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Optimizing a Disease Severity Scale for Evaluating Travelers' Diarrhea in Adults

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Abstract

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by

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MS, George Washington University, 2007

BS, Mary Washington College, 2004

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

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Abstract

Primary efficacy endpoints for interventional products targeting travelers' diarrhea (TD) prevention have been predominately based on stool frequency. However, reliance on stool-based endpoints alone may obscure potentially meaningful differences in illness profiles. A single, standardized scoring system optimized for use in adult travelers is needed to accurately measure TD severity and enable more robust estimates of treatment or intervention effectiveness. The purpose of this quantitative secondary data analysis was to describe the variability in TD signs and symptoms across traditional severity metrics such as stool output, identify which symptoms were significantly associated with a negative impact on activity, and determine whether a TD scoring system that considers other symptomology could be optimized for use in future studies using the IRT and CTT frameworks. Data were obtained from two interventional studies: TrEAT TD, a multi-site TD treatment trial, and OEV-118—a placebo-controlled ETEC vaccine efficacy trial in travelers. Correlation, regression and multiple correspondence analyses were performed across multiple signs and symptoms to assess impact on activity and a TD severity score was established. Conclusions were (a) the new TD score significantly benefits the estimation of impact on activity over any individual sign or symptom, and (b) there was a benefit to reduction in overall TD disease severity when applied to a previously conducted vaccine efficacy trial. The use of a single optimized scoring system may better capture illness severity than commonly utilized metrics and moves the field towards current recommendations for TD management. Additionally, the use of the TD severity score may be an improved efficacy metric than stool frequency for future vaccine trials.

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Chapter 1: Introduction

Study Background

Travelers' diarrhea (TD) is the most common travel-related illness, with an estimated 10 million people afflicted annually and a reported attack rate of 30 to 70% depending on destination and season (Centers for Disease Control and Prevention [CDC], 2015; Shah, DuPont, & Ramsey, 2009; Steffen, Hill, & DuPont, 2015). TD also significantly affects deployed military populations, a unique subset of the travel population, with an estimated attack rate of approximately 30 cases per 100-person months (Jaep, 2016; Riddle, Sanders, Putnam, & Tribble, 2006; Porter, Olson, Hall & Riddle, 2017). Numerous bacterial, viral, and protozoal pathogens cause diarrhea, with approximately 80 to 90% of cases caused from bacterial etiology (Steffen et al., 2015). While generally an acute, self-limiting illness with symptom resolution within 1 to 5 days, increasing evidence has linked significant chronic health conditions to these enteric infections, the most common of which include reactive arthritis and post-infectious functional gastrointestinal disorders (PI-FGDs) such as irritable bowel syndrome (Connor & Riddle, 2013; Nair et al., 2014; Pitzurra, Steffen, Tschopp, & Mutsch, 2010; Porter, Thura, & Riddle, 2013).

A future vaccine against the primary TD etiologic agents remains the most cost-effective approach from a military and long-term traveler perspective (Cachafeiro, Szucs & Behrens, 2008; Tallant et al., 2014). TD prevention in civilian travelers is driven largely by the desire to not be inconvenienced by illness while traveling; however, in military populations the objective is to minimize lost duty days, medical resources

needed within the theater of operations, and post-infectious sequelae in returning troops (PATH & BVGH, 2011). In fact, the US Department of Defense's (DoD) strategy for developing a vaccine for infectious diarrhea was listed among its top three priorities as described in a 2003 technical and policy recommendations report issued by the Institute of Medicine's (IOM; Lemon, Thaul, Fisseha, & O'Maonaigh, 2003). The DoD currently maintains three parallel vaccine development programs focused on *Campylobacter jejuni*, enterotoxigenic *Escherichia coli* (ETEC), and *Shigella* with the goal of developing a mono- or multipathogen vaccine against these common causes of TD (Naval Medical Research Center, 2017; Riddle, Tribble, Cachafiero, Putnam, & Hooper, 2008). With the significant morbidity of TD in travelers, especially long-term travelers and military populations, a critical path to preventing acute cases as well as potential long-term sequelae includes TD vaccine and other prevention development approaches.

TD Epidemiology

Initial symptoms of TD often begin within the first 2 weeks of travel and resolve without treatment within 3 to 5 days (Diemert, 2006; Steffen et al., 2015). Nevertheless, symptoms can force changes in travel/business plans, or more rarely, hospitalization (Kollaritsch, 1998; Peltola & Gorbach, 1997; Diemert, 2006). TD has been traditionally defined as the passage of three or more loose stools in a 24 hour period, often with an associated gastrointestinal symptom such as fever, nausea, vomiting, abdominal pain, tenesmus, fecal urgency, or bloody or mucoid stools (Broor & Singal, 1989; Diemert, 2006; Hill, 2010; Steffen et al., 2015). Conventionally, TD is used to reference an illness that occurs in a resident from a high-income country traveling to a low- to middle-income

country (LMIC), but it is also applied to deployed military populations (Connor, 2017; Steffen et al., 2015). Destination remains the most important TD risk factor, with regional differences in risk and pathogen etiology. More developed areas such as North America, Australia, New Zealand, Japan and Northern and Western Europe represent areas of minimal to no-risk; intermediate risk countries include China, South Africa, some Caribbean islands and countries in Eastern Europe, while highest risk areas include Asia, the Middle East, Africa, and South America. While bacterial etiology accounts for 80 to 90% of TD cases, intestinal viruses and protozoa make up the remaining 5 to 8% and 10% of cases, respectively (Steffen et al., 2015). The leading bacterial causes of TD include diarrheagenic *Escherichia coli*, *Campylobacter jejuni*, *Shigella spp*., and *Salmonella spp*., with regional and population differences (Shah et al., 2009). Common viral causes include rotavirus, astrovirus, and norovirus (Bohnker & Thornton, 2003; Freeland, Vaughan, & Banerjee, 2016).

TD Interventions

There are four main strategies of primary prevention for TD: immunization, nonpharmacological therapy, antibiotic prophylaxis, and avoidance. Vaccines against the most common pathogenic causes of TD hold particular promise for preventing gastroenteritis in individuals traveling to high-risk areas. Studies on the economic burden of diarrheal diseases, the potential for vaccines under development, and the estimated economic value of vaccines justify investment in the development and utilization of candidates against the most common causes of TD (Bartsch & Lee, 2014; PATH & BVGH, 2011; Riddle et al., 2008). Effective prophylaxis such as antibiotics or anti-

diarrheals, like bismuth subsalicylate (BSS) (Pepto-Bismol tablets), are also attractive primary and secondary prevention methods for TD (Taylor, Hamer & Shlim, 2017), with efficacy of marketed agents having been demonstrated in various field and controlled human infection model studies (DuPont et al., 2014; DuPont et al., 2009; Hu, Ren, Zhan, Li, & Dai, 2012; Taylor et al., 2006; Zanger, Nurjadi, Gabor, Gaile, & Kremsner, 2013). However, before interventions such as vaccines or prophylaxes can be used widely, rigorous clinical trials are often required. Additionally, once vaccines are introduced into the market, additional post marketing surveillance studies are typically performed to monitor vaccine effectiveness and changes in disease epidemiology, burden, and duration of protection (López-Gigosos, Segura-Moreno, Díez-Díaz, Plaza, & Mariscal, 2014). With this in mind, well-established and more clinically meaningful endpoints are critical to advancing vaccines through proof of principal studies, licensure, and pre-qualification, and disease scoring algorithms aimed at disease severity should be developed (Porter, Gutierrez & Kotloff, 2019).

Scoring Systems and Challenges

TD vaccines are meant to prevent moderate to severe disease caused by a specific etiology (Lopez-Gigosos et al., 2014; Porter et al., 2019; World Health Organization, 2006). For this reason, efficacy studies focus on moderate to severe gastroenteritis as a primary endpoint, which requires a standardized measure of disease severity. However, the primary outcomes of interest in clinical trials of vaccine candidates and interventions have varied. While most, if not all, TD vaccine and treatment studies have utilized a diarrhea definition based on number of unformed stool in a 24-hour period, some

incorporate an additional symptom such as abdominal pain or nausea to the endpoint (Stoney et al., 2017; Wiwanitkit, 2007), and most do not consider the functional impact (i.e., impact on daily activity). Measuring the efficacy of an intervention in these trials involves accurately defining the clinical endpoint of interest, such as prevention of moderate-to-severe TD. Furthermore, because vaccine efficacy may differ by disease severity, the accuracy of measuring more severe disease impacts vaccine efficacy estimates, thereby influencing future investment towards the continued development and implementation of these interventions. Because conducting vaccine efficacy trials in low-resource settings or in a controlled human infection model is expensive and challenging, the use of well-defined, validated primary endpoints is necessary.

Researchers have addressed a similar issue in pediatric studies of diarrheal disease, and numerous scoring systems have been developed and validated for the purpose of simplifying and standardizing clinical outcome measurements (Clark & Offit, 2004; Friedman, Goldman, Srivastava, & Parkin, 2004; Gorelick, Shaw, & Murphy, 1997; Jauregui et al., 2014; Kinlin & Freedman, 2012; Lee et al., 2016; Ruuska & Vesikari, 1990; WHO and UNICEF Joint Statement, 2004). Furthermore, Porter and colleagues have sought to standardize clinical endpoints and establish disease scoring systems for use in controlled human infection models (CHIMs) for ETEC (Porter et al., 2016), *Shigella* (Porter et al., 2017) and *Campylobacter* (Tribble et al., 2017).

In Chapter 2, I will discuss additional information about the history of TD disease characterization, summary of scoring system challenges as it applies to pediatric populations, and recent efforts to create similar etiologic-specific scoring systems for

application in CHIMs. In the remainder of this chapter, I summarize the problem, research design, and methodology. The purpose of the study, research questions, and hypotheses are briefly presented. Measurement theories are explored as the conceptual frameworks guiding the study. Further, I introduce key variables and identify the assumptions, limitations, and significance of the study.

Problem Statement

The global diarrheal disease burden for travelers and military personnel has prompted the development and assessment of primary and secondary disease prevention efforts. The efficacy of these interventions is often based on the prevention of moderate to severe diarrheal diseases, typically defined by maximum number of loose stools in a 24-hour period (e.g., mild = 1-2 stools; moderate = 3-5 stools; severe ≥ 6-9 stools) (Behrans et al., 2014; Darsley, Harro, Chakraborty, Sack, & DeNearing, 2015; Frech et al., 2008; Sack et al., 2007). However, reliance on stool-based endpoints alone may obscure potentially meaningful differences in illness profiles. Attempts to address this limitation have been advanced for pediatric populations and include the Vesikari and Clark scores—both Rotavirus specific (Clark & Offit, 2004; Ruuska & Vesikari, 1990); the Gorelick Score (Gorelick et al., 1997); the Clinical Dehydration Scale (CDS; Friedman et al., 2004); and the WHO Integrated Management of Childhood Illness (IMCI; WHO & UNICEF, 2004). Furthermore, more recent validation efforts have pointed towards their potential utility on the front line using a more optimized endpoint for interventional studies (Clark & Offit, 2004; Jauregui et al., 2014; Kinlin & Freedman, 2012; Lee et al., 2016). However, these scores are focused on pediatric populations with

limited utility for the adult traveler population. There is no standardized disease score for diarrheal illness in adult travelers, limiting the interpretation of disease severity differences within and across studies.

Purpose

A single, standardized scoring system optimized for use in adult travelers is needed to accurately and consistently characterize TD severity. Such a score will enable more accurate estimates of efficacy, effectiveness, and cost-effectiveness of primary and secondary prevention efforts in adult populations. The purpose of this quantitative secondary analysis was to:

1. Describe the TD disease complex and how the clinical signs and symptoms co-occur.
2. Describe the variability in signs and symptoms of TD across more traditional severity metrics (i.e., stool output).
3. Determine what individual clinical signs and symptoms of TD are significantly associated with a negative impact on activity among adult travelers.
4. Apply the disease complex score to a previously conducted TD vaccine field trial to re-estimate vaccine efficacy.

Research Questions and Hypotheses

The research questions and associated hypotheses are as follows:

Research Question 1: What combination of clinical signs and symptoms best characterizes TD severity in adult travelers?

H_o1: There are no significant differences in the frequency and severity of clinical signs and symptoms across disease severity classification.

H_a1: There are significant differences in the frequency and severity of clinical signs and symptoms of TD on disease severity classification.

Research Question 2: What individual clinical signs and symptoms of TD are significantly associated with a negative impact on activity among adult travelers?

H_o2: Individual clinical signs and symptoms of TD are not significantly associated with a negative impact on activity.

H_a2: Individual clinical signs and symptoms of TD are significantly associated with a negative impact on activity.

Research Question 3: Does a TD disease score better differentiate treatment groups than prior estimates of vaccine efficacy when applied to a previously conducted ETEC vaccine study (Protocol OEV-118)?

H_o3: The estimated vaccine efficacy of the ETVAX vaccine in the OEV-118 Phase 3 trial does not change as a result of using the new disease complex score.

H_a3: The estimated vaccine efficacy of the ETVAX inactivated whole cell vaccine tested in the OEV-118 Phase 3 trial does change as a result of using the new disease complex score.

Conceptual Framework

Two conceptual frameworks guided this research as it relates to scale development and application. They are based on scale development theories and include the item response theory (IRT) and classic test theory (CTT) that researchers often use to

guide scale development through factor analysis and item selection. IRT was first used in the field of psychometrics to evaluate instruments and assess subjects on their abilities, attitudes, or other traits (Xinming & Yung, 2014). Commonly used aptitude tests like the Scholastic Assessment Tests (SATs) and Graduate Record Exams (GREs) have been developed using IRT, which has been shown to improve reliability and measurement accuracy while also improving efficiency in assessment time (Xinming & Yung, 2014). Over the years, IRT methodology has become increasingly popular and applied to health outcomes, clinical research, and quality-of-life research (Hays, Morales, & Reise, 2000; Xinming & Yung, 2014). Researchers have shown that IRT models better reflect actual response patterns in sample populations, resulting in better estimates of self-reported health outcomes that are more sensitive to true cross-sectional differences and more responsive to changes in health over time (Hays et al., 2000). CTT is another psychometric theory researchers use to predict the abilities of subjects, in which a subject's observed score on a test is the subject's true score plus some error (since it is unlikely that a subject will perform perfectly on a test; Tractenberg, 2010). Similar to the IRT, CTS has also been widely used in development and selection of endpoint measurements in clinical trials (Tractenberg, 2010), with advantages and disadvantages cited for using one over the other (Hays et al., 2000). These frameworks have been relevant to the development of various scales for gastroenteritis assessment (Cappelleri et al., 2014) and thus remain an applicable framework for this research.

Nature of the Study

This study consisted of a quantitative secondary data analysis in which I compared two existing clinical trial datasets containing clinical signs and symptoms of TD for consistent variables and subsequently analyzed them to develop and validate a TD severity score for the target population. This study could also be considered methodological research, as it involved the development and evaluation of data collection scales (see Frankfort-Nachmias & Nachmias, 2008), or more specifically a scoring system that better characterizes moderate to severe TD disease. The goal with such research is to make an intangible concept tangible (Dancer, 1994). In this case, my goal was to develop a standardized instrument for measuring a disease profile consisting of both objective and subjective parameters. This optimized scoring system would be available for additional validation and testing with currently existing datasets, in future enteric vaccine or prophylaxis trials, post introduction effectiveness, and surveillance studies, as well as in additional secondary analyses utilizing other vaccine or intervention trial datasets.

Variables

Data for this analysis came from two clinical trial datasets (studies TrEAT TD and OEV-118; Riddle et al., 2017; Bourgeois et al., 2011). The items that comprise both datasets include the maximum number of loose stools in a 24-hour period, total number of stool containing blood, number and duration of vomiting episodes, duration and severity of abdominal cramping, duration and severity of nausea, duration and severity of gas, duration and severity of tenesmus, duration and severity of malaise, highest recorded

fever in a 24-hour period, and duration and severity of fecal incontinence/urgency of defecation. The relative severity category assignment (i.e., mild, moderate, severe) based on impact on activity was also included in the TrEAT TD dataset. I used these data and sources, which I further described in Chapter 3, to determine which signs and symptoms were most relevant for describing TD severity, develop a TD complex scoring system, apply the score to a previously conducted Phase 3 study for re-determination of vaccine efficacy, and ultimately propose an optimized TD scoring system for use in future studies.

Definitions

The below terms are consistent with the clinical research protocol from which the original data were collected and were used in this secondary analysis (see Riddle et al., 2017).

Travelers Diarrhea: Three or more loose stools in 24 hours, or ≥ 2 loose stools in 24 hours with associated symptoms such as nausea, vomiting, abdominal cramps, or tenesmus of ≤ 96 hours duration.

Diarrheal stools: Loose or liquid stools taking the shape of the container.

Diarrhea-associated signs/symptoms: Abdominal cramps, nausea, vomiting, malaise, excessive gas, tenesmus, and fever temporally related to the diarrheal episode. Signs/symptom severity was recorded on subject diary cards and based on functional impact on subject duty performance (i.e., no impact, ≤ 50% impact, > 50% impact, inability to function).

Temperature: Oral temperature obtained by a study clinician during a clinic visit using a thermometer.

Fever: The oral equivalent of a higher than normal temperature (i.e., > 100.4ºF) as recorded upon assessment of temperature using a thermometer by a study clinician during a clinic visit.

Assumptions

One major assumption in this study was that the items captured as part of the original TrEAT TD and OEV-118 studies provide a holistic view of the major clinical symptoms involved with TD gastroenteritis. Because this research was a secondary data analysis, no further information beyond what was captured in the original clinical trial datasets is available. However, as described in Chapter 2, the major clinical symptoms of TD gastroenteritis identified in epidemiological and previous intervention studies remain consistent with those captured in the original two datasets as well as the compiled secondary dataset I used in this research. While both the TrEAT TD and OEV-118 studies allowed for collection of "other symptoms" as part of their daily diary card assessments, providing for capture of signs beyond what has been historically associated with TD, such symptomology was not included in this secondary data analysis.

Limitations

The first major limitation of this study is that it is based on a secondary data analysis and thus limited to the sample size of gastroenteritis episodes experienced by study participants in the TrEAT TD and OEV-118 studies. While both studies were relatively large and contained an approximate combined total of 1800 participants, they

were conducted across four regions (Mexico, Central America, South Asia, and Africa) and six countries (Afghanistan, Djibouti, Kenya, Honduras, Mexico, and Guatemala). The pooled sample size across and between regions is adequate to make some general conclusions; however, the sample size may not be adequate to conduct a sufficiently powered analysis at the country level, or other levels (i.e., by specific pathogens).

As this is a secondary data analysis, participants enrolled in the parent studies were confined to the parameters of the original study protocols, potentially limiting the generalizability of the results to a generally healthy adult population ≥ 18 years old. Nevertheless, because the majority of travelers and military members who travel to high-risk areas are generally comprised of people of similar demographics, this limitation might be considered minor.

While both original studies utilized diary cards to collect symptoms beginning from the first day of an episode, thus limiting the risk of recall bias, it is possible that response bias persisted in reporting of symptoms. For example, a participant who experienced a severe symptom might also have been more likely to report other signs and symptoms as he or she was more focused on what might have been making him or her feel unwell. In contrast, a participant who experienced a mild symptom with little impact on activity might have been less inclined to focus on feeling unwell and therefore record additional symptoms.

Another potential limitation of this study is that the original datasets represent an approximate ten-year time gap in data collection, and the regional variability of TD might have changed over that period of time. However, as the literature review in Chapter 2

highlights, the areas of most risk to potential travelers has remained consistent in the past 10 years and includes the study sites from both TrEAT TD and OEV-118 studies.

Delimitations

Delimitations of this study are partially included in the above limitations section. Furthermore, this research benefitted from the methods used to collect the data contained in each of the secondary analysis datasets. Both original studies (TrEAT TD and OEV-118) utilized diary cards that allowed participants to collect symptoms beginning from the first day of an episode in real-time as symptoms occurred, thus limiting potential for recall bias and an over- or under-estimation of symptoms.

Regardless of the limitations and delimitation outlined in this section, it was important that I conduct this study, as it built upon recent efforts to develop scoring systems for etiology-specific challenge human infection models and provides timely and relevant information to the field for future testing of TD interventions and treatments that are fast approaching or currently undergoing field efficacy evaluation.

Significance

TD is the most common travel-related illness with an estimated 10 million people afflicted annually (CDC, 2015), and is the second leading infectious disease cause of death in children under the age of five in developing countries (PATH & BVGH, 2011). Porter et al. (2016) highlighted the importance of including other clinical signs and symptoms of clinical disease along with traditionally used stool output measures to more accurately characterize ETEC disease as it applied to CHIMs. Similar to the development and utilization of disease severity scales for pediatric diarrhea, using a validated score for

TD could lead to more appropriate outcomes for epidemiological research and interventional studies. Positive results from such efficacy studies would potentially lead to licensure of a vaccine for use in traveler, military, and pediatric populations. Meanwhile, both travelers and military populations represent important segments of a potential TD interventions market that drives demand due to not wanting to be inconvenienced by illness during travel, the desire to minimize lost duty days, and the need to reduce post-infectious sequelae in returning travelers (PATH & BVGH, 2011). Finally, development of a single optimized scoring system provides the field a more clinically meaningful endpoint to utilize for future studies, thereby appropriately setting the bar for advancement and licensure of TD vaccines and treatments.

This chapter provided a brief introduction regarding the epidemiology of TD gastroenteritis, the importance of disease prevention through vaccination and other methods, the use of existing scoring systems for measuring disease severity and vaccine efficacy and associated challenges, the gap in knowledge with existing scoring systems, especially in the context of assessing TD severity, and the benefits of creating a simplified and standardized tool for measuring clinical outcomes. I also introduced the research design, research questions, hypotheses, conceptual frameworks, assumptions, limitations, and delimitations of the study, ending with a description of the social change implications of this research. The following chapters provide (a) the methodology, rationale, and need for this study; (b) the review of literature including more detailed look into TD epidemiology; (c) the history and most current approach to measuring severity of TD gastroenteritis; (d) the utility of measuring additional TD symptomology; (e)

expansion on the conceptual framework; (f) the research methodology, including an in-depth description of the design and analysis that were used in this study; (g) the results; and (h) the conclusion, including recommendations for future research and social implications.

Chapter 2: Literature Review

Introduction

TD is the most common travel-related illness with an estimated 10 million people afflicted annually and a reported attack rate of 30 to 70% depending on destination and season (CDC, 2015; Shah et al., 2009; Steffen et al., 2015). TD typically begins within the first 2 weeks of travel and often resolves without treatment within 3 to 5 days (Diemert, 2006; Steffen et al., 2015). Nevertheless, symptoms can often manifest into more severe sequalae, forcing change in travel plans or more rarely, hospitalization (Kollaritsch, 1998; Peltola & Gorbach, 1997; Diemert, 2006). International tourism represents 7% of the world's exports in goods and services, often ranking as the first export sector for developing countries (UNWTO, 2016). As a result, TD bears significant economic costs to both travelers as well as to developing countries' tourism industries. In addition to the general traveler from a developed to a developing country, TD also significantly affects deployed military populations, a unique subset of the travel population, with an estimated attack rate of 30 cases per 100-person months (Porter et al., 2017; Riddle et al., 2006; Steffen et al., 2015). TD is also one of the principal causes of non-combat-related disease morbidity among deployed U.S. military personnel, including those battling insurgencies in Iraq and Afghanistan (Sanchez, Gelnett, Petruccelli, Defraites, & Taylor, 1998; Sanders et al., 2005). From a military public health standpoint, its impact on troop health and readiness is larger than any other infectious disease syndrome (Diemert, 2006). The impact of TD on both military and travel medicine as well as the public health sector necessitates further investment in sanitation infrastructure,

education about primary prevention, and development of prophylaxis and vaccines against the various etiologic agents attributable to disease.

In the following in-depth analysis of the literature, I will demonstrate the necessity of this research and its implications for social change, showing how it will benefit future TD vaccine and treatment studies to enable more appropriate estimates of efficacy. I (a) explain the background and epidemiology of TD and its clinical presentation; (b) describe the history of TD classification; (c) detail scoring systems used in pediatric populations, controlled human infection models, and earlier TD studies; (d) provide information on the conceptual framework driving this research; (e) explain the methodology used for answering the research questions; and (f) describe the implications for social change resulting from this research.

I completed this literature review using library database searches to gather articles optimally published within the last 5 years; however, original sources older than 5years and seminal publications were also cited. I also reviewed references from each peer-reviewed article for relevant literature. I began research at the PubMed website because it is considered the premier scientific database for accessing articles relevant to the topic of TD epidemiology, vaccine studies, and scoring systems. I also use other databases available via the Walden University Library because they provided access to many articles not otherwise available on PubMed. I used the following search terms alone or in combination with other search terms in order to identify relevant articles: *travelers' diarrhea (travellers' diarrhea), clinical trial, epidemiology, score, scale, clinical scoring system, gastroenteritis, diarrhea (diarrhoea), Vesikari, travelers, challenge,* and *TD*. I

also contacted Walden University library staff in order to help with retrieving the oldest articles describing the first descriptions of travelers' diarrhea for which they were able to provide.

Epidemiology

Place and time. Destination remains the most important risk factor for developing TD, with regional differences dictating both risk for and particular etiologic agents of TD. More developed areas such as North America, Australia, New Zealand, Japan, and Northern and Western Europe represent areas of low-risk (< 8% attack rate); intermediate risk countries include China, South Africa, some Caribbean islands, and countries in Eastern Europe (10-40% attack rate); while highest risk areas (attack rates up to 70%) include Southeast Asia, the Middle East, Latin America, Africa, and Central and South America. Attack rates of up to 75% have been observed in travelers to high-risk areas (Cobelens, Leentvaar-Kuijpers, Kleijnen, & Coutinho, 1998; Peltola & Gorbach, 1997; Diemert, 2006), with incidence rates ranging from 10 to 40% in travelers to areas of intermediate risk (Steffen et al., 2015). There exist regional differences in the etiology of TD, with enterotoxigenic *Escherichia coli* (ETEC) predominating worldwide as the leading cause of TD in most regions except certain areas of Southeast Asia, including Thailand, in which *Campylobacter* and *Aeromonas* are more common (Shah et al., 2009; Steffen et al., 2015; Tribble et al., 2007). Enteroaggregative *Escherichia coli* is the second most common bacterial enteropathogen in Latin America and the Caribbean, as well as in South Asia, with less than 5% reported from Africa. In contrast, *Salmonella, Shigella*, norovirus, and rotavirus are reported in approximately 5 to 25% of reported TD

cases from Africa (Jiang et al., 2002; Riddle, Sanders, Putman, & Tribble, 2006; Steffen et al., 2015). Enhanced diagnostic methods such as polymerase chain reaction (PCR) and TaqMan are continuing to be developed to determine etiology and help identify a broader array of pathogens in a single multiplex assay (Antikainen et al., 2013; Youmans et al., 2014; Lertsethtakarn et al., 2016). However, a potential drawback of this newer technology and the ability to detect multiple viable and non-viable pathogens is the potential difficulty in differentiating the etiologic agent (Connor, 2018).

TD risk is also seasonal, with higher rates due to bacterial pathogens in summer months and rainy seasons (Cobelens et al., 1998; Hoge et al., 1968); whereas dryer seasons are associated with TD of viral etiology (de la Cabada Bauche, & DuPont, 2011). Longer duration of travel has also been significantly associated with TD, with greater than 1 week of stay associated with increased risk (Hill, 2000; Pitzurra et al., 2010; Steffen et al., 2004; Vilkman, Pakkanen, Laaveri, Siikamaki, & Kantele, 2016). However, it should be noted that in a study of Korean travelers visiting Southeast Asia, Ahn et al. (2011) identified shorter duration of travel (less than 7 days) as more significantly associated with higher TD incidence.

Demographic risk factors. Despite prior studies finding no effect of gender on TD risk (Diemert, 2006; Evans, Shickle, & Morgan, 2001; Steffen et al., 2004), more recent studies have shown gender to be a confounding factor in predisposition to acquiring illness. In a study of Finnish travelers visiting outside the Nordic region (77 countries included), Vilkman et al. (2016) observed females were more predisposed to illness than their male counterparts (OR = 1.5; 95% CI = 1.0-2.4, p = .008); however, in a

study of Korean adults traveling to Southeast Asia, being male was associated with higher TD rates (p = .007; Ahn et al., 2011). Age has long been established as playing a significant role in diarrheal disease illness, with highest incidence rates among children under the age of5 in developing countries (approximately 2.9 episodes/child year) and younger adults traveling from industrialized nations to high-endemic areas (approximately 1.6 illnesses/traveler; DuPont & DuPont, 2006; Fischer Walker, Perin, Aryee, Boschi-Pinto, & Black, 2012; Hill, 2000; Steffen, 2005; Steffen et al., 2004; WHO, 2017). The higher rates among the former subpopulation (i.e., endemic children) is likely due to naïve immunity, lack of hygiene and sanitation infrastructure, high pathogen co-infection rates, and increased fecal-oral contamination (Brown, Cairncross, & Ensink, 2013; Fewtrell, & Colford, 2005; Oyekale, 2017; UNICEF/WHO, 2009; WHO, 2017). The higher incidence rates in younger adult travelers is likely attributable to propensity for more adventure travel (Kollaritsch, 1989; Steffen 2005; Steffen et al., 2015) and lack of vigilance in eating contaminated foods (Diemert, 2006; Hoge et al., 1996; Pitzurra et al., 2010). Interestingly, within the military subpopulation, the direction of age effect is inverse to that of other traveler cohorts, in that risk of TD increases with increased age; a finding consistent in military studies (Riddle et al., 2006; Sanders et al., 2004) but in contrast to traditional traveler cohort studies as described above.

