expressing *E. coli* O157:H7 outer membrane protein Intimin induces transient reduction of fecal shedding of E coli O157:H7 in cattle. BMC Veterinary Research. 2010;**6**:35. DOI: 10.1186/1746-6148-6-35

[30] Stamm I, Mohr M, Bridger PS, Schröpfer E, König M, Stoffregen WC, et al. Epithelial and mesenchymal cells in the bovine colonic mucosa differ in their responsiveness to *Escherichia coli* Shiga toxin 1. Infection and Immunity. 2008;**76**:5381-5391

[31] Moussay E, Stamm I, Taubert A, Baljer G, Menge C. *Escherichia coli* Shiga toxin 1 enhances il-4 transcripts in bovine ileal intraepithelial lymphocytes. Veterinary Immunology and Immunopathology. 2006;**113**:367-382. DOI: 10.1016/j.vetimm.2006.06.007

[32] Menge C, Wieler LH, Schlapp T, Baljer G. Shiga toxin 1 from *Escherichia coli* blocks activation and proliferation of bovine lymphocyte subpopulations in vitro. Infection and Immunity. 1999;**67**:2209-2217

[33] Schmidt N, Barth SA, Frahm J, Meyer U, Dänicke S, Geue L, et al. Decreased STEC shedding by cattle following passive and active vaccination based on recombinant *Escherichia coli* Shiga toxoids. Veterinary Research. 2018;**49**:1-15. DOI: 10.1186/s13567-018-0523-0

[34] Martorelli L, Garimano N, Fiorentino GA, Vilte DA, Garbaccio SG, Barth SA, et al. Efficacy of a recombinant intimin, EspB and Shiga toxin 2B vaccine in calves experimentally challenged with *Escherichia coli* O157:H7. Vaccine. 2018;**36**:3949-3959. DOI: 10.1016/j.vaccine.2018.05.059

[35] Isidean SD, Riddle MS, Savarino SJ, Porter CK. A systematic review of ETEC epidemiology focusing on colonization factor and toxin expression. Vaccine. 2011;**29**:6167-6178. DOI: 10.1016/j.vaccine.2011.06.084

[36] Chakraborty S, Harro C, DeNearing B, Ram M, Feller A, Cage A, et al. Characterization of mucosal immune responses to enterotoxigenic *Escherichia coli* vaccine antigens in a human challenge model: Response profiles after primary infection and homologous rechallenge with strain H10407. Clinical and Vaccine Immunology. 2016;**23**:55-64. DOI: 10.1128/CVI.00617-15

[37] Lanata CF, Fischer-Walker CL, Olascoaga AC, Torres CX, Aryee MJ, Black RE, et al. Global causes of diarrheal disease mortality in children

<5 years of age: A systematic review. PLoS One. 2013;**8**:e72788. DOI: 10.1371/journal.pone.0072788

[38] Lamberti LM, Bourgeois AL, Fischer Walker CL, Black RE, Sack D. Estimating diarrheal illness and deaths attributable to *Shigellae* and enterotoxigenic *Escherichia coli* among older children, adolescents, and adults in South Asia and Africa. PLoS Neglected Tropical Diseases. 2014;**8**:e2705. DOI: 10.1371/journal.pntd.0002705

[39] Svennerholm A-M. From cholera to enterotoxigenic *Escherichia coli* (ETEC) vaccine development. The Indian Journal of Medical Research. 2011;**133**:188-196

[40] Zhang W, Sack DA. Current progress in developing subunit vaccines against enterotoxigenic *Escherichia coli*-associated diarrhea. Clinical and Vaccine Immunology. 2015;**22**:983-991. DOI: 10.1128/CVI.00224-15

[41] Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews. 1998;**11**:142-201

[42] Gaastra W, Svennerholm AM. Colonization factors of human enterotoxigenic *Escherichia coli* (ETEC). Trends in Microbiology. 1996;**4**:444-452

