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Mathematical Modelling of Cholera Immunology

by

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A Dissertation Submitted at the

UNIVERSITY OF VENDA

In Partial Fulfilment of the Requirements for the Degree

of

Master of Science

in

Modelling Health and Environmental Linkages Research Group

Applied Mathematics

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April 2016

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Declaration of Authorship

I, MAPHIRI AZWINDINI DELINAH, declare that this thesis titled, 'Mathematical modelling of Cholera Immunology' is my own work that has never been submitted before for any degree or examination in any other university, where I have quoted from the work of others, the source is always acknowledged.

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Abstract

Cholera is still regarded as an illness that causes morbidity and mortality, more especially in developing countries. Cholera is an acute, diarrhoeal illness caused the bacterium Vibrio Cholerae (V. Cholerae), which is found in marine waters. This disease is acquired mainly by ingesting food or water contaminated by faecal material from patients or carriers. Mathematical models are used to outline how infectious diseases progress, to show the likely outcome of an epidemic and help inform public interventions. Mathematicians have used epidemiological models to study more about the dynamics of cholera. Epidemiology is about patterns in space and also time of the disease. Out of the patterns we may infer the causes of the disease. In addition, the future of the disease can be predicted and hence decisions regarding the need for control measures can be made. The immune response to disease agents should also be considered. The immune system is a collection of mechanisms that protect against infection by identifying and killing the invading pathogens. Mathematicians have ignored the study of cholera immunology. The aim of this study was to understand cholera immunology. We have developed the basic mathematical model of cholera immunology. Secondly we extend the model by partitioning the immune system in general to phagocytes and lymphocytes. We have learned that the decay rate of immune cells in an individual is not good. People must have strong immune system so that they can fight cholera infection. We recommend that future studies should focus on the development of immuno-epidemilogical model and also to include treatment on the model.

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Acknowledgements

I thank God Almighty for granting me this great opportunity to write this work. Praise be to God. I thank Prof J. Crafford and Dr S. Moyo for believing in me and granting me an opportunity to register in the Department of Mathematics and Applied Mathematics. My special thanks to Prof Garira, my supervisor. This work would not have been possible without his guidance, patience and support. Without his outstanding knowledge in mathematical modelling in infectious disease, this work would not have been possible. I owe many thanks to my co-supervisor Dr Musie who helped me with the biological view of disease. I thank him for his support and love. He was also the source of encouragement. I would like to express my sincere gratitude to the postgraduates students in the Department of Mathematics and Applied Mathematics. To mention a few, these are Rendani Netshikweta, Dephney Mathebula, Stanley Mulaudzi and Nqobile Muleya. You were there when I needed you the most during the writing of this work. You were never too busy to assist me whenever I needed you. I thank you all for the support you have given me. I would like to thank all the staff members in the Mathematics and Applied Mathematics Department for their support and encouraging words, in particular Mrs R.M. Mukhodobwane, your words were the source of strength. My mother, brothers and sisters, I thank you for the unwavering support during the time of this study, especially my uncle, Sam Ramatshekgisa. My education would not have been possible without his sacrifices. My kids, for your patience, support and love all these years. I would like to express my sincere gratitude to Apostle T.G. Mulima for his endless prayers on the completion of this work, may our good Lord be with you always. I thank everybody for supporting me in any way, either directly or indirectly; for this work to be a success. Your kindness has been my source of strength.

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Dedication:

I dedicate my dissertation work to my late sister Ndandu and her late daughter Ngeletshedzo.

I also dedicate this work to my Mom and my lovely daughters Murendeni and Mufunwa.

This work is also dedicated to my niece/nephews Khumbuleni, Thomani and Takalani.

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Chapter 1

Introduction

1.1 Background of Vibrio Cholerae

Cholera is still regarded as illness that causes morbidity and mortality, more especially in developing countries. Cholera is an acute, diarrhoeal illness caused by the bacterium Vibrio cholera (*V. cholerae*) which is found in marine waters. It can result in severe dehydration and even death within a matter of hours. Seven cholera pandemics around the world have been recorded since 1817, of which six of them started in the densely-populated regions of the Bay of Bengal (King *et al.*, 2008). Deaths caused by cholera are estimated at millions per year, mostly in Asia and Africa (Nelson *et al.*, 2009). This disease is acquired mainly by ingesting food or water which is contaminated by faecal material from patients or carriers.

1.1.1 V. Cholerae

V. cholerae is a facultative pathogen that has both human and environment stages in its life cycles (Koch, 1884). *V. cholerae* was first isolated as the cause of cholera by Italian anatomist, Fillipo Pacini in 1854. However 30 years later Robert Koch, published the knowledge and ways of fighting the disease (Bentivoglio & Pacini, 1995). There are more than 200 serogroups of *V. cholerae* known. These are differentiated by the O antigen of their lipopolysaccharide (LPS). However, only two, namely 01 and 0139

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serogroups, are responsible for all epidemic and endemic of cholera. The 01 serogroup is divided into three serotypes, Ogawa, Inaba, and Hikojima. They are also divided into two biotypes, namely classical and E1 Tor (Faruque *et al.*, 1998). *V. cholerae* has two major virulence factors. These are, cholera toxin (Holmgren, 1981), which is responsible for the diarrhoea, and Toxin Co-regulated Pilus (TCP) (Herrington *et al.*, 1998). The latter is a self-binding pilus that ties bacterial cells together in order to resist shearing forces in the small intestine (Kirn *et al.*, 2000) & (Nelson *et al.*, 2009). The classical biotype is known to have caused earlier pandemics. In a 20 years period since first pandemic E1 Tor is now the cause of cholera pandemic (Longini *et al.*, 2002). The 0139 sub-group first appeared in 1992 and, although it has caused the outbreaks in the 1990s, the E1 Tor remains the dominant strain globally. Below is an illustration of the bacterium *V. cholerae*



Figure 1.1: *Vibrio Cholerae.* : A magnified view of typical *V. cholerae* - scan microscopy

1.1.2 Cholera Toxin (CT)

CT is responsible for the massive, watery diarrhoea. It is composed of two subunits, an enzymatically active A subunit with adonosine-diphosphate (ADP)-ribosyltransferase, which is responsible for toxicity and a pentameric B subunit(CTB), which is necessary for internalization of the globular A subunit after binding to cell-surface receptors. The B subunits bind to the ganglioside receptors on epithelial cells of the intestinal mucosal, after attachment. Then cleavage occurs between the A subunit and the A2 component, facilitating entry of the Al component of the cell. The A1 component stimulates the production

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of the enzyme adenylate cyclase, which is responsible for the production of cyclic AMP (cAMP). cAMP is a cyclic nuecleotide of adenosine that acts at the cellular level to regulate various metabolic processes. High concentrations of cAMP result in the disruption of the active transport of electrolytes across the cell membrane. This makes it impossible for fluid to be absorbed and lead to secretion of fluid into the small intestine. Diarrhoea occurs when the amount of fluid entering the colon from the small intestine is more than the re-absorptive capability (Schofield *et al.*, 2007). Lack of treatment of diarrhoea leads to severe dehydration, electrolyte abnormalities and metabolic acidosis (Karaolis *et al.*, 1998), almost resulting in death. The evolution of cholera is shaped by the transmissible elements as the lysogenic bacteriophage that contains the genes of CT (Waldor & Mekalanos, 1996) and the SXT element that carries antibiotic resistance genes (Huddleston, 2014).

1.2 Signs and symptoms

The symptoms of cholera are profuse, painless vomiting and diarrhoea of clear fluid (Byrne, 2008). These symptoms usually start within a short period of time after ingestion of the bacteria (Azman *et al.*, 2013). The diarrhoea looks like "rice water" in nature and may have a fishy odour (Svennerholm *et al.*, 1984). If a patient does not receive treatment, he may produce 10 to 20 litres of diarrhoea in a day. Without treatment there will be fatal results. Without treatment and with intravenous rehydration, there can be life-threatening dehydration and electrolyte imbalances. The known symptoms of dehydration are; low blood pressure, poor skin turgor for instance, wrinkled hands, sunken eyes, and a rapid pulse (Byrne, 2008).

1.3 Mechanism of action of *V.cholerae*

Upon arrival in the stomach through ingestion of contaminated food or water, V. cholerae colonizes the small intestine for 12 to 72 hours before symptoms could be seen. Another known symptom of cholera is stomach cramps, all of which may lead to fluid loss of up to a litre per hour (Phillips, 1964). Fluid depletion and metabolic acidosis lead to collapse and death (Seas & Gotuzzo, 2000). Rice water stool mostly contains between 10^{10} to 10^{12} vibrios per litre .

Those with symptoms may shed the bacteria before the onset of illness (Cash *et al.*, 1974) and they will continue to do so for 7 to 14 days (Fasano *et al.*, 1995). On the other hand asymptomatic patients shed vibrios for only 1 day and at approximately 10^3 vibrios per gram of stool (Gangarosa, 1974). In

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endemic areas, such as the Ganges River Delta, children aged up to 5 years are more likely to be found with severe symptoms. By contrast, in epidemic patterns of transmission , when *V. cholerae* is introduced into an immunologically naive population, all age groups become equally susceptible to symptomatic infection (Nelson *et al.*, 2009).

V. cholerae releases toxin and colonize the human small intestines (Herrington *et al.*, 1988). They tolerate alkaline media that kill most intestinal commensals. However they are sensitive to acid and most of them die in the stomach due to gastric acid. Bacteria which manage to survive may adhere to and colonize the small bowel, where they secrete the potent cholera enterotoxin (CT, also called "choleragen"). This toxin binds to the plasma membrane of intestinal epithelial cells and releases an enzymatically active subunit that causes a rise in cyclic adenosine 51-monophosphate (cAMP) production. The resulting high intracellular cAMP level causes massive secretion of electrolytes and water into the intestinal lumen which manifest as a diarrhoea (Frey *et al.*, 1996).

1.4 Treatment of cholera

Cholera can be successfully treated with oral rehydration therapy (ORT), which is highly effective, safe, and simple to administer (Sack *et al.*, 2006). In endemic cases with significant dehydration, intravenous rehydration may be considered. Ringer's lactate is the preferred solution, often with added potassium. It can be given in large amount until diarrhoea has subsided (Sithivong *et al.*, 2010). On the other hand patients can be given a solution of 1 liter of boiled water, 1/2 teaspoon of salt, 6 teaspoons of sugar. As the dehydration is corrected, potassium levels may decrease rapidly, and thus need to be replaced . Foods with high potassium like bananas or green coconut water can also be eaten. Antibiotic treatments for one to three days shorten the course of the disease and reduce the severity of the symptoms (Sithivong *et al.*, 2010).

1.5 Transmission cycle

Cholera disease is known to be a water-borne disease (Glass & Black, 1992). It is found in two reservoirs; namely humans and the aquatic environment. *V. cholerae* are taken in by humans when they eat or drink contaminated food or water. Food characteristics which promote the survival of *V. cholerae* are low temperatures, high-organic content, neutral or alkaline pH, high-moisture content and the also if there is no other micro-organisms in the particular food (Seas & Gottuzo, 2000). Eating vegetables that are

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washed with contaminated water can also result in cholera. This can also happen when contaminated water is injected into fruits such as water melons in order to sustain their weight and taste (Rabbani & Greenough, 1999). However *V. cholerae* do not survive in sour fruits with lower pH, that is below 4.5, Examples are fruits like lemons and oranges (Seas & Eduardo, 2000) . Even eating a raw or uncooked seafoods such as shellfish, molluscs, crustaceans, crabs and oysters *V. cholerae* infection (Huq *et al.*, 1984).

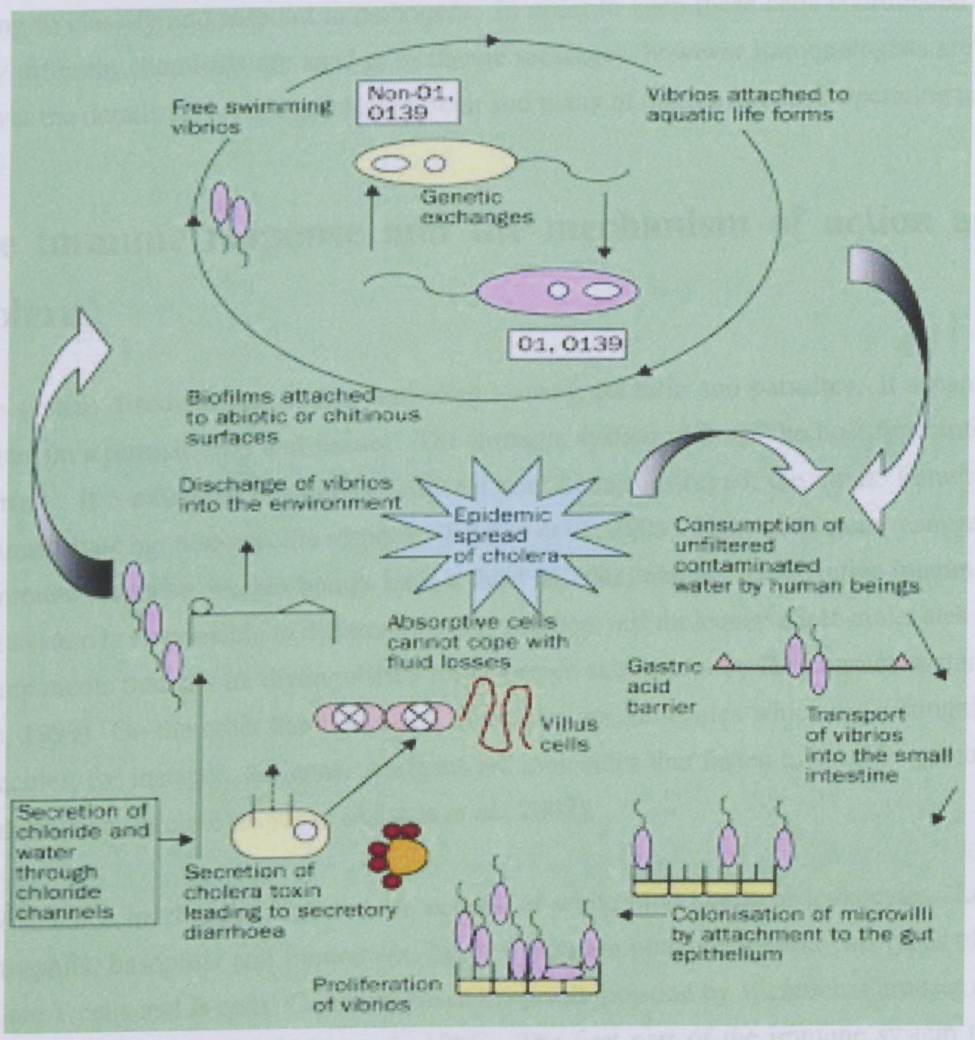


Figure 1.2: *Life cycle of V. Cholerae. 2008 Andhramania. All rights received.*

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1.6 The Immune System

The immune and non-immune mechanisms inside the organism, protect the privileged environment of the lamina propia by identifying and killing invading pathogens. The immune mechanisms protect the hosts in three different sites, in the gut, mucosal surfaces and within the lamina propia (Doe, 1989). The immune system is composed of several interdependent cell types such as T-cells, B-cells, macrophages, mast cells, natural killer (NK) cells, neutrophils, and others, and proteins that have different responsibilities in fighting foreign invaders. These cells are always in motion, patrolling independently and self-organizing to classify and respond to pathogens. In order to keep these cells coordinated and controlled, many different chemicals are used to exchange messages, however immunologists are working hard to uncover the details of this complicated system and many of the fundamental operating principles.