Etiologies/Causative Agents

The most important etiological agents of TD are bacterial, with approximately 80 to 90% of cases across various studies attributable to at least one bacterial agent (Ansdell & Ericsson 1999; Diemert, 2006; Peltola, & Gorbach, 1997; Steffen et al., 1999; Taylor

et al., 2006). Of the more prevalent bacterial causes, diarrheagenic enterotoxigenic *Escherichia coli* (ETEC) remains the leading cause of TD, followed by *Campylobacter jejuni*, *Shigella* spp., and *Salmonella* spp. Other *Escherichia coli* pathotypes, such as enteroinvasive *E. coli* (EAIC) and enteroaggregative *E. coli* (EAEC), have also been increasingly associated with TD (Adachi et al., 2001; Huang, Okhuysen, Jiang, & DuPont, 2004; Steffen et al., 2015; Wanger, Murray, Echeverria, Mathewson, & DuPont, 1988). Emerging prevalence of *Aeromonas* species, *Arcobacter* species, and *Plesiomonas shigelloides* have also been observed (Jiang et al., 2010; Kayman et al., 2012; Yamada, Matsushita, Dejsirilert, & Kudoh, 1997; Steffen et al., 2015), and non-cholera vibrios less commonly isolated in some TD cases (CDC, 2015).

Viruses account for 2 to 27% of TD, with norovirus, rotavirus, astrovirus, and enteric adenovirus being the most commonly isolated pathogens (CDC, 2015; Diemert, 2006). However, their etiological importance is somewhat tempered by the fact that bacterial pathogens are also concomitantly isolated in many TD cases, especially with rotavirus. Norovirus has been implicated in a number of unique settings, such as cruise ships (Bert et al., 2014; Freeland et al., 2016; Morillo et al., 2017; Wang et al., 2017) and military deployments (Ahmed et al., 2012; Hameed et al., 2016; Surveillance Snapshot, 2017; Watier-Grillot et al., 2017), highlighting it as an important pathogen in certain travel populations.

TD caused by parasites is more persistent and results in prolonged duration compared to TD cases of bacterial etiologies (Connor, 2017; Ross & Crips, 2013; Swaminathan et al., 2009). Giardia intestinalis is the most predominant protozoal cause

of TD (Connor, 2017; Fullerton & Yoder, 2017; Hagmann et al., 2014; Harvey et al., 2013; Swaminathan et al., 2009). Entamoeba histolytica, Cryptosporidium and Cyclospora are less commonly isolated (Black, 1990; Diemert, 2006; Steffen et al., 2015), the latter being highly geographically and seasonally dependent with highest risk associated with travel to the mountainous regions of Peru and Nepal (Drenaggi, Cirioni, Giacometti, Fiorentini, & Scalise, 1998; Jelinek, Loze, Eichenlaub, Loscher, & Nothdurft, 1997; Pandey et al., 2011; Thapa & Basnyat, 2017). No pathogen is isolated in 10 to 50% of all TD cases, likely as a result of limited diagnostic methods or self-limiting characteristics of infection (Diemert, 2006).

TD Costs

Though mostly self-limiting, TD can cause significant disruption to travel and/or itineraries, business plans, and tourism revenues (Wang, Szucs, Steffen, 2008). Because tourism is an important industry to many developing countries' economies, associated revenues in those areas with the highest attack rate for TD are significantly affected. Wang et al. (2008) estimate that approximately 1 day of incapacitation per traveler due to TD in developing countries would result in $500 million of missed tourism revenue. The burden of medical costs and productivity losses is exacerbated if TD persists upon returning home. In the United States it is estimated that approximately $300 million and $650 million in medical costs and lost productivity costs, respectively, are incurred from ill-returning travelers. Similarly, in the European Union, €200 million and €450 million are incurred for medical and lost productivity costs, respectively, from travelers returning with TD (Wang et al., 2008). Because of these financial implications, among others, a

future vaccine against the primary TD-attributable etiologic agents remains as one of the most cost-effective approaches from a military and long-term traveler perspective (Cachafeiro et al., 2008; Tallant et al., 2014).

Clinical Syndromes/Presentation

TD has been traditionally defined in studies as the passage of three or more loose stools in a 24-hour period, often with an associated gastrointestinal symptom such as fever, nausea, vomiting, abdominal pain, tenesmus, fecal urgency, or bloody or mucoid stools (Broor & Singal, 1989; Diemert, 2006; Hill, 2010; Steffen et al., 2015). Conventionally, TD is used in reference to illness that develops in a resident from a developed country who travels to a developing country; also applied to military populations deployed from industrialized areas to low-resource regions (Connor, 2017; Steffen et al., 2015). Symptoms typically present within the first week of travel, although 90% of cases are identified within the first two weeks (Diemert, 2006; Steffen, van der, Gyr, & Schar, 1983).

The average course of untreated TD is about 4 to 5 days, often self-limiting and requiring limited hospitalization (Connor, 2017; Diemert, 2006; Steffen et al., 2015). The most commonly reported symptom is abdominal cramping in about 80% of TD cases, followed by vomiting (20%), fever (10 to 25%) and blood and/or mucous in the stools (5 to 10%) (Cobelens et al., 1998; Diemert, 2006; Sanchez et al., 1998).

In addition to the morbidity of acute disease, there are associated chronic post-infectious sequelae. Post-infectious irritable bowel syndrome (PI-IBS) can occur in 3 to 17% of patients after TD (Okhuysen, Jiang, Carlin, Forbes, & DuPont, 2004; Mutsch,

Pitzurra, Hatz, & Steffen, 2014; Schwille-Kiuntke et al., 2015; Stermer, Lubezky, Potasman, Paster, & Lavy, 2006) while higher rates of other gastrointestinal symptoms, such as chronic or persistent diarrhea, are also observed (Nair et al., 2014; Steffen et al., 2015). Finally, reactive arthritis and Guillain-Barre syndrome have also been associated with TD (Connor & Riddle, 2013; Jackson et al., 2014; Steffen et al., 2015). Development of such chronic complications emphasizes the need for further characterization of the risk factors and incidence of these syndromes to more effectively determine prophylactic and/or treatment procedures to reduce their incidence.

Impact on Activity

Impact on activity varies across studies and travel populations. Some studies have reported a significant impact of TD on daily activities, with 20% of travelers requiring bed rest over 1-2 days (Hill, 2000; Sebeny et al., 2012; Soonawala, Vlot, & Visser, 2011; Steffen et al., 2004; Steffen et al., 2015); up to 40% requiring modification to daily activities during travel (Soonawala et al., 2011; Ryan & Kain, 2000; Diemert, 2006) and only 1% requiring hospitalization (Kollaritsch, 1989; Peltola & Gorbach, 1997; Steffen et al., 2015). In a study of healthy adults with TD during travel from the Netherlands to the subtropics, 39% characterized their TD as mild, 34% as moderate and 27% as severe, with those reporting major inconveniences having more severe symptoms and prescribing to treatment protocols (either self-treatment or visiting health facilities). However, travelers with TD reported it less problematic upon their return in country than how they characterized it before departure (Soonawala et al., 2011).

Within the military, travelers' diarrhea remains the leading infectious cause of disease non-battle injury (DNBI), lost duty days and reduced operational readiness (Connor, Porter, Swierczewski, & Riddle, 2012; Riddle et al., 2011). Historically, approximately 80,000 duty-days were lost due to diarrhea in deployed troops during the Korean War; and diarrheal illness accounted more troops being hospitalized and confined to quarters during the Vietnam War than due to Malaria by a 4:1 ratio (Connor & Farthing, 1999). During the First Gulf War, 97% of American troops had TD and 20% of troops suffered from TD that prevented reduced fighting effectiveness (Connor & Farthing, 1999; Putnam et al., 2006). Approximately 70% of U.S. personnel deployed as part of Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) in Iraq and Afghanistan, respectively, experienced at least one episode of TD, with >50% experiencing multiple episodes (Putnam et al., 2006; Sanders et al., 2005; Sanders et al., 2004). The implications of acute diarrhea on operational readiness is further compounded with the abovementioned long-term chronic sequalae.

Most recently, a graded expert panel critically issued recommendations on management of TD for use by health care providers when providing pre- and post-travel consultation to travelers (Riddle et al., 2017). As part of those recommendations, the panel advised accounting for functional impact in characterizing diarrhea severity rather than the more traditional stool frequency-based algorithm that has been utilized in practice and discussed throughout this Chapter (Riddle et al., 2017). Such an assessment of illness based on functional impact as tolerable (mild), distressing (moderate), or incapacitating (severe) could better delineate treatment options or tailor therapies for

affected individuals (Riddle et al., 2017). Historically, TD severity was based on the number of unformed stools passed in 24 hours (e.g., mild = 1-2 stools; moderate = 3-5 stools; severe = ≥ 6-9 stools). However, a traveler could be impacted by having TD with a low frequency of loose stools along with fever or severe cramps compared to a traveler experiencing six loose stools without additional symptoms. Meanwhile, additional research into defining non-frequency-based outcomes in TD is needed. That was the intent of this dissertation.

Prophylaxis and Treatment

General management of TD follows standard guidance, including avoiding dehydration, mitigating associated symptoms (diarrhea, cramps, vomiting, nausea) and reducing impact on interruption of travel plans (Al-Abri, Beeching, & Nye, 2005; Zaidi & Wine, 2015). Antibiotics when taken prophylactically, reduce the incidence of TD by 80 to 90% (DuPont et al., 2009); when used for treatment during illness they shorten the duration of disease by approximately 1.5 days (de Bruyn, Hahn, & Borwick 2000; DuPont et al., 2014). Geographic region influences choice of antibiotic treatment; ciprofloxacin is often recommended but where *Campylobacter spp*. are prevalent the drug of choice is azithromycin (Steffen et al., 2015). Rifaximin and ciprofloxacin are both effective in treatment of TD due to non-invasive agents; however, rifaximin should not be used when invasive agents are suspected due to limited data on its effectiveness against such pathogens.

Prevention

There are four main categories of TD prevention: immunization, nonpharmacological therapy, avoidance and antibiotic prophylaxis. While immunization confers the most cost-effective method of protection, there are no US licensed products for use the causative agents. Only recently has a vaccine against *Vibrio cholerae* serogroup O1 (Vaxchora^{TM}) been licensed by the US Food and Drug Administration (FDA) for adults traveling to cholera-affected areas (Levine et al., 2017), although it should be noted, and as previously described, *Vibrio cholera* is not a leading bacterial cause of TD. For European and Canadian residents, there are two licensed products for use against *Vibrio cholera*, a live attenuated oral candidate (Mutacol, Orochol) and an inactivated whole cell vaccine (Dukoral). The former demonstrated up to 90% efficacy in a challenge study in which participants were challenged with *Vibrio cholerae* three months after vaccination (Tacket et al., 1999); whereas the latter candidate showed 50-71% efficacy against *V. cholerae* as well as to ETEC in two large travelers' studies (Peltola et al., 1991; Sack et al., 2002; Svennerholm & Savarino, 2004). Nevertheless, despite also being licensed in some countries for protection against ETEC, the estimated efficacy of Dukoral against all-cause TD remains low and predicted to protect only approximately 7% of travelers (Steffen et al., 2015). Similarly, vaccines against *Salmonella enterica* serotype Typhi confer modest protection against enteric fever, but this disease may not be associated with diarrhea (Steffen et al., 2015). Because these pathogens are an uncommon cause of TD, available vaccines should only be considered for high-risk populations traveling to endemic areas or used in outbreak campaigns.

Numerous nonantibiotic and antibiotic agents have been evaluated for the prevention of TD, including but not limited to Bismuth subsalicylate (commonly known as Pepto-Bismol), rifaximin, ciprofloxacin, loperamide, and azythromycin. Bismuth subsalicylate is mostly marketed in North America for the prevention of diarrhea and has been shown to provide moderate protection by reducing diarrhea by 65% when taken four times a day (DuPont et al., 1987; DuPont, Ericsson, Johnson, & de la Cabada, 1990). The aforementioned antibiotics are highly effective in TD prevention and treatment; however, their prophylactic use should be restricted to high-risk populations and only under special circumstances (Gorbach & Edelman, 1986; Diemert, 2006; Steffen et al., 2015). Rifaximin is a gut selective antibiotic that is poorly absorbed and has demonstrated significant reduction in the incidence of noninvasive TD in a meta-analysis of four trials (Dupont et al., 2005; Hu, Ren, Zhan, Li, & Dai, 2012). More recent studies continue to show the moderate benefit of this antibiotic, including a Phase 3 study of a new oral formulation of Rifamycin SV MMX (RIF-MMX) in which clinical cure of treated adult travelers to Mexico or Guatemala was 81.4% compared to placebo recipients (DuPont et al., 2014); one study confirming 48% protection against TD in travelers to South and Southeast Asia compared to placebo (Zanger, Nurjadi, Gabor, Gaile, & Kremsner, 2013); and a small challenge study in which the drug prevented shigellosis (Taylor et al., 2006), thus suggesting an effect against this invasive pathogen. Rifaximin is approved for the treatment of TD due to non-invasive enteric pathogens or specifically *E. coli* infections.

Measuring the Severity of TD Gastroenteritis

Vaccines targeting the main bacterial causes of TD, including *Campylobacter*, *Shigella* and enterotoxigenic *Escherichia coli* (ETEC), are currently in development and may be ready for large-scale efficacy trials as soon as 2019 (Walker, 2015). The impact of a vaccine is often best defined by its ability to prevent severe illness where impact of vaccination will greatest on severe outcomes, such as hospitalization (World Health Organization [WHO], 2008); as a result, clinical sponsors and/or need accurate and reliable tools to characterize severity. Failure to properly define the severity endpoint in a clinical trial could result in a reduced statistical power. In the specific case of rotavirus, vaccine efficacy estimates vary by the severity of disease, often increasing with greater severity; consequently, disease severity measurements directly affect vaccine efficacy estimates and impacts the ability for inter-trial comparison when different scoring systems or definitions of clinical disease endpoints are used (Das et al., 2013; WHO, 2008). For this reason, the symptom parameters that are obtained must be carefully considered across studies and should appropriately characterize the illness.

While most, if not all, TD vaccine and treatment studies utilize a diarrhea definition based on number of unformed stools in a 24-hour period, some incorporate an additional symptom such as abdominal pain or nausea to the endpoint (Stoney et al., 2017; Wiwanitkit, 2007). Reliance on stool-based endpoints alone without consideration of other symptoms may obscure meaningful differences in illness profiles and underestimate vaccine efficacy. This study aimed to explore the relationship between clinical signs and symptoms of TD contained in two different clinical trial datasets and

describe the variability in signs and symptoms across stool output, determining which variables are required to accurately measure TD gastroenteritis and propose a single, standardized scoring system optimized for use in adult travelers. The results from this study will impact future TD vaccine and treatment studies enabling more appropriate estimates of efficacy.

Utility of Additional TD Symptomology

TD historically emerged as a significant public health topic in relation to military campaigns, where more soldiers were dying from diarrheal disease than from war-related injury (Butler, Middleton, Earnest, & Strickland, 1973; Cook, 2001; Davison, 1922), or other maladies, such as malaria (Connor & Farthing, 1999). Later TD came to light as a problem for international travelers, students traveling to high-burden areas and expatriates (Dupont, Ericsson, & Steffen, 2008). As mentioned before, the first description of TD emerged through study of military populations engaged in campaigns. An epidemiologic investigation of 2,500 travelers from the United States to Mexico by Kean (1963) provided the first detailed clinical description of the syndrome obtained from 75 participants. In that study, travelers' diarrhea was named 'turista' and poetically described as 'a staccato ballet' of watery bowel movements every 15 minutes during the initial onslaught, accompanied by malaise, severe cramps, nausea and several episodes of vomiting. The turista syndrome was identified as a definite clinical entity by Kean, and hereafter has provided the clinical context on which the clinical profile of travelers' diarrhea is based. In that same study Kean drew an association between outcomes of competition of athletes participating in the Olympic Games to occurrence of diarrhea

(1963). Students traveling to Mexico have served as a popular setting for extensive TD study over the past sixty years (Ericsson, DuPont, & Mathewson, 1995; Kean, 1963; Kean, Schaffner, Brennan, & Waters, 1962; Ko et al., 2005; Paredes-Paredes et al., 2011; Varela, Kean, Barrett, & Keegan, 1959), and studies in persons living overseas in high-risk areas for prolonged periods of time has increased the evidence base to such populations as expatriates, Peace Corps volunteers, and missionaries (Habergerger et al., 1994; Herwaldt, de Arroyave, Roberts, & Juranek, 2000; Shlim et al., 1999). Between 1963 and 1983, epidemiologic features of TD such as risk factors, illness timing and duration were further described. Based on the increased availability of published research, Dupont & Dupont (1981) attempted to delineate the world into low, medium and high-risk areas, after which further research continued to establish region-specific attack rates in high-risk areas (Ericsson et al., 1995; Harvey et al., 2013; Steffen et al., 2004; Steffen, van der, Gyr, & Schar, 1983).

Since the early descriptions of TD and subsequent evaluations of either vaccines, prophylaxis or treatments, slightly different definitions of TD have been utilized as the primary endpoint for determination of efficacy. Table 1 summarizes the variability in clinical endpoints used for field trials testing various vaccines for prevention of TD. What has remained consistent in those evaluations has been setting the primary endpoint definition on stool number, while other clinical signs and symptoms factoring into the primary endpoint definition rarely or not at all. As the leading bacterial cause of TD, vaccines against ETEC have been a primary focus for vaccine developers and thus the most evaluated in late stage efficacy trials to date. The most recent pivotal Phase 3 trial

evaluating the safety and efficacy of a skin-patch vaccine against ETEC's heat labile toxin (LT) utilized a trial endpoint definition of moderate-to-severe diarrhea as four or more unformed stools in a 24-hour period to determine efficacy (Behrens et al., 2014; Steffen et al., 2013). While the study collected detailed symptomology from participants during experienced diarrheal episodes, including their effect on daily activity, those parameters were never factored into vaccine efficacy determination nor even commented on in the trial publication. While the vaccine conferred limited protection against vaccine preventable outcomes (VPO), as well as showed a slight reduction in severity and duration of all-cause diarrhea, it remains unknown from the published literature whether the vaccine had an impact on overall disease profile outside of a clinical definition based on stool number alone.

In an earlier pivotal field efficacy study in which an inactivated whole cell ETEC vaccine was evaluated in adult travelers to Guatemala and Mexico, accounting for other clinical signs and symptoms of TD besides diarrheal stool number as well as impact on activity actually increased vaccine efficacy estimates (Sack et al., 2007). In this study, the primary outcome was a VPO as defined as an episode of ETEC diarrhea with an ETEC organism producing vaccine-relevant antigen(s). In addition to recording the number of loose stools, participants also recorded daily diary card information on other associated TD symptomology that interfered with daily activity, such as cramps, abdominal pain, vomiting, anorexia, nausea, and urgency of defecation. While the vaccine proved inefficient at protecting against mild symptoms associated with VPO-ETEC, it did significantly reduce episodes of more severe VPO diarrhea as defined by more than five

stools in 24 hours (85% protective efficacy, p=0.037) or by symptoms that interfered with daily activities (77% protective efficacy, p=0.039) (Sack et al., 2007). This latter example provides preliminary evidence of the potential impact in accounting for other clinical signs and symptoms of TD and how utilization of a more clinically meaningful endpoint might lead to better evaluation of vaccine efficacy.

Table 1

Clinical Endpoints in TD-Vaccine Field Studies

Publication	Vaccine Candidate	Study Population (n)	Primary Endpoint Definition	Vaccine Efficacy (VE)
Scerpella et al., 1995	Killed whole-cell V. cholerae O1 with a recombinant B-subunit of cholera toxin (WC/rBS)	Student travelers to Mexico (n=502)	≥4 loose stools in 24 hours (or 3 in 8 hours) plus an additional symptom	VE against ETEC = 50% (95% CI, 14-71%) beginning 7 days after the second dose. However, no efficacy was demonstrated within 7 days of the second vaccination when 74% of ETEC cases occurred
Widerman et al., 2000	Inactivated whole cell ETEC and Cholera vaccines plus recombinant B-subunit of cholera toxin (rCTB)	Austrian travelers to tropical or subtropical destinations (44 different countries in Africa, Asia, Latin-America) (n=250)	≥3 liquid stools and ETEC-only pathogen detected in stool	ETEC vaccine VE = 79% (p=0.119) Cholera vaccine VE = 82% (p = 0.0496)
Leyten et al., 2005	Live-attenuated oral cholera vaccine strain CVD 103-HgR	Travelers to Indonesia, India, Thailand and West Africa (n=134)	≥3 loose stools in 24 hours, or 2 loose stools plus additional symptoms	Study terminated early as the primary endpoint ≥50% VE not achieved at point of interim analysis
Sack et al., 2007	Inactivated whole cell ETEC vaccine plus recombinant B-subunit of cholera toxin (rCTB)	Travelers to Mexico and Guatemala (n=672)	Primary VPO: ≥3 loose stools in 24 hours plus ≥1 gastrointestinal symptom caused by homologous ETEC vaccine strain	VE = 24% (n.s.)
Frech et al, 2008	Heat labile toxin LT-patch	Travelers to Mexica and Guatemala (n=170)	Mild TD: 3 loose stools in 24 hours Moderate TD: 4-5 loose stools in 24 hours and ETEC LT, LT/ST or ST positive Severe TD: ≥6 loose stools in 24 hours and ETEC LT, LT/ST or ST positive	VE against moderate-to-severe TD = 75% (p=0.007) VE against severe TD = 84% (p=0.0332)
Bourgeois et al., 2011	Inactivated whole cell ETEC vaccine plus recombinant B-subunit of cholera toxin (rCTB)	Travelers to Mexica and Guatemala (n=1406)	VPO-ETEC TD: ≥5 unformed or liquid stools in 24 hours plus ≥1 gastrointestinal symptom and homologous ETEC vaccine strain isolated within 24 hours of episode	VE = -59 (95% CI, -384-48)
Steffen et al., 2013	Heat labile toxin LT-patch	Travelers to India (n=723)	Mild TD: 3 loose stools in 24 hours Moderate TD: 4-5 loose stools in 24 hours and ETEC LT, LT/ST or ST positive	VE near zero (p=1.000)
Behrens et al., 2014	Heat labile toxin LT-patch	Travelers to Mexica and Guatemala (n=1644)	Severe TD: ≥6 loose stools in 24 hours and ETEC LT, LT/ST or ST positive	VE against moderate-to-severe TD = 34.6% (95% CI, -2.2, 58.9)

Note: Table adapted from various vaccine field trials (Scerpella et al., 1995; Widerman et al., 2000; Leyten et al., 2005; Sack et al., 2007; Bourgeois et al., 2011; Steffen et al., 2013; Behrens et al., 2014) and 2018 VASE Workshop Presentation (Porter, Kotloff, & Gutierrez, 2018)

Existing Scoring Systems

Scoring Systems in Pediatric Populations

Early vaccine studies for pediatric populations, specifically in the context of Rotavirus, utilized different definitions of clinically significant diarrhea to measure vaccine efficacy. These early studies based their primary endpoint on stool output, with subsequent trials gradually including other clinical signs and symptoms. The use of scoring systems has now become the norm for how disease severity is defined in rotavirus vaccine efficacy studies (Bhandari et al., 2014; Clark et al., 1988; Flores et al., 1987; Isanaka et al., 2017; Mahdi et al., 2016; Vesikari et al., 1984; Vesikari et al., 1985). While the Vesikari and Clark scoring systems have emerged as the predominant methods for determining disease severity in vaccine efficacy studies, they utilize different combinations of symptoms and scoring algorithms to assess disease severity, thus resulting in limited comparability of vaccine efficacy estimates between studies (Givon-Lavi et al., 2008). It has been suggested that for more standardized assessment of Rotavirus vaccine efficacy in future clinical trials, a single scoring system should be used (Givon-Lavi et al., 2008).

There has been increased recognition that the suitability of the Vesikari, Clark or modified forms of either scale for trials involving pathogens other than rotavirus can be called into question, particularly considering its limitations in developing countries where diarrhea remains a global health priority. For example, some parameters are irrelevant in many low resource settings, such as hospitalization (children are rarely hospitalized for diarrhea, even if dehydrated), and temperature (rarely measured to diagnose fever at

home, and in many health care settings). Additionally, the cardinal signs of diarrheal diseases (diarrhea, vomiting, dysentery) are heterogeneous even within a single etiology. Finally, such scores may not include clinical parameters that may contribute to the severity of nonviral gastroenteritis. Attempts to address such limitations, especially for etiologies other than rotavirus as new vaccines against ETEC, *Shigella* and other pathogens are at the cusp of field evaluation, have accelerated over the past five to ten years. In addition to the Vesikari and Clark scores, other scales that emerged for the purpose of assessing gastroenteritis in pediatric populations include the Gorelick Score, Clinical Dehydration Scale (CDS), and the WHO Integrated Management of Childhood Illness (IMCI). Six studies have evaluated previously developed and existing diarrheal disease grading scales (Arifeen et al., 2013; Jauregui et al., 2014; Levine et al., 2015; Kinlin & Freedman, 2012; Pringle et al., 2011; Tam et al., 2014), whereas two studies sought to develop a new grading scale for the use in community-based healthcare settings in developing countries (Lee et al., 2016; Levine et al., 2015).

While the clinical profile of diarrheal disease and severity may vary, dehydration status remains a central parameter on which treatment of diarrheal illness is based, especially for pediatric populations. Several scales have been developed for the estimation of dehydration status and clinical signs associated with diarrheal disease; all targeted for slightly different age groups and tested for limited validation outside institutional development origin. The most popular of these include the IMCI scale developed by WHO, the Gorelick Scale created at the Children's Hospital of Philadelphia, and the Clinical Dehydration Scale (CDS) originating from Toronto. The

WHO scale groups severity of symptoms as a means to classify children and meant for children 1 month of age to 5 years old; the CDS is for children 1 to 3 years of age and implements a scoring system for dehydration symptoms; and the Gorelick scale targets a similar age group to the WHO scale but utilizes a binary system to classify dehydration status with severity based on number of symptoms present. Two studies evaluated the effectiveness of the WHO, CDS and Gorelick scores and found none were helpful predictors of children with diarrhea (Jauregui et al., 2014; Pringle et al., 2011), and one study found the CDS to be slightly helpful in the assessment of dehydration but cautioned against its singular use for planning treatment interventions (Kinlin & Freedman, 2012). Similar to the abovementioned CDS, Gorelick and WHO scales, the DHAKA score was empirically derived to assess the most relevant dehydration symptoms for specific use in low-resource settings (Levine et al., 2015). Meanwhile, the Community Diarrheal Assessment (CODA) score incorporated the six clinical signs and symptoms of non-pathogen specific diarrheal disease that most correlated with poorer weight gain – diarrhea, anorexia, vomiting, loose stools and maximum number of loose stools in a 24-hour episode. Similar to the Vesikari and Clark scores, the CODA score incorporates other clinical predictors of diarrheal disease; however, it seeks to expand its applicability beyond just rotavirus disease towards classification of all diarrheal disease (Lee et al., 2014; Rouhani et al., 2016). Nevertheless, with the addition of these newer index tools comes the need for validation and further testing in expanded populations.

Scoring System Use in TD Studies

It has been long-recognized that TD can be associated with varied degrees of unformed stools ranging from semisolid to liquid states, often developing into an explosive illness characterized by nausea, fever, and vomiting; with the early years of TD often leading to dehydration and even death in the most severe cases (Kean & Waters, 1959). The earliest study in which there was an attempt at TD severity delineation was that of Kean and Waters (1959), in which diarrhea was classified into the following categories: mild, defined as three or more bowel movements per day with camps and/or nausea, or diarrhea with three to six bowel movements per day with only mild cramps or no other symptoms and no impact on daily activity; moderate, defined as diarrhea with more than four bowel movements per day with cramps, nausea and/or vomiting, with occasional chill and/or fever with confinement to the room for less than 1 day; or severe, defined as confinement to the room for more than 1 day and with at least five of the seven following symptoms: diarrhea with more than four or five bowel movements per day, nausea, vomiting, cramps, chills, fever, joint pain or back pain (Kean & Waters, 1959).

Building on the above-mentioned study and subsequent research (Kean & Waters, 1959; Kean, 1963) the scoring systems used and publicly available in the peer-reviewed literature use multiple items for defining TD, with a diarrhea episode defined as three or more loose stools within a 24-hour period, with some studies including an additional symptom parameter that may include fever, abdominal pain, nausea, vomiting, tenesmus, blood in stool, etc. Some studies report a definition of ‘Classic TD’ as three unformed stools with at least one accompanying symptom; however, even among those studies the

inclusion of various accompanying symptoms is inconsistent. The WHO and UNICEF similarly utilize a definition of three or more unformed stools over a 24-hour period for diarrheal disease; however, inclusion of an accompanying symptom is not required (WHO, 2017; UNICEF, 2012). It should be noted that both global organizations concentrate on childhood public health priorities, not adult traveler afflictions. Epidemiologic studies characterizing the etiology of TD identified the symptoms most associated with TD gastroenteritis as abdominal pain, fever, nausea, vomiting, tenesmus and malaise. Because of the frequency with which these symptoms consistently occur in illness, they have been consistently included in TD investigations. In contrast, other symptoms/signs like flatulence and borborygmus have been inconsistently included in disease follow-up evaluations. Furthermore, the abovementioned symptoms are inconsistently defined from study to study that could further confuse what impact such symptom has on the clinical endpoint.

