The Benefit of a Plant-Based Cattle Vaccine for Reducing Enterohemorrhagic *Escherichia Coli* Shedding and Improving Food Safety

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Abstract Upon ingestion, enterohemorrhagic *Escherichia coli* (EHEC) can colonize intestinal mucosa and cause hemorrhaging of nearby tissue. The failure to adequately control its contamination of food and water can consequently compromise the health of a population and incur economic losses to all stages of the food supply chain. EHEC is currently one of the foremost foodborne pathogenic threats worldwide because of its virulence across all age groups and demographics, a low infective dose, a relatively high resilience in diverse environments and its widespread prevalence across cattle herds. EHEC primarily colonizes the bovine digestive tract from which it can be transmitted via fecal shedding or during slaughter. Considering its threat to food security and in accord with the 'One Health' framework, the development of a bovine vaccine as a pre-harvest intervention strategy to curtail the transmission of EHEC is of great interest. Although two EHEC vaccines have already been developed using bacterial production platforms, their market penetrance has been markedly low. As an alternative, production in a plant platform may have the potential to redress the reasons for this low penetrance by providing a better economy of scale and a more convenient mode of delivery. This chapter summarizes the scope of the threat posed by EHEC and discusses the prospects for developing a commercial plant-based vaccine for EHEC within the framework of the North American beef industry.

Keywords EHEC · O157 · VTEC · STEC · Shiga · Cattle · Vaccine

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1 Occurrence and Disease Symptoms

1.1 Problem and Context

Diarrhea is the second leading cause of death among toddlers under the age of five globally, with an estimated occurrence of 2.5 billion cases overall, and an estimated mortality of 1.5 million annually (Unicef 2010). While diarrhea may be a common symptom of a broad spectrum of gastrointestinal upsets, a relatively small handful of micro-organisms are the primary causes for most acute diarrheal cases, including *Escherichia coli*. The pathogenic *E. coli* strains that cause diarrheal disease in humans, collectively known as diarrheagenic *E. coli,* are broadly categorized based on clinical symptoms and virulence attributes into: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and Vero toxin-producing/Shiga toxin-producing *E. coli* (VTEC/STEC). The latter category is further divided into enterohemorrhagic *E. coli* (EHEC) and non-enterohemorrhagic subgroups though in practice, the terms EHEC, STEC and VTEC are often used interchangeably. As the name suggests, the EHEC subgroup is typified by hemorrhaging of the intestines but constitutes more than 100 different serotypes that are identifiable based on variations of their O (somatic lipopolysaccharide), H (flagellar) and K (capsular) antigens. While lipopolysaccharides are found in all Enterobacteriaceae, flagellar and capsular antigens are not always present in some strains. Therefore, routine epidemiological surveillance has conventionally screened primarily for O serogroups as the primary biomarker, with subsequent H-subtyping if presumptive pathogenic O strains are detected. Subtyping for the K antigen is not part of routine surveillance since few labs are equipped for the requisite assay. The most prevalent and virulent EHEC serotype in North America is O157:H7 and has been classified as a major food adulterant by the United States Department of Agriculture (USDA) and Canadian Food Inspection Agency (CFIA) for almost 20 years. Although non-O157 strains are individually less prevalent, the collective contribution of non-O157 strains to gastrointestinal illness has as of late been of growing concern, particularly since recent surveillance indicates a 41% increase in the average annual incidence of infection of non-O157 strains over the last five years across the US (Gill and Gill 2010). Six additional EHEC serogroups O26, O45, O103, O111, O121 and O145, known as the "Big Six", generally comprise >90% of non-O157 infections of any given year and have been traced to at least 22 human disease outbreaks in the US since 1990. In the US, national surveillance was only recently enabled in 2012 by the USDA to individually track non-O157 serotypes in human illness (Mathusa et al. 2010). In 2011, Canadian national surveillance by the Public Health Agency of Canada expanded their monitoring of O157 to include all VTEC strains in agricultural, water, retail and human health components (Public Health Agency of Canada 2015).

1.2 Epidemiology of Human Infections

The recognition of EHEC as a discrete and important class of diarrheagenic *E. coli* originally stems from two reports in 1983. The first was a clinical report detailing two separate outbreak events in the United States of a distinctive gastrointestinal illness, subsequently called hemorrhagic colitis (HC), characterized by severe abdominal pain and acute watery diarrhea that later developed into bloody diarrhea (Riley et al. 1983). In both cases, the illness was associated with consuming undercooked hamburger meat from two fast food chains and dubbed by news media as the "hamburger disease". Also, stool cultures sampled from the patients both yielded a previously unidentified *E. coli* strain. The second report provided strong association between fecal cytotoxin producing *E. coli* and the occurrence of hemolytic uremic syndrome (HUS) (Karmali et al. 1983). HUS is characterized by the triad combination of acute renal failure, thrombocytopenia and microangiopathic hemolytic anemia, and was already known to be preceded by a bloody diarrhea that was symptomatically similar to that observed in the two fast food chain outbreak events. This discovered link between EHEC, its enteric disease causing ability and its route of transmission via undercooked beef products has subsequently prompted a series of surveillance efforts in the food industry to curtail the outbreak potential of EHEC (Doyle et al. 2006).

Since then, EHEC, particularly the O157:H7 strain has been detected worldwide. Based on a data mining approach of incidence studies covering 21 countries, a review has conservatively estimated that each year VTEC causes 2,801,000 acute illnesses, 3890 cases of HUS and 230 deaths (Majowicz et al. 2014). Based on these estimates, on a global ranking, VTEC places behind typhoid fever, foodborne trematodes and nontyphoidal salmonellosis in importance. EHEC is estimated to affect approximately 230,000 people in the United States each year, with ∼73,000 of these being caused by O157:H7 (Hale et al. 2012). In terms of most frequently isolated overall food-borne pathogen ranking in North America, it places fourth after *Campylobacter, Salmonella* spp and *Shigella* spp based on stool samples collected from patients (Griffin 1995). However, if restricted to only stool samples with visible blood then EHEC, particularly O157:H7, is the most frequently isolated (Slutsker et al. 1997).