1.7 The immune response and the mechanism of action against cholera.

The immune system discovers pathogens, including viruses, bacteria and parasites. It separates them from the organism's normal cells and tissues. The immune system protects the host from infection by physical barriers. If a pathogen manages to pass without being destroyed, the innate immune system provides an immediate but non-specific response (Litman *et al.*, 2005). If the pathogens manage to evade the innate immune response, human beings have a third defence; namely the adaptive immune system. The immune system is responsible to differentiate self and non-self molecules. Self-molecules are an organism's components that can be distinguished from foreign substances by the immune system, (Smith & Germolec, 1999). On the other hand, non-self molecules are molecules which are distinguished as a foreign molecules, for instance, antigens. Antigens are molecules that fasten to specific immune receptors and stimulates an immune response (Alberts *et al.*, 2002).

The immune defence mechanisms involve the actions of white blood cells or leukocytes. Leukocytes include neutrophils, basophils and monocytes, all of which are phagocytic, and two types of lymphocytes which are T cells and B cells. Colonization of the small intestine by *V. cholerae* arouses a mucosal immune response in the host (Jertborn *et al.*, 1986). The first part of the immune system that meets *V. cholerae* is a group of proteins. These proteins flow into the blood and can quickly reach the small intestines where they can react directly with vibrio molecules that the body recognizes as foreign substances. Being activated, these complement proteins can attract macrophages to the area of infection.

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V. cholerae is considered extracellular pathogen; hence antibodies are the primary defence against it. Antibodies against vibrio function in three major ways, thus they neutralize the bacterium by binding to it, hence blocking the association of the vibrio with its targets. Secondly antibody binding to vibrio can opsonize the material and facilitate its uptake and destruction by phagocytic cells. Lastly, technique that the antibody uses to function through activation of the complement cascade, which results in lysis of *V. cholerae*

Immune responses to cholera at mucosal surfaces are characterized by production and transport across the epithelium of antibodies of the secretory IgA (sIgA) isotype, which is the first line of defence against colonization by *V. cholerae* and other pathogens (Staats *et al.*, 1994). The stimulation of CD4+T cells in the host defence is a regulatory event for both immunity and inflammation (Sher & Coffman, 1992), although vibrio is non-flammatory.

1.7.1 Gastric acid

Gastric acid is considered as a chemical barrier; it protects against infection (Agerberth & Gudhmundsson, 2006). It acts as one of non-immunological defence factors against bacteria such as *V. cholerae* (Howden & Hunt, 1987). *V. cholerae* are sensitive to gastric acid, hence they do not survive the gastric acid barrier of the stomach and are rapidly killed at low pH. A reduction of gastric acid secretion influences the infection with a wide range of organisms, including cholera (DuPont *et al.*, 1972). If there is a reduction in acid secretion, there will be a severe infection in the human host (Nalin *et al.*, 1978). *V. cholerae* introduced in the stomach are destroyed within a period of 15 minutes when the pH is 3.0-4.0 or below (Giannella *et al.*, 1972).

1.7.2 Cells of the innate immune system.

Cells of the innate immune system include phagocytic cells (monocytes or macrophages, neutrophils and dendritic cells), natural killer cells (NK cells), basophils, mast cells, and eosinophils. They identify and eliminate pathogens, by attacking or by engulfing and then killing them (Janeway & Bottomly, 1994). Human intestinal epithelial cells are the initial site of entry for *V. cholerae*, and also provide early signals for the acute mucosal inflammatory response by the release of proinflammatory cytokines and inflammatory mediators. The response of the intestine to infections by vibrios represents a complex interaction between non-specific inflammatory mechanisms and immunologically-specific adaptive events, although

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cholera has traditionally been classified as a non-inflammatory diarrhoeal disease (Bandyopadhaya *et al.*, 2007).

1.7.2.1 Epithelial cells

Gastric acid, mucus secretion, and intestinal mobility are the prime non- specific defences against *V. cholerae*. The mucosal response involves production of effective specific immunity, secretory immunoglobulin (IgA), as well as IgG antibodies, against vibrios, somatic antigen, bacterial lipopolysaccharide (LPS), the enterotoxin and other products (Jertborn *et al.*, 1986). The interaction between the vibrio, the gut epithelium and the host innate defences responses are regarded as the most important factors that control the fate of bacterial infections and disease outcomes (Kim *et al.*, 2010). In defence to vibrio, the epithelium consists of several layers of microbial sensing and natural defence systems which are built in and are against pathogens. The elements that are countermeasures to *V. cholerae* infection include, commensal microbiata, epithelial integrity, rapid epithelial cell turnover, quick epithelial cell exfoliation and sealing, and innate immune systems (Kim *et al.*, 2010). The commensal bacterial flora in the lumen compete with vibrio and interfere with colonization of the epithelial surface. The intestinal flora can help in balancing the immune tolerance with immune activation and influencing epithelial metabolism and production of a mucus layer (Leser & Mølbak, 2009). On the other hand, the innate and acquired immune systems are part of the mucosal immunity which are very important in defending the host against the *V. cholerae* infection.

The epithelial monolayer and the mucosal surface play as physical and biological barriers against pathogens. The reliability of the epithelial monolayer is sustained by tight cell-cell junctions, and the mucosal surface is covered by a mucin layer containing various digestive enzymes, secreted IgA and many other microbial agents, including chemokines (Kim *et al.*, 2010). In order to maintain epithelial integrity and tissue homeostasis, and to avoid the accumulation of damaged or dead cells, gut epithelial cells are always renewed throughout the host's life time through provision of progenitors from cryptic stem cells whereas a well-differentiated epithelial cells constantly undergo cell death and cell shedding, which contributes to epithelial cell turnover and prevents vibrio colonization (Kim *et al.*, 2010).

1.7.2.2 Mechanism of action of Monocytes/Macrophages.

Monocytes/Macrophages are phagocytes that travel throughout the body in tracking down the pathogens (Zen & Parkos, 2003). They initiate the development and outcome of the immune response (Janeway & Bottomly, 1994). They play a very important role in the presentation of *V. cholerae* to $CD4+$ T-cells

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and in the activation of co-signals which initiate and maintain $CD4+$ T-cells activation (Abbas *et al.*, 1996). They are foremost among the cells that "present" antigen, which is a crucial role in initiating an immune response. After digesting vibrio bacterium, macrophages present the antigen of the vibrio to the corresponding helper T cell on MHC class II. Because they are secretory cells, monocytes/macrophages are determined to the regulation of immune responses and the development of inflammation. They are responsible in production of an array of powerful chemical substances which are monokines; these include enzymes, complement proteins, and regulatory factors such as interleukin-1 (Medzhitov & Janeway, 2002). They also act as scavengers, and get rid of the body's exhausted cells and other remains (Mayer *et al.*, 2007). Macrophages are activated by LPS and IFN-gamma, and secrete high levels of IL-12 and low levels of IL10.

1.7.2.3 Neutrophils

Neutrophils are the most phagocytic type of cells which constitutes 50% to 60% of the circulating white blood cells in the human host blood stream (Smith, 1994). They are considered a first-line cellular defence against vibrio cholerae (Queen & Satchell, 2012). Neutrophils play an important role in the protection of the host against cholera; they limit the infection to the intestine and control the spreading of vibrio. The main function of neutrophils during infection is clearing dispersed vibrios (Queen & Satchell, 2012). However research shows that neutrophils may not have a significant impact on*V. cholerae* infection (Mathan *et al.*, 1995).

1.7.2.4 Dendritic cells (DC)

Dendritic cells are unique antigen presenting cells in the sense that, they are the only ones that are able to regulate adaptive immune response (Bell *et al.*, 1999). They are phagocytic cells which are found in tissues that are in contact with the external environment. They are also found in the stomach and intestines (Guermonprez *et al.*, 2002).

1.7.2.5 Natural killer cells (NK)

NK cells are a component of the innate immune system which does not attack the invading vibrio directly; rather, they destroy damaged cells (Janeway & Bottomly, 1994). NK cells form part of the innate immune system. They have a vital role in protecting the host against tumours and virally-infected cells. They are able to distinguish infected cells and tumours from healthy and uninfected cells by identifying changes of MHC class. NK cells are activated in response to a family of interferons cytokines. Upon activation they

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release cytotoxic (cell-killing) granules which destroy the distorted cells (Janeway & Bottomly, 1994). They were named "natural killer cells" because of the initial notion that they do not need to be activated first, rather they kill cells which are missing MHC class I. Below is the summary table of the innate immune cells.

Component of Immune system	Mechanism of action
Macrophages or Monocytes	-They initiates the development and outcome of immune response -They present the vibrio choleare to T cells
Neutrophils	-Their main function is to clear dispersed vibrio choleare. However research shows that neutrophils do not have significant role on V.cholerae infection.
Dendritic cells	-Like Macrophages, they present vibrio choleare to T cells.
Natural Killer cells	Activated NK cells release cytotoxic granules which destroy the distorted cells.

Table 1.1: Cells of the innate immune response.

1.8 Adaptive Immunity to cholera

The adaptive immune system, also known as the acquired immune system or specific immune system, is a subsystem of the overall immune system that consists of highly specialized, systemic cells and processes that eliminate or prevent pathogen growth. The function of adaptive immune responses is to destroy invading pathogens and any toxic molecules they produce. Adaptive immune responses are carried out by white blood cells called lymphocyte The major cells of the adaptive immune system are the B and T lymphocytes (Janeway & Bottomly, 1994). These lymphocytes have receptor molecules that recognize specific targets. However. B cells are involved in the humoral immune response while T cells are involved in the cell-mediated immune response (Holtmeier & Kabelitz, 2005).

1.8.1 T lymphocytes and cholera

T-cells recognize *V. cholerae* only after the antigen has been processed and presented together with a self-receptor called major histocompatibility complex (MHC) molecule (Holtmeier & Kabelitz, 2005). Cytokine production by antigen-specific Th cells clone has shown that there are at least two different Th subsets (Mosmann & Coffman, 1989). These two types of Th cells differ in their cytokine secretion

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pattern and in their effector functions (Sims *et al.*, 1988) and (Abbas *et al.* 1996) Cytokines are proteins which are able to be dissolved and which regulate the growth and activity of immune cells. Upon activation Th1 produce interleukin-2 (IL-2), gamma interferon (IFN-), and tumour necrosis factor beta. On the other hand Th2, cells are unique cells in which IL-4, IL-5, IL-6, IL-10 and IL-13 are produced (Street & Mosmann, 1991). Th1 cell is involved in cell-mediated immunity and delayed-type hypersensitivity, while Th2 cells display anti-flammatory properties and help B-cells response for certain IgG subclasses and IgE as well as IgA-positive B cells to differentiate into IgA-generating plasma cells (Beagley *et al.*, 1989).

1.8.2 B lymphocytes and *V. cholerae*

The B cell recognizes *V. cholerae* and produce antibodies which bind to a specific antigen (Sproul *et al.*, 2000). The B cell take the antigen-antibody combination and process it into peptides through proteolysis. The B cell displays these peptides on its surface, MHC class II. The matching helper T cell is then attracted through the combination of MHC and antigen. After activated, the T cell releases lymphokines and activates the B cells (Kehry & Hodgkin, 1994). Activated B cells begin to divide into plasma cells and memory B cells. The plasma cells will then secrete many copies of the antibody that will recognize this antigen. These antibodies circulate in the blood plasma and lymph. Each B cell has a unique receptor protein (B cell receptor) on its surface that will bind to one vibrio cholerae. Specific antibodies which are secreted in the mucosal cells against V. cholerae are secretory IgA (sIgA) and IgG. Antibodies bind to V. cholerae, expressing the antigen and marking them for destruction by complement activation or for opsonization which promotes phygocytosis (Kermack & McKendrick, 1927).

Memory B cells express a surface of specific antigen receptor and antigen play an important role in triggering their recall response. Memory B cells will differentiate very fast during re-infection by *V. cholerae* and add large amounts of high-affinity antibodies to those which are already in the serum, hence blocking the attack by the bacterium (Yoshida *et al.*, 2010). Long- lived plasma cells maintain a constant level of selected immunoglobulin (Manz *et al.*, 2005). However in the process of re-infection the concentration of useful antibodies specifically increases because memory B cells are called into action.

As *V. cholerae* is non-invasive, serum response takes a few days to respond after ingestion of the bacteria. It is also believed that protection against cholera is conferred by secretory IgA within the intestinal lumen. According to Glass, et al, 1983, patients from moderate to severe cholera produce high levels of

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anti-CT as well as anti-LPS antibodies in the intestines. Activated B cells produce specific antibodies against specific bacteria cell components(anti-LPS, anti-TCPA and anti-CTB). Bacterial LPS is a major molecule recognized by the innate immune system (Brightbill & Modlin, 2002). Below is a summary table of the cells of the adaptive immunity to cholera.

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Component of Immune system	Mechanism of action
T lymphocytess	-They recognize *V. cholerae* after its antigen has been processed and presented together with a self-receptor called major histocompatibility complex (MHC) molecule. -They can be divided into CD8+ and CD4+TH which will then divide into TH1 and TH2. -TH1 produce interlukin-2,gamma interferon(IFN-). TH1 is involved in cell-mediated immunity and delayed-type hypersensitivity. -TH2 produces IL-4, IL-5, IL-6, IL-10 and IL-13. TH2 displays anti-flammatory properties and also helping B cells response for certain IgG subclasses and IgE and also IgA-positive B cells to differentiate into IgA-generating plasma cells.
B lymphocytes	-B cell recognize V. cholerae when antibodies bind to specific antigen. -B cell will then process this antigen-antibody complex. It will then display this complex on its surface MHC class II. -The combination of MHC and antigen attracts a matching helper T cell, which will then release lymphokines and activates the B cells. -Activated B cells divide into plasma and memory B cells. -Plasma cells will secrete many copies of antibodies that will recognize the antigen. -Specific antibodies are sIgA and IgG. These antibodies bind to *V. cholerae* and mark them for destruction by complement activation or by opsonisation or by phygocytosis. -These antibodies also neutralize the cholera toxin or by interfering with receptors that *V. cholerae* use to cause infection.