[43] Wolf MK. Occurrence, distribution, and associations of O and H serogroups, colonization factor antigens, and toxins of enterotoxigenic *Escherichia coli*. Clinical Microbiology Reviews. 1997;**10**:569-584

[44] Qadri F, Svennerholm A-M, Faruque ASG, Sack RB. Enterotoxigenic *Escherichia coli* in developing countries: Epidemiology, microbiology, clinical features, treatment, and prevention. Clinical Microbiology Reviews. 2005;**18**:465-483. DOI: 10.1128/CMR.18.3.465-483.2005

[45] Evans DJ, Evans DG, Opekun AR, Graham DY. Immunoprotective oral whole cell vaccine for enterotoxigenic *Escherichia coli* diarrhea prepared by in situ destruction of chromosomal and plasmid DNA with colicin E2. FEMS Microbiology Immunology. 1988;**1**:9-18

[46] Evans DG, Evans DJ, Opekun AR, Graham DY. Non-replicating oral whole cell vaccine protective against enterotoxigenic *Escherichia coli* (ETEC) diarrhea: Stimulation of anti-CFA (CFA/I) and anti-enterotoxin (anti-LT) intestinal IgA and protection against challenge with ETEC belonging to heter. FEMS Microbiology Immunology. 1988;**1**:117-125

[47] Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic *Escherichia coli*. Clinical Microbiology Reviews. 2013;**26**:822-880. DOI: 10.1128/CMR.00022-13

[48] Turner AK, Terry TD, Sack DA, Londono-Arcila P, Darsley MJ. Construction and characterization of genetically defined aro omp mutants of enterotoxigenic *Escherichia coli* and preliminary studies of safety and immunogenicity in humans. Infection and Immunity. 2001;**69**:4969-4979. DOI: 10.1128/IAI.69.8.4969-4979.2001

[49] Harro C, Sack D, Bourgeois AL, Walker R, DeNearing B, Feller A, et al. A combination vaccine consisting of three live attenuated enterotoxigenic *Escherichia coli* strains expressing a range of colonization factors and heat-labile toxin subunit B is well tolerated and immunogenic in a placebo-controlled double-blind phase I trial in healthy adults. Clinical and Vaccine Immunology. 2011;**18**:2118-2127. DOI: 10.1128/CVI.05342-11

[50] Darsley MJ, Chakraborty S, DeNearing B, Sack DA, Feller A, Buchwaldt C, et al. The oral, live

attenuated enterotoxigenic *Escherichia coli* vaccine ACE527 reduces the incidence and severity of diarrhea in a human challenge model of diarrheal disease. Clinical and Vaccine Immunology. 2012;**19**:1921-1931. DOI: 10.1128/CVI.00364-12

[51] Harro C, Chakraborty S, Feller A, DeNearing B, Cage A, Ram M, et al. Refinement of a human challenge model for evaluation of enterotoxigenic *Escherichia coli* vaccines. Clinical and Vaccine Immunology. 2011;**18**:1719-1727. DOI: 10.1128/CVI.05194-11

[52] Ahrén C, Jertborn M, Svennerholm AM. Intestinal immune responses to an inactivated oral enterotoxigenic *Escherichia coli* vaccine and associated immunoglobulin A responses in blood. Infection and Immunity. 1998;**66**:3311-3316

[53] Jertborn M, Ahrén C, Holmgren J, Svennerholm AM. Safety and immunogenicity of an oral inactivated enterotoxigenic *Escherichia coli* vaccine. Vaccine n.d. 1988;**16**:255-260

[54] Sack DA, Shimko J, Torres O, Bourgeois AL, Francia DS, Gustafsson B, et al. Randomised, double-blind, safety and efficacy of a killed oral vaccine for enterotoxigenic *E. coli* diarrhoea of travellers to Guatemala and Mexico. Vaccine. 2007;**25**:4392-4400. DOI: 10.1016/j.vaccine.2007.03.034