In the US, the national surveillance program for foodborne pathogens, FoodNet, reported that in 2015 (most recent available report) the average incidence rate for that year for O157 was 0.95 per 100,000 persons and for non-O157 strains was 1.65 per 100,000 persons (CDC 2017). Among the approximately 1200 EHEC infections (out of a total sample of ∼49 million), the most common serogroups were O157 (39.8%), O26 (17.6%) and O103 (14.3%) (CDC 2017). Although surveillance for non-O157 strains is still fairly recent, the growth in incidence over the past five years is stark. Compared with the average annual incidence rate 2012–2014, non-O157 incidence has increased by 41% (CDC 2017). For that same period, there has been no significant change for O157 incidence (CDC 2017). This is possibly because most EHEC diagnostic and control measures have historically been specific

for O157, despite the clinical relevance of non-O157 strains. Since discovering O157 in the 1980's, the trend of infection has progressively shown a decreasing incidence in North America, mirrored by a decrease in HUS (CDC 2011). Between 1996 and 2010, the incidence of infection for O157 has decreased by 44% and the number of HUS cases has decreased by 90% (CDC 2017). There are many likely contributing factors such as improved regulatory and biosecurity control, cleaner slaughter methods, better microbial testing and improved food awareness by consumers. In Canada, the national surveillance system for foodborne pathogens, FoodNet Canada, reported an average incidence rate for VTEC to be 3.00 per 100,000 persons (Public Health Agency of Canada 2015). Targeted surveillance on retail ground beef products across Ontario for 2015 indicated VTEC in 2.3% of samples, with a similar prevalence to Salmonella (1.5%), and placing second behind the consistent frontrunner, *Listeria monocytogenes* (25%) (Public Health Agency of Canada 2015). The 10-year trend for VTEC in contamination in retail ground beef reveals that VTEC consistently hovers around 2% positive with the exception of 2010–2011 in Ontario when incidence spiked to ~8% due to large scale outbreak (Public Health Agency of Canada 2015).

Both incidence rates and occurrence of HUS are consistently highest in toddlers <5 years compared with all other age groups. In FoodNet's latest report (2015), toddlers <5 had incidence rates of 3.72 and 6.76 per 100,000 for O157 and non-O157 strains respectively (Gill and Gill 2010). In comparison, all other age groups ranged between 0.33–2.39 and 0.62–2.04 per 100,000 for O157 and non-O157 strains respectively (Gill and Gill 2010). Approximately 1 in 5 toddlers <5 years with an O157 infection will develop HUS. Out of all HUS patients, more than 90% are due to O157, followed by O121 (4.8%) and then O111 (2.4%) (Gill and Gill 2010). Compared with 2006–2008, the incidence of pediatric HUS has decreased by 32%, which likely corresponds to the 30% decrease in O157 infections (Gill and Gill 2010).

Large-scale outbreaks are rare but can affect large numbers of people and may be transmitted from a variety of sources, though most commonly from raw foodstuff or untreated water. For example, the five largest EHEC outbreaks worldwide were from: radish sprouts in Japan (12,680 cases) (Fukushima et al. 1999), drinking water in Canada (2300 cases) (Hrudey et al. 2003), well water in the US (>1000 cases) (Charatan 1999), raw beef in the US (788 cases) (Wendel et al. 2009) and undercooked hamburger meat in the US (>700 cases) (Bell et al. 1994). In comparison, sporadic EHEC infections are more frequent and comprise the major disease burden in a population. The average frequency of sporadic cases has slightly risen over the past five years of surveillance (CDC 2017; Public Health Agency of Canada 2015). Of these sporadic cases, the incidence is distributed unevenly across North America, being more common in Canada and the northern US states than the southern US states and more common in western Canada than eastern Canada (Griffin 1995).

EHEC primarily occupies a bovine intestinal reservoir and correspondingly, its main route of transmission is via cattle's excretion of fecal matter carrying the bacterium, a process known as 'shedding'. Sporadic EHEC incidence can be

affected by seasonality with the most common reports of EHEC shedding occurring during the summer through fall seasons. An investigation by the USDA on the seasonal occurrence of O157 suggests that the increased shedding of *E. coli* O157 during the summer season is strongly associated with an increased likelihood of product contamination and a corresponding increase of enterohemorrhagic cases in humans (Williams et al. 2010). Both O157 and non-O157 serogroups exhibit this trend.

1.3 Disease Symptoms in Humans

Milder forms of EHEC infection are typically associated with watery diarrhea while more aggressive forms may develop into HC or HUS, and in uncommon cases, accompanied by cardiovascular or nervous system abnormalities (Griffin and Tauxe 1991). In humans, the incubation period for EHEC O157:H7 ranges from 1 to 16 days. Symptoms usually become apparent after 3–4 days, typically manifesting as moderate to severe diarrhea. Most resolve without treatment whereas others can progress to HC after a few days, characterized by severe, bloody diarrhea with abdominal tenderness and cramping. Mild fevers, nausea, vomiting and dehydration are also possible accompanying symptoms (Cleary 2004). Although this will typically resolve in approximately 1 week, 16% will develop into HUS, characterized by the triad combination of kidney failure, hemolytic anemia and thrombocytopenia. In more severe cases, paresis, stroke, cerebral edema or coma are accompanying symptoms. Although 65–85% of patients recover from HUS without permanent injury, long term complications including hypertension, renal insufficiency and end-stage renal failure are possible. Certain demographics of patients seem to be more susceptible to the development of the infection into more serious symptoms. Patients who are younger than five, older than 60 or who are immunocompromised are significantly more likely to develop HC or HUS (Gould et al. 2009; Karmali 2004; Tuttle et al. 1999). In the elderly, a form of HUS, known as thrombocytopenia purpura, is more common, characterized by less kidney damage but more severe occurrence of neurologic symptoms such as stroke, seizure and central nervous system deterioration.

1.4 Histopathology

The typical histopathology characteristic of EHEC infection includes hemorrhaging and edema of the lamina propria (Griffin et al. 1990). Biopsy samples taken from the colon of infected patients also show focal necrosis and neutrophil infiltration. One of the hallmarks of EHEC infection is the attaching-and-effacing (A/E) lesion. This histopathology is apparent by microscopy in a variety of animal models and can also be reproduced in in vitro cell cultures (Donnenberg et al. 1993; Ismaili et al. 1995;

Pai et al. 1986). In vitro organ culture of human endoscopic biopsy samples suggests EHEC adhere and form lesions on the terminal ileum (Chong et al. 2007). This distinct phenotype is caused during the EHEC colonisation phase when microvilli become effaced and various secreted proteins enable the intimate adherence between the EHEC pathogen and the outer membrane of the intestinal epithelium. Following attachment, the accumulation and rearrangement of polymerized actin leads to an altered cytoskeleton in which a pedestal-like structure protruding from the epithelium emerges. These structures can extend up to 10 μm in a pod-like formation upon which the bacterium is ensconced (Moon et al. 1983).