Table 1.2: Cells of the adaptive immune response

1.9 Problem statement

Many epidemiological mathematical models of cholera have been developed. However, the transmission dynamics of the disease and the role of immunity are not yet fully understood. This models gave much

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attention to cholera epidemiology by incorporating different compartments in order to find the way to minimise the infections or to find a vaccine. The main problem that hinders researchers from understanding its dynamics is that *V. cholerae* are always found in lower levels than expected (Colwell & Huq, 1994). Even though there are a number of existing cholera epidemiological models, researchers have not yet considered the cholera immunological model to understand the dynamics of *V. cholerae*. In view of the above, in this study we develop cholera immunological models in order to understand the dynamics of the cholera infection.

1.10 Overall Aim

The main aim of this study to understand cholera immunology. Specifically, it is to get more insights into how the immune system responds to *V. cholerae* using mathematical models.

The specific objectives of this thesis are :

- To develop two immunological mathematical models of cholera infection. The first model is the basic model and the second one is the extension of the basic model by partitioning the immune cells to phagocytes and lymphocytes.
- To determine the most crucial aspect that contribute to the development of cholera infection within the human host.
- To assess which parameters of the model needs much attention in order to minimize the disease.

1.11 Methodology

We develop two immunological models of cholera. The models are about the mechanism of immune response to *V. cholerae*. For the developing these models, important aspects which are involved during cholera infection are considered. Aspects such as, the bacteria itself, gastric juice, water within the body, memory cells and others. The first model includes the immune cells in general. In the second model we extend the first model by partitioning the immune cells to phagocytes and lymphocytes. The models will be analysed both mathematically and numerically in order to gain more understanding of *V. cholerae* and the mechanism of immune system.

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1.12 Significance of the study

- The findings of this study will add more knowledge to the existing knowledge about cholera and its dynamics.
- The results will help researchers such as mathematicians and microbiologists to develop other models for further research on cholera.
- The study will help to develop programmes to educate the community on how to reduce infection and transmission of cholera.
- The results may also help suggest study designs to collect data that is currently lacking.

1.13 Outline of the thesis

Chapter 1, presents an overview of infectious disease, in this case the biological background of cholera, *V. cholerae* , the immune system and the mechanism of action against *V. cholerae* and cholera toxin. Chapter 2 presents the literature review on mathematical modelling and a background of mathematical modelling framework immunological models and epidemiological models. Chapter 3 provides the basic model of cholera immunology model that describes the bacterium *V. cholerae* inside the human host. Chapter 4 presents the extended model of the basic model developed in chapter 3, in order to gain more knowledge on immune response to V. cholerae and cholera toxin, in which the immune system is partitioned to lymphocytes and phagocytes. Chapter 5 provides discussion, conclusion and recommendations.

1.14 Summary of the chapter 1

In this section we give a brief summary of chapter 1. A background of the bacteria *V. cholerae* has been given, where it is found and its survival ways. The description of the bacteria and cholera toxin has been explained and moreover how the cholera infection is caused. The signs, symptoms,the transmission cycle and also the treatment of the disease were explained in this chapter. The mechanism on how the bacteria manipulates the host by suppressing the immune system has been given. The description of the immune system response against this pathogen and also the cells which are involved are explained. Finally, this chapter outlines the problem statement of the study, the overall aim, the methodology, the significance of the study and in conclusion the outline of the thesis.

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Chapter 2

Mathematical Preliminaries

2.1 Introduction

Mathematical modelling has been used in evaluating social and economic policies. It can also be used to evaluate health policies as well. Although it is not easy to include all the socio-economic and demographic factors beside climatic factors, mathematical modelling gives a tool to facilitate consensus and action with an iterative and incremental approach to making decisions (Helmerson, 2012). Mathematical models can outline how infectious disease progress to show the likely outcome of an epidemic and help inform public interventions. Epidemiology is about patterns in space and time of occurrence of the disease. Out of the patterns we may infer the cause of the disease. In addition, the future of the disease can be predicted and hence people may decide about the need for control measures. Its approach in research problems increases with the capacity of computer development.

Mathematical Modelling takes four stages to finally come up with a conclusion. The first one is to develop a theoretical framework, to interpret a research problem into a set of system of equations, to find relevant parameters to connect with health reality from empirical data, and to compute the solutions of the equations in numerical and graphic form. Mathematical modelling rely on the assumptions and information on the past events and projected-future conditions to directly predicts the past or future events. A mathematical disease model constitutes a set of causal pathways involved in exposure to disease process

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and simulates disease transmission over time and space (Helmerson, 2012).

Daniel Bernoulli is one of the mathematicians who used mathematical modelling in predicting and rationalizing the spread of infectious disease in a population in 1760. The main aim of modelling the spread of an infectious disease mathematically is to give a framework in order to make predictions about its progress within a population which is susceptible to infection. Bernoulli is also the first mathematician to model infectious disease such as smallpox which was prevalent at the time. He argued that the vaccination of healthy people against the smallpox virus was effective (Bernoulli, 1760). For the system of equations of his model, Bernoulli divided the population into susceptible and immune compartments and assumed an age-specific force of infection and case fatality rate.

In 1854, John Snow observed and identified a single water pump as the source of a cholera outbreak, and this findings contributed to the development of epidemiology as a science (Snow, 1855). In 1882, Koch's postulates established formal criteria to show that specific microbes caused specific diseases, after which scientists identified the bacterial and parasitic causes of many infections and began to understand how infectious agents spread (Falkow, 2004).

On the other hand, Hamer developed and analysed a discrete time model in 1906. He was trying to understand the reappearance of measles epidemics (Hamer, 1906). His model may have been the first to assume that the number of new cases per unit time depends on the product of the densities of the susceptibles and infectives. In 1911, Ross became interested in the incidence and control of malaria. He then developed differential equation models for malaria as a host-vector disease Kermack & McKendrick(1927). By the 1950*s*, mathematical models had explored the stochastic aspects of infectious diseases, especially the stochastic fade out of measles, and the critical factor of community size in sustaining an epidemic (Bartlett, 1956).

Colwell & Huq, (1994) proposed the first basic model for cholera epidemic occurred in the Mediterranean in 1973. Brayton *et al.*, (1987) was the first mathematician to incorporate the environment component, which is the concentration of cholera bacterium in the water supply. This term is denoted by B,into an SIR system to form a combined environment-to-human (SIR-B) epidemiological model. This model has given rise to a number of cholera models.

Hsu & Hsieh,(2008) presented a more general model from Codeco's model. Their model consists of five compartments. These are susceptible, infectious , removed human population, the dynamics of a

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hyper infective state of V. cholerae and the lower infective state of V. cholerae population. Based on the assumption that the total population N is a constant and the birth and death rate are also constant, Hartley et al concluded that cholera infection is caused by drinking contaminated water with either hyper infective or lower infective vibrios. In (Hartley & Morris, 2005), it was shown that cholera bacteria mostly become more infectious for a short period of time just after passing through the human digestive tract.

Dushoff *et al.*, (2004) developed a model by incorporating the human to human factor (interactions between susceptible and infectives) on Codeco's work. They came up with an estimation of the basic reproduction number for the 2008 – 2009 cholera outbreak in Zimbabwe. To formulate their model they used Zimbabwean data which gives insights into the nature of the epidemic in Zimbabwe. Their model aimed at controlling cholera at a global level. They have used the data to estimate the basic reproduction number at a regional level.

Curtis & Danquah,(2009) developed a SIR compartmental model that consists of susceptible, infected class and infectious. Recovered were removed to avoid further infection. They have replaced aquatic environment compartment in Codeco's model and put water compartment . They concluded that cholera is a water-borne disease and that transmission can take place either by ingesting contaminated water or through close contact with infectives.

Jensen *et al.*, (2006) incorporated phage compartment and divide infectives into bacteria and phage, and phage-infected individuals into Codeco's work. They assumed that bacteria go through logistic growth with carrying capacity, and bacteria predation by phage occurs via a Holling I response. The infection term is a Holling II functional response. The sigmoidal was meant to capture the low infectivity of low levels of bacteria, however, there are still infections at very small levels which could overestimate the number of infections in the long run. This model was developed in order to determine the ability of phage to end outbreaks or indirectly cause outbreaks by being reduced in number. However their model did not consider the required dose for V. cholerae to cause infection. They analysed the model but did not examine the role of limit cycles caused by the predator-prey-like relationship of phage and bacteria.

Kong *et al.*, (2014) improved the model developed by Jensen et al.(2006) by including a minimum infectious dose (MID) into the incidence term. This infection term is a piecewise continuous function which is zero below the minimum infectious dose threshold and has a holling II response curve above the threshold. Similar to Jensen et al, they have also allowed bacteria to exist naturally under logistic growth. Kong et al. concluded that it is the bacteria and phage which are the source, and not the reverse

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situation. They observed that if the MID is less than the carrying capacity of the bacteria the bacteria cycles usually fail to overwhelm the MID, as a result there will be no new cases of infections and the system will eventually be disease-free. However if the bacteria's natural carrying capacity is larger than the MID, the disease will persist. They further indicated that if the phage levels could be increased in any way to keep the bacteria below this MID, then the cycles would remain in both the bacteria and the phage (Kong *et al.*, 2014).

2.2 Development of Immunological models

An understanding into immune response against pathogens gained a lot of interest from researchers. Mathematical epidemiologists started to incorporate specific features of the pathogens and terms on how the immune system responds when encountering pathogens when developing their models in order to enhance their predictability, even though it was not too so easy. In the late 80's researchers such as Alan Perelson, Robert May, Robert Nowak, Roy Anderson, and Simon Levin developed a basic within-host models to give a description of the dynamical behaviour of pathogens and specific immune cells and antibodies. The immune system may be regarded as a population whose individuals cooperate. It is a multi-dimensional system in the sense that the population is made up of different types of species(e.g T-cells,B- cells antigens). It also has compartments because different species originate in varying densities in different regions,e.g. the blood, the skin and so on (Andrew *et al.*, 2007).

Mathematical models describing infection dynamics within-hosts have so far proved useful in that they have provided more insights into viral kinetics and disease outcomes. Chronic infectious diseases, such as human immunodeficiency virus (HIV) (Perelson & Weisbuch, 2002) and hepatitis C virus (Neumann *et al.*, 1988) were given much attention by researchers. These developments became the cornerstone of mathematical immunology. Through mathematical models in 1995, it was found that the replication rate of HIV is great in magnitude and hence the antiretrovirals that were given would not be effective enough in eliminating the HIV from infected patients (Ho *et al.*, 1995). From this point, it could be seen that mathematical immunology is a useful tool to uncover the intricacies of an infectious process and in evaluating the adequacy of therapeutic strategies.

Nelson *et al.*, (2009) and Perelson & Weisbuch, (2002),in their HIV-AIDS research work, report that mathematical modelling has shown to be useful in understanding the dynamics of HIV infection at both the population and cellular levels by using both ordinary and delay differential equations, and also the techniques of parameter estimation. Mathematical modelling gives a unique approach to help researchers

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gain basic knowledge in cholera dynamics. It has played an essential role both in immunology and epidemiology. The main goal of immunological research is to understand what controls the ability of the immune system to mount a protective response against pathogen-derived foreign antigens while on the other hand avoiding a pathological response to self-antigens (Andrew *et al.*, 2007).

Ben-Shachar & Koelle, (2005) have developed a suite of within-host mathematical dengue models to describe the dynamics of the virus and to understand better the development of severe dengue disease.They parametrize their minimal models using a combination of literature estimates and described features. They arrived at minimal models able to reproduce characterized viral features for both primary and secondary infections. They have done that by first beginning with a target cell model and adding increasing complexity. They finally showed that only the innate immune response is required to recover the features of a primary dengue infection, and that for a secondary dengue infection to occur, a higher rate of viral infectivity is needed to recover the higher peak viraemia value and a shorter time to peak viraemia, while T-cells are needed to recover the higher viral clearance rate.

Sullivan *et al.*, (2012) have showed that the immune system plays an important role in overpowering the growth of tachyzoites within host cells. This suppression, once the system reaches endemic behaviour, allows for the body to exit the acute infection stage and begin the long-term, virtually symptom-free, state. Without an immune response, the tachyzoites would be free to multiply and invade many different hosts.

The innate immune response and the adaptive immune response have been incorporated into the existing model to assess the outcome of immune responses on viral control Getto *et al.*, (2008). The influenza models have shown the importance of both the innate and the adaptive immune response in regulating viral dynamics (Dobrovolny *et al.*, 2013), and particularly, the role of the innate immune response in contributing to disease symptoms (Saenz *et al.*, 2010).

Mathematical modelling of immune response to pathogens gives an understanding in biology. Modelling of the immune response, for instance, of T-cell receptor (TCR) signalling and the immune synapse have provided a better understanding of infectious diseases. Therefore, mathematics is essential for understanding biological systems. It is used to investigate the behaviours of a biological immune system and it is important to take into consideration what features of the immune system need a mathematical approach for their understanding (Callard & Yates, 2005).

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Infectious diseases such as cholera remain of epidemic nature and endemic mostly in developing countries. Much work still needs to be done in order to limit or eradicate cholera. For the past years mathematical models for cholera have been developed. However many of them focus much on epidemiology and ignore immunology, and yet a significant gap in the knowledge of cholera dynamics still exists. As a result there is no development of a cholera vaccine. To the best of our knowledge there is no work that has been done on mathematical modelling of the immunology of cholera. Therefore this work represents a first attempt to model cholera immunology with all the attendant difficulties of parameterization of the model. In this study we develop the mathematical immunology models of cholera to gain more insights into the disease. We develop the basic cholera model and in the model we consider the rate of change over time of *V. cholerae*, Immune cells, Cholera Toxin, Gastric acid, Memory cells and Water within the human body respectively. In the second model which is an extension of the basic model, we incorporate phagocytes and lymphocytes in the first model.