[55] Savarino SJ, Hall ER, Bassily S, Wierzba TF, Youssef FG, Peruski LF, et al. Introductory evaluation of an oral, killed whole cell enterotoxigenic *Escherichia coli* plus cholera toxin B subunit vaccine in Egyptian infants. The Pediatric Infectious Disease Journal. 2002;**21**:322-330

[56] Svennerholm A-M, Tobias J. Vaccines against enterotoxigenic *Escherichia coli*. Expert Review of Vaccines. 2008;**7**:795-804. DOI: 10.1586/14760584.7.6.795

[57] Holmgren J, Bourgeois L, Carlin N, Clements J, Gustafsson B, Lundgren A, et al. Development and preclinical evaluation of safety and immunogenicity of an oral ETEC vaccine containing inactivated *E. coli* bacteria overexpressing colonization factors CFA/I, CS3, CS5 and CS6 combined with a hybrid LT/CT B subunit antigen, administ. Vaccine. 2013;**31**:2457-2464. DOI: 10.1016/j.vaccine.2013.03.027

[58] Giuliani MM, Del Giudice G, Giannelli V, Dougan G, Douce G, Rappuoli R, et al. Mucosal adjuvanticity and immunogenicity of LTR72, a novel mutant of *Escherichia coli* heat-labile enterotoxin with partial knockout of ADP-ribosyltransferase activity. The Journal of Experimental Medicine. 1998;**187**:1123-1132. DOI: 10.1084/jem.187.7.1123

[59] Pizza M, Giuliani MM, Fontana MR, Monaci E, Douce G, Dougan G, et al. Mucosal vaccines: Non toxic derivatives of LT and CT as mucosal adjuvants. Vaccine. 2001;**19**:2534-2541

[60] Norton EB, Lawson LB, Freytag LC, Clements JD. Characterization of a mutant *Escherichia coli* heat-labile toxin, LT(R192G/L211A), as a safe and effective oral adjuvant. Clinical and Vaccine Immunology. 2011;**18**:546-551. DOI: 10.1128/CVI.00538-10

[61] Norton EB, Branco LM, Clements JD. Evaluating the A-subunit of the heat-labile toxin (LT) as an immunogen and a protective antigen against enterotoxigenic *Escherichia coli* (ETEC). PLoS One. 2015;**10**:e0136302. DOI: 10.1371/journal.pone.0136302

[62] Norton EB, Lawson LB, Mahdi Z, Freytag LC, Clements JD. The A subunit of *Escherichia coli* heat-labile enterotoxin functions as a mucosal adjuvant and promotes IgG2a, IgA, and Th17 responses to vaccine antigens. Infection and Immunity. 2012;**80**:2426-2435. DOI: 10.1128/IAI.00181-12

[63] Glenn GM, Villar CP, Flyer DC, Bourgeois AL, McKenzie R, Lavker RM, et al. Safety and immunogenicity of an enterotoxigenic *Escherichia coli* vaccine patch containing heat-labile toxin: Use of skin pretreatment to disrupt the stratum corneum. Infection and Immunity. 2007;**75**:2163-2170. DOI: 10.1128/IAI.01740-06

[64] Güereña-Burgueño F, Hall ER, Taylor DN, Cassels FJ, Scott DA, Wolf MK, et al. Safety and immunogenicity of a prototype enterotoxigenic *Escherichia coli* vaccine administered transcutaneously. Infection and Immunity. 2002;**70**:1874-1880. DOI: 10.1128/IAI.70.4.1874-1880.2002

[65] Frech SA, DuPont HL, Bourgeois AL, McKenzie R, Belkind-Gerson J, Figueroa JF, et al. Use of a patch containing heat-labile toxin from *Escherichia coli* against travellers' diarrhoea: A phase II, randomised, double-blind, placebo-controlled field trial. Lancet. 2008;**371**:2019-2025. DOI: 10.1016/S0140-6736(08)60839-9