2 Transmission

2.1 Route of Transmission

The intestines of ruminants, especially cattle, are considered the primary reservoirs of EHEC and can transmit EHEC via excreted fecal matter or after slaughter during processing (Beutin et al. 1993; Montenegro et al. 1990). High levels of EHEC colonization have been reported in cattle herds from various countries, ranging typically between 10 and 25%, but can be as high as 60%. Healthy cattle transiently host EHEC in their gastrointestinal tract and can directly or indirectly transmit this pathogen to humans (Rangel et al. 2005a, b). EHEC can persist in various environments that range extensively from soil, to water to the ruminant GI tract. In North America, most cases are caused by ingestion of contaminated food or water (Rangel et al. 2005a, b). When shed in bovine feces, the pathogen can remain viable in the farm environment and may contaminate nearby agricultural crops, other holding pens and ground water (Sanderson et al. 2006). Aside from undercooked or unpasteurized animal products and contaminated fruits and vegetables, exposure may come from contaminated soil, such as at campgrounds or other sites grazed by cattle, or from open water sources, such as swimming lakes or private wells that are drainage sinks from agricultural run-off. O157:H7 has been reported to persist for up to a year in manure-treated agricultural soil and for 21 months in non-composted raw manure (Jiang et al. 2002). Its resilience in water especially is a major factor for its dissemination and persistence across various transmission routes. Culturable O157 has been demonstrated to be able to survive for at least 8 months in contaminated water troughs (Lejeune et al. 2001). Furthermore, O157 strains that survived longer than 6 months still retained the capacity to colonize cattle (Lejeune et al. 2001). EHEC's robustness has implications for crop contamination considering that bovine manure often is used as fertilizer as well as after irrigation when surface water containing EHEC collects in sumps. Even if the use of bovine fertilizer were to be avoided, a recent report indicated that airborne transport of O157:H7 could contaminate leafy greens that were up to 180 m away from a cattle feedlot, particularly when pen surfaces were under arid conditions (Berry et al. 2015). A safe

set-back distance between feedlots and crops has not yet been determined. Additionally, EHEC requires a much lower infectious dose than other foodborne pathogens when ingested, with fewer than 40 bacterial cells being sufficient to cause illness (Strachan et al. 2005). To a lesser degree than contaminated food and water, EHEC can also be transmitted from direct contact between humans as well as from animal to human contact, likely via fecal residues (Heuvelink et al. 2002).

Although infected cattle remain asymptomatic, cattle that have been exposed to EHEC develop a local immune response, an associated inflammatory response and attaching-effacing (A/E) lesions suggesting not only that EHEC is an active bovine pathogen but also that there is a limit to which the bovine host will tolerate pathogen load and after which host resistance mechanisms may actively function to reduce pathogen burden (Baines et al. 2008; Nart et al. 2008).

2.2 Super Shedders

Generally, there are three distinct patterns observed for EHEC carriage in cattle that are characterized in terms of increasing severity of intestinal colonization, duration of shedding and magnitude of shedding. First, some cattle, known as passive shedders, lack colonization, transiently shed for only a few days and in small numbers. Second, cattle that are colonized, shed for approximately 1–2 months (Besser et al. 1997). Third, a small subset of cattle populations, known as "super shedders", are colonized for extended periods, shed EHEC for longer periods at 3–12 months and at significantly higher levels (between 10^4 and 10^4 colony forming units/g of faeces) (Omisakin et al. 2003; Stephens et al. 2009). These super shedders are suggested to be important hubs in a cattle population for maintaining the penetrance of EHEC infection that perhaps would otherwise be transient and short-lived. While there is as of yet no definitive explanation of the causes of the super shedding phenomenon, it is thought to collectively be mediated by factors from the EHEC pathogen, the bovine host and the environment. Hide contamination associated with super shedders rapidly resulted in the transmission of *E. coli* O157:H7 among cattle housed in a common pen (Stanford et al. 2011). An assessment of the link between shedding density and human risk suggested that even though super shedding events were relatively rare, they dominated as the environmental contamination source as well as the relative human risk of acquiring illness (Matthews et al. 2013). Almost half of all EHEC shed from cattle in an Alberta feedlot was due to super-shedders, even though these animals represent less than a tenth of the cattle population (Stephens et al. 2009). While super-shedders are increasingly considered to have a significant role in population-level persistence of EHEC, this small proportion of super-shedding cattle is not a stable, consistent subset of the population but rather varies transiently and dynamically making quarantining of the super-shedding animal an unviable option. Consequently, targeting them for interventions such as vaccination is difficult, unless applied to the entire herd for herd immunity. However, the exception to this is if immediately

prior to slaughter, there were tools available to quickly diagnose and identify these super-shedders, these could be targeted for intervention to reduce the likelihood of meat product contamination.

3 Mechanism of Infection

The ability of EHEC to successfully colonize the gastrointestinal tracts of both humans and cattle despite peristaltic movements and resource competition with neighboring microflora is one of the most defining features across all strains. In particular, although all *E. coli* strains have some form of fimbrial structure to enable surface adherence, EHEC strains express specific fimbrial antigens that seem to specialize in adherence to the gut mucosa, enhancement of colonization of the intestinal epithelium, and defining of host specificity (Vial et al. 1988). In cattle, EHEC principally adheres to and colonizes the lymphoid follicle dense mucosa at the terminal rectum known as the rectoanal junction, whereas in humans, it adheres to and colonizes the follicle-associated epithelium of ileal Peyer's patches (Lim et al. 2007; Naylor et al. 2003; Phillips et al. 2000). Successful colonisation in both humans and cattle will typically be marked by a canonical A/E lesion.

The mechanism of colonization by EHEC of a mucosal site in either cattle or humans is a conserved process requiring the expression of at least 59 genes (Büttner 2012; Dziva et al. 2004). The main virulence genes cluster together on a chromosomal 43-kb pathogenicity island known as the locus of enterocyte effacement (LEE), the presence of which is both necessary and sufficient for showing the A/E phenotype (Perna et al. 1998). The LEE contains 41 open reading frames including genes encoding various subunit proteins that assemble to form a type III secretion system (T3SS), the major adhesin protein known as intimin (Eae) and its cognate Translocated intimin receptor (Tir), a lytic transglycosylase EtgA to remove glycans near to the site of colonisation (Burkinshaw et al. 2015), various effector proteins that are secreted through this system and various chaperones to stabilize the folding and assembly of these proteins (Wong et al. 2011). The T3SS consists of a syringe-like structure that permits the secretion of multiple effector proteins stored within the bacterial cell and into the host cytosol (Jarvis and Kaper 1996).