2.3 Mathematical Preliminaries

In this section we present some definitions and theorems required to study the models developed in this study. Concepts such as positivity of solutions, the boundedness of the model, equilibrium points, stability , sensitivity analysis and the reproductive number R_0 are presented in this chapter. Mathematical modelling is a process of developing models. Mathematical models are used in different disciplines, such as natural sciences, engineering disciplines as well as in the social sciences. A model may help to explain a system and to study the effects of different components. It can also used to make a predictions about a behaviour. Mathematical models take many forms including dynamical systems, differential equations and statistical models. The following is the general definition of ordinary differential equation by (Salle & Solomon, 1961)

$$\begin{aligned}
\dot{x}_1 &= f_1(x_1, x_2, x_3, ..., x_n, t) \\
\dot{x}_2 &= f_2(x_1, x_2, x_3, ..., x_n, t) \\
\dot{x}_3 &= f_3(x_1, x_2, x_3, ..., x_n, t) \\
&\vdots \\
\dot{x}_n &= f_n(x_1, x_2, x_3, ..., x_n, t)
\end{aligned}$$

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If $x_1, ..., x_n$ are components of an n-vector x, and $f_1, ..., f_n$ as components of an n-vector X, we can present the system as

$$\dot{x} = f(x,t).$$

If f depends on x only and not upon time the above system of equations can be presented in vector notation as :

$$\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}) \tag{2.3.1}$$

where **X** and **f** are vectors of n-dimension, with components x_i and f_i respectively.

2.3.1 Boundedness of solution

Definition:

Consider

$$\begin{aligned} \dot{x} &= f(x,t), t \geq 0 \\ x(t_0) &= x_0, \quad t_0 \geq 0 \end{aligned} \tag{2.3.2}$$

where

$$x \in R^n, f : \mathbb{R}^+ \times \mathbb{R}^n \to \mathbb{R}^n$$

is a given non-linear continuous function in t and x, where $t \in \mathbb{R}^+$. We say that solutions of system (2.3.2) are bounded, if any solution $x(t, t_0, x_0)$ satisfies

$$\|x(t, t_0, x_0)\| \leq C(\|x_0\|, t_0), \text{ for all } t \geq 0.$$

where $C : \mathbb{R}^+ \times \mathbb{R}^+ \to \mathbb{R}^+$ is a constant that depends on t_0 and x_0 . We say that solutions of (2.3.2) are uniformly bounded if C is independent of t_0, see (Raffoul, 2007).

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2.3.2 The Basic Reproduction Number

The basic reproductive number R_0, plays an important role in determining the status of the diseases at present and in future. R_0 is defined as the average number of secondary infections that occurs when one infected individual is introduced into a completely susceptible population. It is regarded as the threshold quantity that determines when an infection can raid and persist in a new host population (Heathcote & van den Driessche, 2000). If $R_0 < 1$ it means that on average an infected individual produces less than one infected individual in a period of an attack, which reflects that the infection cannot spread, but if $R_0 > 1$, then each infected individual produces on average more than one new infection and hence the disease will invade the population, which will result in endemic equilibrium point.

2.3.3 Stability of non-linear systems

Theorem 2.1. *: Stability of non-linear systems*

Consider the system

$$x^{'}(t) = f(x, y), \qquad y^{'}(t) = g(x, y), \tag{2.3.3}$$

where f, g are differentiable with continuous partial derivatives and they both vanish at the point (x_0, y_0). Let J denote the Jacobian matrix at that point, namely

$$J = \begin{bmatrix} f_x(x_0, y_0) & f_y(x_0, y_0) \\ g_x(x_0, y_0) & g_y(x_0, y_0) \end{bmatrix}. \tag{2.3.4}$$

If all eigenvalues of J have negative real part, then $(x_0; y_0)$ is asymptotically stable. And if some eigenvalue of J have positive real part, then $(x_0; y_0)$ is unstable.

2.3.4 Routh-Hurwitz Criteria

The Routh-Hurwitz criteria are important tools that provide necessary and sufficient conditions for all the roots of the characteristic polynomial. The Routh-Hurwitz Criteria are used in chapter 3 to determine the local asymptotic stability of an equilibrium for non-linear system of differential equations. The following is the theorem of Routh-Hurwitz criterion:

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Theorem 2.2. *(Routh-Hurwitz Criteria): Given the polynomial,*

$$P(\lambda) = \lambda^n + a_1\lambda^{n-1} + ... + a_{n-1}\lambda + a_n$$

where the coefficients a_i are real constants,$i = 1, ...n, define$ n Hurwitz matrices using the coefficients a_i of the characteristic polynomial:

$$H_1 = \begin{pmatrix} a_1 \end{pmatrix}, \quad H_2 = \begin{pmatrix} a_1 & 1 \\ a_3 & a_2 \end{pmatrix}$$

$$H_3 = \begin{pmatrix} a_1 & 1 & 0 \\ a_3 & a_2 & a_1 \\ a_5 & a_4 & a_3 \end{pmatrix}, \text{ and } \quad H_n = \begin{pmatrix} a_1 & 1 & 0 & 0 & ... & 0 \\ a_3 & a_2 & a_1 & 1 & ... & 0 \\ a_5 & a_4 & a_3 & a_2 & ... & 0 \\ . & . & . & . & ... & . \\ . & . & . & . & ... & . \\ . & . & . & . & ... & . \\ 0 & 0 & 0 & 0 & ... & a_n \end{pmatrix}.$$

where $a_j = 0$ if $j > n$. All the roots of the polynomial $P(\lambda)$ are negative or have negative real part iff the determinants of all Hurwitz matrices are positive: $detH_j > 0, j = 1, 2, ...n$.

When $n = 2$, the Routh-Hurwitz criteria simplify to $detH_1 = a_1 > 0$ and $detH_2 = det\begin{pmatrix} a_1 & 1 \\ 0 & a_2 \end{pmatrix} = a_1a_2 > 0$ or $a_1 > 0$ and $a_2 > 0$. For polynomials of degree $n = 2, 3, 4$ and 5, the Routh-Hurwitz criteria are summarized as follows : Routh Hurwitz criteria for $n = 2, 3, 4$, and 5.

$n = 2 : a_1 > 0$ and $a_2 > 0$

$n = 3 : a_1 > 0, a_3 > 0$,and $a_1a_2 > a_3$.

$n = 4 : a_1 > 0, a_3 > 0, a_4 > 0$, and $a_1a_2a_3 > a_3^2 + a_1^2a_4$.

$n = 5 : a_i > 0 i = 1, 2, 3, 4, 5, a_1a_2a_3 > a_3^2 + a_1^2a_4$. and $(a_1a_4 - a_5)(a_1a_2a_3a_3^2 - a_1^2a_4) > a_5(a_1a_2 - a_3)^2 + a_1a_5^2$.

For a proof of the Routh-Hurwithz criteria. See (Allen, 2007).

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2.3.5 Global stability conditions for the disease-free equilibrium.

For the differential equations when $R_0 < 1$ the disease free equilibrium is locally asymptotic stable and is unstable whenever $R_0 > 1$. In this dissertation we show the global stability of a model by using the method developed by (Castillo-Chavez *et al.*, 2002). They list two conditions that, if satisfied suggests that is a global asymptotic stability of a disease free-state. Consider the epidemiological model that can be written in the form :

$$\begin{cases} \dfrac{dX}{dt} &= f(X,Y,Z), \\ \dfrac{dY}{dt} &= g(X,Y,Z), \\ \dfrac{dZ}{dt} &= h(X,Y,Z) \end{cases} \tag{2.3.5}$$

where

$$X \in \mathbb{R}^r, Y \in \mathbb{R}^s, Z \in \mathbb{R}^n$$

$$r, s, n \geq 0,$$

and

$$h(X,0,0) = 0.$$

and

1. The components of X represent the number of susceptibles , recovered and the other class of non-infected individuals.
2. The components of Y represent the number of infected individuals who are not infectious
3. The components of Z represent the number of infected individuals who are infectious.

The above model can then be written in the form :

$$\begin{aligned} \frac{dX}{dt} &= F(X,Z), \\ \frac{dZ}{dt} &= G(X,Z) \end{aligned} \tag{2.3.6}$$

where $U_0 = (x^*, 0)$ denotes the disease-free equilibrium of this system. Then to guarantee local asymptotic stability the following conditions must be met.

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(H1) For $\frac{dX}{dt} = F(X, 0)$, X^* is globally asymptotically stable $(g.a.s)$,

(H2) $G(X, Z) = AZ - \hat{G}(X, Z)$, $\hat{G}(X, Z) \geq 0$ for $(X, Z) \in \Omega$,

where $A = D_Z G(X^*, 0)$ is the an M-matrix (the off diagonal elements of A are region where the model makes biological sense.

The following theorem holds if the above system satisfies the above two conditions :

Theorem 2.3. *: The fixed point $U_0 = (x^*, 0)$ is a globally asymptotic stable $(g.a.s)$ equilibrium of (2.3.6) above provided that $R_0 < 1$ is a locally asymptotic stable $(l.a.s)$ and that assumptions $(H1)$ and $(H2)$ are satisfied.*

2.3.6 Sensitivity Analysis

To determine how best to reduce cholera infection it is important to understand the relative importance of the various factors responsible for its transmission and prevalence. However disease transmission is direct in relation to R_0. R_0 helps researchers to assess the key parameters of the model. Most often sensitivity analysis is used to determine the robustness of model predictions to parameters because it is easy to make errors in data collection and presumed parameter values. In fact it is used to find parameters that have a high impact on the reproductive number and that will assist in intervention strategies. Sensitivity allows us to find the relative change in the variable to the relative change in the parameter and when the variable is a differentiable function of the parameter , the sensitivity index may be defined through the use of partial derivatives (Chitinis *et al.*, 2008).

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Chapter 3

Development of immunological cholera model

There are many cell types which are involved in the guidelines of the immune system and almost hundreds of soluble mediators and different receptor–ligand interactions. Integrated view of immune system, qualitative and quantitative is needed to develop models that look at the immune system (Perelson, 2002). The dynamics between infections and the immune system involve many different components and are multi-factorial. Hence, the principles governing the dynamics and the outcome of infection cannot be understood through verbal or graphical reasoning. Mathematical models provide an essential tool to capture a set of assumptions and to follow them to their precise logical conclusions. They allow us a chance to find new hypotheses, suggest experiments, and measure crucial parameters (Wodarz & Martin, 2002).

In this chapter we develop a mathematical model of cholera immunology to assess the transmission dynamics of *V. cholerae* inside the human host, taking into consideration factors related to *V. cholerae* and the host and also immune system as a dynamic process. We take into consideration what makes *V. cholerae* replicate after passing through stomach gastric acid. We should also note the importance of how the antigen presenting cells stimulates the immune system. Immune response to *V. cholerae* consists of three major steps. The first one is replication of *V. cholerae* in the small intestines which is followed by awareness of the specific immunity; the second step is how the immunity takes control of *V. cholerae* and lastly the clearance of this pathogen and also the generation of the memory cells. For model formulation, we consider six ordinary differential equations that describe the dynamics of *V. cholerae* in the host V_H, the Immune cells I, Cholera toxin T, Gastric acid G_J, Memory cells M and lastly water inside the human body W_H.

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3.1 Assumptions of the model

1. It is assumed that individuals have consumed contaminated water or food with an infective dose of *V. cholerae* to cause an infection which is at least 10^8 to 10^{11} (Seas & Gotuzzo, 2000), so that it is sufficient to overcome innate immune defences, and hence express virulence factors to colonize the small intestines and also coordinate an exit from the host in order for transmission to continue (Schild *et al.*, 2008).

2. It is also assumed that an individual has ordinary stomach acid which serves as a first line of defence against cholera. Those who have low levels of acid are at a greater risk of getting cholera as gastric acid can kill a large number of *V. cholerae*.

3. It is assumed that *V. cholerae* ingested will secrete cholera toxin enough to cause diarrhoea.

3.2 Mathematical Model

In order to develop a model we consider the following classes. The first one is V_H the bacterium *V. cholerae* inside the human host, this is after an individual has consumed water or food contaminated by *V. cholerae*. I the immune cells of the human host. Upon arrival in the intestines the bacteria secrete the cholera toxin, T which is the main cause of the diarrhoea. G_J gastric juice which the stomach releases aids in digestion in the stomach, and G_0 the initial gastric acid. The activated B cells differentiate to plasma cells and memory cells M which they will wait for reinfection, W_H represents water in the human body . The model is then governed by the following system of six ordinary differential equations.

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$$\begin{cases} \dfrac{dV_H}{dt} = \rho V_H(1-\dfrac{V_H}{\kappa}) - \phi_V G_J V_H - \phi_I I V_H - \mu_V V_H \\ \dfrac{dI}{dt} = \Lambda_I + \rho_I I V_H - (\mu_I + \alpha_I) I - \gamma I V_H \\ \dfrac{dT}{dt} = \beta V_H - \rho_T I T - \mu_T T \\ \dfrac{dG_J}{dt} = G_0 + \sigma G_J V_H - \mu_J G_J \\ \dfrac{dM}{dt} = \alpha_I - \mu_M M \\ \dfrac{dE}{dt} = \Lambda_E - \rho_E E T - \mu_E E \\ \dfrac{dW_H}{dt} = \Lambda_W + \rho_E N_E E T - \mu_W W_H \end{cases} \tag{3.2.1}$$

The model system (3.2.1) represents the dynamics of *V. cholerae* inside the human body and the rate of change of *V. cholerae* over time and the interactions of *V. cholerae* with the host immune system. The first equation gives the dynamics of *V. cholerae* where-in the first term

$$\rho V_H(1-\frac{V_H}{\kappa})$$

is the logistic growth of the *V. cholerae* inside the human host, ρ is the growth rate of cholera bacteria and κ is the carrying capacity and $\kappa \geq V_H$. The second term is amount of *V. cholerae* which are being killed by gastric acid in the human body, and the third term presents the amount of *V. cholerae* which are being killed by the immune system whilst the last term gives the natural decay of the *V. cholerae*.

The second equation of the model system above presents the rate of change of immune cells over time after the availability of the bacteria. The first term is the supply of the immune cells at a rate Λ_I and the second term describes the stimulation of the immune system that adaptive immune forces of antibody-mediated immunity (AMI) and cell-mediated immunity (CMI) are brought into play during the presentation of bacterial antigens to the immunological system. The third term is the natural decay of the immune system at the rate μ_I plus 10% of activated cells which become memory cells at a rate α_I. The last term $\gamma I V_H$ presents the immune system exhaustion during encounter with *V. cholerae*. This process happens

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when macrophages are activated by the presence of the *V. cholerae* they present it to T cells and the T cells produces cytokines in order to activate the B cells, activated B cells differentiate to plasma and memory cells wherein plasma cells will produce different kinds of antibodies such as SIgA, of which they will bind to the bacteria to neutralise or immobilise.