[66] Behrens RH, Cramer JP, Jelinek T, Shaw H, von Sonnenburg F, Wilbraham D, et al. Efficacy and safety of a patch vaccine containing heat-labile toxin from *Escherichia coli* against travellers' diarrhoea: A phase 3, randomised, double-blind, placebo-controlled field trial in travellers from Europe to Mexico and Guatemala. The Lancet Infectious Diseases. 2014;**14**:197-204. DOI: 10.1016/S1473-3099(13)70297-4

[67] Riddle MS, Savarino SJ. Moving beyond a heat-labile enterotoxin-based vaccine against enterotoxigenic *Escherichia coli*. The Lancet Infectious Diseases. 2014;**14**:174-175. DOI: 10.1016/S1473-3099(13)70355-4

[68] Lundgren A, Bourgeois L, Carlin N, Clements J, Gustafsson B, Hartford M, et al. Safety and immunogenicity of an improved oral inactivated multivalent enterotoxigenic *Escherichia coli* (ETEC) vaccine administered alone and together with dmLT adjuvant in a double-blind, randomized, placebo-controlled phase I study. Vaccine. 2014;**32**:7077-7084. DOI: 10.1016/j.vaccine.2014.10.069

[69] Ruan X, Robertson DC, Nataro JP, Clements JD, Zhang W, STa Toxoid Vaccine Consortium Group. Characterization of heat-stable (STa) toxoids of enterotoxigenic *Escherichia coli* fused to double mutant heat-labile toxin peptide in inducing neutralizing anti-STa antibodies. Infection and Immunity. 2014;**82**:1823-1832. DOI: 10.1128/IAI.01394-13

[70] Purcell AW, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. Nature Reviews. Drug Discovery. 2007;**6**: 404-414. DOI: 10.1038/nrd2224

[71] Savarino SJ, Poole S, Sincock SA, McVeigh A, Lee LH, Akay Y. One step beyond: A new approach for vaccines against enterotoxigenic *Escherichia coli*. In: 40th Joint Conference ofthe US-Japan Cooperative Medical Sciences Program on Cholera and Rela 2005; 2005

[72] Ruan X, Knudsen DE, Wollenberg KM, Sack DA, Zhang W. Multiepitope fusion antigen induces broadly protective antibodies that prevent adherence of *Escherichia coli* strains expressing colonization factor antigen I (CFA/I), CFA/II, and CFA/IV. Clinical and Vaccine Immunology. 2014;**21**: 243-249. DOI: 10.1128/CVI.00652-13

[73] Roy K, Hilliard GM, Hamilton DJ, Luo J, Ostmann MM, Fleckenstein JM. Enterotoxigenic *Escherichia coli* EtpA mediates adhesion between flagella and host cells. Nature. 2009;**457**:594-598. DOI: 10.1038/nature07568

[74] Roy K, Hamilton D, Allen KP, Randolph MP, Fleckenstein JM. The EtpA exoprotein of enterotoxigenic

Escherichia coli promotes intestinal colonization and is a protective antigen in an experimental model of murine infection. Infection and Immunity. 2008;**76**:2106-2112. DOI: 10.1128/IAI.01304-07

[75] Nagy B, Fekete PZ. Enterotoxigenic *Escherichia coli* in veterinary medicine. International Journal of Medical Microbiology. 2005;**295**:443-454. DOI: 10.1016/j.ijmm.2005.07.003

[76] Nguyen TD, Vo TT, Vu-Khac H. Virulence factors in *Escherichia coli* isolated from calves with diarrhea in Vietnam. Journal of Veterinary Science. 2011;**12**:159-164

[77] Nagy B, Fekete PZ. Enterotoxigenic *Escherichia coli* (ETEC) in farm animals. Veterinary Research n.d. 1999;**30**:259-284

[78] Casey TA, Nagy B, Moon HW. Pathogenicity of porcine enterotoxigenic *Escherichia coli* that do not express K88, K99, F41, or 987P adhesins. American Journal of Veterinary Research. 1992;**53**:1488-1492

[79] Wilson RA, Francis DH. Fimbriae and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with colibacillosis. American Journal of Veterinary Research. 1986;**47**:213-217