Scoring System Use in Challenge Studies

While randomized, placebo-controlled blinded field efficacy studies remain a gold standard for evaluation of any potential vaccine candidate or TD treatment (Creswell, 2009; Frankfort-Nachmias, & Nachmias, 2008; Riddle et al., 2014), CHIMs enable early evaluation of potential interventions. Consequently, they should be included in this literature review as another example of a) how clinical signs and symptoms play an important role in determining disease profile, and b) how use of only stool output parameters may mislead intervention development efforts to down-select potentially promising candidates too early. The CHIM provides a tool for advancing vaccine

candidates by providing a controlled environment in which to evaluate vaccine efficacy using an established inoculum dose and during which clinical disease is well-defined and managed (Riddle et al., 2016; Porter et al., 2017), and it can even be used in late-stage evaluation efforts. A recent example of CHIM utility for advancing a vaccine candidate towards licensure by the US Food and Drug Administration (FDA) is that of Vaxchora™ (PaxVax Redwood City, CA; Chen et al., 2016), in which proof of concept vaccine efficacy was demonstrated in a human challenge model (Chen et al., 2016). In addition to cholera, other enteric challenge models have been developed for ETEC (DuPont et al., 1971; Harro et al., 2011; Levine et al., 1977), *Shigella* (Porter, Thura, Ranallo, & Riddle, 2013), and Norovirus (Bernstein et al., 2015; Frenck et al., 2012; Riddle & Walker, 2016) to support vaccine, treatment and therapeutic development efforts. Furthermore, often CHIMs are used as a down-selection stage-gate mechanism to advance potential candidates to further field efficacy evaluation and licensure, thus elevating their importance in the overall development pathway for developers.

For any clinical trial, including CHIMs, standardization of clinical endpoints is challenging and further complicates interpretation of results across studies and interventions. Ensuring consistency across objective measures such as diarrhea, vomiting and fever has been challenging; standardizing definitions of subjective measures such as myalgia, headache and abdominal pain have proven even more difficult (Porter et al., 2017).

While the benefits and utility of the CHIM is widely recognized, there remains challenges with utilization of this tool that extend to clinical disease definitions and

prioritization of symptomology, highlighting the need for a scoring system that could be applied to such studies. Potential development of disease-specific scores that incorporates the conglomeration of signs and symptoms, both objective and subjective in nature, has been discussed as necessary given the specificity of clinical outcomes as it relates to the pathogen of interest (Porter et al., 2017). As ETEC is the leading bacterial cause of childhood diarrheal morbidity and mortality in developing countries for which there is not yet a licensed vaccine, Porter et al. (2016) attempted to address the aforementioned challenge and derived a three-parameter composite score based on previously conducted ETEC CHIM. Akin to the CODA score for endemic pediatric population use, the ETEC score supported use of additional clinical signs and symptoms more than just stool output parameters as a more comprehensive measure of clinical disease in an experimental human challenge model setting. Porter et al. (2018) has most recently followed up with the development of a *Shigella* disease complex score, which has been applied to preliminary immunological analysis of a *S. sonnei* model refinement CHIM (Frenck, 2018; Clarkson, 2018) and an immunization-challenge trial with a *Shigella flexneri 2a* bioconjugate vaccine (Porter et al., 2018). Shimanovich et al., (2016) used a categorical outcome-based disease index (DI) of four symptoms (maximum body temperature, bloody stools, loose stools and stool volume) experienced post *Shigella flexneri* 2a (2457T) challenge to characterize severity of clinical disease experienced by subjects in a Phase 2/2b safety, immunogenicity and efficacy study of two different *Shigella* vaccine candidates. These characterizations were then used to assess utility of two different functional assays to predicting protection against shigellosis. Finally, the Vesikari scale

persists in the CHIMs field for use in norovirus challenge studies, with a modification to the dehydration and IV treatment point system resulting in a reduction from the original 20-point scale as applied to rotavirus trials to 17-point scale more representative of norovirus illness (Atmar et al., 2011; Bernstein et al., 2015)

Conceptual Framework

There are two types of instruments most commonly utilized to measure constructs using a composite score: scales and indexes (DeVellis, 2003). Both instruments incorporate multiple variables, or items, within the tools. A scale is unidimensional tool in which all items reflect, or cause, one construct, such as traveler's diarrhea (DeVellis, 2003; Streiner, 2003). As a result, all items are correlated to some degree; thus, not every item needs to be represented as one item that is missed will be picked up by another's correlated item (Streiner, 2003). The Vesikari and Clark scales are examples of such instrumentation as it applies to assessment of pediatric rotavirus gastroenteritis. In contrast, an index is a multidimensional instrument comprised of different non-correlated items that determine the level of the construct; thus, omit an item and the entire construct changes (Bollen & Lennox, 1991; Devellis, 2003). Because the variables, or items, within the secondary datasets to be compared and contrasted in this study are caused by traveler's diarrhea, scale development theory is an applicable theoretical framework to this research.

Scale Development Theories

Scales are an instrumentation method consisting of a cluster of questions to measure more complex social science concepts having several properties, such as

socioeconomic status (Frankfort-Nachmias & Nachmias, 2008). Unlike an index that is constructed by adding up the scores assigned to individual items, a scale utilizes the differences in intensity among the individual items to suggest that there are varying degrees to a variable (Frankfort-Nachmias & Nachmias, 2008). The most commonly used scale in sociological investigation is the Likert Scale, which consists of response categories including “strongly disagree,” “disagree,” “agree” and “strongly agree” (Frankfort-Nachmias & Nachmias, 2008). In enteric research and as previously described, scales have been utilized to characterize the severity of clinical disease, particularly for pediatric studies (Ferdous et al., 2013; Lee et al., 2016; Levine et al., 2015; Pringle et al., 2011). The Vesikari Clinical Severity Scale is the most commonly used example of a composite measure considering specific parameters that constitutes the clinical profile of rotavirus disease (Lewis, 2011). For the evaluation of acute gastroenteritis in pediatric populations in both developed and developing countries research into clinical severity scales has largely focused on the use of the Vesikari Scale Score (Lewis, 2011; Schnadower, et. al., 2013); however, its applicability to both non-rotavirus diarrheal disease and the adult population is limited. Symptoms such as fever, abdominal pain, tenesmus with diarrheal stools accompanied by pathological elements such as blood and mucus typically orient to a bacterial etiology, whereas more frequent vomiting and aqueous stools tend to orient to a viral etiology, as is the case of rotavirus (CDC, 2015; Simona et al., 2015). With this clinical profile in mind, the Vesikari Score highlights commonly observed rotavirus-specific symptoms observed such as vomiting, watery diarrhea and level of dehydration. Moreover, both commonly used Vesikari and

Clark score were developed and have since been modified for use in only pediatric populations, with parameters such as fever, behavioral symptoms and dehydration based on biological ranges and characteristics of children less than 2 years of age. As a result, use of the Vesikari or Clark Scores would be inappropriate for assessment of non-viral disease or adult use in populations.

The two most predominant scale development theories include the item response theory (IRT) and classic test theory (CTS); often used to guide scale development through factor analysis and item selection. IRT was first used in the field of psychometrics to evaluate instruments and assess subjects on their abilities, attitudes, or other traits (Xinming, & Yung, 2014). Commonly used aptitude tests like the Scholastic Assessment Tests (SATs) and Graduate Record Exams (GREs) are developed using IRT as it has been shown to improve reliability and measurement accuracy while improving efficiency in assessment time (Xinming, & Yung, 2014). Over the years IRT methodology has become increasingly popular and applied to health outcomes, clinical research and quality-of-life research (Hays et al., 2000; Xinming, & Yung, 2014). IRT models have been shown to better reflect actual response patterns in the sample population, resulting in better estimates of self-reported health outcomes that are more sensitive to true cross-sectional differences and more responsive to changes in health over time (Hays et al., 2000). CTS is another psychometric theory used to predict the abilities of subjects, in which a subject's observed score on a test is the subject's true score plus some error (since it is unlikely that a subject will perform perfectly on a test) (Tractenberg, 2010). Similar to the IRT, CTS has also been widely used in development

and selection of endpoint measurements in clinical trials (Tractenberg, 2010), with advantages and disadvantages cited for using one over the other (Hays et al., 2000). These frameworks have been relevant to the development of various scales for gastroenteritis assessment (Cappelleri et al., 2014) and thus remains an applicable framework for this research to consider.

Conclusion

This chapter began by characterizing TD gastroenteritis and reviewing the epidemiology and disease burden. The major symptoms of TD vary depending with etiology; although usually includes diarrhea, vomiting, fever, nausea, and abdominal pain. The utility of measuring severity of TD in addition to accounting for other clinical symptomology, history of scoring systems and use of them for use in pediatric populations and experimental human challenge studies were described. The chapter closed with a discussion of the conceptual research chosen to guide this research and its implications for social change.

In summary, this chapter outlines the need to develop an optimized and simplified scoring system for TD that can be used to better define clinical outcomes for use in epidemiological research and intervention evaluation. The literature reviewed in this chapter highlights the potential for better classifying disease outcome through the use of an optimized scoring system including clinical signs and symptoms in addition to stool number. It also suggests that research has been completed to further explore this issue in both pediatric populations and to a more limited extent in experimental human challenge studies for ETEC, but additional research on the development of a scoring system for TD

should be conducted. The next chapter focuses on the methods used to conduct this research and test the study hypotheses outlined in Chapter 1.

Chapter 3: Research Method

As I outlined in the previous two chapters, TD is the most common travel-related illness, prompting the development and assessment of primary and secondary disease prevention efforts such as vaccines and antibiotic treatment. The efficacy of these interventions is often based on the prevention/management of moderate to severe diarrheal disease, predominately based solely on the maximum number of loose stools in a 24-hour period (Behrens et al., 2014; Darsley, Harro, Chakraborty, Sack, & DeNearing, 2015; Frech et al., 2008; Sack et al., 2007). However, reliance on stool-based endpoints alone may obscure or over-inflate the efficacy of potentially meaningful differences in illness profiles. Attempts to address this limitation have been advanced for pediatric populations as well as, most recently, in CHIMs for ETEC and *Shigella* (Porter et al., 2016; Porter et al., 2018). However, there is no standardized disease score for diarrheal illness in adult travelers limiting epidemiologic and interventional studies. In this chapter, I explain the research methodology used for this quantitative secondary analysis in detail. The appropriateness of the methodology chosen for this research is justified below. I detail the sampling methods, study population, inclusion and exclusion criteria, and data analysis procedures and also present the research questions, hypotheses, and ethical considerations.

Research Design and Rationale

In this quantitative study, I aimed to answer three research questions and test the corresponding hypotheses using secondary data analysis. Secondary data analysis is a common technique of using data previously collected to answer a different research

question (Cheng & Phillips, 2014). The original trials were designed to estimate the efficacy of combination antibiotic therapy in treating TD cases (TrEAT TD) and an inactivated whole cell ETEC vaccine (OEV-118). Both datasets contain information on common clinical signs and symptoms of TD, as well as duration, severity of symptoms, and impact on activity. As such, they were ideal for testing hypotheses related to the role of clinical signs and symptoms on TD severity and assessing whether an optimized scoring system can be developed for TD intervention studies.

Research Questions and Hypotheses

The research questions are as follows:

Research Question 1: What combination of clinical signs and symptoms best characterizes TD severity in adult travelers?

H_o1: There are no significant differences in the frequency and severity of clinical signs and symptoms across disease severity classification.

H_a1: There are significant differences in the frequency and severity of clinical signs and symptoms of TD on disease severity classification.

Research Question 2: What individual clinical signs and symptoms of TD are significantly associated with a negative impact on activity among adult travelers?

H_o2: Individual clinical signs and symptoms of TD are not significantly associated with a negative impact on activity.

H_a2: Individual clinical signs and symptoms of TD are significantly associated with a negative impact on activity.

Research Question 3: Does a TD disease score better differentiate treatment groups than prior estimates of vaccine efficacy when applied to a previously conducted ETEC vaccine study (Protocol OEV-118)?

H_o3: The estimated vaccine efficacy of the ETVAX vaccine in the OEV-118 Phase 3 trial does not change as a result of using the new disease complex score.

H_a3: The estimated vaccine efficacy of the ETVAX inactivated whole cell vaccine tested in the OEV-118 Phase 3 trial does change as a result of using the new disease complex score.

Methodology

Participants and Datasets

Participants included in this quantitative secondary analysis included healthy male and female adults aged ≥ 18 years from two previously conducted clinical trials, OEV-118 and TrEAT TD, both described in greater detail below.

OEV-118. Participants included in the OEV-118 dataset were healthy adults, aged ≥ 18 years at the time of enrollment in a double-blind, randomized, placebo-controlled, Phase 3 study designed to evaluate the safety and protective efficacy of a two-dose oral candidate vaccine against enterotoxigenic *E. coli* induced TD. The candidate vaccine was prepared from formalin-killed whole cell ETEC strains expressing colonization factor antigens CFA/I and CS1 through CS5, supplemented with 1 mg of the B subunit of cholera toxin (CTB) per dose. Study participants were vaccinated twice at 2-week intervals in the United States 7 days prior to travel to Mexico and Guatemala, both countries with an intermediate to high TD risk, for a period of at least 7 days and

followed for 2-4 weeks after arrival. Participants underwent clinical and microbiological surveillance for diarrhea and other illness while traveling. The oral ETEC vaccine used in this study had been proven safe in a predecessor trial (OEV-114, VTU no. 982, unpublished) which was conducted at the same sites in Guatemala and Mexico in May 1998-August 1999 and December 1998-August 1999, respectively. At the time of the OEV-118 study, approximately 700 participants had been enrolled in the OEV-114 study with no safety issues reported; OEV-118 was an additional Phase 3 study to address points raised by regulatory agencies (SBL Vaccin AB/Active Biotech, 2001). The OEV-118 vaccine trial was conducted from November 1999 to April 2002.

Inclusion criteria for OEV-118 included healthy adult males and females aged ≥ 18 years at the time of enrollment and receipt of the first study vaccination, planning to travel to one of the clinical trial sites (Cuernavaca, Mexico or Antigua, Guatemala) and stay for at least 14 days, mostly for language study. Participants had to provide written documentation from a physician that they had undergone a physical exam within 12 months of enrollment, and they had to (a) live in a household with a telephone in the United States, (b) sign a consent form, (c) pass a protocol comprehension exam, and (d) be able and willing to comply with protocol specified procedures. Participants had to be willing and able to participate in nurse-supervised health counseling sessions as well as nurse-supervised study product dosing sessions, all conducted over the telephone. Females could not be pregnant, as verified by urine-pregnancy test, and were willing to use birth control for the duration of the study. Exclusion criteria included participants who had (a) clinically significant acute or chronic gastrointestinal disease; (b) recent

exposure to ETEC; (c) previous travel to a developing country within a year of study enrollment; (d) an immunodeficiency medical condition; (e) any serious medical condition; or (f) planned use of antibiotics during the trip (not pertaining to per-protocol use of antibiotics for the purpose of treating severe diarrhea during travel). Criteria that excluded participants from being in the per-protocol analysis included (a) use of another investigational drug or vaccine other than the one evaluated in the trial; (b) administration of chronic immunosuppressants (inhaled or topical steroids allowed); (c) chronic or planned intake of antibiotics during the study period, and (d) administration of any vaccine not foreseen by the study protocol during the study period.

Participants completed a symptom diary card after each vaccination over the course of 5 days, beginning 1 day prior to each dosing, in which information regarding severity of gastrointestinal and general symptoms were recorded. The questionnaire also documented the impact of their symptoms on daily activities in addition to inquiring about symptoms not specifically solicited.

A total of 1458 subjects received vaccine or placebo and 1435 completed 14-28 days of in-country surveillance. As in the previous OEV-114 field trial with the first-generation oral inactivated killed product (Sack et al., 2007), the commercial scale vaccine produced for the OEV-118 study was extremely well tolerated. Overall there were no significant differences between the rates of solicited general symptoms in the 3 days after vaccination with the exception of vomiting, which was more common in vaccinees versus placebos after both Dose 1 ($p = 0.03$) and Dose 2 ($p = 0.001$; Bourgeois et al., 2011).

Two study offices were established at the field sites to facilitate sample collection, meet with participants on a weekly basis to discuss any symptoms recorded on the diary cards, and to report any illness experienced during their stay in-country. Participants were provided fecal specimen collection containers and diary cards to record daily symptoms. Diary cards were collected on a weekly basis at the study site. Cards were reviewed with study staff weekly, at departure and during all TD episodes. Similar to the vaccination phase of the study, the diary cards collected information on specific gastrointestinal symptoms as well as general symptoms, associated severity and impact on activity. TD was defined as ≥ 3 loose stools in a 24-hour period plus one of the following: abdominal pain/cramps, nausea, vomiting, urgency, gas, and fever. Stools were characterized as Grade 1-5 where Grade 1 and 2 stools were considered normal, firm, and retaining shape. Thick liquid stools taking the shape of a container were considered Grade 3, opaque water stools were graded 4, and rice water stools were graded 5. Grades 3, 4, and 5 loose or watery stools were all considered "loose." A diarrhea episode was considered complete on the last day after which 72 hours passed symptom free with no additional loose stools. Other solicited gastrointestinal and systemic symptoms were graded as mild (Grade 1) if they were noticed but did not impact activity; moderate (Grade 2) if they caused some limitation in activity; and severe (Grade 3) if they impacted activity to the point of non-participation (Bourgeois et al., 2011). The primary study outcome was a vaccine preventable outcome (VPO-ETEC TD) which was defined as ≥ 5 unformed or liquid stools in a 24-hour period accompanied by abdominal pain/cramps, nausea and/or vomiting of any intensity, plus ETEC sharing vaccine antigens as the sole pathogen and

isolated in a window of 24 hours before to 72 hours after onset. Secondary endpoints considered in a *post hoc* analysis included ETEC TD and more severe ETEC TD. ETEC TD was like VPO-ETEC TD, except that only ≥ 3 unformed or liquid stool in a 24-hour period accompanied by abdominal pain/cramps, nausea and/or vomiting of any intensity were needed to qualify as a case, with ETEC isolated as the sole pathogen anytime during the diarrhea episode unless otherwise noted. Cases associated with moderate to severe GI symptoms or changes in activity were considered more severe ETEC TD (MS-ETEC TD).

Among the participants in the study, 412 of 1435 (29%) experienced TD overall, including 31% of participants traveling to Guatemala, and 22% traveling to Mexico. Overall TD incidence was 11.6 cases per 100 person-weeks at risk; in Guatemala the TD incidence was significantly higher than that observed in Mexico, with 13.3 cases per 100 person-weeks at risk, versus 9.5 cases per 100 person-weeks at risk, respectively (Bourgeois et al., 2011). The vaccine had a negative point estimate of efficacy in the primary analysis, yielding a *PE* of -59 with 95% CI (-384 – 48). Post-hoc point estimate efficacy was improved against ETEC TD, with a *PE* of 15 with 95% CFI (-83 – 60). About half TD cases met the definition of MS-ETEC TD; post-hoc efficacy analysis against MS-ETEC TD further improved the vaccine efficacy point estimate, as fewer vaccine than placebo recipients developed MS-ETEC TD (4 of 705 vs. 10 of 701, *PE* = 60%, $p = 0.10$). The most common ETEC phenotype outcome (35% of cases) were ST strains expressing CS6, two antigens not covered by the vaccine formulation (Bourgeois et al., 2011).

Dosing was not directly observed for the vast majority of study participants. A majority of subjects received their vaccine or placebo product under telephonic supervision by a study nurse, with no direct observation of actual product intake (SBL Vaccin AB, 2003). Consequently, it was difficult to determine whether all doses were actually taken by the volunteers as intended (SBL Vaccin AB, 2003). Following completion of the primary analysis for the trial, a secondary analysis was performed to assess the impact of vaccine "take" on vaccine efficacy. Serological studies in subjects providing sera before and after immunization suggested that most mounted strong IgA responses (~88%) to the CTB component of the vaccine but only modest responses against the CFA components (15-20%). These observations revealed that the vaccine take rate may have been more variable than in prior studies using direct-observed vaccination (Bourgeois et al., 2011). Among vaccine takes (n = 162) there was a strong trend toward protection against ETEC TD of any intensity (PE = 58%; p = 0.09), with greater protection seen against MS-ETEC based on change in activity or symptom severity (PE range 88-100%, $p \leq 0.02$ for both comparisons; Bourgeois et al., 2011).

Trial Evaluating Ambulatory Therapy of Travelers' Diarrhea (TrEAT TD) study. Participants in the TrEAT TD Study were active-duty US or UK military personnel or beneficiaries, aged ≥18 years of age, deployed to one of four countries (Kenya, Djibouti, Afghanistan, or Honduras) who presented with acute-watery diarrhea, febrile or dysentery illness and who were ambulatory at the time of enrollment (Riddle et al., 2017). Participants presenting with acute-watery diarrhea were randomized into one of three treatment groups in a double-blind manner to evaluate the comparative efficacy

of single-dose azithromycin, levofloxacin, or rifaximin in combination with loperamide. Participants who presented with febrile or dysentery illness were randomized to receive azithromycin with or without loperamide. Personnel presenting with suspected TD, febrile or dysentery illness were evaluated for potential trial inclusion, randomized to receive one of the aforementioned treatment regimens for direct observed dosing. The vaccine trial began September 2012 and ended in July 2015.

Inclusion criteria for this study included (a) active-duty (or beneficiaries) adult males and females aged ≥18 years at the time of enrollment who had to meet the definition of TD (≥ 3 loose stools in 24 hours or ≥ 2 loose stools in 24 hours with associated symptoms) of ≤ 96 hours duration; (b) able to comply with protocol specific procedures; and (c) remain eligible for follow-up 5 days or more after treatment. Exclusion criteria included participants who (a) reported allergies to any one or more of the study drugs; (b) received antibiotic therapy (including malaria prophylaxis) within 72 hours of enrollment; (c) reported history of seizures; (d) were taking medications with known drug-interactions with the study investigational products (IPs); (e) had a positive urine pregnancy test at enrollment (females of child-bearing potential only); (f) had dysentery or fever; and (g) used a total of > 4 mg loperamide or any amount of loperamide for > 24 hours prior to enrollment (Riddle et al., 2017).

The primary efficacy endpoint of this study was the proportion of participants who achieved clinical cure of their TD within 24 hours of receiving their first treatment dose; clinical cure was defined as 1) no reported diarrheal stools >24 hours after initiation of treatment, 2) diarrhea had no impact on activity, and 3) any diarrhea-associated

symptom present at 24 hours were graded no higher than ‘mild’ (Riddle et al., 2017). Treatment failure was defined as follows: 1) recurrence of TD diarrhea (utilizing the study definition) within 72 hours after clinical cure, 2) worsening of symptoms after 24 hours of treatment, or 3) continuing illness after 72 hours. Participants were provided diary cards on which to record their daily treatment usage, form and number of loose stools passed, presence of gastrointestinal (abdominal cramping, excessive gas, nausea, vomiting, fecal urgency, constipation) and systemic symptoms (fever, malaise/fatigue), and impact of illness on activity (no impact, ≤ 50% impact, > 50% impact, inability to function).

While randomization occurred based on clinical presentation (acute watery-diarrhea or acute dysentery/febrile diarrhea), only results of the acute watery-diarrhea portion of the study is published and results summarized in detail here. Of 844 participants assessed for eligibility, 339 were enrolled with acute-watery diarrhea, randomized to receive either azithromycin (n = 106), rifaximin (n = 107), or levofloxacin (n = 111) and took the full treatment dose as prescribed for inclusion in the per protocol analysis (Riddle et al., 2017). Approximately 130, 104, 50, 42 participants were enrolled in Kenya (40%), Djibouti (32%), Afghanistan (15%) and Honduras (13%), respectively. Most participants presented for care of diarrheal illness at around 26.8 hours, with 12.6% presenting with diarrhea between 48 and 72 hours and 4.7% presenting with diarrhea between 72 and 96 hours, with no difference in proportions between treatment arms (Riddle et al., 2017). The average number of loose stools reported by participants regardless of treatment arm who had diarrheal illness ≥ 24 hours was 6.9 (Riddle et al.,

2017). The most common symptoms reported included cramping (74.3%), vomiting (18%), fecal incontinence (12.7%), and fever (9.6%) (Riddle et al., 2017). Approximately three quarters of participants (76.5%) reported some impact of illness on activity, with approximately 30% reporting significant or complete disability associated with illness (Riddle et al., 2017).

In regards reaching clinical cure at 24 hours, single-dose levofloxacin, rifaximin and azithromycin were shown to be comparable for treatment of AWD with 81.4%, 74.8% and 78.3% efficacy, respectively (Riddle et al., 2017). Furthermore, efficacy among regimens at 48 and 72 hours were essentially equivalent with no differences in post dose adverse events between treatment arms (Riddle et al., 2017).

Power Calculations

The post hoc power analyses were conducted using G*Power 3.1.9.2. G*Power is a power and sample size calculation software with capabilities to perform calculations for a variety of tests, including logistic regression analyses and t-tests (Faul, Erdfelder, Buchner, & Lang, 2009). A minimum sample size approximately 360 subjects from the TrEAT TD dataset was included to address research questions 1 and 2 and to develop the optimized scoring system. Sampling frames and specific power calculations are discussed further in the data analysis summary for each performed statistical test.

Instrumentation and Operationalization of Constructs

Two diary card instruments for determining severity of TD gastroenteritis episodes and impact on activity were included in analysis procedures described further below. These diary cards existed in questionnaire format filled in by the participants and

verified by study clinicians during clinic follow-up visits. Diary cards from both trials contained continuous and categorical independent and dependent variables, which are further outlined in Table 2.

Table 2

Clinical Information Collected in TrEAT TD and OEV-118 Diary Cards

TrEAT Diary Card			OEV-118 Diary Card		
Symptom	Present?	Information Collected	Symptom	Present?	Information Collected
Diarrhea	Yes/No	Max # loose stools/24 hours Number of loose stools in past 8 hours Number of loose stools in past 24 hours Number of loose stools onset to presentation	Unformed Stool	Yes/No	Number of unformed stools per hour
Subjective Fever	Yes/No	Duration (# hours) Mild Moderate Severe	Temperature	Yes/No	Record temperature
Dysentery	Yes/No	Date/Time of Onset	Blood in stool	Yes/No	0: Normal/No visible blood 1: minute traces of visible blood in stool or TP 2: One stool with abundant blood (clots or liquid) 3: >1 stool with abundant blood (clots or liquid)
IV Fluids given	Yes/No	# liters			
Impact of illness on activity level		Normal Decreased ≤ 50% Decreased > 50% Completely unable to function	Did you change your activity for gastrointestinal symptoms	Yes/No	
Vomiting	Yes/No	Duration (# of hours) # of episodes	Vomiting	Yes/No	0: Normal/no vomiting 1: one episode of vomiting 2: 2 or more episodes of vomiting 3: 2 or more episodes of vomiting severe enough to prevent normal daily activities
Abdominal Cramps	Yes/No	Duration (# hours) Mild Moderate Severe	Cramping	Yes/No	1: Mild 2: Moderate 3: Severe
Excessive Gas/Flatulence	Yes/No	Duration (# hours) Mild Moderate Severe	Gas	Yes/No	1: Mild 2: Moderate 3: Severe

TrEAT Diary Card			OEV-118 Diary Card		
Symptom	Present?	Information Collected	Symptom	Present?	Information Collected
Nausea	Yes/No	Duration (# hours) Mild Moderate Severe	Nausea	Yes/No	1: Mild 2: Moderate 3: Severe
Ineffective and/or Painful Straining to pass stool	Yes/No	Duration (# hours) Mild Moderate Severe			
Tenesmus	Yes/No	Duration (# hours) Mild Moderate Severe	Tenesmus: painful, ineffectual	Yes/No	1: Mild 2: Moderate 3: Severe
Malaise/Fatigue	Yes/No	Duration (# hours) Mild Moderate Severe	Weakness	Yes/No	1: Mild 2: Moderate 3: Severe
Fecal Incontinence	Yes/No	Duration (# hours) Mild Moderate Severe	Urgency of defecation	Yes/No	1: Mild 2: Moderate 3: Severe
Constipation	Yes/No	Duration (# hours) Mild Moderate Severe			
Other symptoms	Yes/No	Duration (# hours) Mild Moderate Severe	Other Symptoms	Yes/No	1: Mild 2: Moderate 3: Severe
			Headache	Yes/No	1: Mild 2: Moderate 3: Severe
			Lightheadedness	Yes/No	1: Mild 2: Moderate 3: Severe
			Muscle aches	Yes/No	1: Mild 2: Moderate 3: Severe
			Chills	Yes/No	1: Mild 2: Moderate 3: Severe
			Abdominal Pain	Yes/No	1: Mild 2: Moderate 3: Severe

(table continues)

TrEAT Diary Card			OEV-118 Diary Card		
Symptom	Present?	Information Collected	Symptom	Present?	Information Collected
			Gurgling stomach	Yes/No	1: Mild 2: Moderate 3: Severe
			Belching	Yes/No	1: Mild 2: Moderate 3: Severe
			Decreased appetite	Yes/No	1: Mild 2: Moderate 3: Severe
			Seek medical advices for symptoms?	Yes/No	1: Mild 2: Moderate 3: Severe

(table continues)

Data Analysis Plan

All analyses except the Multiple Correspondence Analysis (MCA) were conducted using SPSS 24.0 (IBM Corp, 2012). MCA was performed utilizing SAS and all graphs depicting TD Score distributions were generated with Microsoft Office 365 Excel software. Table 3 provides a summary of the hypothesis testing analyses that were conducted.