The third equation of the system describes the rate of change over time of cholera toxin. The first term is the rate of the production of cholera toxin by *V. cholerae*, the second term is the rate of the toxin which becomes ineffective due to antitoxin produced by B cells. The last term of the equation is the natural decay at rate μ_T of the cholera toxin. The fourth equation describes the rate of change over time of the gastric juice (G_J) in the human body, wherein G_0 is the initial gastric acid, the second term $\sigma G_J V_H$, is the rate of the gastric juice secreted upon ingested contaminated water and the last term $\mu_J G_J$ presents the natural decay of gastric juice.

The fifth equation gives the rate of change over time of the memory cells M. The first term $\alpha_I I$ models the rate at which the memory cells are produced by the immune system, B cells to be specific. After the encounter with the *V. cholerae*, the memory cells wait for reinfection by the *V. cholerae*. The last term is the natural decay at a rate μ_m of the memory cells. The last equation is the equation of the epithelial cells wherein the first term gives the supply of epithelial cells, the second term is the reduction of these cells and the last term $\mu_E$gives the decay rate of the epithelial cells. The last equation shows the rate of change over time of water inside the human body. The first term Λ_W is the supply of water within the body, the second term $\rho_E N_E ET$ is the addition rate of water within an individual due to the damage of epithelial cells caused by toxin and the last term is the loss of water through sweat and urine. The following is the summary of variable and the parameters used on the model.

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Parameter	Description
Λ	Replication rate of Vibrios
μ_v	Death rate of vibrios due to natural death.
ϕ_v	Death of vibrios due to gastric acid
Λ_I	Supply rate of immune cells
μ_I	Natural decay rate of immune cells
γ	Exhaustion rate of activated immune cells
β	Rate of cholera toxin production
ρ_I	Multiplication rate of immune cells during encounter with vibrios
ρ_T	Rate of cholera toxin becoming ineffective by immune cells
μ_T	Natural decay of cholera toxin
σ	Rate of secretion of gastric acid upon ingesting of food and water.
μ_J	Natural decay of gastric juice.
μ_M	Natural decay of memory cells
Λ_E	Supply rate of epithelial cells
ρ_E	Rate for water added within due to the damaged cells.
μ_E	Natural decay rate of epithelial cells.
Λ_W	Supply of water
ρ_W	Loss of water due to the amount cholera toxin
μ_W	Loss of water rate through sweat and urine

Table 3.1: Description of parameter value

3.3 Basic Properties

3.3.1 Positivity of solutions

We let the initial condition be positive, thus $V_H(0) = V_0 \geq 0, I(0) = I_0 \geq 0, T(0) = T_0 \geq 0, G_J(0) = G_0 \geq 0, M(0) = M_0 \geq 0, E(0) = E_0 \geq 0$ and $W_H(0) = W_0 \geq 0$ for all $t \geq 0$ since we are modelling disease.

$$\frac{dV_H}{dt} = \rho V_H \left(1 - \frac{V_H}{\kappa}\right) - \phi_V G_j V_H - \phi_I I V_H - \mu_V V_H \tag{3.3.2}$$

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Then

$$\frac{dV_H}{dt} \geq (-\phi_V G_j - \phi_I I - \mu_V) V_H \tag{3.3.3}$$

separation of variables yields

$$\frac{dV_H}{V_H} \geq (-\phi_V G_j - \phi_I I - \mu_V) dt \tag{3.3.4}$$

integrating both sides

$$\int \frac{dV_H}{V_H} \geq -\int (\phi_V G_j + \phi_I I + \mu_V) dt \tag{3.3.5}$$

which gives

$$lnV_H \geq -\left(\mu_V(t) + \int_0^t (\phi_V G_j(t) + \phi_I I)\, dt\right) + C \tag{3.3.6}$$

C is a constant of integration.

Taking exponents both sides gives

$V_H \geq e^C \bar{E}$ where $\bar{E} = e^{-(\mu_V(t) + \int_0^t (\phi_V G_J + \phi_I I) d_t}$

Therefore V_H is always positive for all $t \geq 0$. The same approach can be applied for the remaining equations and they will show that they are positive for all $t \geq 0$.

We can also show for the second equation of (3.2.1), thus,

$$\frac{dI}{dt} = \Lambda_I + \rho_I I V_H - (\mu_I + \alpha_I) I - \gamma I V_H \tag{3.3.7}$$

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then

$$\frac{dI}{dt} \geq [(\rho_I - \gamma)V_H - (\mu_I + \alpha_I)]I$$

by separation of variables we have

$$\frac{dI}{I} \geq [(\rho_I - \gamma)V_H - (\mu_I + \alpha_I)]dt \tag{3.3.8}$$

Integrating both sides yields

$$\int \frac{dI}{I} \geq \int [(\rho_I - \gamma)V_H - (\mu_I + \alpha_I)]\, dt \tag{3.3.9}$$

$$\ln I \geq -(\mu_I + \alpha_I)(t) + \left(\int_0^t (\rho_I - \gamma)V_H \right) dz + C \tag{3.3.10}$$

Taking exponents

$$I \geq Be^{-(\mu_I+\alpha_I)(t)+\int_0^t((\rho_I-\gamma)V_H)dz}, \tag{3.3.11}$$

where $B = e^C$, It can be seen that I is always positive for all $t \geq 0$.

Positivity of solution can also be shown for the last equation of (3.2.1),thus

$$\begin{cases} \dfrac{dW_H}{dt} = \Lambda_W - \rho_W T W - \mu_W W_H \\ \dfrac{dW_H}{dt} \geq -(\rho_W T + \mu_W) W_H \end{cases} \tag{3.3.12}$$

Separation of values gives

$$\frac{dW_H}{W_H} \geq -(\rho_W T + \mu_W)\, dt \tag{3.3.13}$$

We integrate both sides as follows,

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$$\int \frac{dW_H}{W_H} \geq - \int_0^t (\rho_W T - \mu_W)\, dt \tag{3.3.14}$$

$$\ln W_H \geq \mu_W(t) - \int_0^t \rho_W T dt + C \tag{3.3.15}$$

taking exponents we get

$$W_H \geq D e^{\mu_W(t) - \int_0^t \rho_W T dt} \tag{3.3.16}$$

where

$$D = e^C$$

3.3.2 The boundedness of the model

The Boundedness of the system of equations implies that the system is biologically well-behaved. Then we must show the biological validity of the model by finding the boundedness of the solution of the model.

Theorem 3.1. *All solutions of the system of equations (3.2.1) are uniformly bounded.*

Let us consider the second equation of the model ,thus

$$\begin{aligned} \frac{dI}{dt} &= \Lambda_I + \rho_I I V_H - (\mu_I + \alpha_I) I - \gamma I V_H \\ &= \Lambda_I + (\rho_I - \gamma) I V_H - (\mu_I + \alpha_I) I \\ &\leq \Lambda_I - (\mu_I + \alpha_I) I \end{aligned}$$

Therefore

$$\frac{dI}{dt} + (\mu_I + \alpha_I) I \leq \Lambda_I \tag{3.3.17}$$

Integrating factor is $e^{\int (\mu_I + \alpha_I) dt} = e^{(\mu_I + \alpha_I)t}$, multiplying both sides of (3.3.17) we obtain,

$$\frac{dI}{dt} e^{(\mu_I + \alpha_I)t} + \mu I e^{(\mu_I + \alpha_I)t} \leq \Lambda_I e^{(\mu_I + \alpha_I)t}$$

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Integrating both sides

$$\int d\left[Ie^{(\mu_I+\alpha_I)t}\right] \leq \int \Lambda_I e^{(\mu_I+\alpha_I)t}dt \tag{3.3.18}$$

$$Ie^{(\mu_I+\alpha_I)t} \leq \frac{\Lambda_I e^{(\mu_I+\alpha_I)t}}{(\mu_I+\alpha_I)} + C \tag{3.3.19}$$

where C is a constant of integration, therefore

$$\lim_{t\to\infty}(sup(I(t)) \leq \frac{\Lambda_I}{\mu_I+\alpha_I} + Ce^{-(\mu_I+\alpha_I)t} \tag{3.3.20}$$

which implies that

$$\lim_{t\to\infty} \sup\left(I\left(t\right)\right) \leq \frac{\Lambda_I}{\mu_I+\alpha_I} \tag{3.3.21}$$

also implies that the solution is bounded for $0 \leq I\left(t\right) \leq \frac{\Lambda_I}{\mu_I+\alpha_I}$.

Now we consider the following equation:

$$\frac{dM}{dt} = \alpha_I - \mu_M M \tag{3.3.22}$$

This is equals to :

$$\frac{dM}{dt} + \mu_M M = \alpha_I I \tag{3.3.23}$$

Integrating factor is $e^{\mu_M t}$ and multiplying the above we obtain:

$$\frac{dM}{dt}e^{\mu_m t} + \mu_m M e^{\mu_m t} = \alpha_I e^{\mu_m t} I \tag{3.3.24}$$

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Then

$$\frac{d}{dt}Me^{\mu_m t} = \alpha_I e^{\mu_m t} I \tag{3.3.25}$$

Integrating both sides of (3.3.10) we obtain

$$Me^{\mu_m t} = \frac{\alpha_I e^{\mu_m t} I}{\mu_m} + C. \tag{3.3.26}$$

Dividing both sides of (3.3.26) by $e^{\mu_m t}$ we get:

$$M = \frac{\alpha_I I}{\mu_m} + Ce^{-\mu_m t} \tag{3.3.27}$$

Therefore

$$\lim_{t\to\infty} \sup\left(M\left(t\right)\right) = \frac{\alpha_I I}{\mu_m} \tag{3.3.28}$$

But

$$I\left(t\right) \leq \frac{\Lambda_I}{\mu_I + \alpha_I} \tag{3.3.29}$$

Therefore

$$M(t) \leq \frac{\Lambda_I \alpha_I}{\mu_m(\mu_I + \alpha_I)} \tag{3.3.30}$$

Therefore it can be shown that all solutions of (3.2.1) in R_+^7 are bounded in the region

$$\begin{cases} \Omega = (V_H(t), I(t), T(t), G_J(t), M(t), E(t), W_H(t)) \in \mathbb{R}_6^+ : \\ 0 \leq V_H \leq \dfrac{\kappa}{\rho}\left(\rho - \mu_V\right), \ 0 \leq I \leq \dfrac{\Lambda_I}{\mu_I + \alpha_I}, \ 0 \leq T \leq \dfrac{\beta}{\mu_T}, \ 0 \leq G_J \leq \dfrac{G_0}{\mu_J}, \\ 0 \leq M \leq \dfrac{\Lambda_I \alpha_I}{\mu_M(\mu_I + \alpha_I)}, 0 \leq E \leq \dfrac{\Lambda_E}{\mu_E}, 0 \leq W_H \leq \dfrac{\Lambda_W}{\mu_W}. \end{cases} \tag{3.3.31}$$

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3.4 The Disease Free Equilibrium and its Stability

Disease-free equilibrium depends on V_H and T.Thus if $V_H = T = M = 0$, then the population is said to be free of cholera. To find the DFE of the system of equations (3.2.1) , we put these equations equal to zero. This means that at DFE there is no cholera bacterium. Thus the DFE will be given by,

$$\begin{aligned} E_0 &= [V_H, I, T, G_J, M, E, W] \\ &= \left[0, \frac{\Lambda_I}{\mu_I + \alpha_I}, 0, \frac{G_0}{\mu_J}, 0, \frac{\Lambda_E}{\mu_E}, \frac{\Lambda_W}{\mu_W}\right]. \end{aligned}$$

3.4.1 The Reproductive Number (R_0)

The reproductive number R_0 defined as the average number of secondary infections produced by single infectious host, introduced into a totally susceptible population. The most important tool in the analysis of disease outbreak. For most disease outbreaks, if $R_0 < 1$, then the outbreak will disappear with time, whereas if $R_0 > 1$, the outbreak will persist at endemic levels. The basic reproduction number of the system is calculated by using next generation operator described in Castillo-Chávez et al.,(2002). Thus the system can also written in the form

$$\begin{cases} \frac{dX}{dt} = f(X, Y, Z), \\ \frac{dY}{dt} = g(X, Y, Z), \\ \frac{dZ}{dt} = h(X, Y, Z). \end{cases} \tag{3.4.32}$$

Where

- $X = (I, G_J, M, E, W_H)$ represent all compartment which are not infected.
- $Y = (T)$ represent compartment which is not capable of infecting.
- $Z = (V_H)$ represent compartment which is infected and capable of infecting.

We also let the disease free-equilibrium of the model to be denoted by the following expression

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$$\bar{U}_0 = \left[0, \frac{\Lambda_I}{\mu_I + \alpha_I}, 0, \frac{G_0}{\mu_J}, 0, \frac{\Lambda_E}{\mu_E}, \frac{\Lambda_W}{\mu_W}\right] \tag{3.4.33}$$

In this case

$$\tilde{g}(X^*, Z) = (\tilde{g}_1(X^*, Z),) \tag{3.4.34}$$

Having that

$$h(X, Y, Z) = (h_1(X, Y, Z)) \tag{3.4.35}$$

with

$$h_1(X, Y, Z) = \rho V_H - \frac{\rho V_H^2}{k} - \phi_V G_J V_H - \phi_I I V_H - \mu_V V_H \tag{3.4.36}$$

Now as V_H approaches zero

$$A = D_Z h(X^*, \tilde{g}(X^*, 0), 0) = \rho - (\phi_V G_J + \phi_I I + \mu_V) \tag{3.4.37}$$

This can be expressed in the form

$$A = M - D \tag{3.4.38}$$

so that

$$M = \rho \tag{3.4.39}$$

and

$$D = (\phi_V G_J + \phi_I I + \mu_V) \tag{3.4.40}$$

Since the reproductive number is given by

$$R_0 = MD^{-1}. \tag{3.4.41}$$

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Therefore

$$R_0 = \frac{\rho}{\phi_V G_J + \phi_I I + \mu_V} \tag{3.4.42}$$

which ca be expressed as

$$R_0 = \frac{\rho\mu_J(\mu_J + \alpha_I)}{\phi_V(\mu_I + \alpha_I)G_0 + \mu_J\phi_I\Lambda_I + \mu_V\mu_J(\mu_I + \alpha_I)} \tag{3.4.43}$$

Considering the expression of the basic reproductive number above we make the following deductions. The growth rate of the Vibrios within the human host has a very crucial role in cholera disease. Reduction of the bacteria either by gastric acid, Immune cells or natural death can help an individual in fighting cholera disease.

3.4.2 Local stability of the Disease Free Equilibrium

The stability of DFE can be obtained by eigenvalues of the Jacobian matrix of the linearized system.