The first step of colonization is likely through contact to an intestinal epithelial membrane by an extended hollow, filamentous structure consisting of multiple polymerized subunits of *E. coli* secreted protein A (EspA) (Delahay et al. 1999; Knutton et al. 1989). Upon initial contact, two other LEE-encoded proteins, EspB and EspD, are translocated via the EspA filament into the host cell where they will assemble along with EspA to form a translocon pore stabilizing the entry point (Fivaz and Van Der Goot 1999; Kenny and Finlay 1995; Lai et al. 1997; Warawa et al. 1999). At least 39 other effector proteins are then secreted into the host cell, altering a variety of host cell processes that ultimately improve the likelihood of the bacterium's survival and replication (Tobe et al. 2006; Wong et al. 2011). Several of these effectors along with components of the T3SS are potential vaccine

candidates because of their efficacy in engaging the host's active immune response. One of these effector proteins known as the non-Lee encoded effector A (NleA) protein is also secreted into the host cell where it may have a role in disruption of intestinal tight junctions and inhibition of intercellular protein trafficking (Gruenheid et al. 2004; Kim et al. 2007). Another effector known as Tir integrates into the host cell membrane where it allows docking of the adhesin protein, intimin (Kenny et al. 1997). Docking enables intimate attachment of the bacterium to the host cell and signals the recruitment and polymerization of actin at the pore resulting in a protrusion of the membrane toward the bacterium forming the canonical A/E lesion (Garmendia et al. 2004).

Subsequent to colonization, EHEC will produce a variety of virulence factors including verocytotoxins, also called Shiga-like toxins (Stx) because of their similarity to toxins produced by *Shigella dysenteriae*. In humans, the production of Stx is the primary cause of the microvascular endothelial damage associated with HUS and HC. There are two major immunologically distinct types of Shiga-like toxins, Stx1 and Stx2, that are encoded by separate phage-derived *stx* genes on the bacterial chromosome (Wagner and Waldor 2002). Although Stx1 tends to be highly conserved across serotypes, there are many variants for Stx2. Nonetheless, all Shiga toxins form a basic A-B5 subunit structure. Typically, the 32-kDa A subunit is cleaved to yield an enzymatically active 28-kDa A1 peptide that is bridged via a 4-kDa A2 peptide to a pentamer consisting of five 7.7-kDa B subunits. The B subunit pentamer is able to bind to a specific glycolipid receptor, globotriaosylceramide (Gb3) that is found on the cell membrane surface of intestinal epithelial cells. A Gb4 receptor may also be targeted by some Stx2 variants. Upon successful binding to a receptor, the toxin is endocytosed via clathrin coated pits. The internalized toxin is then delivered to endosomes where they are primarily targeted to lysosomes for degradation. However, a fraction can be delivered to the trans-Golgi network, followed by retrograde transport via Golgi cisterns into the ER. Similar to the effects of ricin, the A1 peptide of the cytotoxin is an N-glycosidase that catalytically removes a single adenine residue from the 28S RNA of 60S ribosomal subunits to effectively suppress protein synthesis by preventing binding of tRNAs to the ribosome and consequently triggering apoptosis in affected cells (Endo et al. 1988). The presence of the Gb3 receptor on the cell surface is required for Stx toxicity (Jacewicz et al. 1995). Although Stx production occurs in both humans and cattle, the former exhibit Stx-related pathophysiology primarily because of vascular expression of the Gb3 receptor in intestinal epithelial cells while the latter lack vascular Gb3 receptor expression in their GI tracts (Pruimboom-Brees et al. 2000). Although the Gb3 receptor is expressed in the bovine brain and kidney, cells in the recto-anal junction do not permit Stx to be endocytosed and transported across the GI tract vasculature and consequently, the toxin is isolated from susceptible cells (Pruimboom-Brees et al. 2000). In contrast, EHEC's colonisation of human ileal tissue is proximal to the intestinal epithelial cells that express Gb3. The selective apoptosis of absorptive villus tip intestinal epithelial cells, carrying the Gb3 receptor, and the preservation of Gb3-absent secretory crypt cells may then lead to the osmotic dysregulation that manifests as diarrhea (Kandel et al. 1989).

The development to HUS is assumed to be based on the translocation of Stx across the epithelial cell layer and into the bloodstream. The Gb3 receptor is abundant in human renal tissue (Boyd and Lingwood 1989). Upon contact, Stx is cytotoxic to the glomerular endothelial cells leading to blocking of the glomerular microvasculature with platelets and fibrin (Louise et al. 1997). This disrupted ability to filter fluid through the glomerulus may lead to the acute renal failure characteristic of HUS.

The significance of Stx in intestinal pathology can vary depending on the animal model used. In cattle, which lack the Gb3 receptor, the occurrence of the diarrhea is independent of the presence or absence of Stx but is rather determined by the extent and distribution of the A/E lesions. This pattern is similar across cattle, sheep, goats, chickens and rabbits that do not display clinical symptoms despite the formation of A/E lesions in their GI tracts, presumably due to a lack of Gb3 receptors (Best et al. 2005; La Ragione et al. 2005, 2006; Tzipori et al. 1989; Tzipori et al. 1995; Woodward et al. 2003). Overall, reports from various animal models suggest that the occurrence of the A/E lesions is sufficient to cause non-bloody diarrhea but the cellular entry of the Stx is essential for inducing clinically relevant symptoms such as bloody diarrhea, HUS and HC.

4 Interventions

4.1 Pre-harvest and Post-harvest Interventions Against EHEC

EHEC be transmitted to humans via multiple routes such as crops, water and meat products. Towards the implementation of strategies to prevent EHEC infection of humans, the prevailing train of thought is to curtail its colonization of cattle and to minimize its spread from fecal shedding and at harvest. These strategies are broadly grouped into pre-harvest and post-harvest interventions with the former typically being adopted by beef producers and the latter by meat processors. Intervention strategies that are most commonly used or are most promising have been summarized in Table 1.