Table 3

Summary of Quantitative Data Analysis

Research Question	**Hypothesis**	**Independent Variables**	**Dependent Variables**	**Statistical Test**
What severity and combination of clinical signs and symptoms best characterize TD severity in adult travelers?	There are significant differences in association between clinical signs and symptoms of TD on disease severity classification.	a) Fever b) Vomiting c) Abdominal Cramps d) Excessive flatulence e) Nausea f) Tenesmus g) Malaise/fatigue h) Fecal Incontinence	Diarrhea severity a) Mild b) Moderate c) Severe Complex Disease Score	Spearman Correlation Univariate linear regression Multiple Correspondence Analysis (MCA)
What individual clinical signs and symptoms of TD are significantly associated with a negative impact on activity among adult travelers?	Individual clinical signs and symptoms of TD are significantly associated with impact on activity.	a) Loose stools b) Fever c) Vomiting d) Abdominal Cramps e) Excessive flatulence f) Nausea g) Tenesmus h) Malaise/fatigue i) Fecal incontinence	Impact on activity a) Normal b) Decreased ≤ 50% c) Decreased > 50% d) Completely unable to function	Ordinal logistic regression
Does a TD disease score better differentiate treatment groups than prior estimates of vaccine efficacy when it is applied to a previously conducted ETEC TD vaccine study (Protocol OEV-118)?	The estimated vaccine efficacy of the ETEC inactivated whole cell vaccine tested in the OEV-118 Phase 3 trial does change as a result of using the new disease complex score.	N/A	N/A	Independent samples t-test Mann-Whitney U test

For the purpose of developing the score, detailed clinical information on the signs and symptoms associated with TD were obtained from the TrEAT TD dataset. To subsequently validate the complex disease score, the OEV-118 dataset was utilized. To be included in the dataset for score development, subjects must have complied with eligibility criteria and enrolled in the TrEAT TD study.

While signs and symptoms were originally defined by each study independently (see Table 2), for standardization the following signs and symptoms within the final dataset for analysis were re-defined as follows (based on maximum severity recorded):

- Abdominal cramps, nausea, tenesmus, malaise/fatigue, excessive flatulence (gas) and fecal incontinence:
    - 0 – none
    - 1 – mild (no interference with routine activity)
    - 2 – moderate (symptoms cause interference but do not preclude from participating in routine activity)
    - 3 – severe (symptoms prevent routine activity)
- Fever:
    - 0 – None - <100.4° F
    - 1 – Mild - 100.4°F – 101.1°F
    - 2 – Moderate – 101.2°F – 102.0°F
    - 3 – Severe – ≥102.1°F
- Vomiting:
    - 0 - None – 0 episodes in 24 hours

  - 1 - Mild – 1 episode in 24 hours
  - 2 - Moderate – 2 episodes
  - 3 - Severe – ≥3 episodes
- Diarrhea (Only for OEV-118)
  - 1 - Mild – 1 loose/liquid stool of ≥300 g or ≥2 loose/liquid stools totaling ≥200 g and ≤400 g during a 24-hour period)
  - 2 - Moderate – 4 to 5 loose/liquid stools or >401 to 800 g in a 24-hour period
  - 3 - Severe – 6 or more loose/liquid stools or ≥800 g in a 24-hour period

Correlation between signs and symptoms. To test the first hypothesis and determine if there was significant correlation between clinical signs and symptoms of TD, Spearman's correlations were utilized. The Spearman's rank-order correlation coefficient is a nonparametric measure of the direction and strength of the association that exists between two variables measured on an ordinal, interval or ratio scale (Laerd Statistics, 2013f). As all clinical signs and symptoms were originally classified in the original TrEAT TD study as none, mild, moderate and severe, the critical assumption that the included variables fit an ordinal level of measurement was already satisfied prior to moving forward with analysis.

The sample size for this analysis was calculated in G*Power using an alpha of 0.05, a power of 0.95 and a medium effect size ($p = 0.03$) for a two-tailed test (Faul et al., 2009). Because Spearman's rank correlation coefficient is computationally identical to Pearson's product-moment coefficient (Statistics Solutions, 2010), the

calculation was performed using software for estimating power of a Pearson's correlation (Faul et al., 2009). A minimum total sample size of 111 was sufficient to ensure at least 95% power; the total sample size included in the final analysis was 363 participants.

Correlation between signs and symptoms and frequency of loose stool. As most, if not all, TD definitions utilized across epidemiologic and interventional studies incorporate stool output as the primary endpoint (see also Chapter 2), univariate regression models were conducted to describe the strength of association between the number of loose stools over a 24-hour period and the TD-attributable signs and symptoms. While multiple linear regression was originally proposed for this analysis, that approach resulted in violations of both assumptions of multicollinearity and homoscedasticity. The risk for multicollinearity between the variables to be analyzed in this dataset was not unexpected, as Porter et al. (2016 and 2018) found a high degree of multicollinearity across many of the same clinical signs and symptoms within those respective datasets when developing a disease complex score for ETEC CHIM. Therefore, I proceeded with the same approach as Porter et al. (2016) in which univariate linear regression was utilized. As a critical assumption of linear regression is that there should be no significant outliers in the data which can impact the regression line and lead to inaccurate results (Laerd Statistics, 2013c), I identified such datapoints for maximum 24-hour stool frequency in any 24-hour period prior to presentation that fell outside the first or third quartile; mathematically represented as Q1 - (1.5*IQR) and Q3 + (1.5*IQR), respectively. I ran each univariate regression model with and without outliers to

determine if there were differences in the associations between clinical signs and symptoms and stool output, with detailed results presented in Chapter 4 and Appendix 1.

The sampling frame for testing this hypothesis included participants from the TrEAT TD study who received treatment, presented with acute-watery diarrhea and had gastroenteritis severity data collected and stool results available, regardless of treatment allocation. The sample size for the proposed analysis was calculated in G*Power by setting the test family to F tests; selecting 'Linear multiple regression: Fixed model, R^2 deviation from zero,' and selecting to compute required sample size (Faul et al., 2009). Then, the effect size f^2 was set to 0.15 (medium), alpha error probability was set to 0.05, power was set to 0.95, and the number of predictors was set to one (Faul et al., 2009). The minimum total sample size for this analysis was calculated to be 89 participants; the total sample size included in the final analysis for each univariate regression model was 363 participants.

Impact of clinical signs and symptoms in activity level. Ordinal logistic regression is often used to predict an ordinal dependent variable (i.e., impact on activity) given one or more predictor variables (i.e., clinical signs and symptoms; Laerd Statistics, 2013b). To test the second hypothesis, separate cumulative odds ordinal logistic regressions with proportional odds were run to determine the effect of each individual clinical sign and symptom of TD (e.g., abdominal cramps, nausea, tenesmus, gas, incontinence, malaise, vomiting, and tenesmus) as well as the traditional TD-illness metric of stool output (i.e., maximum number of loose/liquid stools in any 24 hours prior to presentation) to determine the effect of each of those parameters on impact on activity.

A Classification and Regression Tree (CART) analysis was conducted to determine optimal cut points of the maximum number of loose stools in 24 hours for this analysis as well as inclusion in the scoring system. Briefly, CART maximizes the distribution of observations into different categories that are predicted by another category (Lewis, 2000). For each logistic regression analysis, the data were checked to assess assumptions of proportional odds (tested with a full likelihood ratio comparing the fit of the proportional odds model to a model with varying location parameters), and multicollinearity through generation of a collinearity matrix to determine if multicollinearity was present (correlation > .90).

In addition to performing this with the individual predictor variables (i.e., clinical signs and symptoms), I re-ran this analysis utilizing the disease complex score further described below via multinomial logistic regression to assess whether the new score improved the estimation of impact on activity over any single individual sign or symptom.

The sampling frame for testing this hypothesis included participants from the TrEAT TD study who received treatment, presented with acute-watery diarrhea and had gastroenteritis severity data collected and stool results available, regardless of treatment allocation. The sample size for the proposed analysis was calculated in G*Power by setting the test family to F tests; selecting 'Linear multiple regression: Fixed model, R^2 deviation from zero,' and selecting to compute required sample size (Faul et al., 2009). Then, the effect size f^2 was set to 0.15 (medium), alpha error probability was set to 0.05, power was set to 0.95, and the number of predictors was set to eight (Faul et al., 2009).

The minimum required sample size for this analysis was 160 participants; the total sample size included was 363 participants.

Development of the disease complex score. To further and graphically describe the overlap of the severity of all the symptoms in the dataset, a multiple correspondence analysis (MCA) was performed. MCA, like exploratory factor analysis, is a variable-reduction technique that aims to reduce a larger set of variables into a smaller set of principle components that account for most of the variance in the original variables (Laerd Statistics, 2013e). There are a number of uses for MCA, including but not limited to: a) when there are many variables in a dataset and some of the variables may be measuring the same underlying construct; if these variables are highly correlated, only those variables most closely representing the construct should be included in the overall measurements scale; b) in creating a new measurement scale, MCA helps one understand whether some of the variables chosen are not sufficiently representative of the construct of interest and whether certain variables should be removed from the new measurement scale; c) for testing whether an existing measurement scale can be shortened to include fewer items (Laerd Statistics, 2013e). From the MCA analysis, relevant clusters of symptoms were identified and then amalgamated in a way to ensure equal distribution across an ordinal spectrum of illness. This was then combined with an ordinal score developed from the distribution of stool output (frequency) using the cutpoints established from the aforementioned CART analysis, resulting in a single, combined composite ordinal disease severity score.

Utilizing this methodology for development of the disease complex score for TD was in line with the same methodology utilized for development of a *Campylobacter*, ETEC and *Shigella*-specific disease complex score, the latter two of which have been published and validated (Porter et al., 2016; Porter et al., 2018).

Application of complex disease score to OEV-118. The new TD disease complex score was 'externally validated' by applying it to a previously conducted Phase 3 vaccine field efficacy trial (OEV-118) to test the hypothesis that disease score significantly differentiates interventions. This analysis was conducted for the entire dataset as well as multiple subgroups representing different endpoints and definitions of interest within the OEV-118 dataset, defined in Table 4.

Table 4

Travelers' Diarrhea (TD) Endpoints (OEV-118 Dataset) for Hypothesis 3 Testing

Endpoint	Abbreviation	N	Definition	Rationale for Testing
All	ALL	1435	Subjects enrolled in the OEV-118 study who received one or two doses of vaccine, traveled to Mexico or Guatemala and had symptom data available for analysis	Full database analysis
Travelers' Diarrhea (TD)	TD	412	≥3 loose or watery stools in a 24-hour period accompanied by ≥1 accompanying gastrointestinal (GI) symptom	Classic TD Endpoint
All ETEC Infections	ALL-ETEC	188	ETEC as sole pathogen isolated in any subject who received one or two doses of vaccine, traveled to Mexico or Guatemala, and had symptom data available for analysis	To determine vaccine efficacy against infection with any ETEC
ETEC TD	ETEC TD	27	≥3 loose or watery stools in a 24-hour period accompanied by abdominal pain or cramps, nausea, or vomiting of any intensity, plus ETEC sharing antigens[1] with the vaccine as sole pathogen isolated	Recommended by OEV-118 Data Safety Monitoring Board*
Vaccine Preventable ETEC TD	VPO-ETEC TD	13	≥5 loose/watery stools in 24 hours plus ≥1 of abdominal pain/cramps, nausea and/or vomiting, plus ETEC sharing antigens[1] with the vaccine as sole pathogen and isolated in window of 24 hours before to 72 hours after illness onset among subjects completing 2-dose regimen, traveling during window of 7 to 14 days post 2nd dose and completing 14-28 days surveillance.	Original Study Endpoint
ETEC with Mixed Infections	ETEC-MX	14	ETEC along with (an)other pathogen(s) isolated by culture in any subject who received one or two doses of vaccine, traveled to Mexico or Guatemala and had symptom data available for analysis	To determine vaccine efficacy against infection with any ETEC plus one or more enteric pathogens

[1] LT, LTST, CFA/I, CS1, CS2, CS3, CS4 or CS5

*AL Bourgeois, personal communications

To do this, a disease score was calculated for each subject in the OEV-118 study that met one of the definitions outlined in the table above, and the mean score between placebo and vaccine recipients was compared utilizing the independent samples t-test for each subgroup. The independent samples t-test compares the means between two unrelated groups on the same continuous dependent variable (Green & Salkind, 2014). For each subgroup analysis, all critical assumptions of the independent t-test were met except the assumption of normality. If this assumption is violated, there are four options available to continue with analysis. First, the data can be transformed to be normally distributed and then the independent t-test run on this transformed data. The second option is to run a non-parametric test such as the Mann-Whitney U-test. The third option is to proceed with analysis despite the violation, as the independent t-test is robust to deviations from normality (Laerd Statistics, 2013a). Fourth and finally, test comparisons can be run in which an independent samples t-test on transformed and non-transformed data is performed, analyzed and upon comparison if they appear similar the non-transformed original data is used for final interpretation (Laerd Statistics, 2013a). I proceeded with conducting an independent t-test analysis for each subgroup in addition to performing a Mann-Whitney U test as the nonparametric alternative to the independent samples t-test to ensure due diligence was achieved with this research. Results of both statistical approaches are presented in detail in Chapter 4. Finally, I re-calculated estimates of vaccine efficacy within each subgroup using the formula below:

$$\frac{\text{MeanScore}_{\text{placebos}} - \text{MeanScore}_{\text{vaccinees}}}{\text{MeanScore}_{\text{placebos}}}$$

The sampling frame for the validation of the disease score and assessment of utility as applied to the ETEC vaccine included various subgroups of participants from the OEV-118 trial who had clinical gastroenteritis severity data collected and stool results available regardless of whether they received a vaccination with inactivated ETEC vaccine or placebo, with primary consideration to the original per-protocol VPO endpoint. As the sample size was already known for the original study endpoint analysis (i.e., among the participants in the study, 14 met the criteria as a VPO event), G*Power was utilized to calculate what statistical power would be obtained given a sample size of 26, an effect size of 0.3 (medium) or 0.5 (large), and a 2-sided alpha of 0.05. The post-hoc test for achieved power for this particular analysis was calculated in G*Power by setting the test family to t tests; selecting 'Means: differences between two independent means (two groups) for the statistical test; and selecting 'Post hoc: Compute achieved power – given alpha, sample size, and effect size' (Faul et al., 2009). Then, the number of tails was set to one, effect size set to either 0.5 or 0.8 (two calculations performed), alpha error probability was set to 0.05, sample size of group 1 was set to 8 (to represent the vaccinees group) and the sample size of group 2 was set to 5 (to represent the placebo recipients) (Faul et al., 2009). The calculated power for a set medium effect size of 0.5 was 0.205, or 21%; the calculated power for a set large effect size of 0.8 was 0.371, or 37%.

Threats to Validity

Internal validity is a means to measure whether or not research is sound and conducted appropriately (Frankfort-Nachmias & Nachmias, 2008). The higher the

internal validity, the more confidence there is in the observed changes to the dependent variable being attributable to the independent variables rather than other possible causes, or confounders (Frankfort-Nachmias & Nachmias, 2008). Threats to internal validity of this study as applicable to the dataset that were utilized from the original TrEAT study are automatically minimized via the experimental and randomized nature of the clinical trial. For example, threats to internal validity include but are not limited to changing instruments during the study, participants dropping out, and failure for participants to complete protocols (Cheng & Phillips, 2014; Frankfort-Nachmias & Nachmias, 2008). Because eligibility criteria for participants enrolling in both the original TrEAT and OEV-118 studies required adherence to all protocol-specified procedures, the attrition rate and risk of participants failing to complete the study was reduced at study initiation. Furthermore, a standardized diary card was utilized for the collection of TD-associated clinical signs and symptoms for all enrolled participants in both trials with no change of that critical instrument nor the data it collected, thus minimizing the threat to internal validity of the original datasets and carrying over to this secondary analysis.

External validity refers to the degree to which results can be applied across settings, individuals, times and other investigations (Frankfort-Nachmias & Nachmias, 2008). Similar to internal validity, external validity of this study was assured through the randomized placebo-controlled experimental nature of the original TrEAT and OEV-118 clinical trials by minimizing the risk of selection biases and the experimental treatment.

Validity of a new measurement tool such as an optimized TD disease scoring system is assessed by evaluating three components: content, criterion, and construct

(Frankfort-Nachmias & Nachmias, 2008). Criterion validity was not assessed as there is not an existing scoring system for measuring TD (Streiner & Norman, 2008). Content validity is based on the content of the scale as assessed by experts (Streiner & Norman, 2008). Since the clinical signs and symptoms that were considered in the new scoring system have been included as common and expected solicited symptoms in the original clinical trial and as established throughout multiple TD trials as described in Chapter 2, it is expected that the newly proposed scale has adequate content validity.

Construct validity seeks to establish if the instrument measures what it is intended to measure (Frankfort-Nachmias & Nachmias, 2008). As this study followed similar methodology as that to establish ETEC and *Shigella*-specific disease severity scores (Porter et al., 2016; Porter et al., 2018), beginning with ensuring empirical validity through estimation of predictive validity through determining correlation coefficients between criterion included in the scale (Frankfort-Nachmias & Nachmias, 2008), followed by a multiple correspondence analysis that has been demonstrated to result in high construct validity for their respective disease-specific gastroenteritis, it is anticipated that this study resulted in an optimized TD severity score with adequate construct validity.

Ethical Procedures

Protection of Participants. Before collecting information regarding TD gastroenteritis, the adults included in both OEV-118 and TrEAT TD datasets all signed informed consent forms to participate in the respective trials. All study documentation for the OEV-118 trial was approved by the Joint Committee on Clinical Investigation at

Johns Hopkins University (SBL Vaccin AB, 2003); the TrEAT TD study was approved by the Uniformed Services University's Infectious Disease Institutional Review Board, the UK Ministry of Defense Research Ethics Committee, and the Kenyan Medical Research Institute's Institutional Review Board (Riddle et al., 2017). As this analysis does not involve direct contact with the participants to collect additional data and dose not increase the risk to study participant's rights, safety, welfare or affect the integrity of the data as originally collected, this secondary analysis does not require special participant protection.

Risk to Participants. As the data used in this secondary analysis were already coded using identification codes and I do not have access to these identification codes, the risk to study participants that their individual level data would be disclosed is nonexistent.

Ethical Committee Review. This research was reviewed and approved by two ethical committees: the Naval Medical Research Center Institutional Review Board (IRB) and the Walden University IRB. I was added identified as a co-Investigator for the purpose of utilizing the TrEAT TD dataset as owned by Dr. Chad K. Porter at the Naval Research Medical Center, and this research was considered "Exempt" human participants research from that IRB. The OEV-118 dataset was obtained from the study Principal Investigator Dr. Lou Bourgeois. As this research was completed using existing datasets with deidentified data, I pursued archival research review by the Walden University IRB and completed the IRB application according to guidance outlined in the Guide for

Archival Researchers Completing the Walden IRB Application (IRB Approval #10-29-18-0491914).

Summary

This chapter further elaborated on the proposed research design and rationale behind this secondary data analysis. Summaries of the population, instrumentation and procedures used in the original TrEAT TD and OEV-118 clinical trials were described. Detailed data analysis plans were presented to answer each of the proposed research questions, including sampling frames and power calculations as applicable to each statistical test. This chapter closed with an exploration into threats to validity and strategies to address the various ethical considerations inherent to this research. Chapter 4 addresses the results from the study and Chapter 5 provides a synthesis of the results, including recommendations and implications for social change.

Chapter 4: Results

While efforts to expand beyond solely stool-based endpoints to assess the efficacy of treatments and/or prophylactics against moderate to severe diarrheal disease have advanced for pediatric populations and certain CHIMs by inclusion of other symptomology into a clinical disease score, there remains no standardized disease score for TD. In addition, a TD disease score has not been previously studied to assess its potential utility in differentiating illness among a population receiving treatment(s) or intervention(s) for TD. The quantitative secondary data analyses presented below enabled the following:

1. An evaluation of which TD associated signs and symptoms were most strongly associated with negative impact of illness on activity.
2. Development of a complex TD disease score accounting for the most relevant signs and symptoms and their associated illness severity.
3. Determination of whether the TD disease score proved a useful tool for potential use in intervention studies as a measure of protective efficacy or reduced disease severity in cases.

In Chapter 4, I present the results of the analysis in order of generation: (a) the determination of correlation between individual TD signs and symptoms, including output of loose stools; (b) examination of which TD signs and symptoms impact most negatively on activity level; (c) generation of a proposed TD disease complex scoring system; and (d) validation of the resulting model. In Chapter 5, I discuss the results presented below.

Secondary Data Analysis

I obtained datasets from two trials from Dr. Chad Porter of the Naval Medical Research Center (NMRC) and Dr. Lou Bourgeois, Principal Investigator of the OEV-118 study, and used them to generate the results presented below. Chapter 3 includes a full description of both secondary datasets. Briefly, the TrEAT TD dataset contained basic demographic information and all the variables necessary to generate a disease scoring system for TD, whereas the OEV-118 dataset contained the basic demographic information and TD symptom variables needed to validate the disease severity score. The analysis I completed includes the precursor analyses conducted to check assumptions as outlined in Chapter 3 as well as to address each of the three research questions.

Quality Control

Prior to analysis, I received the TrEAT TD and OEV-118 datasets from Dr. Chad Porter and Dr. Lou Bourgeois, respectively, in the form of excel spreadsheets and accompanying data dictionaries. In each dataset, one row of data corresponded to all data obtained for each participant during the study. Because I received the complete datasets from both trials, I determined which data fields were pertinent for answering the research questions and hypothesis testing. Since my analysis included re-analysis of the optimized scoring system on both the TrEAT TD and OEV-118 datasets, I calculated the hypothesis-defined TD severity score for each study participant.

Precursor Analyses

Demographic variables and symptoms experienced by participants enrolled in the TrEAT TD and OEV-118 studies. While separately and partially published,

Table 5 shows the collated demographic characteristics of the participants enrolled in the two datasets. Briefly, the mean age of participants enrolled in the TrEAT TD and OEV-118 studies were 29.3 and 34.7 years, respectively. An overwhelming majority of the participants in the TrEAT TD study were male (*n* = 300, 92%) whereas there was a more equal distribution of gender in the OEV-118 study, with approximately 66% females (*n* = 945) and 34% males (*n* = 490). Both studies enrolled approximately an equal proportion of white participants (84.0% and 86.8% in TrEAT TD and OEV-118, respectively), with 9.4 to 2.5% of participants identifying as 'black' and 6.6 and 10.5% of participants identifying their race as "other" in the TrEAT TD and OEV-118 studies, respectively.

Table 5

Demographic Characteristics of Participants Enrolled in TrEAT TD and OEV-118

Characteristic	TrEAT TD (*n* = 363)	OEV-118 (*n* = 1435)
Mean age, (std. dev)	29.3 (8.5)	34.7 (14.2)
Gender		
Male, No. (%)	300 (92.0)	490 (34.1)
Female, No. (%)	26 (8.0)	945 (65.9)
Race		
White, No. (%)	89.0 (84.0)	1246 (86.8)
Black, No. (%)	10 (9.4)	36 (2.5)
Other, No. (%)	7 (6.6)	151 (10.5)

As shown in Table 6, the most commonly observed subjective symptoms among those enrolled in TrEAT TD were abdominal cramps (75.4%) and malaise (64.3%), followed by nausea reported in approximately half the subjects (52.5%), gas (38.9%), tenesmus (29.1%), and fecal incontinence (14.3%). In contrast, the more objective signs of vomiting and fever were less frequently observed (20.4% and 15.8%, respectively). In OEV-118, among those subjects who experienced travelers' diarrhea (≥ 3 loose or

watery stools in a 24-hour period accompanied by ≥ 1 GI symptom), the most commonly observed subjective symptoms were gas (75.0%), malaise (69.0%) and fecal incontinence (66.2%), followed by abdominal cramps (61.9%), and nausea reported in approximately half the subjects. In contrast to the TrEAT TD study, reports of tenesmus were low (4.8%). Meanwhile, the more objective signs of vomiting and fever were less frequently observed than all subjective symptoms except tenesmus (14.6% and 11.9%, respectively).

Table 6

Frequency (%) of Signs and Symptoms in the TrEAT TD and OEV-118 Datasets

	Cramps	Malaise	Nausea	Vomiting	Fever**	Tenesmus	Incontinence	Gas
TrEAT TD (*n* = 363)								
None	24.6	35.7	47.5	79.6	84.3	70.8	85.7	61.2
Mild	29.8	23.4	30.2	5.2	5.8	14.3	5.8	16.8
Moderate	35.4	33.2	18.7	5.8	7.2	12.9	6.3	18.5
Severe	10.2	7.7	3.6	9.4	2.8	1.9	2.2	3.6
Any Severity	75.4	64.3	52.5	20.4	15.8	29.1	14.3	38.9
OEV-118 Dataset (TD endpoint* only, *n* = 412)								
None	38.1	31.1	51.9	85.2	88.1	95.1	33.7	25.0
Mild	28.2	22.1	25.5	7.5	5.8	2.2	27.4	55.6
Moderate	25.2	28.9	15.8	2.7	2.9	2.4	26.7	15.0
Severe	8.5	18.0	6.8	4.4	3.2	0.2	12.1	4.4
Any Severity	61.9	69.0	48.1	14.6	11.9	4.8	66.2	75.0

Note. *Classic Traveler's Diarrhea (TD) endpoint defined per protocol as ≥3 loose or watery stools in a 24-hour period accompanied by ≥1 gastrointestinal (GI) symptom

Note. **Temperature was recorded on a daily basis in the OEV-118 study; severity classification for the purpose of this table and the severity score for OEV-118 subjects were made using the same severity classifications as the TrEAT TD study as follows: mild = 100.4-101.1°F; moderate = 101.2-102.0°F; severe = 102.1-104°F.

The majority of subjects enrolled in the TrEAT TD study presented with acute watery diarrhea (*n* = 320; 87.4%), whereas only 46 (12.6%) enrolled with acute dysentery or febrile illness (Table 7).

Table 7

Frequency (%) of Diarrhea Based on Protocol-Specific Classifications (TrEAT TD dataset; N = 363)

Classification	Frequency (%)
TrEAT TD	
Acute watery diarrhea	87.4
Acute dysentery or febrile illness	12.6

When stratified by age (Table 8), the two youngest cohorts (18-25 and 26-35 years old) experienced the highest proportion of TD, presenting with acute watery diarrhea (39.4% and 40.7%, respectively) or acute dysentery or febrile illness (56.5% and 26.1%, respectively). Incidence of TD (acute watery diarrhea, acute dysentery or febrile illness) consistently declined as age increased, with only three cases of acute watery diarrhea (0.8%) and no cases of acute dysentery or febrile illness in the oldest age cohort (56-65 years old). Of the 317 cases of acute watery diarrhea, 93.1% were in males compared to 6.9% in females (*n* = 22); and of the 46 cases of acute dysentery or febrile illness, 95.7% were in males (*n* = 44) compared to only 4.3% in females (*n* = 2; Table 8).

Table 8

Distribution of Age and Gender by Diarrhea Classification (TrEAT TD dataset; N = 363)

	Diarrhea classification	
	Acute watery diarrhea (*n* = 317)	Acute Dysentery or febrile illness (*n* = 46)
Age		
18-25 years	125 (39.4%)	26 (56.5%)
26-35 years	129 (40.7%)	12 (26.1%)
36-45 years	41 (12.9%)	7 (15.2%)
46-55 years	19 (6.0%)	1 (2.2%)
56-65 years	3 (0.9%)	0 (0.0%)
Gender		
Male	295 (93.1%)	44 (95.7%)
Female	22 (6.9%)	2 (4.3%)

Of the 1435 subjects enrolled in the OEV-118 study, 412 met the defintion of TD. When stratifed by age, 40% of those cases occurred in the youngest age cohort of 18-25 year old travelers, followed by 27.4% in 26-35 year olds (Table 9). The 46-55 year old group had a slightly higher proportion of TD cases (12.1%) compared to 36-45 year olds (10.7%); and 8.3% and 1.5% of TD cases were experienced in the two oldest cohorts of 56-65 year olds and ≥ 66 years old, respectively. When stratified by gender, the majority of TD cases was observed in females (66.7%) compared to males (33%). Distribution of TD cases were approximately equal between vaccinees and placebos for all age groups and by gender (Table 9).

Table 9

Distribution of Age and Gender with TD by Treatment Group (OEV-118; N = 412)

	Treatment group		
Age	Placebo (*n* = 200)	Vaccine (*n* = 212)	Total (*N* = 412)
18-25 years	78 (39.0%)	87 (41.0%)	165 (40.0%)
26-35 years	56 (28.0%)	57 (26.9%)	113 (27.4%)
36-45 years	18 (9.0%)	26 (12.3%)	44 (10.7%)
46-55 years	30 (15.0%)	20 (9.4%)	50 (12.1%)
56-65 years	17 (8.5%)	17 (8.0%)	34 (8.3%)
≥ 66 years	1 (0.5%)	5 (2.4%)	6 (1.5%)
Gender			
Male	67 (33.7%)	69 (32.5%)	136 (33.0%)
Female	132 (66.3%)	143 (67.5%)	275 (66.7%)

Note. *Classic TD endpoint defined per protocol as ≥ 3 loose or watery stools in a 24-hour period accompanied by ≥ 1 gastrointestinal (GI) symptom.