To determine the local stability we evaluate the Jacobian matrix at disease-free equilibrium point

$$E_0 = \left[0, \frac{\Lambda_I}{(\mu_I + \alpha_I)}, 0, \frac{G_0}{\mu_J}, 0, \frac{\Lambda_E}{\mu_E}, \frac{\Lambda_W}{\mu_W}\right]. \tag{3.4.44}$$

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The Jacobian matrix at DFE is given by

$$J(E_0) = \begin{bmatrix} -b_1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{(\rho_I - \gamma)\Lambda_I}{(\mu_I + \alpha_I)} & -(\mu_I + \alpha_I) & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -b_2 & 0 & 0 & 0 & 0 \\ \frac{\delta G_0}{\mu_J} & 0 & 0 & -\mu_J & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -\mu_M & 0 & 0 \\ 0 & 0 & -\frac{\rho_E \Lambda_E}{\mu_E} & 0 & 0 & -\mu_E & 0 \\ 0 & 0 & \frac{-\rho_E N_E \Lambda_E}{\mu_E} & 0 & 0 & 0 & -\mu_W \end{bmatrix} \tag{3.4.45}$$

where

$$\begin{cases} b_1 = \phi_V \dfrac{G_0}{\mu_J} + \dfrac{\phi_I \Lambda_I}{(\mu_I + \alpha_I)} + \mu_V - \rho \\ b_2 = -(\dfrac{\rho_T \Lambda_T}{\mu_I + \alpha_I} + \mu_T) \end{cases} \tag{3.4.46}$$

If all eigenvalues of the Jacobian matrix are negative then we conclude the locally asymptotic stability and if one of the eigenvalues is negative then the equilibrium is unstable. The characteristic equation is given by

$$(-b_1 - \lambda)(-(\mu_I + \alpha_I) - \lambda)(-b_2 - \lambda)(-\mu_J - \lambda)(-\mu_M - \lambda)(-\mu_E - \lambda)(-\mu_W - \lambda) = 0 \tag{3.4.47}$$

It can be seen from the characteristic equation above that there are six eigenvalues. For the determination of local stability we consider equation (3.5.47) above. For b_1, the eigenvalue is negative provided

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$$\phi_V \frac{G_0}{\mu_J} + \frac{\phi_I \Lambda_I}{(\mu_I + \alpha_I)} + \mu_V < \rho \tag{3.4.48}$$

Therefore all the roots of the polynomial are either negative or have negative real parts. This proves the local asymptotic stability.

3.4.3 Global stability of DFE

We use the next generation operator to determine the global stability, we then present our system of equations as follows :

$$\begin{cases} \dfrac{dX}{dt} &= F(X,Z), \\ \dfrac{dY}{dt} &= G(X,Z) \end{cases} \tag{3.4.49}$$

where

- $X = (I, G_0, M, E, W)$ represents all uninfected components, and
- $Z = (V_H, T)$ represents infectious components.

The following represent the disease-free equilibrium of the system. The following conditions should be met X^* to be globally asymptotic stable (g.a.s).

H1. for $\dfrac{dX}{dt} = F(X,0)$ is globally asymptotically stable ,

H2. $G(X,Z) = AZ - \hat{G}(X,Z)$, $\hat{G}((X,Z) \geq 0$ for $(X,Z) \in \mathbb{R}^7_+$ where $A = D_Z G(X^*, 0)$ is an M-matrix and $\mathbb{R}^7_+$ and is the region where the model have a biological meaning.

now we have

$$F(X,0) = \begin{bmatrix} \Lambda_I - \mu_I \\ G_0 - \mu_J G_J \\ 0 \\ \Lambda_E - \mu_E E \\ \Lambda_W - \mu_W W \end{bmatrix}, \tag{3.4.50}$$

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and

$$A = \begin{bmatrix} \rho - \phi_V G_J - \phi_I - \mu_V & 0 \\ \beta & -\rho_T I - \mu_T \end{bmatrix} \tag{3.4.51}$$

Therefore

$$AZ = \begin{bmatrix} \rho - \phi_V G_J - \phi_I - \mu_V & 0 \\ \beta & -\rho_T I - \mu_T \end{bmatrix} \tag{3.4.52}$$

and

$$\hat{G}(X, Z) = \begin{bmatrix} \frac{\rho V_H}{\kappa} \\ \\ 0 \end{bmatrix} \tag{3.4.53}$$

It can be seen that $\hat{G}(X, Z) \geq 0$ for all $(X, Z) \in \mathbb{R}^7_+$. It is also clear that matrix A is M-matrix, we therefore conclude by stating the following theorem which summarises the results found.

Theorem 3.2. *The fixed point*

$$U_0 = (X^*, 0) = E_0 = (0, \frac{\Lambda_I}{(\mu_I + \alpha_I)}, 0, \frac{G_0}{\mu_J}, 0, \frac{\Lambda_E}{\mu_E}, \frac{\Lambda_W}{\mu_W}.) \tag{3.4.54}$$

is globally asymptotically stable equilibrium of model system (3.2.1) if $R_0 \leq 1$ and then assumptions $(H1)$ and $(H2)$ are satisfied.

3.5 The endemic equilibrium point

At endemic equilibrium state *V. cholerae* are ingested in large amount and they cause diarrhoea in the human host and are shed to the water again. We equate the derivatives of the equation systems (3.2.1) to zero and solve the system algebraically. At endemic V_H, T and M are not equal to zero. All the variables of our model system are expressed in terms of V_H. We denote the endemic points of our system of equations by :

$$E_1 = (V_H^*, I^*, T^*, G_J^*, M^*, E^*, W_H^*,) \tag{3.5.55}$$

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The value of the immune system at endemic equilibrium point is given by;

$$I^* = \frac{\Lambda_I}{(\mu_I + \alpha_I) + (\gamma - \rho_I)V_H^*} \tag{3.5.56}$$

We learn that at endemic state when $\rho_I < \gamma$, the immune system is inversely proportional to the supply of immune cells, the natural decay rate μ_I of the immune cells , the exhaustion rate γ of the immune cells and the stimulation rate ρ_I by the presence of *V. cholerae* in the body for ρ_I less than γ. The value of the gastric acid at endemic is given by,

$$G_J^* = \frac{G_0}{\mu_J - \sigma V_H^*} \tag{3.5.57}$$

The above equation defines the value of the gastric acid found in the human host. The expression requires that $\mu_J > \sigma V_H$ in order for this expression to have biological meaning. This implies that the stimulation of gastric acid due to the arrival of *V. cholerae* is less than the natural decay of gastric acid. At the endemic equilibrium state the cholera toxin is shown by the following expression,

$$T^* = \frac{[(\beta\mu_I + \alpha_I) + \beta(\gamma - \rho_I)]V_H^*}{\rho_I\Lambda_I + \mu_T[\mu + (\gamma - \rho_I)]V_H^*} \tag{3.5.58}$$

The amount of cholera toxin at endemic is inversely proportional to the amount of *V. cholerae* ingested provided $\rho_I < \gamma$, the immune cells recruited, the amount of toxin which runs ineffective naturally and also cholera toxin which runs ineffective through the secretion of the antibodies. The memory cells at endemic state are given by

$$M^* = \frac{\alpha_I}{\mu_M} \tag{3.5.59}$$

Tt endemic state memory cells are inversely proportional to the activated immune cells after the ingestion of the *V. cholerae* and the natural decay of the memory cells. About 10 per cent of activated immune

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cells become memory cells. The following is the equation for epithelial cells at endemic,

$$E^* = \frac{\Lambda_E}{\rho_E T + \mu_E} \tag{3.5.60}$$

During endemic times, the epithelial cells are inversely proportional the supply of epithelial cells, the rate of damage of cells caused by cholera toxin.

The water in the body during the endemic is given by

$$W_H^* = \frac{(\Lambda_W + N_E\Lambda_E)\rho_E T + \mu_E}{(\rho_E T + \mu_E)\mu_W} \tag{3.5.61}$$

The above equation represents water inside the human body during a cholera outbreak. Water is inversely proportional to the water supplied by the human body following the fact that 60% of the human weight is water, the water released by damaged epithelial cells and the natural loss of water through sweat and urine. The following is the *V. cholerae* in the endemic state.

$$V_H^* = \frac{(\rho - \phi_V G_J + \phi_I I + \mu_V)k}{\rho} \tag{3.5.62}$$

At endemic times, *V. cholerae* is inversely proportional to the product of growth rate of vibrios, their death rate due to gastric acid, immune cells, natural decay and the carrying capacity.

3.6 Sensitivity Analysis of R_0

In the model we apply the following definition : The normalised forward sensitivity index of a variable, u, that depends differentiably on a parameter, p, is defined as :

$$A_\rho^u = \frac{\partial u}{\partial p} \times \frac{p}{u}$$

The sensitivity indices of R_0 with respect to each parameter are as follows :

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$$\begin{aligned} A_{\phi_v}^{R_0} &= \frac{\partial R_0}{\partial \phi_v} \times \frac{\phi_v}{R_0} \\ &= \frac{G_0}{\rho\mu_J} \times \frac{\phi_v}{\phi_V \frac{G_0}{\rho\mu_J} + \frac{\phi_I\Lambda_I}{\rho\mu} + \frac{\mu_V}{\rho}} \\ &= \frac{\phi_v\mu G_0}{\phi_v\mu G_0 + \mu_J\phi_I\Lambda_I + \mu_v\mu_J\mu} \end{aligned}$$

$$\begin{aligned} A_{\rho}^{R_0} &= \frac{\partial R_0}{\partial \rho} \times \frac{\rho}{R_0} \\ &= -\frac{1}{\rho^2}\left(\frac{\phi_V G_0}{\mu_J} + \frac{\phi_I\Lambda_I}{\mu} + \mu_V\right) \times \frac{\rho^2\mu_J\mu}{\mu\phi_v G_0 + \mu_J\phi_I\Lambda_I + \mu_J\mu_v\mu} \\ &= \frac{\mu\phi_v G_0 + \mu_J\phi_I\Lambda_I + \mu_J\mu_v\mu}{\mu_J\mu} \times \frac{\mu_J\mu}{\mu\phi_v G_0 + \mu_J\phi_I\Lambda_I + \mu_J\mu_v\mu} \\ &= -1 \end{aligned}$$

$$\begin{aligned} A_{\mu_J}^{R_0} &= \frac{\partial R_0}{\partial \mu_J} \times \frac{\mu_J}{R_0} \\ &= -\frac{\phi_v G_0\mu_J^{-2}}{\rho} \times \frac{\mu_J^2\rho\mu}{\mu\phi_v G_0 + \mu_J\phi_I\Lambda_I + \mu_J\mu_v\mu} \\ &= -\frac{\mu\phi_v G_0}{\phi_v G_0 + \mu_J\phi_I\Lambda_I + \mu_J\mu_v\mu} \end{aligned}$$

$$\begin{aligned} A_{\phi_I}^{R_0} &= \frac{\partial R_0}{\partial \phi_I} \times \frac{\phi_I}{R_0} \\ &= \frac{\Lambda_I}{\rho\mu} \times \frac{\phi_I\rho\mu\mu_J}{\mu\phi_v G_0 + \mu_J\phi_I\Lambda_I + \mu_J\mu_v\mu} \\ &= \frac{\Lambda_I\phi_I\mu_J}{\mu\phi_v G_0 + \mu_J\phi_I\Lambda_I + \mu_J\mu_v\mu} \end{aligned}$$

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$$\begin{aligned} A_{\Lambda_I}^{R_0} &= \frac{\partial R_0}{\partial \Lambda_I} \times \frac{\Lambda_I}{R_0} \\ &= \frac{\phi_I}{\rho\mu} \times \frac{\Lambda_I \rho \mu \mu_J}{\mu\phi_v G_0 + \mu_J \phi_I \Lambda_I + \mu_J \mu_v \mu} \\ &= \frac{\phi_I \Lambda_I \mu_J}{\mu\phi_v G_0 + \mu_J \phi_I \Lambda_I + \mu_J \mu_v \mu} \end{aligned}$$

$$\begin{aligned} A_{\mu}^{R_0} &= \frac{\partial R_0}{\partial \mu} \times \frac{\mu}{R_0} \\ &= -\frac{\phi_I \Lambda_I \mu^{-2}}{\rho} \times \frac{\mu^2 \mu_J}{\mu\phi_v G_0 + \mu_J \phi_I \Lambda_I + \mu_J \mu_v \mu} \\ &= -\frac{\phi_I \Lambda_I \mu_J}{\mu\phi_v G_0 + \mu_J \phi_I \Lambda_I + \mu_J \mu_v \mu} \end{aligned}$$

$$\begin{aligned} A_{\mu_v}^{R_0} &= \frac{\partial R_0}{\partial \mu_v} \times \frac{\mu_v}{R_0} \\ &= \frac{1}{\rho} \times \frac{\rho \mu_v \mu_J \mu}{\mu\phi_v G_0 + \mu_J \phi_I \Lambda_I + \mu_J \mu_v \mu} \\ &= \frac{\mu_J \mu_v \mu}{\mu\phi_v G_0 + \mu_J \phi_I \Lambda_I + \mu_J \mu_v \mu} \end{aligned}$$

3.7 Parameter values and their sensitivity

Mathematical modelling of many diseases of immunology is limited because of lack of knowledge of the biological system being studied. and also the numerical values of some or all of the model parameters may be unknown and, more importantly, the structure of the model itself may be unknown. If the structure of the model is known, it is still possible to describe the qualitative features of the model that would be seen with different parameter values. However, if the model is uncertain, then it is not easy to make predictions (Jakeman *et al*, 2006).

The most sensitive parameter is the natural decay of immune system which is given by $\Upsilon_{\mu_I}^{R_0} = -1,515$, when μ_I increases by 10% the basic reproductive number is decreased by 10%. If the natural decay of immune system is too high there will be severe cases of cholera in the community. We also learn that $\Upsilon_{\rho}^{R_0} = -1$, reducing the growth rate of *V. cholerae* inside the human host reduces the amount of cholera

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Parameter	Value	Sensitivity Index
ϕ_V	0.035	0.091
ρ	0.8	-1
μ_J	0.006	0.3
ϕ_I	0.010	0.9567
Λ_I	3000	0.9567
μ_I	1.1	-1.515
μ_V	0.0685	0.00005

Table 3.2: Sensitivity Indices of model reproduction number R_0

.

toxin, thus reducing the diarrhoea. Therefore people must have a normal gastric acid levels, as this will lead to a large amount of *V. choleare* ingested being killed before their attachment and replication. The parameters μ_I should be given more attention in order to minimise or eradicate the cholera disease. If people have a strong immune system the life span of *V. cholerae* inside the human host will be very limited because some vibrios will be killed by gastric juices and some will be killed by immune cells.