Post-harvest interventions involve removing contamination from the hide and/or carcass with various antimicrobial agents such as organic acids, oxidizing agents, heat exposure, irradiation or high pressure systems. Hide contamination can occur during skinning of the animal and to a lesser degree rupturing of the intestines. As an initial step, the carcass is often rinsed or steamed and visibly contaminated parts removed by knife trimming. Subsequently, a combination of treatments is typically used to reduce the contamination. Acid treatment is the most commonly employed method in North America likely due to its cost effectiveness. Promising newer methods such as high pressure and electron beam irradiation are twice as effective as acid treatment and have the highest efficacy amongst known interventions,

Table 1 A summary of intervention strategies that have been investigated in mitigating EHEC carriage in cattle

Strategy	Description
Pre-harvest interventions	
(1) Exposure reduction	Modulates rearing conditions to minimize transmission to cattle
Treatment of drinking water	Destroys bacteria residing in drinking water, typically by chlorination, electrolysis or ozonation
Feed strategies	Reduces ingested bacteria by change of standard grain-based feed a few days before slaughter, usually by fasting or replacement with forage or hay
Maintaining closed herds	Prevents cross-contamination across herds by quarantining of cattle herds and facilities
Pest and wildlife management	Prevents transmission from various pests and wildlife which can act as EHEC transmission vectors
Sanitation practices	Ensures clean pens, bedding and transport to prevent EHEC growth in immediate environment
(2) Exclusion strategies	Alters the mucosal site of colonisation within the GI tract to either interrupt or displace attachment and colonisation
Vaccination	Engages host active mucosal immunity by immunization with an EHEC specific antigen
Probiotics	Alters the gut microbiota by a viable preparation of microorganisms that outcompete EHEC at the ecological niche needed for colonization
Prebiotics	Enriches native competitive microbiota species by providing selectively digestible organic compounds
Competitive exclusion	Competes for EHEC binding to sterically block EHEC access
(3) Direct anti-pathogen strategies	Live animal treatments that specifically target and kill EHEC
Sodium chlorate	metabolized by an EHEC-specific nitrate reductase to chlorite, a bactericidal metabolite
Antibiotics (Neomycin sulfate)	A broad spectrum compound that binds 30S ribosomal subunit and inhibits protein translation
Bacteriophages	Viruses specific for a narrow bacterial host range that infect and lyse the EHEC bacteria
Colicins	Antimicrobial proteins that bind EHEC outer membrane receptors and subsequently translocate to the cytoplasm where they exert various cytotoxic effects
Post-harvest interventions	
Physical removal	Removes visibly contaminated parts and rinses excess unattached EHEC off carcass, usually by knife trimming, steam-vacuuming and ambient temperature water washing
Acid antimicrobials	Disrupts proton motive force and substrate transport mechanisms leading to bacteriostasis, usually acetic, citric and lactic acids
Oxidizer antimicrobials	Generates oxidative damage to a broad array of cellular structures leading to cell death, usually by peracetic acid, acidified sodium chlorite, ozone or hypobromous acid

(continued)

Table 1 (continued)

Strategy	Description
Heat exposure	Uses heat treatment to denature bacterial enzymes and nucleic acid degradation, usually by hot water sprays or steam pasteurization
Irradiation	Uses a stream of high energy electrons or UV light to damage bacterial genetic material leading to cell death
High pressure	Uses hydrostatic pressure to damage bacterial cell membranes causing lysis

This list is not intended to be exhaustive but describes the most commonly used or most promising strategies currently used

though they require specialized equipment for implementation (Wheeler et al. 2014).

Pre-harvest interventions are further sub-grouped into 3 categories: (1) exposure reduction, (2) exclusion, and (3) direct anti-pathogen strategies. Exposure reduction strategies involve management of the rearing conditions of the herd to minimize EHEC exposure such as by water and feed hygiene, by limiting exposure to pests, wildlife, and other cattle herds and by sanitation of living and transport conditions. Exclusion strategies seek to interrupt or displace attachment and colonisation of EHEC to the GI tract by altering the site of colonisation such as by engaging active immunity with vaccination, outcompeting niches with prebiotics and/or probiotics or sterically hindering access with competitive exclusion. Direct anti-pathogen strategies are live animal treatments that directly kill EHEC such as by sodium chlorate, antibiotics, bacteriophages and colicins. Based on systematic reviews of published reports, only three methods of pre-harvest interventions for EHEC have been validated to be reliably efficacious in reducing colonisation in cattle—the feeding of the probiotic combination *Lactobacillus acidophilus* NP51 (NPC 747) and *Propinionibacterium freudenreichii*, feeding of sodium chlorate and vaccination with T3SS proteins or Siderophore Receptor and Porin proteins (SRPs) (Sargeant et al. 2007; Snedeker et al. 2012). Meta-analysis also indicated no consistent association of antimicrobials with degree of shedding, and indicated that there are still an insufficient number of studies to confirm efficacy of other promising interventions such as bacteriophages and colicins.

4.2 Vaccine Products that Have Reached Market

Only two vaccine products have successfully transitioned from research to market: a T3SS formulation known as Econiche® (Bioniche Life Sciences Inc., Belleville, Ontario, Canada) and a SRP formulation known as Epitopix® (Epitopix LLC, Willmar Poultry Company (WPC), Minnesota, USA). EHEC secrete T3SS proteins during colonisation and when injected directly through a host cell wall, these secreted proteins enable a receptor-mediated bacterial adhesion event to firmly anchor the bacterium to the site of the A/E lesion. The plausibility of using T3SS

proteins as a vaccine was first reported on by the Finlay lab which demonstrated the secretion of extracellular proteins via a putative T3SS in both EHEC and EPEC (Jarvis et al. 1995; Jarvis and Kaper 1996). After partnering with the Vaccine and Infectious Diseases Organization (VIDO) in Saskatchewan, they demonstrated in a pilot study using a bacterial production platform that these attachment proteins reduced shedding of O157:H7 in cattle. With the intent of moving this product to market, Bioniche Life Sciences Inc. was contacted for scale-up and commercial manufacture of the vaccine. The product, called Econiche™, obtained full licensure by the CFIA in 2008 after clearing safety and efficacy requirements but has since been discontinued due to poor market penetration, likely because of the cost and the frequency of animal handling that fell outside of regular handling schedules. The vaccine required three doses and in Phase II and Phase III studies using about 30,000 cattle, the vaccine efficacy was demonstrated to reduce duration (by 64%) and magnitude of shedding (2.3 $\log_{10}$ reduction), reduce mucosal colonization (by 98%) and reduce hide contamination (by 54%) (Smith et al. 2009a, b).