A total of 77.4% (*n* = 284) of subjects enrolled in TrEAT TD reported some negative impact on activity due to their illness, with 45.2% (*n* = 166) reporting a mild decrease in activity (less than or equal to 50%), 26.2% (*n* = 96) reporting a decrease in activity of > 50%, and 6% (*n* = 22) of subjects reporting TD illness precluding the subject's ability to function (Table 10). In the OEV-118 study, among those subjects with classic travelers' diarrhea (*n* = 412), one-third (33.1%) indicated they would change their plans due to either their gastrointestinal or general symptoms. When further stratified by treatment group, the proportion of subjects who would have changed their plans due to the severity of their symptoms was roughly equal between vaccine and placebo recipients (Table 10).

Table 10

Number and Frequency (%) of Impact on Activity

Impact on Activity	*N* (%)
TrEAT TD Dataset (*n* = 363)	
Normal	79 (21.5)
Decreased ≤ 50%	166 (45.2)
Decreased > 50%	96 (26.2)
Completely unable to function	22 (6.0)
OEV-118 Dataset (TD endpoint* only, *n*=412)	
Vaccinees	
Change	72 (34.0)
No change	140 (66.0)
Placebos	
Change	64 (32.2)
No change	135 (67.8)

Note. *Classic TD endpoint defined per protocol as ≥ 3 loose or watery stools in a 24-hour period accompanied by ≥1 GI symptom.

The maximum stool output in the TrEAT TD dataset as indicated by the maximum number of loose/liquid stools in a 24 hour period prior to presentation is shown in Figure 1. Stooling was not normaly distributed, positively skewed (2.187, *SE* = 0.128) and leptokurtic (7.16, *SE* = 0.26), with the highest proportion of subjects (*n* = 214; 58.9%) producing between ≥ 3 and ≤ 6 loose/liquid stools per 24 hours with a median of 6 loose stools (IQR: 4, 8). Figure 2 confirms the non-normal and right-skewed distribution of stooling frequency via Q-Q plot. The percent of subjects reporting 7 (*n* = 31), 8 (*n* = 28) or 10 (*n* = 26) loose stools over 24 hours prior to presentation were similar (8.5%, 7.7% and 7.2%, respectively) whereas only 8 subjects reported 9 loose stools (2.2%). 2-4% of subjects reported 12 (*n* = 13; 3.6%), 15 (*n* = 10; 2.8%) or 20 (*n* = 8; 2.2%) loose stools in a 24 hour period, while less than 1% of subjects reported 11 (*n* = 3; 0.8%), 13 (*n* = 1; 0.3%), 16 (*n* = 1; 0.3%), 18 (*n* = 1; 0.3%) or 30 (*n* = 2; 0.6%) loose stools in any 24 hour period prior to enrollment. It is important to note that a relatively

high proportion of subjects reported for care within 24 hours of illness onset. Therefore, these estimates may be lower than what would be observed if subjects waited longer than 24 hours before reporting illness and subsequently being treated as part of the study.

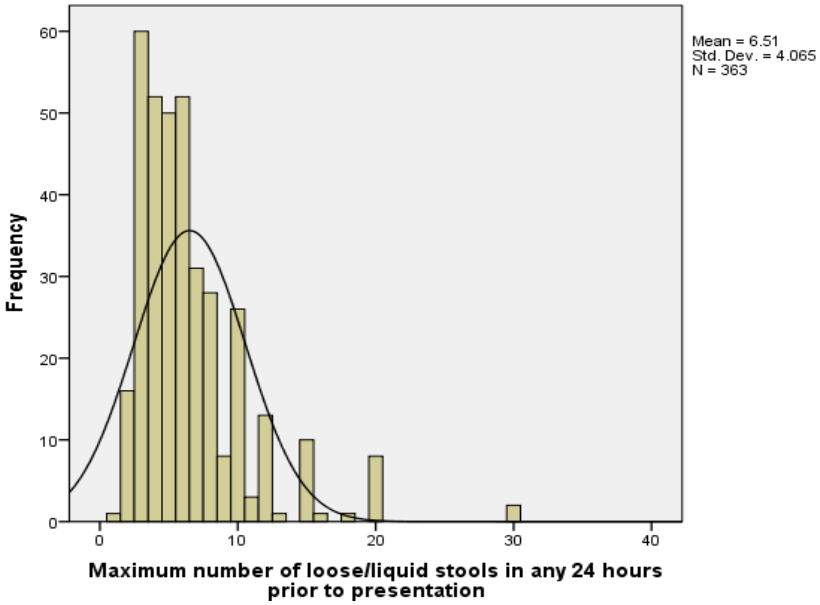

Figure 1. Maximum stool output (frequency; TrEAT TD dataset).

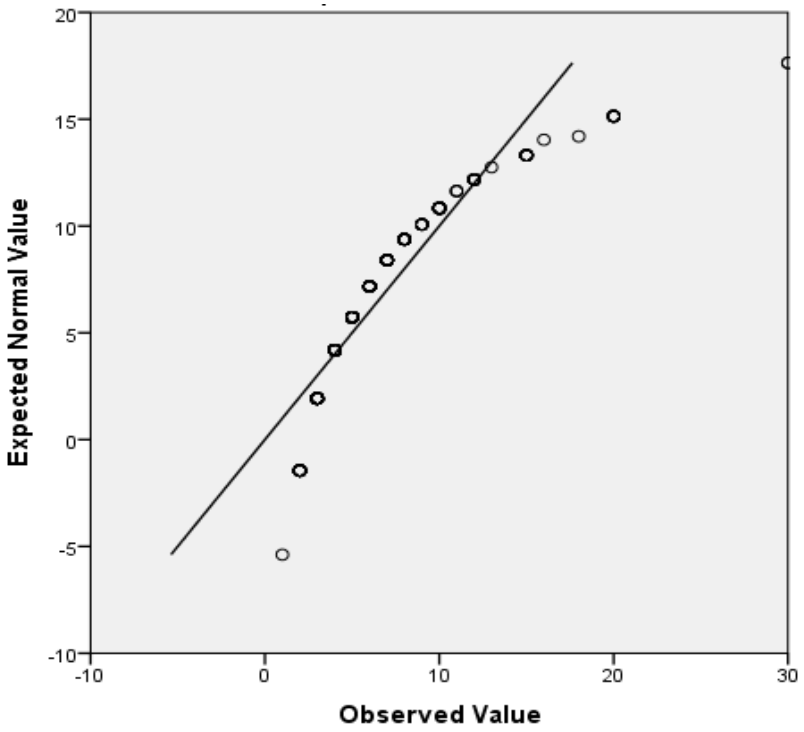

Figure 2. Maximum stool output (frequency; TrEAT TD dataset) Q-Q plot.

The maximum stool output of TD cases in the OEV-118 dataset as indicated by the number of loose/liquid stools in a 24 hour period during the period of overseas travel is shown in Figure 3. Stooling frequency was not normally distributed, positively skewed (3.87, *SE* = 0.12) and leptokurtic (23.37, *SE* = 0.24), with the highest proportion of travelers (*n*=313; 76%) producing between ≥3 and ≤6 loose/liquid stools per 24 hours with a median of 4 loose stools (IQR: 3, 6). Figure 4 confirms the non-normal and right-skewed distribution of stooling frequency via Q-Q plot. The percent of subjects reporting 7 (*n* = 25), 8 (*n* = 20) or 9 (*n* = 21) loose stools over 24 hours were similar (6.1%, 4.9 and 5.1%, respectively); and the number of subjects reporting a maximum of 10 (*n* = 7), 11 (*n* = 5) or 12 (*n* = 6) loose stools over any 24 hour period during travel were similar (1.7%, 1.2% and 1.5%, respectively). Less than 1% of subjects reported ≥13 loose stools in any

24 hour period during travel, with 1 subject (0.2%) experiencing a maximum stool output of 38 stools, the highest reported stooling frequency in the study.

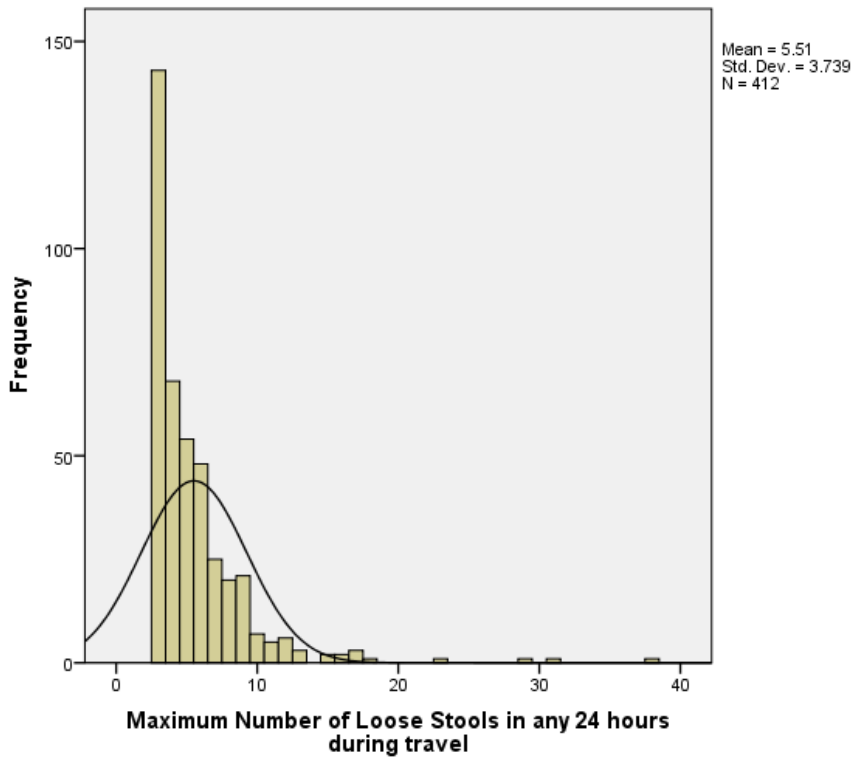

Figure 3. Maximum stool output (frequency; OEV-118 dataset, TD cases).

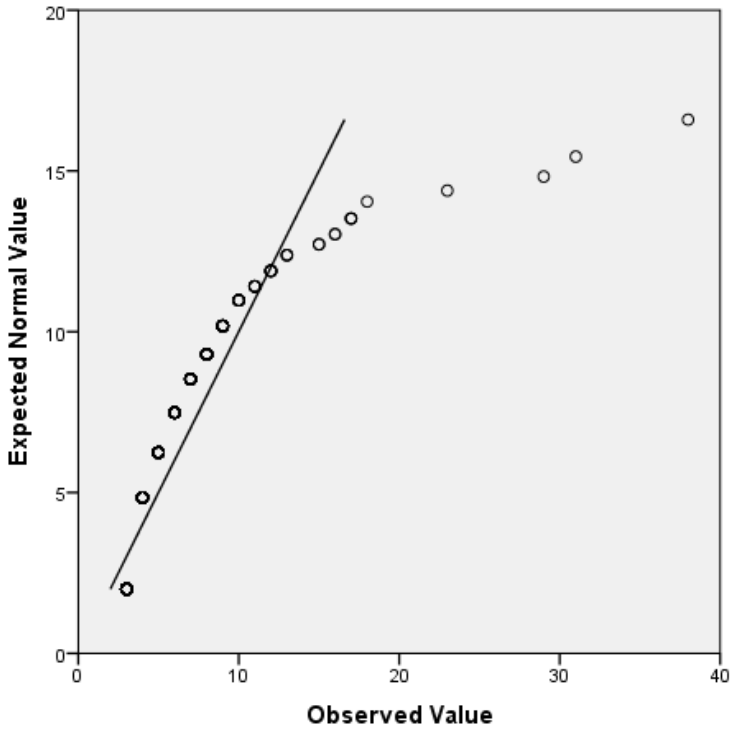

Figure 4. Maximum stool output (frequency; OEV-118 dataset, TD cases) Q-Q plot.

The results presented in the above descriptive statistics analyses for the TrEAT TD and OEV-118 datasets provided good background information to the hypothesis testing analysis. Hypothesis testing was conducted as originally outlined in Chapter 3 and is presented below.

Quantitative Hypothesis Testing Analyses

Hypothesis 1: There are no significant differences in the frequency and severity of clinical signs and symptoms across disease severity classification. The relationship between objective signs (i.e., vomiting, fever) and subjective symptoms (i.e., nausea, malaise, tenesmus, abdominal cramps, fecal incontinence) was assessed using Spearman's correlation. Statistically significant correlations were observed between various signs and symptoms of TD-attributable illness; and among those signs and symptoms that were significantly correlated, the strength of correlation varied (Table 11). The strongest correlation observed was between nausea and vomiting ($\rho = 0.49$; $p < 0.001$) although only 21.3% of participants reported vomiting. Malaise was positively correlated with all signs and symptoms, with the strongest correlation with nausea ($\rho = 0.43$; $p < 0.001$), vomiting ($\rho = 0.34$; $p < 0.001$), fever ($\rho = 0.30$; $p < 0.001$) and abdominal cramps ($\rho = 0.31$; $p < 0.001$). Similarly, abdominal cramps were positively correlated with all analyzed signs and symptoms, with smaller correlations observed between nausea ($\rho = 0.25$; $p < 0.001$) and tenesmus ($\rho = 0.21$; $p < 0.001$). Gas was only significantly correlated with malaise ($\rho = 0.13$; $p = 0.01$) and fecal incontinence was only significantly correlated with malaise ($\rho = 0.13$; $p = 0.01$), nausea ($\rho = 0.11$; $p = 0.03$) and abdominal cramps ($\rho = 0.14$; $p = 0.007$). Tenesmus showed small statistically significant correlations with all signs and symptoms except fever and fecal incontinence.

Table 11

Spearman Correlations of Ordinal Signs and Symptoms (TrEAT TD Dataset; N = 363)

Signs/Symptoms		Correlation estimate (ρ)	Correlation estimate (p) 95% confidence limits		p-value
			Lower	Upper	
Fever	Vomiting	0.24	0.12	0.37	<0.001
	Abdominal Cramps	0.12	0.01	0.22	0.03
	Gas	-0.04	-0.14	0.06	0.43
	Nausea	0.29	0.20	0.38	<0.001
	Tenesmus	0.07	-0.04	0.19	0.16
	Malaise/fatigue	0.30	0.21	0.38	<0.001
	Fecal Incontinence	0.06	-0.06	0.18	0.25
Vomiting	Abdominal Cramps	0.13	0.03	0.23	0.011
	Gas	-0.07	-0.17	0.04	0.188
	Nausea	0.49	0.40	0.57	<0.001
	Tenesmus	0.14	0.03	0.25	0.007
	Malaise/fatigue	0.34	0.25	0.42	<0.001
	Fecal Incontinence	0.08	-0.03	0.12	0.15
	Fever	0.24	0.12	0.37	<0.001
Abdominal Cramps	Gas	0.14	0.04	0.24	0.007
	Nausea	0.25	0.14	0.35	<0.001
	Tenesmus	0.21	0.11	0.30	<0.001
	Malaise/fatigue	0.31	0.22	0.41	<0.001
	Fecal Incontinence	0.14	0.04	0.23	0.007
	Fever	0.12	0.01	0.22	0.03
	Vomiting	0.13	0.03	0.23	0.01
Gas	Nausea	0.37	-0.07	0.14	0.48
	Tenesmus	0.12	0.002	0.23	0.32
	Malaise/fatigue	0.13	0.03	0.24	0.01
	Fecal Incontinence	0.04	-0.06	0.14	0.45
	Fever	-0.42	-0.14	0.06	0.43
	Vomiting	-0.07	-0.17	0.04	0.19
	Abdominal Cramps	0.14	0.04	0.24	0.01

Signs/Symptoms		Correlation estimate (ρ)	Correlation estimate (ρ) 95% confidence limits		p-value
			Lower	Upper	
Nausea	Tenesmus	0.14	0.03	0.24	0.01
	Malaise/fatigue	0.43	0.33	0.52	<0.001
	Fecal Incontinence	0.11	-0.01	0.24	0.03
	Fever	0.29	0.20	0.38	<0.001
	Vomiting	0.49	0.40	0.57	<0.001
	Abdominal Cramps	0.25	0.14	0.35	<0.001
	Gas	0.04	-0.07	0.14	0.48
Tenesmus	Malaise/fatigue	0.23	0.13	0.32	<0.001
	Fecal Incontinence	-0.03	-0.12	0.09	0.64
	Fever	0.07	-0.04	0.19	0.16
	Vomiting	0.14	0.03	0.25	0.007
	Abdominal Cramps	0.21	0.11	0.30	<0.001
	Gas	0.11	0.002	0.23	0.03
	Nausea	0.14	0.03	0.24	0.01
Malaise/fatigue	Fecal Incontinence	0.13	0.03	0.24	0.01
	Fever	0.30	0.21	0.38	<0.001
	Vomiting	0.34	0.25	0.42	<0.001
	Abdominal Cramps	0.31	0.22	0.41	<0.001
	Gas	0.13	0.03	0.24	0.01
	Nausea	0.43	0.33	0.52	<0.001
	Tenesmus	0.23	0.13	0.32	<0.001
Fecal incontinence	Fever	0.06	-0.06	0.18	0.25
	Vomiting	0.08	-0.03	0.19	0.15
	Abdominal Cramps	0.14	0.04	0.23	0.007
	Gas	0.40	-0.06	0.14	0.45
	Nausea	0.11	-0.01	0.24	0.03
	Tenesmus	-0.03	-0.12	0.09	0.64
	Malaise/fatigue	0.13	0.03	0.24	0.01

Note. Ordinal values of signs and symptoms: 0, none; 1, mild; 2, moderate; 3, severe

(table continues)

Correlation between signs and symptoms and frequency of loose stool.

Univariate regression models (Table 12) showed statistically significant associations between the maximum 24-hour stool output and each of the signs and symptoms except gas and tenesmus. Variability in these signs and symptoms accounted for 2% to 6% of the variability in the maximum 24-hour stool output.

Table 12

Univariate Regression between TD-attributable Signs and Symptoms and Maximum 24-hour Stool Output (N = 363)

Sign/symptom	Maximum number of loose/liquid stools in any 24-hour period prior to presentation		
	Adjusted R^2	β	p-value
Abdominal cramps	0.04	0.20	<0.0001
Fever	0.04	0.22	<0.0001
Nausea	0.04	0.19	<0.0001
Vomiting	0.02	0.16	0.003
Fecal incontinence	0.05	0.23	<0.0001
Gas	-0.30	0.003	0.95
Tenesmus	-0.20	0.09	0.3
Malaise/fatigue	0.06	0.25	<0.0001

Note. These results represent analyses with all datapoints. Removal of outliers, defined as those values being greater than three standard deviations away from the mean, were removed for maximum 24 hour stool frequency in any 24-hour period prior to presentation, resulted in a greater number of statistically significant associations between each of the signs and symptoms and stool output (results presented in Appendix 1).

Based on the results presented in this section, I reject the null hypothesis that there are no significant differences in the frequency and severity of clinical signs and symptoms across disease severity classification.

Hypothesis 2: Individual clinical signs and symptoms of TD are not significantly associated with a negative impact on activity. Separate cumulative odds ordinal logistic regression models with proportional odds were developed to estimate the association between individual clinical signs and symptoms of TD (e.g., abdominal

cramps, nausea, tenesmus, gas, incontinence, malaise, vomiting, and tenesmus) as well as the traditional TD-illness metric of stool output (i.e., maximum number of loose/liquid stools in any 24-hours prior to presentation) on activity impact. The association between the severity gas and activity level is shown in Table 13; gas was the only solicited symptom that was not significantly associated with activity in TrEAT TD ($X^2 = 5.16$, $p = 0.160$) (Table 14).

Table 13

Proportion of Gas Severity at Presentation and Impact on Activity (N = 363)

	Impact on Activity			
Gas severity at presentation	Normal	Decreased ≤ 50%	Decreased > 50%	Completely unable to function
None	57 (72.2%)	100 (60.2%)	49 (51.0%)	16 (72.7%)
Mild	14 (17.7%)	25 (15.1%)	20 (20.8%)	2 (9.1%)
Moderate	8 (10.1%)	32 (19.3%)	24 (25.0%)	3 (13.6%)
Severe	0 (0.0%)	9 (5.4%)	3 (3.1%)	1 (4.5%)

Table 14

Ordinal Logistic Regression Analysis of the Relationship between Gas Severity and Impact on Activity (TrEAT TD Dataset) (N = 363)

Variable	β	Standard Error	Adjusted OR	95% CI for OR	p-value
Impact on Activity (ref: completely unable to function)					
Normal	-1.13	0.15	0.32	0.24-0.43	<0.0001
Decreased≤50%	0.90	0.14	2.47	1.88-3.25	<0.0001
Decreased≥50%	2.92	0.24	18.60	11.65-29.71	<0.0001
Gas (ref: None)					
Mild	0.19	0.27	1.21	0.72-2.05	0.47
Moderate	0.54	0.26	1.72	1.04-2.86	0.06
Severe	0.58	0.53	1.78	0.63-5.01	0.27

The association between the severity of fever at presentation and activity level is depicted in Table 15. Increasing fever severity was associated with an increasing negative effect on activity level, $X^2 = 32.875$, $p < 0.0001$, as shown in Table 16. The deviance goodness-of-fit test also indicated the model was a good fit for the observed data, $X^2(6) = 9.762$, $p = 0.135$. The odds of having one's activity impacted by experiencing mild and severe fever were 4.3 (95% *CI* 1.857-9.905) and 3.867 (95% *CI* 1.193-12.537), times greater, respectively, than those experiencing no fever; both with statistically significantly effects, $X^2(1) = 11.743$, $p = 0.001$ for mild and $X^2(1) = 5.078$, $p = 0.024$ for severe fever. The odds of those experiencing moderate fever and reporting a greater impact on activity was the highest compared to those reporting no fever, with an OR of 5.622 ($X^2(1) = 19.446$, $p < 0$ .0001).

Table 15

Proportion of Fever Severity at Presentation and Impact on Activity (TrEAT TD Dataset; N = 363)

Fever severity at presentation	Impact on Activity			
	Normal	Decreased ≤ 50%	Decreased > 50%	Completely unable to function
None	78 (98.7%)	143 (86.1%)	75 (78.1%)	10 (45.5%)
Mild	1 (1.3%)	8 (4.8%)	8 (8.3%)	4 (18.2%)
Moderate	0 (0.0%)	10 (6.0%)	10 (10.4%)	6 (27.3%)
Severe	0 (0.0%)	5 (3.1%)	3 (3.1%)	2 (9.1%)

Table 16

Ordinal Logistic Regression Analysis of the Relationship between Fever Severity and Impact on Activity (TrEAT TD Dataset; N = 363)

Variable	β	Standard Error	Adjusted OR	95% CI for OR	p-value
Impact on Activity (ref: completely unable to function)					
Normal	-1.11	0.13	0.33	0.26-0.43	<0.0001
Decreased≤50%	1.01	0.137	2.74	2.14-3.52	<0.0001
Decreased≥50%	3.15	0.24	23.29	14.50-37.44	<0.0001
Fever (ref: None)					
Mild	1.46	0.43	4.30	1.86-9.91	0.001
Moderate	1.73	0.39	5.62	2.61-12.11	<0.0001
Severe	1.35	0.60	3.87	1.19-12.54	0.02

The association between the severity of abdominal cramps at presentation and activity level is depicted in Table 17. The severity of abdominal cramps is significantly associated with a negative effect on activity, $X^2 = 28.585$, $p < 0.0001$, as shown in Table 18. The odds of having one's activity impacted by abdominal cramps increased approximately two-fold as the severity level category increased. Specifically, the odds of those experiencing moderate abdominal cramps compared to those experiencing none and reporting a greater degree of impact on activity was 2.764 (95% *CI*, 1.649-4.632) with a statistically significant effect, $X^2(1) = 14.879$, $p < 0.0001$. Subjects with mild abdominal cramps (compared to no) abdominal cramps reporting a greater impact on activity was 0.676 (95% *CI*, 0.661-1.894), $X^2(1) = 0.174$ and not statistically significant ($p = 0.676$). Meanwhile, the odds of those with severe abdominal cramps were 4-fold more likely to report a greater impact on activity compared to those without abdominal cramps, with an OR of 4.401 (95% *CI*, 2.126-9.109; $p < 0.0001$).

Table 17

Proportion of Abdominal Cramps Severity at Presentation and Impact on Activity (TrEAT TD Dataset; N = 363)

	Impact on Activity			
Abdominal Cramps severity at presentation	Normal	Decreased ≤ 50%	Decreased > 50%	Completely unable to function
None	34 (43.6%)	30 (18.1%)	19 (19.8%)	6 (27.3%)
Mild	32 (41.0%)	50 (30.1%)	24 (25.0%)	2 (9.1%)
Moderate	11 (13.1%)	68 (41.0%)	40 (41.7%)	9 (40.9%)
Severe	1 (1.3%)	18 (10.8%)	13 (13.5%)	5 (22.7%)

Table 18

Ordinal Logistic Regression Analysis of the Relationship between Abdominal Cramps Severity and Impact on Activity (TrEAT TD Dataset; N = 363)

Variable	β	Standard Error	Adjusted OR	95% CI for OR	p-value
Impact on Activity (ref: completely unable to function)					
Normal	-0.80	0.21	0.45	0.30-0.68	<0.0001
Decreased≤50%	1.35	0.22	3.87	2.53-5.923	<0.0001
Decreased≥50%	3.42	0.30	30.51	17.05-54.59	<0.0001
Abdominal Cramps (ref: None)					
Mild	.11	0.27	1.12	0.66-1.89	0.68
Moderate	1.02	0.26	2.76	1.65-4.63	<0.0001
Severe	1.48	0.37	4.40	2.13-9.11	<0.0001

The association between severity of fecal incontinence at presentation and activity level is depicted in Table 19. Increasing fecal incontinence severity was significantly associated with a negative effect on activity, $X^2 = 24.251$, $p < 0.0001$, as shown in Table 20. The deviance goodness-of-fit test also indicated the model was a good fit for the observed data, $X^2(6) = 10.361$, $p = 0.110$. Interestingly, the odds of those who experienced mild or severe fecal incontinence and reporting a negative impact on activity were higher (OR = 3.534, 95% CI 1.544-8.090, $X^2 = 8.923$, $p = 0.003$ and OR = 10.801, 95% *CI* 2.816-41.429, $X^2 = 12.036$, $p = 0.001$, respectively) than the odds of those who experienced moderate incontinence and reporting a negative impact on activity (OR = 2.617, 95% *CI*, 1.189-5.762, $X^2 = 5.710$, $p = 0.017$).

Table 19

Proportion of Fecal Incontinence Severity at Presentation and Impact on Activity (TrEAT TD Dataset; N = 363)

	Impact on Activity			
Fecal incontinence severity at presentation	Normal	Decreased ≤ 50%	Decreased > 50%	Completely unable to function
None	78 (98.7%)	143 (86.4%)	76 (79.2%)	14 (63.6%)
Mild	1 (1.3%)	9 (5.4%)	7 (7.3%)	4 (18.2%)
Moderate	0 (0.0%)	12 (7.2%)	10 (10.4%)	1 (4.5%)
Severe	0 (0.0%)	2 (1.2%)	3 (3.1%)	3 (13.6%)

Table 20

Ordinal Logistic Regression Analysis of the Relationship between Fecal Incontinence Severity and Impact on Activity (TrEAT TD Dataset; N = 363)

Variable	β	Standard Error	Adjusted OR	95% CI for OR	p-value
Impact on Activity (ref: completely unable to function)					
Normal	-1.15	0.13	0.33	0.25-0.41	<0.0001
Decreased≤50%	0.94	0.13	2.57	2.01-3.28	<0.0001
Decreased≥50%	3.06	0.24	21.30	13.32-34.08	<0.0001
Incontinence (ref: None)					
Mild	1.26	0.69	3.53	1.54-8.09	0.003
Moderate	0.96	0.40	2.62	1.19-5.76	0.02
Severe	2.38	0.42	10.80	2.82-41.43	0.001

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The association between severity of malaise at presentation and activity level is depicted in Table 21. Increase in severity of malaise was significantly associated with an increasing negative effect on activity, $X^2 = 127.864$, $p < 0.0001$, as shown in Table 22. The odds of having one's activity impacted by malaise increased approximately three-fold as the severity level category of the solicited symptom increased. Specifically, the odds of those who experienced mild malaise and reporting a negative impact on activity compared to no malaise and reporting a negative impact on activity was 5.851 (95% *CI* 3.291-10.402), $X^2 = 36.213$, $p < 0.0001$, and the odds of those who experienced moderate malaise compared to none and reporting a negative impact on activity was 14.110 (95% *CI* 8.009-24.858, $X^2 = 83.924$, $p < 0.0001$). Those who experienced severe malaise were three-times more likely than those who experienced moderate malaise, and 44 times more likely than those who experienced no malaise to report a negative impact on activity (OR=44.290, 95% *CI* 18.477-106.165, $X^2 = 72.225$, $p < 0.0001$).