[80] Veilleux S, Dubreuil JD. Presence of *Escherichia coli* carrying the EAST1 toxin gene in farm animals. Veterinary Research. 2006;**37**:3-13. DOI: 10.1051/vetres:2005045

[81] Bertin Y, Martin C, Girardeau JP, Pohl P, Contrepois M. Association of genes encoding P fimbriae, CS31A antigen and EAST 1 toxin among CNF1-producing *Escherichia coli* strains from cattle with septicemia and diarrhea. FEMS Microbiology Letters. 1998;**162**:235-239

[82] Gyles CL. Pathogenesis of Bacterial Infections in Animals, Fourth Edition. In: Prescott JF, Songer JG, Thoen Ch O, editers. Ames, Iowa, USA: Blackwell Publishing; 2010. ISBN: 978-0-813-81237

[83] Mainil JG, Bex F, Jacquemin E, Pohl P, Couturier M, Kaeckenbeeck A. Prevalence of four enterotoxin (STaP, STaH, STb, and LT) and four adhesin subunit (K99, K88, 987P, and F41) genes among *Escherichia coli* isolates from cattle. American Journal of Veterinary Research. 1990;**51**:187-190

[84] Contrepois M, Dubourguier HC, Parodi AL, Girardeau JP, Ollier JL. Septicaemic *Escherichia coli* and experimental infection of calves. Veterinary Microbiology. 1986;**12**:109-118

[85] Deprez P, Van den Hende C, Muylle E, Oyaert W. The influence of the administration of sow's milk on the post-weaning excretion of hemolytic *E. coli* in the pig. Veterinary Research Communications. 1986;**10**:469-478

[86] Foster DM, Smith GW. Pathophysiology of diarrhea in calves. The Veterinary Clinics of North America. Food Animal Practice. 2009;**25**:13-36. DOI: 10.1016/j.cvfa.2008.10.013

[87] Moon HW, Bunn TO. Vaccines for preventing enterotoxigenic *Escherichia coli* infections in farm animals. Vaccine. 1993;**11**:213-200

[88] Wittum TW, Dewey CE. Partial budget analysis of sow *Escherichia coli* vaccination. Swine Health Production. 1996;**1**:9-13

[89] Nesta B, Pizza M. Vaccines *against Escherichia coli*. Current Topics in Microbiology and Immunology. 2018;**416**:213-242. DOI: 10.1007/82_2018_111

[90] Uehling DT, Hopkins WJ, James LJ, Balish E. Vaginal immunization of monkeys against urinary tract infection

with a multi-strain vaccine. The Journal of Urology. 1994;**151**:214-216

[91] Palaszynski S, Pinkner J, Leath S, Barren P, Auguste CG, Burlein J, et al. Systemic immunization with conserved pilus-associated adhesins protects against mucosal infections. Developments in Biological Standardization. 1998;**92**:117-122

[92] O'Hanley P, Lalonde G, Ji G. Alpha-hemolysin contributes to the pathogenicity of piliated digalactoside-binding *Escherichia coli* in the kidney: Efficacy of an alpha-hemolysin vaccine in preventing renal injury in the BALB/c mouse model of pyelonephritis. Infection and Immunity. 1991;**59**:1153-1161

[93] Cryz SJJ, Cross AS, Sadoff JC, Wegmann A, Que JU, Furer E. Safety and immunogenicity of *Escherichia coli* O18 O-specific polysaccharide (O-PS)-toxin A and O-PS-cholera toxin conjugate vaccines in humans. The Journal of Infectious Diseases. 1991;**163**:1040-1045

[94] Cross A, Artenstein A, Que J, Fredeking T, Furer E, Sadoff JC, et al. Safety and immunogenicity of a polyvalent *Escherichia coli* vaccine in human volunteers. The Journal of Infectious Diseases. 1994;**170**:834-840