A SRP vaccine developed by Pfizer and marketed by Zoetis, known as Epitopix™, was granted a conditional marketing license by the USDA in 2009 and is currently the only licensed vaccine available on the market. Siderophore receptor proteins are highly conserved outer membrane proteins that use high affinity ferric iron chelators, known as siderophores, to transport iron inside the bacterial cell. The vaccine consisted of multiple types of purified SRPs, of molecular weights of about 72–96 kDa, extracted from the outer bacterial membrane. By engaging immunity against cell-surface SRP proteins, the vaccine was suggested to possibly restrict iron acquisition and thus competitively disadvantage the bacterium from finding a foothold in the gut. In the initial field study using three doses, efficacy was demonstrated to reduce fecal shedding (by 39% magnitude), reduce mucosal colonization (by 48%) and reduce hide contamination (by 70%). Like Econiche™, recommended usage is for three doses applied subcutaneously over the course of 8–10 weeks with an annual revaccination.

4.3 Plant-Based Vaccines for EHEC

Both Econiche™ and Epitopix™ vaccines when placed on the market required three injections to the animals. This required skilled labor and handling of the animals outside of their normal handling and vaccination schedules, which usually are only twice per individual cow. With additional injections, the risk of infection is also increased and the area around the injection site can sometimes become adulterated. A valuable advantage of plant-based vaccines is the utility of oral delivery with edible plant tissue containing the bioactive therapeutic. The plant matrix has been shown to confer protection against low gastric pH to recombinant proteins stored within the cell's interior (Kolotilin et al. 2012; Kwon et al. 2013; Pelosi et al. 2012). However, while oral immunization offers more convenience, a larger dose is usually required to effectively generate an active immune response, requiring

milligram to gram quantities versus the microgram quantities needed for injectable delivery (Rybicki 2010). A viable plant-based EHEC vaccine therefore needs to be of high yield and stability to meet these requirements. On a general level, a plant-based method of vaccine production may be uniquely advantageous in offering a safer and easier mode of administration, and a better cost-benefit ratio for scaling up production. Table 2 summarizes all reports of plant-based subunit vaccines for EHEC to date.

While production of a SRP vaccine in plants has not yet been reported, a number of T3SS antigens have successfully been produced in plants. Perhaps the greatest technical hurdle at the moment for developing a plant-based T3SS vaccine is improving accumulation. Because many of the T3SS proteins are membrane proteins and partially intrinsically disordered, aggregation and solubilisation are technical problems that need remedying. The choice of subcellular localization in the plant cell can drastically affect the folding and accumulation of T3SS antigens and screening is often needed to select the most optimal compartment. Recently, it was demonstrated that co-expressing the native *E. coli* chaperone for recombinant Tir improved its accumulation and its in vivo and ex vivo stability when both were targeted to the chloroplast (Table 2) (Macdonald et al. 2017). This is of great value because most T3SS proteins require chaperone-mediated folding inside EHEC and suggests the possibility that post-translational regulation may be significant in causing low accumulation of T3SS proteins in heterologous hosts. Another viable strategy is to fuse the vaccine to another protein such as green fluorescent protein (GFP), elastin-like polypeptide (ELP) or hydrophobin (HFBI) which can impart added solubility, stability or accumulation and has been used effectively for EspA, NleA and Tir (Table 2) (Macdonald et al. 2017; Miletic et al. 2017).

While Econiche^{TM} has focused on producing a cocktail of various T3SS proteins for immunization, higher yields in plants may be possible if production is focused on a few individual antigens. Among the T3SS proteins, a select few have been demonstrated to induce higher immune responses than others, namely the 24-kDa EspA and the 37-kDa EspB proteins, and to a lesser degree, intimin. Sera taken from HUS patients contain antibodies that react strongly to these proteins, compared to control patient sera which had no reactivity (Jarvis and Kaper 1996). In addition to reactivity from O157:H7 strains, antigens prepared from O26 strains also show strong reactivity. Therefore, these proteins are great candidates for the possibility of engendering multi-strain protection (Mckee and O'brien 1996).

When lyophilized plant tissue containing a 5 mg dose of a chimeric EspA vaccine (expressed transplastomically in *Nicotiana benthamiana*) was administered to sheep three times over a six week period, five of the six animals inoculated stopped shedding O157:H7 after 48 days with about a 95% reduction in magnitude compared to control animals which persisted in shedding. Of the plant-based EHEC vaccines developed thus far, this chimeric EspA seems to be the most promising candidate based on highest efficacy, yield and and has been the only candidate tested on ruminants. Due to the recent increase in non-O157 EHEC infections, market value of EHEC vaccines could be increased by either incorporating multivalency in vaccine design such as by epitope fusions as well as testing vaccine

Table 2 Accumulation profiles of all plant-based subunit vaccines against EHEC that have been reported to date

Antigen	Serotype	Transformation type	Production system	Subcellular localization	Fusion partner	Accumulation (%TSP)	Accumulation (mg/kg)	Efficacy	References
Chimeric EspA fusion	O157:H7, O26:H11	Transient	*N. benthamiana* leaves	ER	–	0.13	14.8	–	(Miletic et al. 2017)
Chimeric EspA fusion	O157:H7, O26:H11	Transient	*N. benthamiana* leaves	ER	ELP	0.8	87.8	–	(Miletic et al. 2017)
Chimeric EspA fusion	O157:H7, O26:H11	Transient	*N. benthamiana* leaves	ER	HFBI	0.5	52.7	–	(Miletic et al. 2017)
Chimeric EspA fusion	O157:H7, O26:H11	Transient	*N. benthamiana* leaves	Chloroplast	–	0.12	13	–	(Miletic et al. 2017)
Chimeric EspA fusion	O157:H7, O26:H11	Transplastomic	*N. tabacum* leaves	Chloroplast	–	–	480	sheep: ↓ shedding. 5 mg dose lyophilized tissue; parenteral	(Miletic et al. 2017)
EspA	O157:H7	Transient	*N. benthamiana* leaves	Chloroplast	± GFP	–	–	–	(Macdonald et al. 2017)
NleA	O157:H7	Transient	*N. benthamiana* leaves	ER	–	0.003	0.0002	–	(Miletic et al. 2017)
NleA	O157:H7	Transient	*N. benthamiana* leaves	ER	ELP	0.09	0.01	–	(Miletic et al. 2017)
NleA	O157:H7	Transient	*N. benthamiana* leaves	ER	HFBI	0.01	0.001	–	(Miletic et al. 2017)
NleA	O157:H7	Transient	*N. benthamiana* leaves	Chloroplast	–	0.01	0.001	–	(Miletic et al. 2017)

(continued)

Table 2 (continued)