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ϕ_V	0.035	0.091
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limited because some vibrios will be killed by gastric juices and some will be killed by immune cells.

3.8 Numerical Simulations

In this section we provide some numerical simulations to show the dynamics of model (3.2.1). We solved the equations of the basic model numerically. Graphs of the numerical solution are used to understand the effects of some parameters of our model (3.1-3.5). In order to perform this task we used the estimated parameter values given in Tables 3.4 below because of lack of published literature. The system model (3.2.1) was solved numerically using a Python programme version V 2.6 on the linux operation system (Ubuntu 14.04). The programme uses a package odeint function in the scipy.integrate for solving any system of differentiated equations. The initial conditions used for simulation are given by $V_H(0) = 10000,\ I(0) = 0,\ T(0) = 0,\ G_J(0) = 0\ M(0) = 0$ and $W_H(0) = 0$.

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Parameter	Description	Initial values	Units	Source
ρ	Vibrios growth rate	0.9	day^{-1}	Estimated
κ	Concentration rate of vibrios	0.03	day^{-1}	Estimated
μ_v	Natural death rate	0.001	day^{-1}	Estimated
ϕ_v	Vibrios death rate due to gastric	0.1	day^{-1}	Estimated
ϕ_I	Death rate of vibrios killed by immune cells	0.0010	day^{-1}	Estimated
Λ_I	Supply rate of Immune cells	0.1	day^{-1}	Estimated
μ_I	Natural decay rate of immune cells	0.003	day^{-1}	Estimated
γ	Exhaustion rate of activated immune cells	0.000001	day^{-1}	Estimated
β	Rate of cholera toxin production	0.003	day^{-1}	Estimated
ρ_I	Multiplication rate of immune cells	0.003	day^{-1}	Estimated
ρ_T	Rate of toxin becoming ineffective	0.00025	day^{-1}	Estimated
μ_T	Natural decay of Toxin	0.0024	day^{-1}	Estimated
σ	Gastric acid secretion rate	0.0004	day^{-1}	Estimated
G_0	Initial gastric juice	3	day^{-1}	Estimated
μ_J	Natural decay of gastric juice	0.000005	day^{-1}	Estimated
μ_M	Natural decay of memory cells	0.0001	day^{-1}	Estimated
Λ_W	Supply of water	0.97	day^{-1}	Estimated
ρ_W	Loss of water due diarrhoea	0.000072	day^{-1}	Estimated
μ_W	Loss of water through sweat and urine	0.8	day^{-1}	Estimated
Λ_E	Supply rate of epithelial cells	0.005	day^{-1}	Estimated
ρ_E	Damaged rate of epithelial cells	0.0003	day^{-1}	Estimated
N_E	Water released by damaged epithelial cells	0.8	day^{-1}	Estimated

Table 3.4: Estimated human host parameter values used in simulation

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3.9 Results

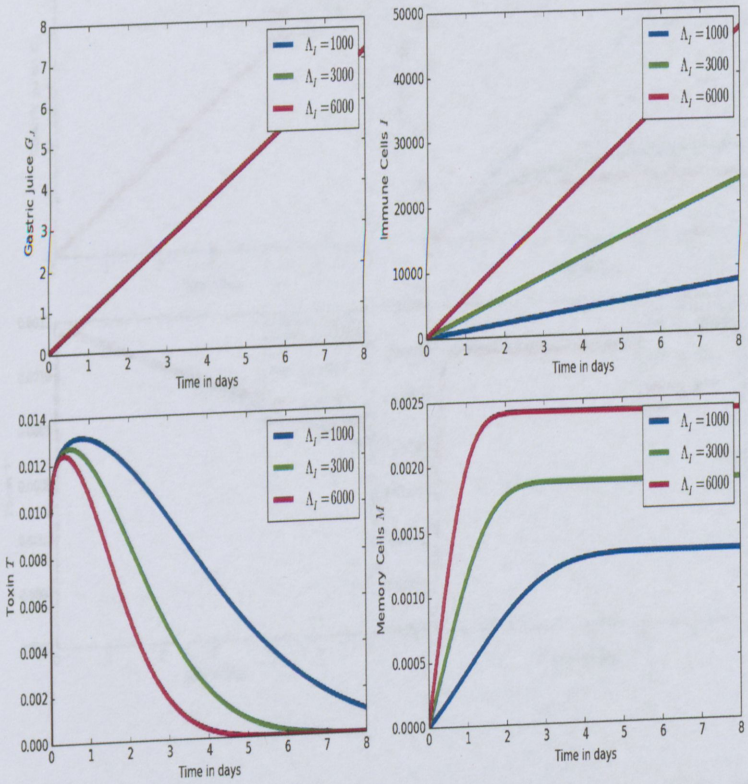


Figure 3.1: *Graphs showing the amount of Gastric Juice inside the human host over a period of time, the amount of Immune cells I,upon arrival of the V_H,Toxin T secreted and Memory cells M, for different values of $\Lambda_I = 1000, \Lambda_I = 3000$ and $\Lambda_I = 6000$*

From figure 3.1, we observe that the amount of activated immune cells have an effect on cholera toxin T. The effectiveness of toxin is inversely proportional to activated immune cells. When the activated immune cells are 1000, the effectiveness of cholera toxin is high. Increasing the activated immune cells to 6000 the toxin drops drastically. Toxin does not last for a day. About 10% 0f the activated immune cells become memory cells and wait for reinfection. We can see from the graph that they rise at some point they are at constant and wait for a second infection.

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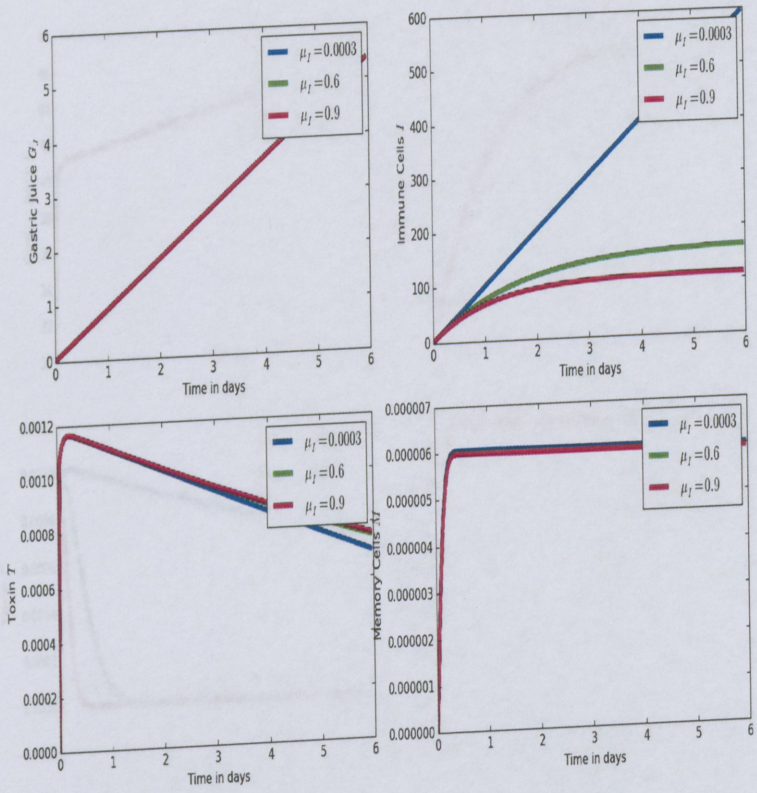


Figure 3.2: *V. Cholerae inside the human host over a period of time. The graphs represent gastric acid G_J, Immune cells I, Toxin T and Memory cells M for a different values of decay rate of immune cells, $\mu_I = 0.0003$, $\mu_I = 0.6$ and $\mu_I = 0.9$.*

Figure 3.2 above shows that the decay of immune cells have an effect on cholera toxin. When immune cells are decaying cholera toxin lasts for a longer period. It means without immune cells the cholera disease will manifest or develop. Memory cells always depend on the activated immune cells, the natural decay of immune cells do not have effect on memory cells.

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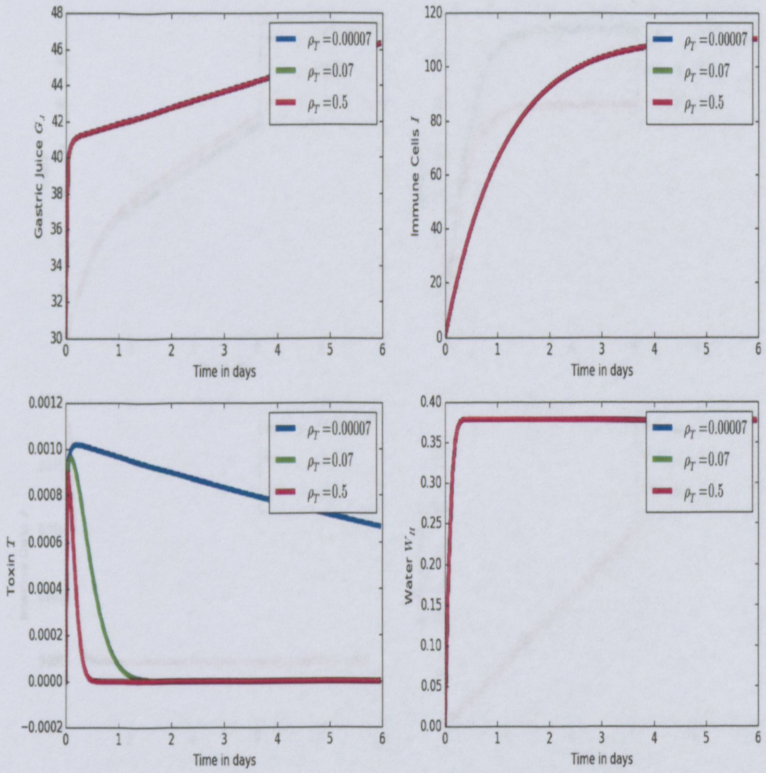


Figure 3.3: *V. Cholerae inside the human host over a period of time.The graphs represent gastric acid G_J, Immune cells I, Toxin T and Water W for a different values of decay rate of toxin, $\rho_T = 0.00005$, $\rho_T = 0.05$ and $\rho_T = 0.7$.*

In figure 3.3 we observe that the decay rate of toxin due to the activation of the immune cells is small and the toxin becomes ineffective very slowly. When the decay rate increases the cholera toxin decreases very fast. If the decay rate of toxin is greater, the toxin becomes ineffective very fast. This means that activated immune cells are important for protecting the human host against cholera.

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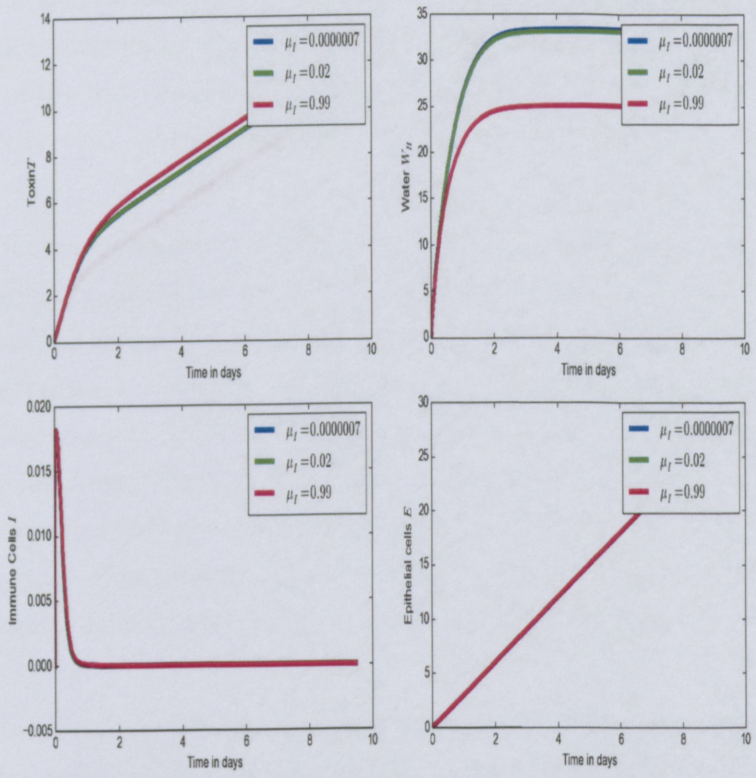


Figure 3.4: *V. Cholerae inside the human host over a period of time. The graphs represent Cholera Toxin T, Immune cells I, Water W_H and Epithelial cells E for a different values of decay rate of immune cells, $\mu_I = 0.0000007$, $\mu_I = 0.02$ and $\mu_I = 0.99$.*

In the above figure we observed that as the decay rate of immune cells due to vibrio cholerae changes, the water volume within an individual also change. As the decay rate of immune cells increases the water levels within the body lowers. This means that the vibrio and toxin overcomes immune cells and it can be seen from the graph of Toxin that it increases or becomes more effective as the decay rate of immune cells increase. This means the diarrhoea will results leading to the loss of water within the body.

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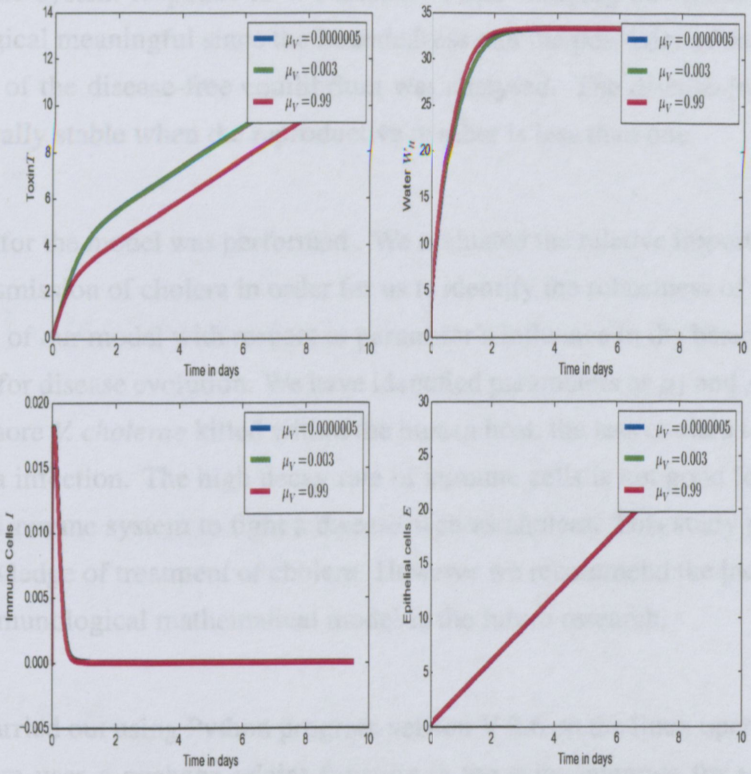


Figure 3.5: *V. Cholerae inside the human host over a period of time. The graphs represent water* W_H*, Immune cells* I*, Toxin* T *and Epithelial cells* M *for a different values of decay rate of vibrios,* $\mu_V = 0.0000005$*,* $\mu_V = 0.003$ *and* $\mu_V = 0.99$*.*

Figure 3.5 above shows different rate of vibrios decay. As the vibrios decay rate increases we observe the water level remain at high level. This indicates that as many vibrios are killed within an individual even the cholera toxin becomes less and as a results there will no diarrhoea or loss of water.