[95] Russo TA, Johnson JR. Extraintestinal isolates of *Escherichia coli*: Identification and prospects for vaccine development. Expert Review of Vaccines. 2006;**5**:45-54. DOI: 10.1586/14760584.5.1.45

[96] Lorenzo-Gómez MF, Padilla-Fernández B, García-Criado FJ, Mirón-Canelo JA, Gil-Vicente A, Nieto-Huertos A, et al. Evaluation of a therapeutic vaccine for the prevention of recurrent urinary tract infections versus prophylactic treatment with antibiotics. International Urogynecology Journal. 2013;**24**:127-134. DOI: 10.1007/s00192-012-1853-5

[97] Bauer H, Darji A, Chakraborty T, Weiss S. *Salmonella*-mediated oral DNA vaccination using stabilized eukaryotic expression plasmids. Gene Therapy. 2005;**12**:364-372. DOI: 10.1038/sj.gt.3302423

[98] Uehling DT, Hopkins WJ, Elkahwaji JE, Schmidt DM, Leverson GE. Phase 2 clinical trial of a vaginal mucosal vaccine for urinary tract infections. The Journal of Urology. 2003;**170**:867-869. DOI: 10.1097/01.ju.0000075094.54767.6e

[99] Uehling DT, Hopkins WJ, Beierle LM, Kryger JV, Heisey DM. Vaginal mucosal immunization for recurrent urinary tract infection: Extended phase II clinical trial. The Journal of Infectious Diseases. 2001;**183**:S81-S83. DOI: 10.1086/318839

[100] Hopkins WJ, Elkahwaji J, Beierle LM, Leverson GE, Uehling DT. Vaginal mucosal vaccine for recurrent urinary tract infections in women: Results of a phase 2 clinical trial. The Journal of Urology. 2007;**177**:1349-1353. DOI: 10.1016/j.juro.2006.11.093

[101] Frey C, Obolensky W, Wyss H. Treatment of recurrent urinary tract infections: Efficacy of an orally administered biological response modifier. Urologia Internationalis. 1986;**41**:444-446. DOI: 10.1159/000281253

[102] Bauer HW, Alloussi S, Egger G, Blümlein H-M, Cozma G, Schulman CC, et al. A long-term, multicenter, double-blind study of an *Escherichia Coli* extract (OM-89) in female patients with recurrent urinary tract infections. European Urology. 2005;**47**:542-548. DOI: 10.1016/j.eururo.2004.12.009

[103] Bauer HW, Rahlfs VW, Lauener PA, Blebmann GS. Prevention of recurrent

urinary tract infections with immunoactive *E. coli* fractions: A meta-analysis of five placebo-controlled double-blind studies. International Journal of Antimicrobial Agents. 2002;**19**:451-456

[104] Wagenlehner FME, Ballarini S, Pilatz A, Weidner W, Lehr L, Naber KG. A randomized, double-blind, parallel-group, multicenter clinical study of *Escherichia coli* lyophilized lysate for the prophylaxis of recurrent uncomplicated urinary tract infections. Urologia Internationalis. 2015;**95**: 167-176. DOI: 10.1159/000371894

[105] Magistro G, Stief CG. Vaccine development for urinary tract infections: Where do we stand? European Urology Focus. 2018; pii: S2405-4569(18)30218-302189. DOI: 10.1016/j.euf.2018.07.034

[106] Huttner A, Gambillara V. The development and early clinical testing of the ExPEC4V conjugate vaccine against uropathogenic *Escherichia coli*. Clinical Microbiology and Infection. 2018;**24**:1046-1050. DOI: 10.1016/j.cmi.2018.05.009

[107] Mobley HLT, Alteri CJ. Development of a vaccine against *Escherichia coli* urinary tract infections. Basel, Switzerland: Pathogens; 2016;**5**:1. DOI: 10.3390/pathogens5010001

[108] Habibi M, Asadi Karam MR, Bouzari S. Evaluation of prevalence, immunogenicity and efficacy of FyuA iron receptor in uropathogenic *Escherichia coli* isolates as a vaccine target against urinary tract infection. Microbial Pathogenesis. 2017;**110**:477-483. DOI: 10.1016/j.micpath.2017.07.037