Antigen	Serotype	Transformation type	Production system	Subcellular localization	Fusion partner	Accumulation (%TSP)	Accumulation (mg/kg)	Efficacy	References
NleA	O157:H7	Transient	*N. benthamiana* leaves	Cytoplasm	–	0.02	0.001	–	(Miletic et al. 2017)
NleA	O157:H7	Transient	*N. benthamiana* leaves	Chloroplast	± GFP	–	–	–	(Macdonald et al. 2017)
Stx2 (B subunit only)	O157:H7	Transient	*N. benthamiana* leaves	ER	ELP	0.3	28	–	(Miletic et al. 2017)
Tir	O157:H7	Transient	*N. benthamiana* leaves	Chloroplast	± GFP	–	–	–	(Macdonald et al. 2017)
Chimeric Tir fusion	O157:H7, O26:H11, O45:H2, O111:H8	Transient	*N. benthamiana* leaves	Chloroplast	± GFP	–	–	–	(Macdonald et al. 2017)
Tir	O157:H7	Transplastomic	*N. tabacum* leaves	Chloroplast	–	–	–	–	(Macdonald et al. 2017)
Chimeric EspA-Intimin-Tir fusion	O157:H7	Stable	*N. tabacum* leaves	Cytoplasm	–	0.3	–	mice: ↓ shedding. 15 μg dose purified; oral & parenteral	(Amani et al. 2011)
Chimeric EspA-Intimin-Tir fusion	O157:H7	Stable	*B. napus* seeds	Cytoplasm	–	0.3	–	mice: ↓ shedding. 15 μg dose purified; oral & parenteral	(Amani et al. 2011)

(continued)

Table 2 (continued)

Antigen	Serotype	Transformation type	Production system	Subcellular localization	Fusion partner	Accumulation (%TSP)	Accumulation (mg/kg)	Efficacy	References
Stx2 toxoid (inactivated A subunit + B subunit)	O157:H7	Stable	*N. tabacum* cell culture	Cytoplasm	–	–	7.35	mice: ↑IgA;↑ survivability against toxin challenge; cytotoxic neutralization. 5 g dose cell culture. oral	(Wen et al. 2006a, b)
Truncated Intimin (C-terminal peptide)	O157:H7	Stable	*N. tabacum* cell culture	Cytoplasm	–	–	11.5	mice: ↑IgA; ↑IgG;↓ duration of colonization. 7.5µg dose purified. oral	(Judge et al. 2004)

ER Endoplasmic reticulum, *HFBI* Hydrophobin, *GFP* (enhanced) Green Fluorescent Protein, *ELP* Elastin-like polypeptide, *Ig* Immunoglobulin

candidates for cross-reactivity during animal trials. Accordingly, fusions of EspA epitopes from both O157 and non O157 strains, produced both transiently and transplastomically in leaves of *Nicotiana tabacum,* show promise as multivalent candidates (Miletic et al. 2017). Another candidate, an EspA-Intimin-Tir fusion, has been demonstrated to accumulate in leaves of *N. tabacum* and seeds of *Brassica napus* at about the same yield and reduce shedding when administered to a mouse model (Amani et al. 2011). In considering *B. napus* as a platform, there is some appeal as it is a much more familiar feed component than *N. tabacum* to producers if oral application is to be considered. However, *N. tabacum* has conventionally been the platform of choice primarily because it is neither a food- nor feed- crop and is less likely to contaminate a food supply. On the other hand, *N. tabacum* cell cultures show promise as a platform because they can be grown in a closed, sterile system isolated from the external environment. For example, this is the platform of choice for Protalix Biotherapeutics in their production of glucocerebrosidase in carrot cells. Further development of a cell-culture based EHEC vaccine towards a similar direction may be of value considering the recent trend of public attitudes and restrictive policy making with regards to containment of genetically engineered crops. An inactivated form of the stx2 toxin has been shown to accumulate in *N. tabacum* cell cultures and when administered to mice, IgA production is triggered and the mice have enhanced survivability against toxin challenge (Wen et al. 2006a, b). A truncated form of intimin has also been shown to accumulate in *N. tabacum* cell cultures and when administered to mice, triggers both IgA and IgG production as well as reduces the duration of EHEC colonization (Judge et al. 2004).

5 Pathways to Commercialization/Implementation for a Plant-Based EHEC Vaccine: Learning from Econiche's Business Model in the Canadian Beef Industry

Despite Econiche™ having Canada-wide availability, marketing as a robust pre-harvest control and multiple validations of its efficacy, its adoption by the Canadian beef industry after product launch was marginal at an estimated level of adoption of only about 5% (Grier and Schmidt 2009). Beef producers, the primary target market, were reluctant to adopting the product, likely because the direct benefits are realized elsewhere along the supply chain, namely processors and consumers. Additionally, aside from the direct cost of the product, vaccination required extraneous labour and veterinary costs to implement. Was the Econiche™ business model flawed? Can the barriers that hampered adoption be addressed to facilitate effective market transition for a similar product?

The economic story of the Econiche™ product in the Canadian market has implications for the general prospects of any future EHEC vaccine to be considered for commercialization. Following proof of concept and efficacy studies of T3SS

proteins, Bioniche Life Sciences Inc. was contracted for scale-up and commercialization (Jarvis et al. 1995; Jarvis and Kaper 1996). The project was financed via a substantial $25 million investment sourced from the Ontario government, Agriculture Canada, Industry Canada and the Business Development Bank of Canada (Bioniche 2012). In a 2012 letter to shareholders prior to the release of Econiche™, management disclosed that the company was suffering from a monthly burn rate of $1 million per month operating on a net income loss in prior years and that one of its foremost strategies for remedying this was from increased revenue anticipated from its new products to be released that year, including Econiche (Bioniche 2012). In the two years prior, revenue had stalled for the company at approximately $27 M. Despite this, a 2012 initiating report by Eresearch, a Canadian independent equity research corporation, recommended considerable upside potential for Bioniche's share price citing the release of Econiche™ as a main reason and forecasted Econiche™-specific revenue as bringing in $1.5 M and $3.75 M in 2013 and 2014 respectively, with steady growth in later years (Eresearch Corporation 2012). Collectively, this implies that (1) there was substantial financial capital available from multiple sources to develop the Econiche™ vaccine for market (2) Bioniche considered it a high priority revenue earner to be developed to counter its looming burn rate and (3) market research also corroborated the belief it would do well in the market. Considering that Econiche™ was announced as the world's first vaccine against EHEC with full licensure by the CFIA and provisional licensure by the USDA, its first commercial batch entered the Canadian market in mid-2012 without competition in its market niche. Epitopix™, the subsequent and only rival to-date in this market niche, obtained conditional USDA licensure a year later, and was restricted to US cattle markets. By 2014, Bioniche decided to refocus its efforts on solely human health, putting up its vaccine development unit up for sale, suspending operations and laying off most of its employees. In a statement released by Michael Berendt, CEO: "While the vaccine is an innovative and valuable product, (Bioniche) has been unable to convince the beef or dairy industries, or the federal and provincial governments, that vaccinating cattle to help reduce the human infection and deaths caused by *E. coli* is something they should support or pay for."