Table 21

Proportion of Malaise Severity at Presentation and Impact on Activity (TrEAT TD Dataset; N = 363)

Malaise severity at presentation	Impact on Activity			
	Normal	Decreased ≤ 50%	Decreased > 50%	Completely unable to function
None	66 (83.5%)	47 (28.3%)	16 (16.7%)	0 (0.0%)
Mild	8 (10.1%)	54 (32.5%)	21 (21.9%)	2 (9.1%)
Moderate	5 (6.35)	55 (33.1%)	51 (53.1%)	10 (45.5%)
Severe	0 (0.0%)	10 (6.0%)	8 (8.3%)	10 (45.5%)

Table 22

Ordinal Logistic Regression Analysis of the Relationship between Malaise Severity and Impact on Activity (TrEAT TD Dataset; N = 363)

Variable	β	Standard Error	Adjusted OR	95% CI for OR	p-value
Impact on Activity (ref: completely unable to function)					
Normal	-3.07	0.17	0.93	0.66-1.30	0.67
Decreased≤50%	2.61	0.25	13.61	8.41-22.03	<0.0001
Decreased≥50%	4.91	0.33	135.37	70.94-258.32	<0.0001
Malaise (ref: None)					
Mild	1.77	0.29	5.85	3.29-10.40	<0.0001
Moderate	2.65	0.29	14.11	8.01-24.86	<0.0001
Severe	3.79	0.45	44.29	18.48-106.17	<0.0001

The association between severity of nausea at presentation and activity level is depicted in Table 23. Increasing severity of nausea was associated with a significantly decrease in activity, $X^2 = 84.375$, $p < 0.0001$, as shown in Table 24. The deviance goodness-of-fit test also indicated the model was a good fit for the observed data, $X^2(6) = 6.681$, $p = 0.351$. Those who experienced mild nausea were almost three times as likely to report a negative impact on activity (OR = 2.745, 95% *CI* 1.724-4.399) compared to those experiencing no nausea ($X^2 = 17.968$, $p < 0.0001$). Those who experienced moderate nausea were approximately 9 times more likely to report a negative impact on activity (OR = 9.193, 95% *CI* 5.164-16.363) compared to those who experienced no nausea, $X^2 = 56.860$, $p < 0.0001$; and those who experienced severe nausea were 37 times more likely to report a decrease in normal activity (OR = 37.073, 95% *CI* 11.781-116.661) compared to those reporting no nausea ($X^2 = 38.154$, $p < 0.0001$).

Table 23

Proportion of Nausea Severity at Presentation and Impact on Activity (TrEAT TD Dataset; N = 363)

	Impact on Activity			
Nausea severity at presentation	Normal	Decreased ≤ 50%	Decreased > 50%	Completely unable to function
None	62 (78.5%)	79 (47.6%)	28 (29.2%)	3 (13.6%)
Mild	14 (17.7%)	61 (36.7%)	32 (33.3%)	3 (13.6%)
Moderate	3 (3.8%)	23 (13.9%)	32 (33.3%)	10 (45.5%)
Severe	0 (0.0%)	3 (1.8%)	4 (4.2%)	6 (27.3%)

Table 24

Ordinal Logistic Regression Analysis of the Relationship between Nausea Severity and Impact on Activity (TrEAT TD Dataset; N = 363)

Variable	β	Standard Error	Adjusted OR	95% CI for OR	p-value
Impact on Activity (ref: completely unable to function)					
Normal	-0.66	-0.15	0.52	0.38-0.70	<0.0001
Decreased≤50%	1.69	0.18	5.44	3.79-7.79	<0.0001
Decreased≥50%	4.05	0.30	57.11	31.99-101.96	<0.0001
Nausea (ref: None)					
Mild	1.01	0.24	2.75	1.72-4.40	<0.0001
Moderate	2.22	0.29	9.19	5.16-16.36	<0.0001
Severe	3.61	0.59	37.07	11.78-116.66	<0.0001

The association between severity of vomiting at presentation and activity level is depicted in Table 25. The severity of vomiting was associated with a significant decrease in activity, $X^2 = 55.697$, $p < 0.0001$, as shown in Table 26. The deviance goodness-of-fit test also indicated the model was a good fit for the observed data, $X^2(6) = 3.420$, $p = 0.755$. The odds of those experiencing mild and moderate vomiting and reporting a negative impact on activity were very similar – 5.422 and 5.344, respectively – compared to those experiencing no vomiting ($X^2 = 14.001$, $p < 0.0001$, mild vomiting; $X^2 = 15.079$, $p < 0.0001$, moderate vomiting). Those experiencing severe vomiting were approximately 8 times more likely to report a negative impact of illness on activity level than those who experienced no vomiting (OR 8.38, 95% *CI* 4.135-16.989) ($X^2 = 34.784$, $p < 0.0001$).

Table 25

Proportion of Vomiting Severity at Presentation and Impact on Activity (TrEAT TD Dataset; N = 363)

	Impact on Activity			
Vomiting severity at presentation	Normal	Decreased ≤ 50%	Decreased > 50%	Completely unable to function
None	76 (96.2%)	144 (86.7%)	60 (62.5%)	9 (40.9%)
Mild	0 (0%)	7 (4.2%)	10 (10.4%)	2 (9.1%)
Moderate	1 (1.3%)	7 (4.2%)	10 (10.4%)	3 (13.6%)
Severe	2 (2.5%)	8 (4.8%	16 (16.7%)	8 (36.4%)

Table 26

Ordinal Logistic Regression Analysis of the Relationship between Vomiting Severity and Impact on Activity (TrEAT TD dataset; N = 363)

Variable	β	Standard Error	Adjusted OR	95% CI for OR	p-value
Impact on Activity (ref: completely unable to function)					
Normal	-1.04	0.13	0.35	0.27-0.46	<0.0001
Decreased≤50%	1.17	0.14	3.23	2.48-4.22	<0.0001
Decreased≥50%	3.44	0.23	31.20	18.79-51.78	<0.0001
Vomiting (ref: None)					
Mild	1.69	0.45	5.42	2.24-13.14	<0.0001
Moderate	1.68	0.43	5.34	2.29-12.45	<0.0001
Severe	2.13	0.36	8.38	4.14-16.99	<0.0001

The association between severity of tenesmus at presentation and activity level is depicted in Table 27. The severity of tenesmus was associated with a significant decrease in activity, X^2=13.031, p = 0.005, as shown in Table 28. Only those who experienced moderate tenesmus in the TrEAT TD trial reported were statistically significantly more likely to report a negative impact on activity (OR = 2.790, 95% *CI* 1.557-4.998, X^2 = 11.898, p = 0.001) compared to those who experienced no tenesmus. The odds of those who experienced mild tenesmus and reporting a negative impact on activity (OR = 1.289, 95% *CI* 0.741-2.243; X^2 = 0.808, p = 0.369) was similar to that of those who experienced severe tenesmus and reported a negative impact on activity (OR = 1.745, 95% *CI* 0.435-7.001; X^2 = 0.617, p = 0.432).

Table 27

Proportion of Tenesmus Severity at Presentation and Impact on Activity (TrEAT TD Dataset; N = 363)

	Impact on Activity			
Tenesmus severity at presentation	Normal	Decreased ≤ 50%	Decreased > 50%	Completely unable to function
None	67 (84.8%)	114 (68.7%)	63 (65.6%)	13 (59.1%)
Mild	10 (12.7%)	24 (14.5%)	17 (17.7%)	1 (4.5%)
Moderate	1 (1.3%)	24 (14.5%)	16 (16.7%)	6 (27.3%)
Severe	1 (1.3%)	4 (2.4%)	0 (0%)	2 (9.1%)

Table 28

Ordinal Logistic Regression Analysis of the Relationship between Tenesmus Severity and Impact on Activity (TrEAT TD Dataset; N = 363)

Variable	β	Standard Error	Adjusted OR	95% CI for OR	p-value
Impact on Activity (ref: completely unable to function)					
Normal	-1.12	0.14	0.33	0.25-0.43	<0.0001
Decreased≤50%	0.94	0.13	2.55	1.97-3.32	<0.0001
Decreased≥50%	2.98	0.24	19.68	12.37-31.30	<0.0001
Tenesmus (ref: None)					
Mild	0.25	0.28	1.29	0.74-2.24	0.37
Moderate	1.03	0.30	2.79	1.56-5.00	0.001
Severe	0.56	0.71	1.75	0.44-7.00	0.43

The association between number of loose stool output in any 24-hour period prior to presentation and activity level is depicted in Table 29. A Classification and Regression Tree Analysis (CART) was conducted to quickly determine the optimal cut points of the maximum number of loose stools in 24-hours for inclusion in the scoring system. The maximum number of loose stools in 24-hours period prior to presentation as defined by the following three categories: 2-4 stools/24 hours, 5-7 loose stools/24 hours, ≥8 loose stools/24 hours, is significantly associated with a negative effect on activity statistically significant effect on activity, $X^2(2) = 62.703$, $p < 0.0001$ (Table 30). For this analysis utilizing an ordinal distribution of stool maximum stool frequency, the assumption of proportional odds was met, as assessed by a full likelihood ratio test comparing the fit of the proportional odds location model to a model with varying location parameters, $X^2(4) = 8.401$, $p = 0.078$. The deviance goodness-of-fit test indicated that the model was a good fit to the observed data, $X^2(4) = 8.401$, $p = 0.078$. The final model statistically significantly predicted the dependent variable over and above the intercept-only model, $X^2(2) = 67.627$, $p < 0.0001$. Subjects who reported 5-7 loose stools in 24-hours were approximately 4-fold more likely to report a greater impact on activity compared to those who reported 2-4 loose/liquid stools in 24-hours, with an OR of 3.848 (95% *CI*, 2.371-6.243; $p < 0.0001$). As stooling increased with 8 or more loose stools in a 24-hour period, the odds of reporting a negative impact on activity also increased dramatically, with an OR of 8.508 (95% *CI*, 4.963-14.585; $p < 0.0001$). This analysis further supports the proposed stool output categories (i.e., 2-4 stools/24 hours, 5-7 loose stools/24 hours, ≥8 loose stools/24 hours) are appropriate cut points for inclusion in the TD disease complex score. Finally, and as demonstrated throughout this section, I reject the null hypothesis

that there are no significant individual clinical signs and symptoms of TD associated with a negative impact on activity in adult travelers.

Table 29

Proportion of Loose Stool Output in any 24-hour Period Prior to Presentation and Impact on Activity (TrEAT TD Dataset; N = 363)

	Impact on Activity			
Maximum number of loose stools in any 24-hour period prior to presentation	Normal	Decreased ≤ 50%	Decreased > 50%	Completely unable to function
2-4 loose/liquid stools	56 (70.9%)	53 (31.9%)	15 (15.6%)	5 (22.7%)
5-7 loose/liquid stools	17 (21.5%)	71 (42.8%)	41 (42.7%)	4 (18.2%)
≥8 loose/liquid stools	6 (7.6%)	42 (25.3%)	40 (41.7%)	13 (59.1%)

Table 30

Ordinal Logistic Regression Analysis of the Relationship between Stool Frequency (Maximum Number of Loose Stools in any 24-hour Period Prior to Presentation – Ordinal) and Impact on Activity (TrEAT TD Dataset; N = 363)

Variable	β	Standard Error	Adjusted OR	95% CI for OR	p-value
Impact on Activity (ref: completely unable to function)					
Normal	-0.37	0.17	0.69	0.49-0.97	0.03
Decreased≤50%	1.98	0.21	7.27	4.80-10.99	<0.0001
Decreased≥50%	4.13	0.30	62.23	34.81-111.24	<0.0001
Maximum number of loose stools (ref: 2-4 loose stools in 24-hours prior to presentation)					
5-7 loose stools in 24-hours prior to presentation	1.34	0.25	3.85	2.37-6.24	<0.0001
≥8 loose stools in 24-hours prior to presentation	2.14	0.28	8.51	4.96-14.59	<0.0001

Development of the disease complex score. Multiple Correspondence Analysis was performed using SAS and all observations within the TrEAT TD dataset (no outliers removed) and showed covariability in multiple signs and symptoms with severity being the most common factor associated with similar dimensions in a two-dimensional space (Figure 5). It should be noted that the CART-defined stool classifications were included in the MCA analysis, with the two lowest categories of stool frequency (0-1 loose stools/24 hours and 2-4 loose stools/24 hours) clustered with the lack of any objective signs and symptoms. Meanwhile, the highest loose stool category (≥8 loose stools/24 hours) appeared most proximal to mild and moderate symptoms. Mild fever, vomiting, fecal incontinence, nausea and malaise clustered tightly with moderate abdominal cramps and fecal incontinence; whereas moderate nausea and vomiting clustered with more severe abdominal cramps and ≥8 loose stools/24 hours. Interestingly, mild tenesmus grouped with the aforementioned cluster with more moderate to severe symptoms, resulting in its elevated scoring of '2' in the final disease severity score. As expected, most severe signs and symptoms (with the exception of abdominal cramps and ≥8 loose stools/24 hours) tended to cluster together, with moderate fever and tenesmus also included in this grouping, the latter two parameters receiving a maximum score of '3' in the final disease severity score. Based on the grouping of clinical outcomes in the MCA and taken together with the results of the correlation, univariate logistic regression analyses, a three-component disease score was developed utilizing the objective signs, subjective symptoms and stool frequency yielding a score ranging from 0 (no disease) to 9 (most severe disease) (Table 31).

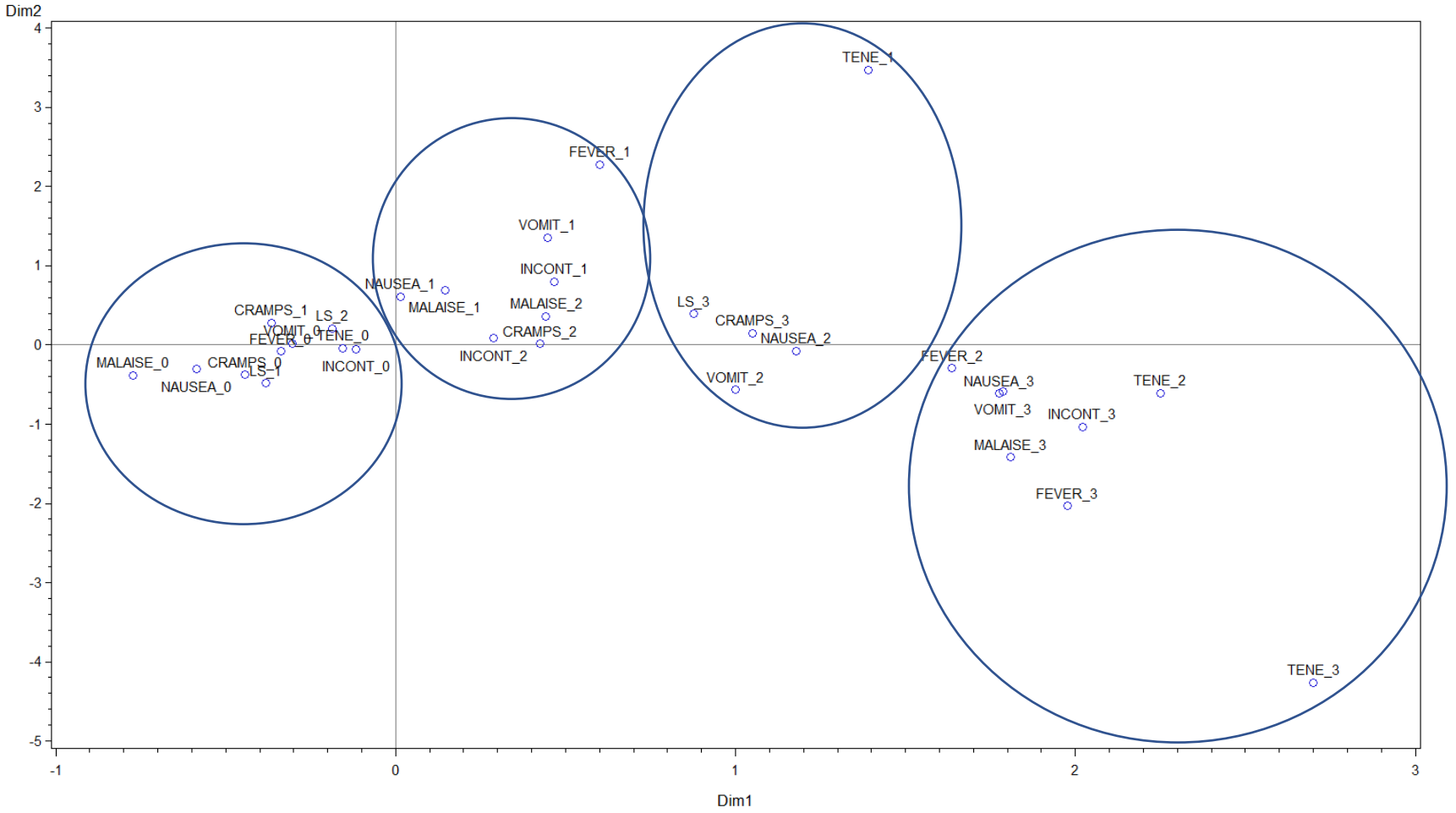

Figure 5. Multiple correspondence analysis of signs and symptoms of travelers' diarrhea (TrEAT TD dataset).

Table 31

Travelers' Diarrhea (TD) Disease Complex Score

Parameter	Outcome	Score
Objective Signs	Severe: ≥3 episodes vomiting **OR**	3
	Moderate to severe fever	3
	2 episodes vomiting	2
	1 episode vomiting **OR** Mild fever	1
	No objective symptoms	0
Subjective Symptoms	Severe: Tenesmus, Malaise, Nausea, Fecal Incontinence **OR**	3
	Moderate Tenesmus	3
	Severe Abdominal cramps **OR**	2
	Moderate Nausea **OR**	2
	Mild Tenesmus	2
	Moderate: Abdominal cramps, Fecal Incontinence, Malaise **OR**	1
	Mild: Abdominal cramps, Nausea, Malaise, Fecal Incontinence	1
	No subjective symptoms	0
Loose stool output (max 24 hour freq)	≥8 loose stools/24 hours	3
	5-7 loose stools/24 hours	2
	2-4 loose stools/24 hours	1
	0-1 loose stools/24 hours	0

Utilizing this new disease complex score and prior to application to the OEV-118 dataset as part of Hypothesis 3, an ordinal logistic regression was performed to assess whether the new score better estimated of TD impact on activity than any single individual sign or symptom.

Assessment of whether the new score benefits the estimation of impact on activity over any single individual sign or symptom. The distribution of subjects by TD severity and impact on activity is depicted in Table 32 and Figure 6. The new TD disease complex score was associated with a significant decrease in activity, X^2 = 127.156, $p < 0.001$, as shown in Table 33. The assumption of proportional odds was met, as assessed by a full likelihood ratio test comparing the fit of the proportional odds location model to a model with varying location parameters, $X^2(16) = 16.28$, $p = 0.434$. The deviance goodness-of-fit test indicated the model was a good fit to the observed data, $X^2(16) = 15.971$, $p = 0.455$, with the final model statistically significantly predicting the dependent variable over and above the intercept-only model, $X^2(8) = 164.997$, $p < 0.001$. As TD disease complex score increased, so did the odds of reporting a negative impact on activity, with a slight exception between those with a TD score of 6 versus 7, in which the latter resulted in a slightly lower odds ratio compared to the former (OR = 167.54, 95% *CI* 40.97-685.19 and OR = 126.00, 95% *CI* 29.66-535.23, respectively) (Table 33). Those scoring highest (TD Score = 9) were 1423 times as likely to report a negative impact on activity (OR = 1422.84, 95% *CI* 244.69-8273.63) compared to those with the lowest score (TD Score = 1). Even those who scored a 2 were 6.5 times more likely to report a negative impact on activity (OR = 6.53, 95% *CI* 1.78-23.86) compared to those with the lowest score (TD Score = 1). It should be noted a TD Score of 0 was not

included in this analysis, as all participants who enrolled in the TrEAT TD study were required to have at least 2 loose stools within 24 hours and subsequently a minimum score of ‘1’.

Table 32

Proportion of TD Disease Score and Impact on Activity (TrEAT TD Dataset; N = 363)

TD Score	Impact on Activity			
	Normal	Decreased ≤ 50%	Decreased > 50%	Completely unable to function
1	20 (86.9%)	2 (8.7%)	1 (4.3%)	0 (0.0%)
2	29 (50.9%)	24 (42.1%)	4 (7.0%)	0 (0.0%)
3	17 (20.7%)	49 (59.8%)	15 (18.3%)	1 (1.2%)
4	6 (9.1%)	39 (59.1%)	19 (28.8%)	2 (3.0%)
5	6 (14.6%)	18 (43.9%)	16 (39.2%)	1 (2.4%)
6	1 (2.9%)	12 (34.3%)	17 (48.6%)	5 (14.3%)
7	0 (0.0%)	14 (58.3%)	6 (25%)	4 (16.7%)
8	0 (0.0%)	7 (29.2%)	14 (58.3%)	3 (12.5%)
9	0 (0.0%)	1 (9.1%)	4 (36.4%)	6 (54.5%)

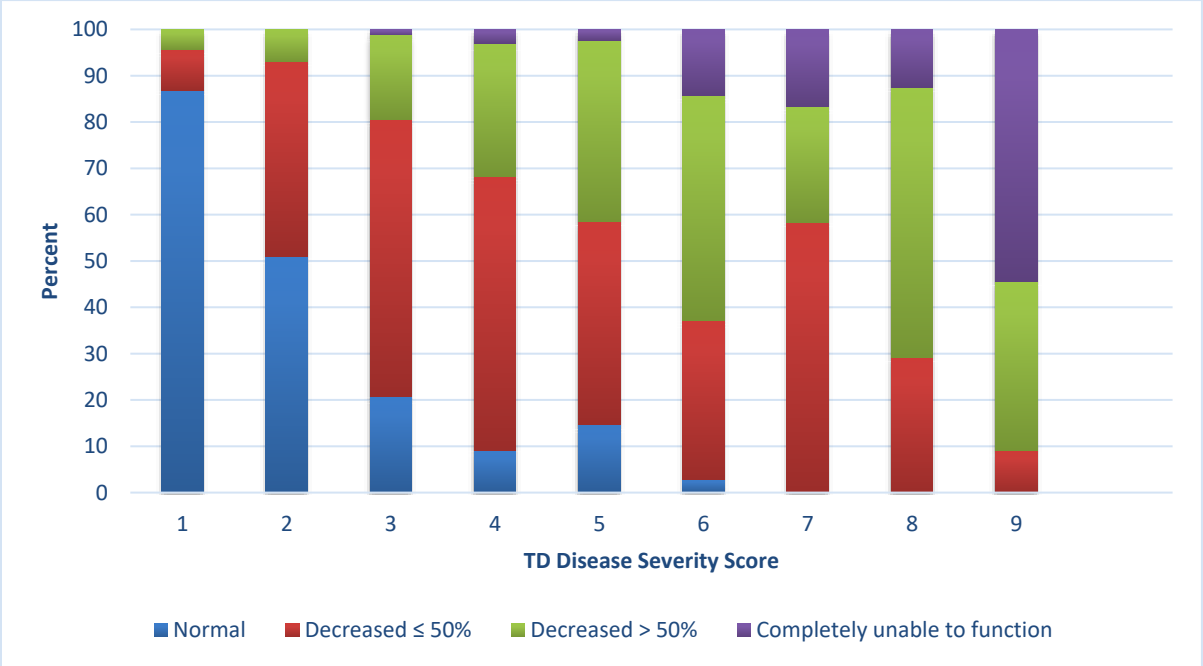

Figure 6. Proportion of TD disease score and impact on activity (TrEAT TD dataset).

Table 33

Ordinal Logistic Regression Analysis of the Relationship between TD Disease Score and Impact on Activity (TrEAT TD Dataset; N = 363)

Variable	β	Standard Error	Adjusted OR	95% CI for OR	p-value
Impact on Activity (ref: completely unable to function)					
Normal	1.857	0.6097	6.407	1.939-21.167	0.002
Decreased≤50%	4.631	0.6415	102.667	29.20-360-98	<0.0001
Decreased≥50%	7.124	0.6872	1241.159	322.77-4772-69	<0.0001
TD Score (ref: TD Score 1)					
TD Score 2	1.876	0.6615	6.525	1.78-23.86	0.005
TD Score 3	3.200	0.6549	24.532	6.77-88.56	<0.0001
TD Score 4	3.929	0.6714	50.842	13.64-189.55	<0.0001
TD Score 5	4.093	0.6978	59.947	15.27-235.38	<0.0001
TD Score 6	5.121	0.7186	167.539	40.97-685.19	<0.0001
TD Score 7	4.836	0.7380	126.001	29.66-535.23	<0.0001
TD Score 8	5.417	0.7573	225.169	51.04-993.39	<0.0001
TD Score 9	7.260	0.8982	1422.835	244.69-8273.63	<0.0001

Hypothesis 3: The estimated vaccine efficacy of the ETVAX vaccine in the OEV-118 Phase 3 trial does not change as a result of using the new disease complex score. In an attempt to assess TD severity score's potential utility, it was applied to a previously conducted Phase 3 vaccine field efficacy trial (OEV-118) to determine whether the disease score significantly differentiated illness between vaccine and placebo recipients. This analysis was conducted for the entire OEV-118 dataset, as well as multiple subgroups within OEV-118, defined in Chapter 3, Table 4 (see also Table 40).

In the dataset, comprised of subjects enrolled in the OEV-118 study who received one or two doses of vaccine, traveled to Mexico or Guatemala and had symptom data available for analysis (N = 1435) ('ALL'), there were 722 vaccinees and 713 placebos. The distribution of the TD score by treatment group is depicted in Table 34 and Figure 7. TD scores for neither treatment group were normally distributed, as assessed by Shapiro-Wilk's test ($p < 0.05$); however, there was homogeneity of variances, as assessed by Levene's test for equality of variances ($p = 0.17$). While the placebo mean TD score (1.82±1.93) was slightly higher than vaccinees mean TD score (1.73±1.82), the difference was not statistically significant, = -0.10, 95% *CI* [-0.29, 0.10], $t(1433) = -0.97$, $p = 0.33$ (Table 40).

Table 34

Distribution of TD Disease Score in All Subjects Enrolled in OEV-118 by Treatment Group (OEV-118 Dataset; N = 1435)

TD Score	Treatment Group (*N* = 1435)	
	Vaccinees (*n* = 722)	Placebos (*n* = 713)
0	206 (28.5%)	202 (28.3%)
1	192 (26.6%)	185 (25.9%)
2	155 (21.5%)	142 (19.9%)
3	68 (9.4%)	63 (8.8%)
4	37 (5.1%)	56 (7.9%)
5	26 (3.6%)	18 (2.5%)
6	16 (2.2%)	18 (2.5%)
7	12 (1.7%)	16 (2.2%)
8	6 (0.8%)	8 (1.1%)
9	4 (0.6%)	5 (0.7%)

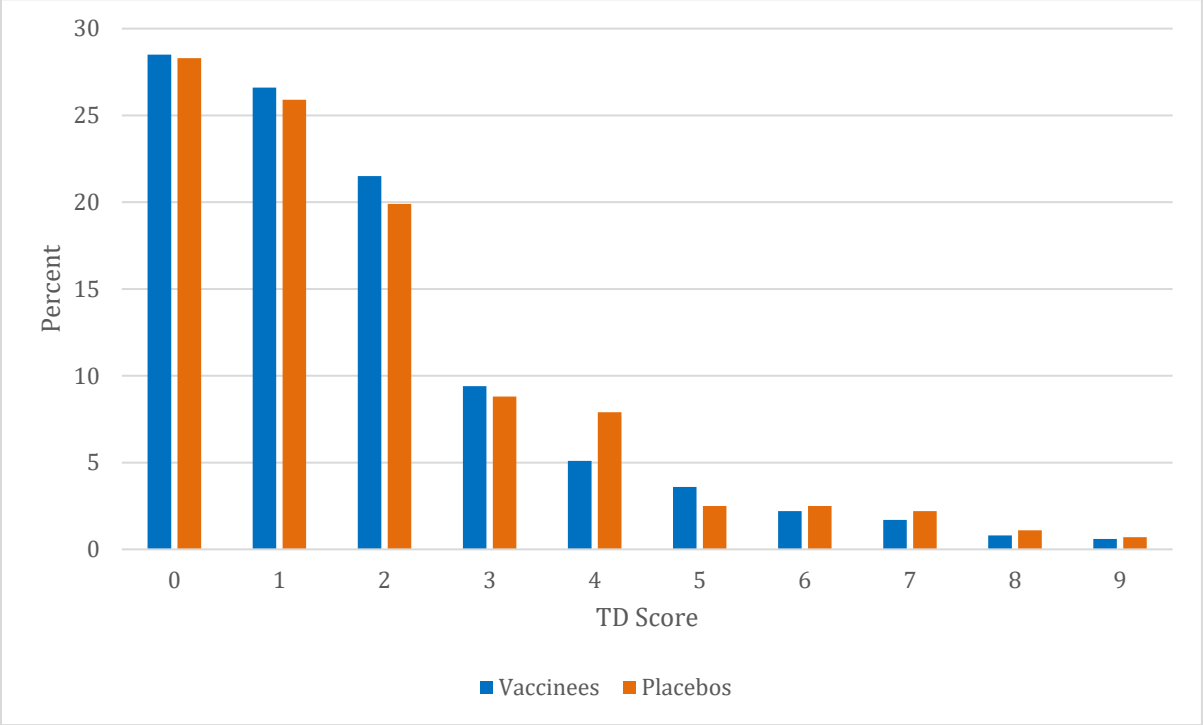

Figure 7. Proportion of TD disease score and subjects meeting TD endpoint by treatment group (OEV-118 dataset).