[109] Asadi Karam MR, Habibi M, Bouzari S. Use of flagellin and cholera toxin as adjuvants in intranasal vaccination of mice to enhance protective immune responses against uropathogenic *Escherichia coli* antigens. Biologicals. 2016;**44**:378-386. DOI: 10.1016/j.biologicals.2016.06.006

[110] Ghunaim H, Abu-Madi MA, Kariyawasam S. Advances in vaccination against avian pathogenic *Escherichia coli* respiratory disease: Potentials and limitations. Veterinary Microbiology. 2014;**172**:13-22. DOI: 10.1016/j.vetmic.2014.04.019

[111] Li L, Thofner I, Christensen JP, Ronco T, Pedersen K, Olsen RH. Evaluation of the efficacy of an autogenous *Escherichia coli* vaccine in broiler breeders. Avian Pathology. 2017;**46**:300-308. DOI: 10.1080/03079457.2016.1267857

[112] Han Y, Liu Q, Willias S, Liang K, Li P, Cheng A, et al. A bivalent vaccine derived from attenuated *Salmonella* expressing O-antigen polysaccharide provides protection against avian pathogenic *Escherichia coli* O1 and O2 infection. Vaccine. 2018;**36**:1038-1046. DOI: 10.1016/j.vaccine.2018.01.036

[113] Chaudhari AA, Matsuda K, Lee JH. Construction of an attenuated *Salmonella* delivery system harboring genes encoding various virulence factors of avian pathogenic *Escherichia coli* and its potential as a candidate vaccine for chicken colibacillosis. Avian Diseases. 2013;**57**:88-96. DOI: 10.1637/10277-061312-Reg.1

[114] Van Goor A, Stromberg ZR, Mellata M. A recombinant multi-antigen vaccine with broad protection potential against avian pathogenic *Escherichia coli*. PLoS One. 2017;**12**:e0183929. DOI: 10.1371/journal.pone.0183929

[115] Uotani Y, Kitahara R, Imai T, Tsutsumi N, Sasakawa C, Nagai S, et al. Efficacy of an avian colibacillosis live vaccine for layer breeder in Japan. The Journal of Veterinary Medical Science. 2017;**79**:1215-1219. DOI: 10.1292/jvms.17-0189

[116] Nagano T, Kitahara R, Nagai S. An attenuated mutant of avian pathogenic *Escherichia coli* serovar O78: A possible live vaccine strain for prevention of avian colibacillosis. Microbiology and Immunology. 2012;**56**:605-612. DOI: 10.1111/j.1348-0421.2012.00482.x

[117] La Ragione RM, Woodward MJ, Kumar M, Rodenberg J, Fan H, Wales AD, et al. Efficacy of a live attenuated *Escherichia coli* O78:K80 vaccine in chickens and turkeys. Avian Diseases. 2013;**57**:273-279. DOI: 10.1637/10326-081512-Reg.1

[118] Kwaga JK, Allan BJ, van der Hurk JV, Seida H, Potter AA. A *carAB* mutant of avian pathogenic *Escherichia coli* serogroup O2 is attenuated and effective as a live oral vaccine against colibacillosis in turkeys. Infection and Immunity. 1994;**62**:3766-3772

[119] Kariyawasam S, Wilkie BN, Gyles CL. Construction, characterization, and evaluation of the vaccine potential of three genetically defined mutants of avian pathogenic *Escherichia coli*. Avian Diseases. 2004;**48**:287-299. DOI: 10.1637/7093

[120] Gregersen RH, Christensen H, Ewers C, Bisgaard M. Impact of *Escherichia coli* vaccine on parent stock mortality, first week mortality of broilers and population diversity of *E. coli* in vaccinated flocks. Avian Pathology. 2010;**39**:287-295. DOI: 10.1080/03079457.2010.495744