So, what went wrong? Perhaps the largest assumption could be that with full control over their market niche, Bioniche anticipated high demand—a far-removed prediction from its dismal 5% penetration (Grier and Schmidt 2009). The barriers to this demand directly relate to the requirements of its target market. In North America, vaccination needs to be done at least three months before slaughter and for most other pathogens, is the responsibility of beef producers. Therefore, the target market for the Econiche™ product comprises a potential total of 75,000 and 913,000 cattle/calf operations for Canada and the United States respectively (inclusive of beef farms, ranches, feedlots and dairy operations) (Statistics Canada 2017; United States Department of Agriculture 2017). Correspondingly, there are currently an estimated 13 million cattle in Canada and 103 million in the United States (Statistics Canada 2017; United States Department of Agriculture 2017). Of the 75,000 beef producers in Canada, 86.7% currently vaccinate their calves

against some form of disease so producers are no strangers to the technology (Ochieng' and Hobbs 2017). However, although these producers are accustomed to the benefits of vaccination, it is considered in many respects an insurance policy. Most Canadian producers routinely sacrifice ~10% of their profit margin to ensure against the risk that the health and productivity of their herd be diminished from the most common bovine diseases. In most herds in Canada, this allocation usually goes toward protecting against infectious bovine rhinotracheitis (IBR), bovine respiratory disease (BRD), parainfluenza-3 virus (PI3V), clostridials, hemophilus and bovine respiratory syncytial virus (BRSV). However, since cattle are asymptomatic carriers of EHEC, whether or not they harbor the bacterium is independent of their health and productivity, or risk thereof. Since EHEC does not pose a risk to the health or viability of cattle, producers are less incentivised to purchase an EHEC vaccine since such an investment would not provide any direct returns.

Towards addressing what were the barriers for EHEC vaccine adoption, a survey of Canadian cattle producers indicated that only 15% of respondents believed they bear the primary responsibility for EHEC risk reduction and only 21% of respondents believed they benefited from an EHEC vaccine (Ochieng' and Hobbs 2017). While there were many perceived barriers to adoption that were reported by respondents, the issues that most agreed to be relevant included: (1) uncertainty over benefits, as indicated by 76.8% respondents, (2) meeting buyer needs, as indicated by 71.4% respondents and (3) efficacy of the vaccine, as indicated by 68.5% respondents (Ochieng' and Hobbs 2017). Additionally, 58% of the beef producers surveyed had not previously heard about a EHEC vaccine (Ochieng' and Hobbs 2017). Given that estimated marginal effects predict an average 16.1% increased willingness to adopt given prior awareness of an EHEC vaccine, this suggests appropriate marketing of this product is an essential component of its commercialization (Ochieng' and Hobbs 2017).

Additionally, many producers were resistant to adopting Econiche™ as a stand-alone technology which could not be easily incorporated into their routine vaccination schedules. For example, the recommended dose regimen was two doses in the initial year of life plus a subsequent annual dose. This required skilled labor and handling of the animals outside of their normal handling and vaccination schedules, which usually are only twice per individual calf. Application was also required to be by injection, a procedure that both carries safety hazards for the handler and the possibility of infection to the calf.

EHEC's prevalence in retail beef products has maintained a steady 2% over the last ten years in Canada and recalls of beef contaminated with EHEC are generally infrequent, though quite costly to processors when they do occur. Indeed, a food recall can sometimes lead to the closing of a meat processing plant if it fails subsequent safety inspections. However, incorporating a vaccination program in a market-driven economy begs the question of whether or not the cost of doing so is worth the added insurance against meat recalls. A cost-benefit analysis of industry adoption of Econiche™ indicated an overall approximate savings of CAD$68 M per year comprising an estimated benefit/cost ratio of 3:1 (Grier and Schmidt 2009). In particular, the analysis indicated a total approximate annual benefit of CAD

$103 M including: CAD$21 M in reduced medical costs, CAD$4 M in reduced recalls and industry costs and CAD$78 M from loss in demand. Conversely, the annual cost to the industry was estimated at CAD$3 M, which scaled directly with the dose regimen and herd numbers. Overall, the study suggested that with implementation, this technology could be both socially beneficial and financially prudent for the beef industry on a whole. Despite this, Canadian federal or provincial sponsored incentives for EHEC pre-harvest control are low or nonexistent at the farm level though processors are well motivated to reduce EHEC contamination in order to avoid recalls. Conversely, producers do not directly benefit from lowering the chance of a food recall, despite the expectation that they pay for vaccination.

Overall, if a similar product was to be considered for commercialization, its success would be dependent on: (1) more availability of sufficient adoption incentives for beef producers by government and supply chain (2) better awareness of EHEC and vaccine technology by producers and their veterinarians (3) development of a vaccine with a high economy of scale and that is easily accommodated into producers' typical vaccination schedule. Whereas the first two requirements will need a concerted dialogue between industry and government, the requirements of the latter can be met with technical innovation that scales well with producing large quantities with minimal investment. In this regard, a plant-based platform for an EHEC vaccine is arguably a competitive solution.

The production of an EHEC vaccine in plants that could be administered orally by incorporating it into livestock feed would bypass the extraneous time and labor that made the Econiche™ vaccines unappealing to producers. The prospect of oral immunization offers a strategic competitive advantage for a plant-based EHEC vaccine because of the increased safety and convenience for the producer. However, this mode of administration may require a much larger dose than parenteral to be effective since much of the protein is degraded during its movement through the animal's gut prior to reaching cells in the distal intestinal epithelium that can generate an immune response (Rybicki 2010). Therefore, if this selling point were to be developed, key research targets could arguably be: (1) better yield of the protein (2) improved protein stability to reduce the amount lost through degradation (3) designs or formulations geared toward adjuvancy such that the threshold required for the production of an immune response may be crossed with a lower concentration of therapeutic. Additionally, the efficient cost scaling of the technology lends itself well to a widespread vaccination program—which may likely prove necessary to enable consistent herd immunity against EHEC. This technology is still in its early development stage with major milestone requirements before commercialization being proof of efficacy across environments and improving yield to enable better scaling. Yet, we are optimistic that this technology will be of value to the Canadian beef industry and toward the control of food safety.

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