Among those with TD independent of etiology (subset 'TD'), there were 212 vaccine and 200 placebo recipients. The distribution of the TD score among this subset stratified by treatment group is depicted in Table 35 and Figure 8. There were no outliers in this subset, as assessed by inspection of a boxplot. TD scores for each treatment group were not normally distributed, as assessed by Shapiro-Wilk's test ($p < 0.05$); however, there was homogeneity of variances, as assessed by Levene's test for equality of variances ($p = 0.73$). While the placebo mean TD score (3.77±1.93) was slightly higher than vaccinees mean TD score (3.60±1.86), the difference was not statistically significant, M= -0.17, 95% CI [-0.54, 0.20], $t(410) = -0.91$, $p = 0.36$ (Table 40). Based on this analysis the TD severity scores distribution in both groups were comparable.

Table 35

Distribution of TD Disease Score and Subjects Meeting TD Endpoint by Treatment Group (OEV-118 Dataset; N=412)

TD Score	Treatment Group (N = 412)	
	Vaccinees (n = 212)	Placebos (n = 200)
1	8 (3.8%)	7 (3.5%)
2	67 (32.1%)	58 (28.5%)
3	51 (23.6%)	43 (22%)
4	31 (14.6%)	37 (18.5%)
5	23 (10.8%)	16 (8.0%)
6	10 (4.7%)	16 (8.0%)
7	11 (5.2%)	11 (5.5%)
8	7 (3.3%)	7 (3.5%)
9	4 (1.9%)	5 (2.5%)

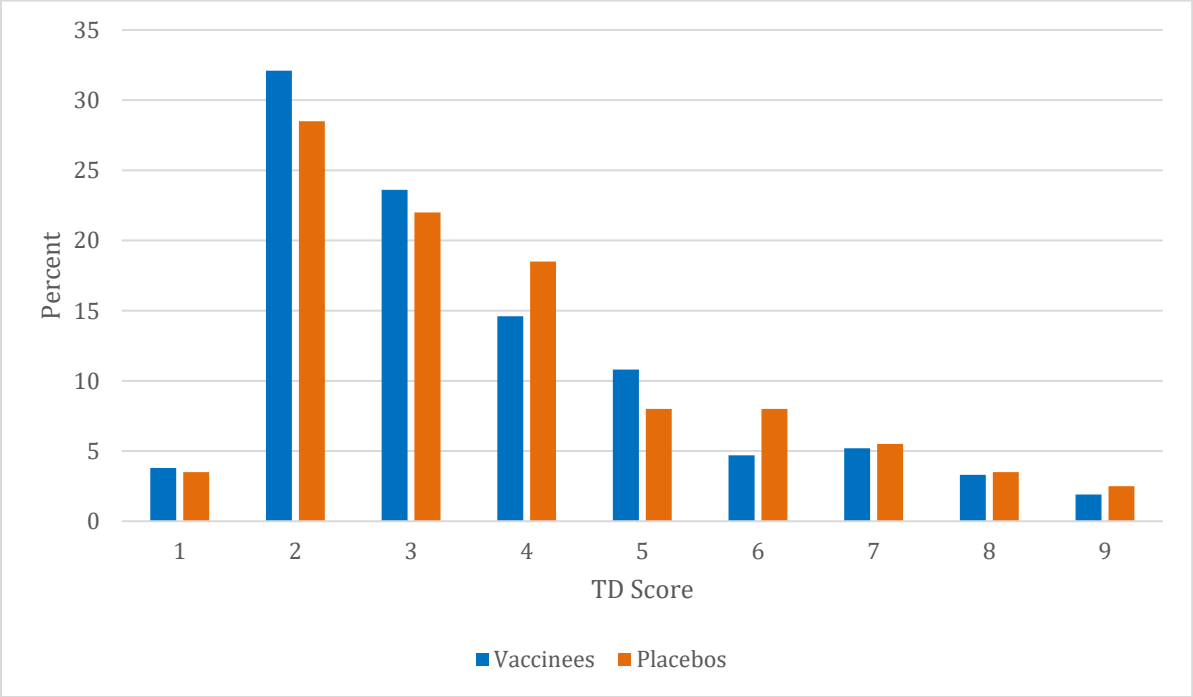

Figure 8. Proportion of TD disease score and subjects meeting TD endpoint by treatment group (OEV-118 dataset).

In the 'Vaccine Preventable ETEC TD (VPO-ETEC TD)' subset, defined as those experiencing ≥5 loose/watery stools in 24 hours plus ≥ of abdominal pain/cramps, nausea and/or vomiting, plus ETEC sharing antigens with the ETVAX vaccine as the sole pathogen and isolated in a window of 24 hours before to 72 hours after illness onset among subjects completing the full 2-dose regimen and traveling during the window of 7 to 14 days post 2nd dose and completing 14 to 28 days surveillance, there were 8 vaccine and 5 placebo recipients. The proportion of subjects by treatment groups across the new TD score is depicted in Table 36 and Figure 9. In an independent t-test, there was homogeneity of variances, as assessed by Levene's test for equality of variances (p = 0.73), but the TD scores for vaccinees were not normally distributed with a Shapiro-

Wilk's value of $p = 0.03$. TD scores between placebos ($M = 4.80$, $SD = 1.64$) and vaccinees ($M = 4.13$, $SD = 1.25$) who met the VPO-ETEC TD endpoint definition did not show a statistically significant difference, $M = -0.68$, 95% CI [-2.44, 1.09], $t(11) = -0.84$, $p = 0.42$ (Table 40).

Table 36

Distribution of TD Disease Score and Subjects Meeting VPO-ETEC TD Endpoint by Treatment Group (OEV-118 Dataset; N = 14)

TD Score	Treatment Group ($N = 14$)	
	Vaccinees (n=8)	Placebos (n=5)
1	0 (0.0%)	0 (0.0%)
2	0 (0.0%)	0 (0.0%)
3	3 (37.5%)	1 (25.0%)
4	3 (37.5%)	2 (40.0%)
5	0 (0.0%)	0 (0.0%)
6	2 (25%)	1 (33.3%)
7	0 (0.0%)	1 (33.3%)
8	0 (0.0%)	0 (0.0%)
9	0 (0.0%)	0 (0.0%)

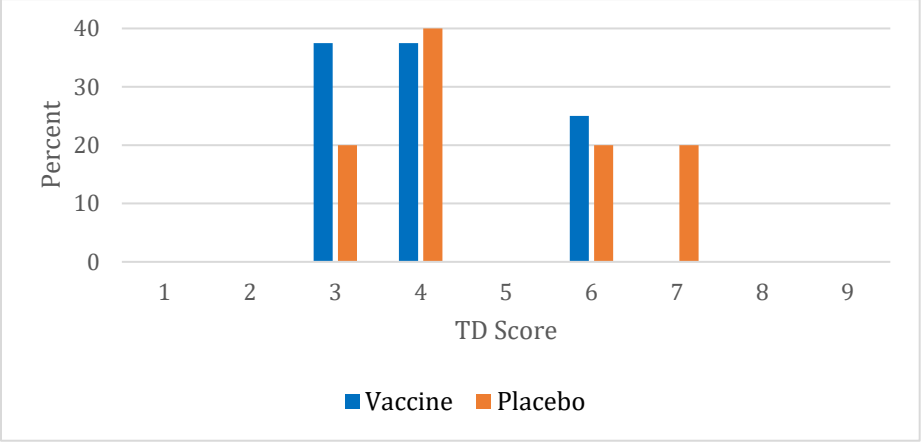

Figure 9. Proportion of TD disease score and subjects meeting VPO-ETEC TD endpoint by treatment group (OEV-118 dataset).

In the ‘ETEC TD’ subset, defined as those experiencing ≥3 loose or watery stools in a 24 hour period accompanied by abdominal pain or cramps, nausea, or vomiting of any intensity, plus ETEC sharing antigens with the vaccine as the sole pathogen isolated anytime during the TD episode, there were 10 vaccinees and 19 placebos who met this particular endpoint as recommended by the OEV-118 Data Safety Monitoring Board (DSMB). The proportion of subjects by treatment group across the new TD score is depicted in Table 37 and Figure 10. An independent t-test to assess differences in the scores between vaccine and placebo recipients within this subset demonstrated TD scores for each treatment group were normally distributed, as assessed by Shapiro-Wilk’s test ($p>.05$), and there was homogeneity of variances, as assessed by Levene’s test for equality of variances ($p = 0.182$). The mean disease score for vaccinees ($M = 3.40$, $SD = 1.174$) was lower than the mean disease score for placebos ($M = 4.74$, $SD = 1.851$), a statistically significant difference, M = -1.34, 95% CI [-2.67, -0.01], $t(27) = -2.07$, $p = 0.05$ (Table 40).

Table 37

Distribution of TD Disease Score and Subjects Meeting ETEC TD endpoint by Treatment Group (OEV-118 Dataset; N = 29)

TD Score	Treatment Group (*N* = 29)	
	Vaccinees (*n* = 10)	Placebos (*n* = 19)
1	0 (0%)	0 (0.0%)
2	2 (20.0%)	3 (15.8%)
3	4 (40.0%)	1 (5.3%)
4	3 (30.0%)	5 (26.3%)
5	0 (0.0%)	5 (26.3%)
6	1 (10.0%)	1 (5.3%)
7	0 (0.0%)	2 (10.5%)
8	0 (0.0%)	2 (10.5%)
9	0 (0.0%)	0 (0.0%)

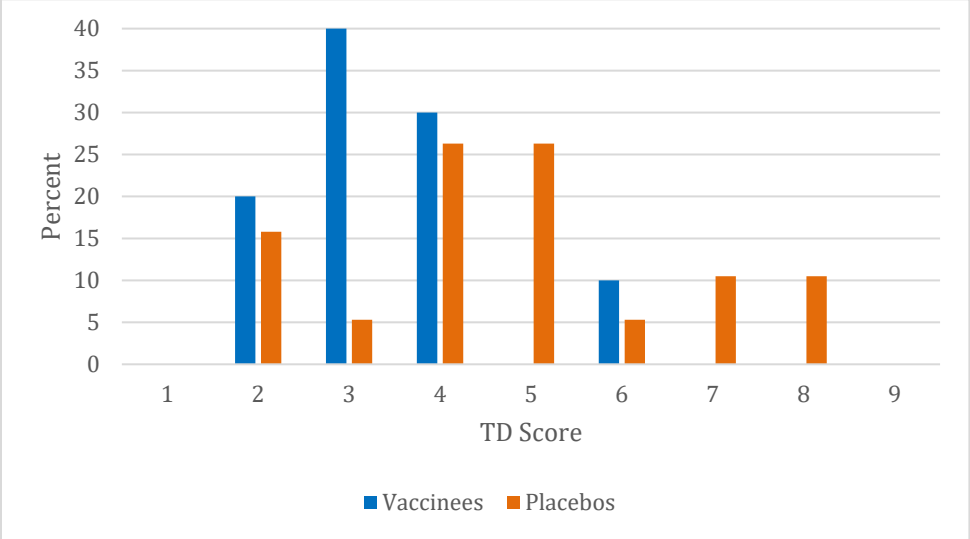

Figure 10. Proportion of TD disease score and subjects meeting ETEC TD endpoint by treatment group (OEV-118 dataset).

In the 'ALL ETEC TD' subset, defined as those who had any ETEC-attributable TD, there were 100 vaccinees and 87 placebos who met this particular criterion. The proportion of subjects by treatment group across the new TD score is depicted in Table

38 and Figure 11. There was a homogeneity of variances as assessed by Levene's test for equality of variances ($p = 0.59$). However, the TD scores for both vaccine and placebo groups were not normally distributed with a Shapiro-Wilk's value of $p < 0.05$. TD scores between placebos ($M = 2.61$, $SD = 1.90$) and vaccinees ($M = 2.59$, $SD = 1.90$) who met the ALL-ETEC TD endpoint definition did not show a statistically significant difference, $M = -0.19$, 95% CI [-0.57, 0.53], $t(185) = -0.07$, $p = 0.95$ (Table 40).

Table 38

Distribution of TD Disease Score and Subjects Meeting ALL-ETEC TD Endpoint by Treatment Group (OEV-118 Dataset; N = 187)

TD Score	Treatment Group (*N* = 187)	
	Vaccinees (*n* = 100)	Placebos (*n* = 87)
0	10 (10.0%)	10 (11.5%)
1	17 (17.0%)	18 (20.7%)
2	31 (31.0%)	18 (20.7%)
3	19 (19.0%)	16 (18.4%)
4	9 (9.0%)	13 (14.9%)
5	7 (7.0%)	6 (6.9%)
6	2 (2.0%)	1 (1.1%)
7	2 (2.0%)	3 (3.4%)
8	1 (1.0%)	2 (2.3%)
9	2 (2.0%)	0 (0.0%)

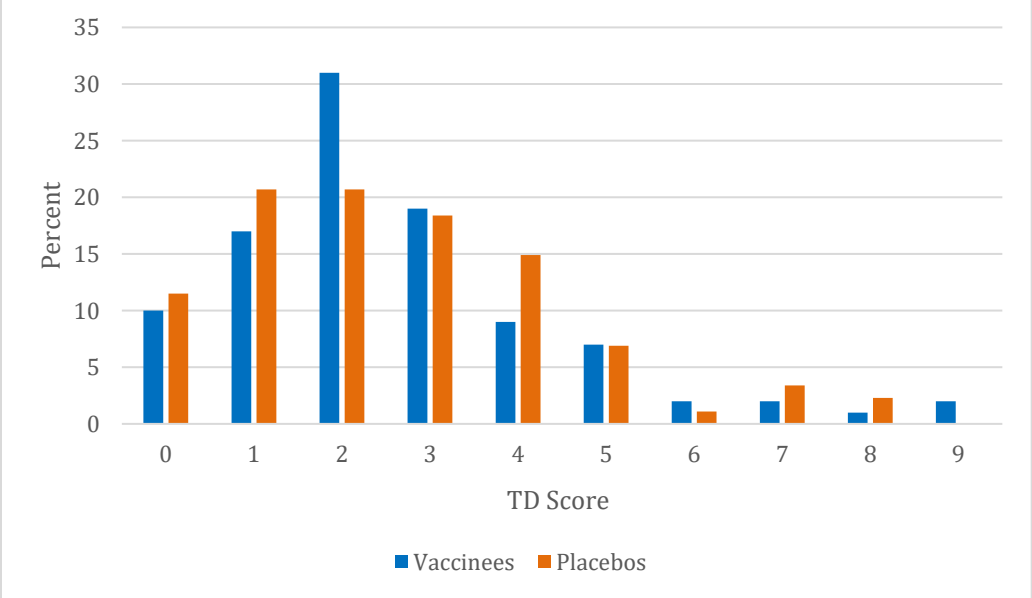

Figure 11. Proportion of TD disease score and subjects meeting ALL-ETEC TD endpoint by treatment group (OEV-118 dataset).

In the ‘ETEC-MX’ subgroup, defined as those who received one or two doses of vaccine, traveled to Mexico or Guatemala, had symptom data available for analysis and had ETEC as well as another enteric pathogen isolated in their stool by culture methods per protocol, there were only 6 vaccinees and 7 placebos who met this endpoint. The proportion of subjects by treatment group across the new TD score is depicted in Table 39 and Figure 12. TD scores for each treatment group were normally distributed, as assessed by Shapiro-Wilk’s test ($p > 0.05$), and there was a homogeneity of variances as assessed by Levene’s test for equality of variances ($p = 0.89$). TD scores for vaccinees ($M = 4.83$, $SD = 1.72$) and placebos ($M = 3.86$, $SD = 2.27$) did not show a statistically significant difference $M = 0.98$, 95% CI [-1.52, 3.48], $t(11) = 0.89$, $p = 0.41$ (Table 40).

Table 39

Distribution of TD Disease Score and Subjects Meeting ETEC-MX Endpoint by Treatment Group (OEV-118 Dataset; N = 13)

TD Score	Treatment Group (*N* = 13)	
	Vaccinees (*n* = 6)	Placebos (*n* = 7)
0	0 (0.0%)	0 (0.0%)
1	0 (0.0%)	1 (14.3%)
2	0 (0.0%)	1 (14.3%)
3	2 (33.3%)	1 (14.3%)
4	1 (16.7%)	2 (28.6%)
5	0 (0.0%)	1 (14.3%)
6	2 (33.3%)	0 (0.0%)
7	1 (16.7%)	0 (0.0%)
8	0 (0.0%)	1 (14.3%)
9	0 (0.0%)	0 (0.0%)

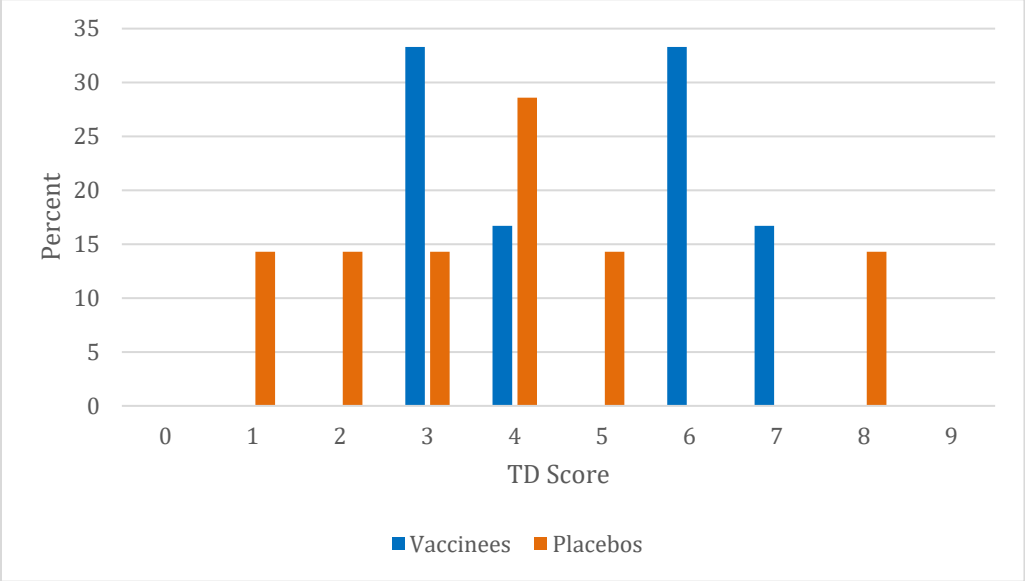

Figure 12. Proportion of TD disease score and subjects meeting ETEC-MX endpoint by treatment group (OEV-118 dataset).

As the assumption of normality was violated for some of the above-analyzed OEV-118 population subsets, I performed a Mann-Whitney U test for each subset as the nonparametric alternative to the independent samples t-test. Consistent with the results of the independent t-test, the Mann-Whitney U test revealed similar results for each group and are summarized in Table 40. For the entire analysis dataset (N = 1435), the median TD score for vaccinees (1.00) and placebos (1.00) was not statistically significantly different, U = 262,140, z = 0.61, p = 0.54. For the TD endpoint population (n = 412), the median TD score for vaccinees (3.00) and placebos (3.00) was not statistically significantly different, U = 20,010.5, z = -1.01, p = 0.31 (Table 38). For the VPO-ETEC TD subset (n = 13), the median TD score for vaccinees (4.00) and placebos (4.00) was not statistically significantly different, U = 25.5, z = 0.846, p = 0.44 (Table 39). For the ETEC TD subset (n = 28), the median TD score for vaccinees (3.29) and placebos (4.60) was statistically significantly different, U = 139, z = 2.06, p = 0.05; the only statistically significant comparison seen amongst all analysis subsets. For the ALL-ETEC TD subset (n=187), the median TD score for vaccinees (1.00) and placebos (2.00) was not statistically significantly different, U = 4298.5, z = -0.142, p = 0.87; and for those 13 subjects who met the ETEC-MX definition, the median TD score for vaccinees (5.00) and placebos (4.00) was not statistically significantly different, U = 27, z = 0.87, p = 0.45 (Table 40).

Table 40

Comparison of 3-Component TD Disease Complex Score in Placebo and Vaccinated Subjects (OEV-118 dataset)

Endpoint Population	Group	*N*	Independent t-test		Mann-Whitney U Test	
			Mean (std) Score	2 sided *p*-value	Median Score	2 sided *p*-value
ALL	Placebo	713	1.82	---	1.00	---
	Vaccine	722	1.73	0.33	1.00	0.54
TD	Placebo	200	3.77±1.93	---	3.00	---
	Vaccine	212	3.60±1.86	0.36	3.00	0.31
ALL-ETEC TD	Placebo	87	2.61±1.90	---	2.00	---
	Vaccine	100	2.59±1.90	0.95	1.00	0.89
ETEC TD	Placebo	19	4.74±1.85)	---	4.60	---
	Vaccine	10	3.40±1.17)	0.05	3.29	0.05
VPO-ETEC-TD	Placebo	5	4.80±1.64	---	4.00	---
	Vaccine	8	4.13±1.25	0.42	4.00	0.44
ETEC MX	Placebo	7	3.86±2.67	---	4.00	---
	Vaccine	6	4.83±1.72	0.41	5.00	0.45

Percent reduction of disease severity (PE) was calculated for the entire dataset as well as each OEV-118 endpoint subgroup. The results are summarized in Table 41. When the TD disease complex score was utilized and applied to the entire dataset, a PE of 9 was observed. When the score was applied to those who met the classic TD definition, a PE of 4.5 was observed. When the new score was applied to the per-protocol VPO-ETEC TD primary analysis subset, a PE of 14 was observed; a major improvement over the original point estimate of efficacy of -59. PE calculations for the ETEC-TD, ALL-ETEC TD, and ETEC-MX subgroups were 28.3, 0.7 and -25.1, respectively (Table 41). Excluding the ETEC-TD subanalysis, there were no statistically significant differences in TD score between treatment groups in the various subpopulation analyses (i.e., ALL, TD, ALL-ETEC TD, VPO-ETEC-TD, and ETEC MX). However, the TD score better predicted reduction in overall clinical disease severity in vaccine versus placebo recipients compared to previous PE estimates, especially as it pertained to the primary VPO endpoint (VPO-ETEC TD). For this reason, as well as others provided in this section, I reject the null hypothesis that a TD disease score does not better differentiate treatment groups than prior estimates of vaccine efficacy when applied to a previously conducted ETEC vaccine study (OEV-118).

Table 41

TD Score Impact on Severity (PE) Estimates for Various Population Subsets in OEV-118

Endpoint	Abbreviation	N	Definition	Rationale for Inclusion	TD Score PE Estimate
All	ALL	1435	Subjects enrolled in the OEV-118 study who received one or two doses of vaccine, traveled to Mexico or Guatemala and had symptom data available for analysis	Full database analysis	9
Travelers' Diarrhea (TD)	TD	412	≥3 loose or watery stools in a 24-hour period accompanied by ≥ 1 accompanying gastrointestinal (GI) symptom	Classic TD Endpoint	4.5
All ETEC	ALL-ETEC	188	ETEC as sole pathogen isolated in any subject who received one or two doses of vaccine, traveled to Mexico or Guatemala, and had symptom data available for analysis	To determine percent reduction in disease severity against infection with any ETEC	0.8
ETEC TD	ETEC TD	29	≥3 loose or watery stools in a 24-hour period accompanied by abdominal pain or cramps, nausea, or vomiting of any intensity, plus ETEC sharing antigens[1] with the vaccine as sole pathogen isolated	Recommended by OEV-118 Data Safety Monitoring Board*	28.3
Vaccine Preventable ETEC TD	VPO-ETEC-TD	13	≥5 loose/watery stools in 24 hours plus ≥1 of abdominal pain/cramps, nausea and/or vomiting, plus ETEC sharing antigens[1] with the vaccine as sole pathogen and isolated in window of 24 hours before to 72 hours after illness onset among subjects completing 2-dose regimen, traveling during window of 7 to 14 days post 2nd dose and completing 14-28 days surveillance.	OEV-118 Study Endpoint	14
ETEC with Mixed Infections	ETEC-MX	14	ETEC along with another pathogen(s) isolated by culture in any subject who received one or two doses of vaccine, traveled to Mexico or Guatemala and had symptom data available for analysis	To determine percent reduction in disease severity against infection with any ETEC plus one or more enteric pathogens	-25.1

Note. [1] LT, LTST, CFA/I, CS1, CS2, CS3, CS4 or CS5

Note. *AL Bourgeois, personal communication

Summary of Results

The above results answer the quantitative research questions and objectives outlined in Chapter 3. Three hypotheses were tested by the secondary quantitative analysis of two clinical trial datasets. Descriptive statistics, ordinal logistic regression, univariate linear regression, multinomial logistic regression, Spearman's correlation coefficients, multiple correspondence analysis, parametric *t* tests and non-parametric Mann-Whitney U tests were used for data analysis. The findings presented in this chapter are discussed in-depth in Chapter 5.

Chapter 5: Summary, Conclusions, and Recommendations

Chapter 5 is a summary of the study and findings, recommendations for actions, implications for social change, and a concluding statement. After reading this chapter, the reader will have a clear understanding of how this research may impact future TD research and areas of additional potential research.

Overview

Primary and secondary outcomes for TD interventions have been predominately limited to loose stool output. However, reliance on stool-based endpoints alone may minimize meaningful differences in illness profiles. In addition to obtaining further information on how TD symptomology correlate with stool output metrics, as well as determining to what extent TD signs and symptoms negatively impact activity, it would be advantageous to have a single standardized and validated disease severity score (Porter et al., 2018). I designed the quantitative secondary data analysis presented here to describe the distribution of TD signs and symptoms, determine their association with impact on activity, develop a TD severity score, and determine whether the TD severity score could be utilized to differentiate illness by treatment arms in an interventional study. It was also performed to inform ongoing and future TD vaccine and therapeutic trials.

I utilized two clinical trial datasets. TrEAT TD data were used to analyze the correlation between TD signs and symptoms and stool output, determine which of the clinical signs and symptoms most negatively impacted activity, and develop a TD score. The OEV-118 dataset was used to validate the developed TD score by applying it to a previously conducted vaccine trial to re-estimate reduction in illness among vaccine and

placebo recipients. Specifically, I used the TrEAT TD dataset to answer two research questions related to the testing of two hypotheses (Steps 1 and 2). I also performed a multiple correspondence analysis to facilitate TD score development (Step 3). This disease complex score was then used to assess whether the disease score could be utilized to predict reduction in activity level beyond individual signs and symptoms (Step 4). The final research step was conducting an analysis utilizing the OEV-118 dataset in order to answer the third research question and test one hypothesis (Step 5). The five research steps and the three research questions/objectives and hypotheses are summarized below:

- Step 1: Research Question 1 and Hypothesis.
    - Research Question 1: What combination of clinical signs and symptoms best characterizes TD severity in adult travelers?
        - Hypothesis 1. There are significant differences in the frequency and severity of clinical signs and symptoms of TD impacting on disease severity classification.
- Step 2: Research Question 2 and Hypothesis
    - Research Question 2: What individual clinical signs and symptoms of TD are significantly associated with a negative impact on activity among adult travelers?
        - Hypothesis 2. Individual clinical signs and symptoms of TD are significantly associated with a negative impact on activity.
- Step 3: Development of the TD disease complex score
- Step 4: Re-assessment of impact on activity utilizing the TD disease complex score

- Step 5: Research Question 3 and Hypothesis
    - Research Question 3: Does a TD disease score better differentiate treatment groups than more traditional estimates of vaccine efficacy when applied to a previously conducted ETEC vaccine study (Protocol OEV-118)?
        - Hypothesis 3. The estimated vaccine efficacy of the ETVAX inactivated whole cell vaccine tested in the OEV-118 Phase 3 trial does change as a result of using the new disease complex score.

Summary of Findings

In the following section I discuss the quantitative results presented in Chapter 4. Before conducting analyses to answer each research question, I performed descriptive statistical analyses using the TrEAT TD secondary dataset in order to determine how the major data features sorted and determined if key assumptions were met. These descriptive statistics and precursor analyses are also discussed below.

Descriptive Statistics and Precursor Analyses

Demographic characteristics and symptoms experienced by participants enrolled in the TrEAT TD and OEV-118 datasets. While partially published elsewhere (Riddle et al., 2017) and as presented in Chapter 4, collated demographic characteristics of the participants enrolled in the two secondary datasets used for this study show male gender dominance in the TrEAT TD dataset, with a more equal gender distribution in the OEV-118 dataset (Table 4). Despite the higher proportion of men enrolled in the TrEAT TD study, the gender distribution and overall demographic characteristics of that study population is reflective of deployed military personnel (Hameed et al., 2016; Porter et al.,

2015; Trivedi et al., 2011). The heavy skew toward males may be important as the disease severity score was developed based on the TrEAT TD dataset. However, the new score was applied using a population more generalizable to the adult travel population, thereby lending more credibility to its potential application to both target traveler groups. The role of gender in TD risk is unclear. While some studies have shown no association between gender and TD risk (Diemert, 2006; Evans, Shickle & Morgan, 2001; Steffen, 2017; Steffen et al., 2004), others have reported an apparent confounding effect of gender on acquiring illness (Schlagenhauf et al., 2010; Vilkman et al., 2016). Meanwhile, the OEV-118 study gender distribution among classic TD cases was more consistent with what has been observed recently in the published literature. It should also be noted that CHIMs studies with the B7A and H10407 strains of ETEC have identified some gender differences in illness profiles, with females complaining of gastrointestinal disturbances such as nausea and abdominal cramps ($p = 0.026$, $p = 0.034$, respectively) more often than males (Coster et al., 2007). In contrast, the incidence of fever for males was significantly higher for males than females ($p = 0.04$; Coster et al., 2007). After further stratifying the symptomology for OEV-118 by gender, females experienced all symptoms at a greater frequency than males across all severity levels (data not shown), with the most striking gender differences between genders in reporting of abdominal cramps, nausea, and vomiting. There was also a statistically significant difference in mean ($p = 0.03$) and median ($p = 0.003$) TD score between males and females when analyzed via independent *t*-test and Mann-Whitney U test, respectively, with females exhibiting a propensity to more severe disease.

Age has long been established as an important risk factor for diarrheal disease, with highest incidence rates among children under the age of five in developing countries and younger adults traveling from industrialized nations to high-endemic areas (approximately 1.6 illnesses/traveler; DuPont & DuPont, 2006; Fischer Walker, Perin, Aryee, Boschi-Pinto, & Black, 2012; Hill, 2000; Steffen, 2005; Steffen et al., 2004; WHO, 2017). The age distribution of classic TD cases in the OEV-118 dataset is consistent with what has been see in published literature, with highest rates in the youngest age cohort (18-25 years; 40%), followed by a 27% burden in 26-35-year-old travelers (Table 9). TD burden within the OEV-118 study decreased as age increased, with lowest frequency in the oldest group and relatively equal distribution between vaccine and placebo recipients (Table 9), perhaps attributable to what has been seen before with younger travelers' proclivity for more adventure travel (Kollaritsch, 1989; Steffen 2005; Steffen et al., 2015) and lack of vigilance in avoiding high risk foods (Diemert, 2006; Hoge et al., 1996; Pitzurra et al., 2010). Meanwhile, the age distribution of TD cases in the TrEAT TD dataset is consistent with what has been previously seen in traditional travelers' studies, for which younger age cohorts tend to have a greater risk of acquiring TD. However, published studies in the military subpopulation have shown that the direction of age effect is inverse to that of other traveler cohorts, in that risk of TD increases with increased age (Riddle et al., 2006; Sanders et al., 2004). Although the age distribution across TD cases in the TrEAT TD contrasts with prior studies in this population, because I developed the disease complex score using this dataset with the intention to apply it across all traveler populations, it seems apropos that the age distribution of cases more align to the more traditional traveler subgroup.

The most commonly reported gastrointestinal symptom was abdominal cramps, reported in approximately 75% of enrolled participants in TrEAT TD (Table 6) and 62% of participants in OEV-118. It should be noted (as outlined in Chapter 3) that in OEV-118 "abdominal pain" and "cramps" were captured as two separate and distinct TD symptoms, and only abdominal cramps were considered. Abdominal cramps are the most consistently and commonly reported gastrointestinal symptom in TD studies (Bourgeois et al., 2011; Riddle et al., 2011; Sack et al., 2007; Stoney et al., 2017), followed closely by nausea, vomiting, and fever (Putnam et al., 2006; Riddle et al., 2011). These data are similar to the signs and symptoms in these secondary datasets. Reported symptomology within both datasets reflected similar trends to those observed in previous TD studies, especially with regards to nausea, vomiting, and fever. Malaise, tenesmus, gas, and fecal incontinence have not been as consistently reported across TD studies, yet approximately 64%, 29%, 39% and 14% of participants in TrEAT TD reported experiencing those symptoms, respectively. Malaise was reported in OEV-118 participants at a similar frequency, with higher reporting of fecal incontinence and gas compared to TrEAT TD and published literature. While tenesmus of varying severity was reported in 29% of participants in the TrEAT TD study, it was only reported in 4.2% of OEV-118 study participants. Tenesmus is a common symptom of infectious gastroenteritis often associated with infection from *Shigella* sp., *Salmonella* sp., and *E.coli* (Adachi et al., 2001; Jensen et al., 2014; McGregor & Wright, 2015). As the burden of ETEC and EAEC infections in the TrEAT TD dataset was relatively high—24.6% and 38.6% isolated as the sole pathogen in 114 subjects, respectively (Riddle et al., 2017)—perhaps it is unsurprising that with the higher proportion of infections from these etiologies comes

a higher reporting of tenesmus as a significant symptom. Meanwhile, in the OEV-118 study, the burden of ETEC infections was not as high (13.1%), with no isolation of EAEC, which might have resulted in a lower reporting of tenesmus as a significant TD symptom.

While impact on activity varies across studies and subpopulations, both TrEAT TD and OEV-118 highlight the negative effects of TD on activity. As shown in Table 10, 6% of participants in the TrEAT TD dataset were completely unable to function as a result of their illness. This is a much lower proportion of inconvenience attributed to severe TD than what was seen by Soonawala, Vlot & Visser (2011), but slightly higher than studies in which 1% of participants required hospitalization (Kollaritsch, 1989; Peltola & Gorbach, 1997; Steffen et al., 2015). Nevertheless, 78.5% of participants within the TrEAT TD dataset reported some degree of negative effect on activity, a comparable proportion to what has been seen in some studies (Tribble et al., 2007; Sanders et al., 2007; Sanders et al., 2002) and even higher than what has been presented in other published literature (Soonawala et al., 2011; Ryan & Kain, 2000; Diemert, 2006; Hill, 2000; Sebeny et al., 2012; Steffen et al., 2004; Steffen et al., 2015). This negative impact on activity is especially important for a military population, since it could result in loss of duty days and reduced operational readiness. Also, consistent with previous studies (Lalani et al., 2015; Olson, Hall, Riddle & Porter, 2019; Soonwala et al., 2011; Steffen et al., 2015), approximately 33% of participants in OEV-118 reported a need to change their activity due to illness. For the business or leisure traveler, the impact of TD has potential large implications for business and tourism industries, resulting in financial loss and increased economic burden (Steffen, 2017; Wang et al., 2008).

Quantitative Hypothesis Testing Analysis

Hypothesis 1: There are significant differences in the frequency and severity of clinical signs and symptoms of TD on disease severity classification. As noted in Table 11, there were positive significant correlations between various clinical signs and symptoms of TD and an individual's disease severity classification. Malaise was positively correlated with all signs and symptoms, perhaps unsurprising given its very definition of "a general feeling of discomfort, illness or uneasiness whose exact cause is difficult to identify" (Oxford University Press, 2019). Abdominal cramps were similarly significantly and positively correlated with all other signs and symptoms as has been previously reported (Bourgeois et al., 2011; DuPont & DuPont, 2006; Fischer Walker, Perin, Aryee, Boschi-Pinto, & Black, 2012; Hill, 2000; Riddle et al., 2011; Sack et al., 2007; Stoney et al., 2017; Steffen, 2005; Steffen, et al., 2004). Gas was only correlated with malaise and was excluded as a parameter from the TD disease complex score.

Correlation between signs and symptoms and frequency of loose stool. Each sign and symptom (except gas and tenesmus) was significantly associated with the maximum 24-hour stool output as measured by frequency (Table 12). The lack of significant association between gas and stool frequency was consistent with its negligible effect on activity (see Hypothesis 2 analyses). Gas was subsequently excluded as a parameter in the TD disease complex score.

In contrast, while tenesmus was prevalent in TrEAT TD and significantly associated with a negative impact on activity (see Hypothesis 2 analyses) it was not significantly associated with stool output, perhaps unsurprising given its clinical definition. Because of the prevalence of tenesmus in the TrEAT TD its significant

association with subject activity, and its significant clustering with other more severe symptoms in the MCA, tenesmus was shown to be an important clinical parameter to include in the overall disease complex score. Furthermore, despite not being significantly associated with stool output but shown in correlation, various regression and multiple correspondence analyses to be a meaningful clinical TD parameter tenesmus is an excellent example of how other symptoms, independent of stool frequency, might play an important role in TD severity.

Meanwhile, all other signs and symptoms were significantly correlated with stool frequency. These primary analyses, as presented in Chapter 4, include all TrEAT TD subjects who met the sampling frame for this research, including 'outlier' subjects (maximum 24-hour stool frequency >3 standard deviations from the mean), as it was considered important to still include these participants since such people would exist in a real-world scenario setting. Nevertheless, when these outliers were removed and the analysis re-run (data not shown), there was a significant association between tenesmus and maximum 24-hour stool frequency, while gas remained not significant.

Hypothesis 2: Individual clinical signs and symptoms of TD are significantly associated with a negative impact on activity. All signs and symptoms (excluding gas) were significantly associated with a negative impact on activity. Generally, as the severity of the sign or symptom increased so did the negative impact on activity; however, there were a few interesting exceptions. Moderate tenesmus was associated with a higher likelihood of reporting a negative impact on activity compared to mild or severe tenesmus, which were not significantly associated with impact on activity. Similarly, experiencing moderate fever (temperature 101.2°F – 102.0°F) resulted in a

higher likelihood of reporting a negative impact on activity compared to experiencing either mild or severe fever; however, all fevers were statistically significant. Meanwhile, experiencing mild vomiting (≥1 episode in 24-hours) resulted in a slightly higher likelihood of reporting a negative impact on activity (OR = 5.42) compared to experiencing moderate vomiting (≥2 episodes in 24-hours; OR = 5.34), although the confidence intervals overlap. As expected, those experiencing severe vomiting (≥3 episodes in 24-hours) were times more likely to report a negative impact on activity compared to mild or moderate vomiting. However, mild vomiting resulted in a slightly higher likelihood of reporting a negative impact on activity (OR= 5.42) compared to moderate vomiting (OR = 5.34), both highly significant. While the grading scale for vomiting classifies severity as mild, moderate and severe, the actual numerical difference between the severity levels is a factor of 1, which may not manifest as two distinct clinical pictures when experiencing only one or two episodes of vomiting. This may be the reason behind mild and moderate vomiting having similar likelihoods of reporting a negative impact on activity compared to severe vomiting. Nevertheless, this definition has been the standard definition for all TD studies conducted to date and is even consistent with the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (US DHHS, FDA CBER, 2007), a commonly used grading scale in many clinical trials. Similar to the results for vomiting, those experiencing moderate fecal incontinence were less likely to report a negative impact on activity (OR = 2.62) than those who experienced mild fecal incontinence (OR = 3.53), although both were statistically significant and with overlapping confidence intervals. Regardless, severe fecal incontinence increased the

odds of a participant reporting a negative impact on activity, a trend seen with every other analyzed sign and symptom graded as ‘severe’ (except fever and tenesmus).

Another interesting observation is that the likelihood of reporting a negative impact on activity among those experiencing severe nausea was 37 times that of those experiencing no nausea; however, severe vomiting only yielded an odds ratio of 9. One interpretation could be that nausea is more detrimental to one’s ability to function than actually vomiting. This may be because vomiting potentially alleviates the feeling of being nauseous and enables one to resume activity.

Optimal cut points for stooling frequency over a 24-hour period were established and assigned to an ordinal scale for inclusion into the TD disease complex score. Via a CART analysis, these categories are as follows: None = 0-1 loose stool/24 hours; Mild = 2-4 stools/24 hours; moderate = 5-7 loose stools/24 hours; severe = ≥8 loose stools/24 hours. As expected, an increase in stooling frequency was significantly associated with a greater likelihood to report a negative impact on activity. As stooling frequency is the single most consistent TD disease parameter utilized, these results confirm its importance; however, based on the results described above, its use as the sole parameter to define TD illness is likely sub-optimal.

As this analysis was conducted using data from a predominantly active-duty military population, with some beneficiaries, it is important to consider that there may be risk of reporting bias by participants underemphasizing the effect their TD illness had on their ability to function. As Mary Roach stated in her 2016 *New York Times* magazine article describing the TrEAT TD study, “For every person who shows up at the morning sick call, four tough it out.” As a result, it should be considered that such a population

might be ‘different’ than the normal population and thus their symptom profile may not be generalizable to a broader traveler population. While reporting bias may be considered a potential limitation, previous studies with military participants report a moderate rate of care-seeking behavior for their TD (Olson et al., 2019; Putnam et al., 2006). Furthermore, a recent review revealed an increase in care-seeking behavior for the treatment of TD illness by affected individuals in both military and long-term travel populations, up from 16% in studies published between 1996-2005 to 38% in studies published between 2006-2015 (Olson et al., 2019).

Association of TD Disease Severity Sore and impact on activity. There was a statistically significant and consistently increasing trend between higher TD disease severity scores and likelihood of reporting negative functional impairment, with a slight exception between those with a TD score of 6 versus 7, in which the latter resulted in a slightly lower odds ratio compared to the former (OR = 167.54, and OR = 126.00, respectively), but with greatly overlapping confidence intervals (Table 33). As presented in Chapter 4, those who reported a TD score of 9 were 1423 times more likely to experience functional impairment compared to those who scored a 1, an exponentially larger likelihood than observed for any individual signs or symptom. In fact, a TD score of ≥5 resulted in odds ratios that were in the hundreds or thousands, compared to a TD score < 5 that yielded comparable odds ratios to some individual signs and symptoms. This further suggests that the TD disease severity score might be a more useful tool for a more refined assessment on functional impact, especially for more severe disease.

These data begin to address a relevant research topic in the field of travel medicine (Riddle et al., 2017). Assessment of whether or not the most commonly

solicited signs and symptoms of TD actually contribute to a negative impact on activity facilitates understanding of the TD as a complex syndrome, and assists with evolving the field away from stool frequency-based definitions (Riddle et al., 2017). These results support the current guidelines for the prevention and treatment of TD. Specifically, all signs and symptoms should be classified by an individual's assessment that his/her illness is tolerable, distressing, or incapacitating (Riddle et al., 2017). Furthermore, as the TD disease severity score is a predictor of impact on activity, its application to the field is aligned with the new recommendations to adopt definitions based on functional impact.

Hypothesis 3: The estimated vaccine efficacy of the ETVAX inactivated whole cell vaccine tested in the OEV-118 Phase 3 trial does change as a result of using the new disease complex score. There was no significant difference ($p = 0.42$) in the mean or median ($p = 0.44$) disease scores between vaccine and placebo groups meeting the original per-protocol primary endpoint definition (VPO ETEC TD). This is consistent with the per-protocol analysis demonstrating no significant efficacy of the vaccine in the population (PE = -59, - 95% *CI* -384 – 48) (Bourgeois et al., 2011). There was a 14% reduction in overall TD disease severity among vaccinees, a finding not observed in the per protocol analysis, suggesting that the vaccine might have had reduced overall clinical illness.

As described in Chapter 3 dosing was not directly observed for the vast majority of study participants, making it difficult to assess whether all doses were actually taken as intended (SBL Vaccin AB, 2003). In reviewing the initial PE results, the Data Safety Monitoring Board (DSMB) recommended exploration of other endpoint definitions in a set of *post-hoc* secondary analyses, including those that were limited to potential ETEC

cases (Bourgeois, personal communication), one of which (ETEC TD) is included in this dissertation analysis. When the analysis was limited to ETEC TD (considered in *post-hoc* analysis and recommended by the OEV-118 DSMB), the disease score indicated a more readily apparent vaccine effect, compared to the *a priori* analysis. For the ETEC TD subgroup, there was a 28% reduction ($p = 0.05$) in the mean disease scores between vaccine (3.40) and placebo (4.74) recipients. This finding is especially important as the ETEC-TD endpoint definition is focused on a more realistic scenario for which this vaccine candidate would be considered beneficial. The VPO-definition may have been unnecessarily limiting to more severe disease and a tighter window around ETEC isolation. In contrast, ETEC-TD included milder TD cases and an expanded window for ETEC isolation. Thus, the fact that the vaccine demonstrated an approximately 30% reduction in disease severity compared to a 14% reduction in disease severity (VPO endpoint) exhibits the potential utility of the TD score, but also the efficacy of the vaccine.

When percent reduction of disease severity (PE) was calculated for the entire OEV-118 dataset, there was a 9% reduction in TD disease severity in vaccine recipients compared to placebo recipients, an important finding given the vaccine was intended only for circulating ETEC strains that matched the vaccine and not all participants became ill. When the analysis was limited to the subgroup meeting the classic TD definition, there was no statistically significant difference ($p = 0.36$) in the mean disease scores between vaccine (3.60) and placebo (3.77) groups, nor the median disease scores (3.00, $p = 0.31$). However, when PE was re-calculated using the new TD score, there was a 4.5% reduction in disease severity among vaccinees compared to placebos, offering a first look

into how the vaccine fared against all-cause TD illness. While perhaps not unexpected that the vaccine did not perform as well for all-cause TD as it would for VPO-ETEC-TD, as similar observations were noted with the LT-patch against all-cause diarrhea (defined as ≥4 unformed stools in 24 hours), the ETVAX vaccine performed better in reducing overall TD illness than the LT-patch technology did in protecting against all-cause TD (PE = -12.3, 95% CI -40 to 10) (Behrens et al., 2014). While Behren's et al. (2014) noted significant reductions in duration of all-cause diarrheal episodes and frequency of unformed stools per all-cause diarrheal episodes, those secondary endpoints still represent the more traditional outcome measures with no consideration for the TD illness spectrum for which the LT-patch may have benefited.

For the ALL-ETEC TD subgroup, another definition of interest in seeing how the vaccine differentiated TD illness regardless of whether or not the vaccine matched antigens isolated from the field, there was no significant difference in the mean ($p = 0.95$) or median ($p = 0.89$) disease scores between vaccine and placebo recipients; and there was only an 0.8% reduction in TD disease severity between the two treatment arms. While it is recognized that there were a significant number of ETEC infections that were ST-only or ST-CS6 strains ($n = 101$), two antigens not covered by the vaccine, it is perhaps unsurprising that the vaccine did not reduce disease severity in this particular subpopulation. Behrens et al (2014) observed similar results in their Phase 3 evaluation of the LT-patch, which did not confer significant protection against strains expressing ST.

Finally, for the ETEC-MX subgroup for which ETEC along with other pathogens (e.g., *Salmonella*, *Shigella*, *Campylobacter*, Giardia, etc.) were isolated during the TD episode, there was no statistically significant difference in the mean ($p = 0.41$) or median

(p = 0.45) TD scores. Furthermore, there was a 25% increase in disease severity among vaccinees with mixed ETEC infections compared to placebos.

Despite minimal or insignificant differences in the disease scores between vaccine and placebo recipients, there was a trend towards lower TD scores in most analyses. Utilization of the TD score allowed for consideration of important clinical parameters (i.e., tenesmus, malaise, fever), not previously considered, enabling further differentiation of illness between treatment groups. For example, while the ETEC-TD subgroup included subjects with ≥3 loose/watery stools in 24 hours with abdominal pain/cramps, nausea or vomiting of any intensity and ETEC as the sole pathogen isolated, TD scores were based on inclusion of other symptoms not otherwise specified in the ETEC-TD definition. As a result, it appeared that the vaccine had a significant impact on reducing overall illness. Furthermore, and perhaps most importantly, re-estimating protective efficacy using the TD score demonstrated a reduction in overall TD illness in vaccine recipients compared to placebo recipients, indicating some vaccine effect against various categories of ETEC TD illness. These results suggest that utilization of a TD disease severity score could prove a more useful tool for assessing an intervention's efficacy compared to reliance on a more narrowly focused outcome.

Recommendations for Action

Results obtained from Hypothesis 1 and 2 testing and the MCA analysis led to the development of a disease severity score for TD to characterize TD severity. The new scoring system was identified using symptom data from a recently completed TD study conducted in adults ≥18 years of age deployed to four high-risk countries. An initial attempt to externally validate the score on a different traveler population was performed

using an existing clinical trial dataset evaluating the field efficacy of an inactivated whole cell ETEC vaccine. Before this new TD disease severity score is routinely used, it should be assessed in other clinical trials to determine if a similar utility is achieved.

Although the TD Score yielded only a single statistically significant difference in scores between treatment groups in OEV-118, there was a benefit to re-estimation of vaccine efficacy using the entire dataset as well as alternate considered definitions. While this is only one attempt at external validation, there is historic evidence that disease severity scores have been useful for application in pediatric studies (Arifeen et al., 2013; Jauregui et al., 2014; Lee et al., 2016; Levine et al., 2015; Kinlin & Freedman, 2012; Pringle et al., 2011; Tam et al., 2014) and more recently there is accumulating evidence that disease severity scores are proving valuable in CHIM studies (Porter et al., 2016; Porter et al., 2018). Moreover, disease severity scores have a demonstrated value in application to in-depth analysis of immune response profiles and identification of potential correlates of protection (Clarkson, 2018; Porter et al., 2018). It is hoped that this TD disease score could also be applied in a number of ways beyond just estimating impact on activity, as scores for other syndromes have demonstrated broad utility beyond just illness characterization. For example, the Rome Criteria for the diagnosis of IBS were originally established to guide researchers, but have undergone several revisions with the intent of making them clinically useful and relevant for both patients and health care providers. The most recent revision included further classification of IBS subtypes, which now helps focus treatment plans for patients in addition to focusing clinical trials for IBS in collecting more relevant information to more accurately categorize IBS subtypes (Drossman, 2016; Lacy & Patel, 2017). Another example includes the coronary

artery calcium score, which not only plays an important role in showing significant association with occurrence of major cardiovascular events, but has also been shown to demonstrate utility in further stratifying coronary risk in various populations (e.g., asymptomatic or diabetic patients) (Neves, Andrade & Moncao, 2017) thus informing more accurate treatment options (American College of Cardiology, 2018).

As has been acknowledged in the development of scoring systems for ETEC and *Shigella* (Porter et al., 2016; Porter et al., 2018), a potential solution to mitigate unequal distribution of stool output as a measure of disease severity is to establish new stool frequency/volume cut points based on existing data. Such analyses were performed here. Interestingly, when these new stool frequency classifications were included in the MCA analysis, the two lowest categories of stool frequency (0-1 loose stools/24 hours and 2-4 loose stools/24 hours) clustered with the lack of any objective signs and symptoms. Meanwhile, the highest loose stool category (≥8 loose stools/24 hours) appeared most proximal to mild and moderate symptoms. This not only suggests that stool frequency alone may not be as useful as a predictive measure of more serious disease, but also lends support that symptoms outside of stooling frequency contribute as much, if not more, to a more severe illness profile. While this score proposes new stool frequency cut points for the TD score, it was on a single dataset and might benefit from further study to see if these cut points are consistent.

If future trials are to utilize this proposed TD scoring system, there will be inherent caveats. Given the relatively small community conducting TD studies, some consistency in endpoints has occurred; which have been further reinforced by national regulatory authorities such as FDA through the provision of Guidances for Industry such

as toxicity grading scales for use in clinical trials (FDA, 2007). Nevertheless, variations exist in that some studies have related severity classifications to interference with daily activities (Bourgeois et al., 2007; Sack et al., 2007), while some have only used stool-based endpoints with one or more individual symptoms and no consideration of functional impact in the primary outcome assessment (Behrens et al., 2014; Steffen et al., 2013). It is recognized that the field would benefit from more standardization of definitions of TD disease signs symptoms, with official recommendations to utilize definitions based on functional impact (Riddle et al., 2017). As the scoring system has been shown to better predict the likelihood of reporting a negative impact on activity than any individual sign or symptom, its utility in interventional studies warrants further exploration (Riddle et al., 2017).

Public health professionals, enteric disease experts, clinical trial scientists, and policy makers need to be aware of these results and the recommendations resulting from this research. As these three audiences are reached through different media, I recommend disseminating results via peer-reviewed journal publications to target enteric disease, public health and clinical trial experts; while policy makers are likely best reached through key public health conferences.

Study Limitations

As referenced in Chapter 1, while the sample size of the dataset on which the TD score was developed was relatively large and covered four high-risk countries, it was based on self-reported data from a military population, thus potentially limiting its generalizability to a civilian travel population. Although the clinical signs and symptoms included in the scoring system are common and expected solicited symptoms consistent

with the published literature, there is recognition that content validity of the proposed score might be reduced if there are other relevant symptoms that were not included. Finally, host-specific factors such as baseline stooling habits, microbiome and diet were not included in these analyses which may impact disease outcome measures, and it is acknowledged that such factors may play an important role in both stool output and non-stool related outcomes not currently captured in the current scoring system.

Recommendations for Further Research

Despite an effort in this research to validate the new TD disease complex score on another dataset, I recommend focusing future research on application of the TD score to additional clinical trial and epidemiologic settings. While it has been proposed that use of a standardized TD scoring system is ideal, the optimized disease score as a result of this research requires additional use prior to relying solely on it for identification of TD gastroenteritis in a study setting. As it was developed using a single database from one study, it would be important to validate its accuracy against other travel populations in different study settings to increase its generalizability.

As it pertains to the OEV-118 study, specifically, further analysis on stratified data could yield other important insights as to the utility of the proposed TD severity score to characterize disease and re-estimating vaccine efficacy for additional subpopulations, including those with more severe disease. Additionally, limiting analysis to those with immunological “take” would be especially interesting and consistent with DSMB recommendations. Finally, as this study collected important health card information regarding impact of symptoms on daily activities and inclination to seek medical advice due to illness, an analysis similar to what was done with TrEAT TD could

be performed to see if the TD score benefits estimation of impact on activity for this travel group.

As the proposed scoring system is intended for use across all-cause travelers' diarrhea, future assessment of the TD score's usefulness in characterizing disease across different etiologies is increasingly necessary. If there is limited utility of this score due to the inherent differences in clinical disease profiles across diarrheal pathogens, then there may benefit to focusing on developing pathogen-specific scoring systems, similar to what has been done for pediatric Rotavirus gastroenteritis, ETEC and *Shigella* CHIMs. As an ETEC CHIM severity score has already been developed and validated, and the proposed TD score has been preliminary applied to an ETEC vaccine study as part of this research, it would be interesting to compare the two different scores and how they might differentiate ETEC disease in a controlled experimental setting versus more realistic field exposure.

Implications for Social Change

Building upon recent efforts to better quantify disease severity in ETEC and Shigella CHIMs as well as extensive prior research to characterize pediatric diarrhea, a similarly developed and validated score for all-cause TD could be applied to future field studies evaluating new preventive interventions for TD. Positive results from such efficacy studies would potentially lead to licensure of a vaccine for use in travelers. Development of a single optimized scoring system provides a better metric to standardize endpoints, thereby appropriately setting the bar for advancement and licensure of TD vaccines and treatments which can reduce morbidity of TD. Reduction in TD disease burden would confer substantial economic benefit at both the country- and individual-

level, as it relates to decreased lost revenue for tourism countries, lowered medical costs associated with overseas medical care or hospitalizations, and reduced health-care costs and productivity losses due to ill-returning travelers. Additionally, this research proves especially relevant at this time as a recent convening of various enteric vaccine intervention stakeholders held a workshop regarding clinical endpoints for efficacy trials during which the attendees agreed that development of disease scoring algorithms, such as the one proposed here, would be important for the field (Porter, Gutierrez & Kotloff, 2019). Finally, if such a scoring system could aid in a more accurate characterization of TD gastroenteritis, result in a more clinically meaningful endpoint, and be implemented for more comparable measurements of intervention efficacy across trials, it could aid funders, policy makers and manufacturers as they attempt to prioritize their valuable resources in vaccine development and implementation efforts.

Conclusions

This quantitative secondary data analysis aimed to determine which combination of clinical signs and symptoms best characterizes TD severity in adult travelers, determine whether there were significant differences in frequency and severity of clinical signs and symptoms on disease severity classification, assess whether those individual symptoms were significantly associated with impact on activity, ascertain whether the new score benefits the estimation of functional impairment over the individual parameters, and determine whether a standardized TD scoring system could differentiate treatment groups when applied to a previously conducted ETEC vaccine study. The item response theory and classic test theory provided the conceptual frameworks for this research as they provide explanations for the various challenges, advantages and

methodologies associated with assessment of self-reported health outcomes and scale development. The literature demonstrated the variability in TD endpoint definitions, a gap in knowledge with existing scoring systems (especially in the context of assessing TD severity), and that the development of a simple and standardized tool for measuring disease outcomes severity could benefit the travel medicine field. Finally, these results support the potential utilization of a single optimized scoring system that may provide the field a better metric for future studies to use a more clinically meaningful endpoint; thereby more appropriately setting the bar for advancement and licensure of TD vaccines and treatments which may ultimately lead to reduced TD-attributable morbidity in travelers.

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Appendix: Ordinal Logistic Regression with Stool Frequency as Continuous Variable

When the ordinal logistic regression analysis was performed utilizing maximum number of loose stools in any 24 hour period prior to presentation as a continuous variable (Table 35), the final model statistically significantly predicted the dependent variable over and above the intercept-only model, $X^2(2) = 46.525$, $p < .0001$ and the independent variable has a statistically significant effect on the prediction of whether activity will be impacted, $X^2(2) = 43.094$, $p < .0001$. An increase in stool number was associated with an increase in the odds of experiencing a more negative impact on activity, with an odds ratio of 1.190 (95% CI, 1.130-1.254), $X^2(1) = 43.094$, $p < .0001$.

Table A1

Ordinal Logistic Regression Analysis of the Relationship between Stool Frequency (Maximum Number of Loose Stools in any 24-hour Period Prior to Presentation – Continuous) and Impact on Activity (TrEAT TD dataset; N = 363)

Variable	B	Standard Error	Adjusted OR	95% CI for OR	*p*-value
Impact on Activity (ref: completely unable to function)					
Normal	-0.252	0.197	0.777	0.528-1.143	0.201
Decreased≤50%	1.940	0.217	6.961	4.553-10.640	<0.0001
Decreased≥50%	4.122	0.329	61.695	32.408-117.450	<0.0001
Maximum number of loose stools in any 24 hour period prior to presentation	0.174	0.027	1.190	1.130-1.254	<0.0001