3.10 Discussion and conclusion

An understanding into immune response against pathogens gained a lot of interest from researchers. Mathematical epidemiologists started to incorporate specific features of the pathogens and terms on how the immune system responds when encountering pathogens when developing their models in order to enhance their predictability. A basic cholera immunological model was developed in this study. The

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model was developed with the view of understanding the insights of *V. cholerae* inside the human hosts and how the immune system responds to *V. cholerae*. After studying this model, we have found that the system is biological meaningful since the boundedness and the positivity of solutions of the systems hold. The stability of the disease-free equilibrium was analysed. The disease-free state is locally and globally asymptotically stable when the reproductive number is less than one.

Sensitivity analysis for the model was performed . We evaluated the relative importance of the model parameters in the transmission of cholera in order for us to identify the robustness of the model parameters with the predictions of our model with respect to parameter's influence in the basic reproductive number which is the source for disease evolution. We have identified parameters as μ_I and ρ as the most sensitive to the model. The more *V. cholerae* killed within the human host, the less cholera toxin produced, which results in no cholera infection. The high decay rate of immune cells is not good for individuals. People must have a strong immune system to fight a disease such as cholera. This study presents the emphasis on the existing knowledge of treatment of cholera. However we recommend the incorporation of cholera treatment in this immunological mathematical model in the future research.

Simulations were carried out using Python program version V 2.6 on the linux operation system (Ubuntu 14.04). The program uses a package odeint function in the scipy.integrate for solving any system of differentiated equations. The graphs are in line with sensitivity analysis made. These sensitive parameters will inform policy makers and public health officials to consider new strategies to minimise the infections.

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Chapter 4

Extended cholera immunological model

4.1 Introduction

The immune response to pathogens depends on innate and adaptive components (Hoffmann *et al*, 1999). The innate immune cells consists of cells and proteins that are ready to mobilise and fight microbes during infection. These cells include neutrophils and macrophages, cells which are ready to phagocytes and to kill the pathogens (Aderem & David, 1999) such as *V. cholerae*. We incorporate these cells in the basic model in chapter 3 as phagocytes (P). Regarding macrophages, the pathogen is killed and its components are presented to T-cells and therefore the activation of the adaptive immune response at the same time establishes the protective immunity (Aderem & David, 1999). In this extended model we incorporate this cells as lymphocytes (L).

4.2 The development of the model

In this chapter we present the extended model of the basic immunological cholera model developed in chapter 3. The extended model is introduced in order for us to gain more understanding of immune response against *V. cholerae*. The extension is done by including the phagocytes and lymphocytes cells in the basic cholera model developed in the previous chapter.
The model includes *V. cholerae* as in the basic model, phagocytes, lymphocytes, Cholera toxin, Gastric

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juice, memory cells and water within the human body. The model is then presented as follows :

$$\begin{cases} \frac{dV_H}{dt} = \rho V_H(1-\frac{V_H}{\kappa}) - \phi_I V_H\,(G_J + P + L) - \mu_V V_H \\ \frac{dP}{dt} = \lambda_P + \gamma_1 P V_H - \mu_1 P - \phi_P P V_H \\ \frac{dL}{dt} = \lambda_L + \gamma_2 L V_H - \phi_L L V_H - (\mu_2 + \alpha) L \\ \frac{dT}{dt} = \beta V_H - \rho_T L T - \mu_T T \\ \frac{dG_J}{dt} = G_0 + \sigma G_J V_H - \mu_J G_J \\ \frac{dM}{dt} = \alpha L - \mu_M M \\ \frac{dE}{dt} = \Lambda_E - \rho_E E T - \mu_E E \\ \frac{dW_H}{dt} = \Lambda_W + \rho_E N_E E T - \mu_W W \end{cases} \tag{4.2.1}$$

The first equation describes the rate of change over time of *V. cholerae* inside the human host, wherein the first term is the growth of the *V. cholerae* ρ being the growth rate and κ the carrying capacity, the second term relates to the amount of vibrios killed by gastric juice, phagocytic cells and also lyphocytes. The last term is the natural death rate of vibrios.

The second equation represents the rate of change over time of phagocytes cells during encounter with vibrios. The first term is the supply of phygocytes by the body, second term represents phagocytic cells which are activated by the presence of the vibrios at a rate γ_1. The third term represents natural decay of phagocytes at a rate μ_1 and the last term is the rate of exhaustion of the phagocytic cells.

The third equation is the rate of change over time of lymphocytic cells. The first term is the supply rate of lymphocytes, the second term is the activation of lymphocytes upon the arrival of vibrios in the stomach, the third term is the exhaustion of the cells together with the vibrios, while the third term is the natural decay rate of lymphocytes μ_2 plus 10% rate that will become memory cells given by α

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The fourth equation is rate of change of cholera toxin, the first term gives the presence of the vibrios resulting in the availability of cholera toxin. The second term shows that cholera toxin runs ineffective after the production of antibodies by B cells and the last term is the natural decay of cholera toxin.

The fifth equation describes the rate of change over time of the gastric juice (G_J) in the human body, wherein G_0 is the initial gastric acid, the second term $\sigma G_J V_H$, is the rate of the gastric juice secreted upon ingested contaminated water and the last term $\mu_J G_J$ presents the natural decay of gastric juice.

The sixth equation of the model gives the rate of change over time of the memory cells. The first term describes the memory cells which are produced by the immune system, B cells specifically, after fighting the *V. cholerae*, the memory cells wait for reinfection by the *V. cholerae*. The last term is the natural decay of the memory cells.

The seventh equation of the model is the dynamic of epithelial cells. The first term is the supply of the cells within the small intestine, the second term is the damage rate of the epithelial cells due to cholera toxin and the last term represent the natural decay rate of the epithelial cells. The last equation shows the dynamics of water inside the human body. The first term Λ_W is the supply of water within the body, the second term $\rho_W TW$ is the rate of loss of water through diarrhoea due to the amount of cholera toxin T, and the last term is the loss of water through sweat and urine. Below is the summary of the state variables and their initial values.

The equations for the gastric acid and water within the human are the same as those developed in chapter three.

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State	Description	Initial Variables	values
$V_H(t)$	The V. cholera bacteria consumed	10000	Estimated
$P(t)$	Phagocytic cells	0	Estimated
$L(t)$	Lymphocytes	0	Estimated
$T(t)$	The cholera toxin	0	Estimated
$G_J(t)$	Gastric Juice	3	Estimated
$M(t)$	Memory cells	0	Estimated
$E(t)$	Epithelial cells	600	Estimated
W_H	Water within the host	60	Estimated

Table 4.1: Description of the state variables of the model system (3.3)

4.3 Positivity of so solutions

Mathematical model system equations (4.2.1) represent *V. cholerae* and human cells reaction, all parameters are positive and it is necessary to prove that all the variables are non-negative all the time. For biological reasons, the system of equations (4.2.1) will be studied in the following region

$$\left\{ \Omega = (V_H, P, L, T, G_J, M, E, W) \in \mathbb{R}^7_+ : \right. \tag{4.3.2}$$

We state the following theorem that assures that the system of equations (4.2.1) is well-posed such that the solutions with non-negative initial conditions remain non-negative for all $t < 0 < \infty$ and hence have biological meaning.

Theorem 4.1. *Let the initial data be* $V_H(0) = V_{H_0} \geq 0, P(0) = P_0 \geq 0, L(0) = L_0 \geq 0, T(0) = T_0 \geq 0, G_J(0) = G_{J(0)} \geq 0, M(0) = M_0 \geq 0, E(0) = E_0 \geq 0$ *and* $W_H(0) = W_{H(0)} \geq 0$. *Then the solutions of the equations in* Ω *are always positive for all* $t \geq 0$.

From the first equation of (4.2.1):

Proof.

$$\frac{dV_H}{dt} = \rho V_H(1 - \frac{V_H}{\kappa}) - \phi_I V_H (G_j + P + L) - \mu_V V_H \tag{4.3.3}$$

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then

$$\frac{dV_H}{dt} \geq -\left(\phi_I\left(G_j + P + L\right) + \mu_V\right) V_H$$

Separation of variables yields

$$\frac{dV_H}{V_H} \geq -\left(\phi_I\left(G_j + P + L\right) + \mu_V\right) dt$$

Integrating both sides gives

$$\int \frac{dV_H}{V_H} \geq -\int \left(\phi_I\left(G_j + P + L\right) + \mu_V\right) dt \tag{4.3.4}$$

$$\ln V_H \geq -\left(\mu_V\left(t\right) + \int_0^t \left(\phi_I\left(G_j + P + L\right) + \mu_V\right) d\tau\right) + C \tag{4.3.5}$$

(4.3.6)

taking exponents

$$V_H = Ae^{-\left(\mu_V(t) + \int_0^t (\phi_I(G_j + P + L) + \mu_V) d\tau\right)} \tag{4.3.7}$$

where

$$A = e^C.$$

The same can be done for second equation of (4.2.1)

$$\frac{dP}{dt} = \lambda_P + \gamma_1 P V_H - \mu_1 P - \phi_P P V_H \tag{4.3.8}$$

$$\begin{aligned} \frac{dP}{dt} &\geq \gamma_1 P V_H - \mu_1 P - \phi_P P V_H \\ \frac{dP}{dt} &\geq \left(\gamma_1 V_H - \mu_1 - \phi_P V_H\right) P \end{aligned}$$

Separation of values gives

$$\frac{dP}{P} \geq \left(\gamma_1 V_H - \mu_1 - \phi_P V_H\right) dt \tag{4.3.9}$$

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Integration both sides

$$\int \frac{dP}{P} \geq \int (\gamma_1 V_H - \phi_P V_H)\, dt \tag{4.3.10}$$

$$\ln P \geq -\mu_1(t) + \int_0^t (\gamma_1 V_H - \phi_P V_H)\, d\tau + C \tag{4.3.11}$$

taking exponents

$$P = D_1 e^{-\mu_1(t) + \int_0^t (\gamma_1 V_H - \phi_P V_H) d\tau}$$

where $D_1 = e^C$. To conclude the proof the same approach could be used for

$$L > 0, T > 0, G_J > 0, M > 0, E > 0 \text{ and } W_H > 0 \text{ for all } t > 0.$$

□

4.4 The boundedness of the model

For biological reasons we show the biological validity of the model by proving the boundedness of the solutions of system of equations (4.2.1)

Theorem 4.2. *All solutions of the equations (4.2.1) of the model are uniformly bounded.*

Proof. We consider the first equation of the model

$$\frac{dP}{dt} = \lambda_P + \gamma_1 P V_H - \mu_1 P - \phi_P P V_H \tag{4.4.12}$$

$$= \lambda_P - \mu_1 P + (\gamma_1 P - \phi_P P) V_H \tag{4.4.13}$$

$$\leq \lambda_P - \mu_1 P \tag{4.4.14}$$

$$\frac{dP}{dt} + \mu_1 P \leq \lambda_P \tag{4.4.15}$$

Then we use the integrating factor method to obtain the solution. Let the integrating factor be

$$e^{\int \mu_1 dt} = e^{\mu_1 t}. \tag{4.4.16}$$

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Multiplying equation (4.4.14) by integrating factor we obtain :

$$\frac{dPe^{\mu_1 t}}{dt} + e^{\mu_1 t}\mu_1 P \leq e^{\mu_1 t}\lambda_P \tag{4.4.17}$$

Integrating both sides of equation (4,4.16)

$$\begin{cases} \int dPe^{\mu_1 t} & \leq & \int e^{\mu_1 t}\lambda_P dt \\ Pe^{\mu_1 t} & \leq & \dfrac{e^{\mu_1 t}\lambda_P}{\mu_1} + C \\ P(t) & \leq & \dfrac{\lambda_P}{\mu_1} + Ce^{-\mu_1 t} \end{cases} \tag{4.4.18}$$

This implies that

$$\lim_{t\to\infty} Sup\left(P(t)\right) \leq \frac{\lambda_P}{\mu_1} \tag{4.4.19}$$

Therefore the solution is bounded for $0 \leq P(t) \leq \dfrac{\lambda_P}{\mu_1}$.

Now for the equation:

$$\begin{cases} \dfrac{dL}{dt} & = & \lambda_L + (\gamma_2 - \phi_L)LV_H - (\mu_2 + \alpha)L \\ & = & \lambda_L - (\mu_2 + \alpha)L + (\gamma_2 - \phi_L)LV_H \\ \dfrac{dL}{dt} & \leq & \lambda_L - (\mu_2 + \alpha)L \\ \dfrac{dL}{dt} + (\mu_2 + \alpha)L & \leq & \lambda_L \end{cases}$$

Integrating factor is $e^{\int(\mu_2+\alpha)dt} = e^{(\mu_2+\alpha)t}$, and multiplying both sides by the integrating factor we get

$$\frac{dL}{dt}e^{(\mu_2+\alpha)t} + e^{(\mu_2+\alpha)t}(\mu_2 + \alpha)L \leq e^{(\mu_2+\alpha)t}\lambda_L \tag{4.4.20}$$

Integrating both sides yields

$$\begin{aligned} \int dLe^{(\mu_2+\alpha)t} &\leq \int e^{(\mu_2+\alpha)t}\lambda_L dt \\ Le^{(\mu_2+\alpha)t} &\leq \frac{e^{(\mu_2+\alpha)t}\lambda_L}{(\mu_2+\alpha)} + C \end{aligned}$$

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Dividing both sides by $e^{(\mu_2+\alpha)t}$ gives

$$L(t) \leq \frac{\lambda_L}{(\mu_2+\alpha)} + Ce^{-(\mu_2+\alpha)t} \tag{4.4.21}$$

Therefore

$$\lim_{t\to\infty} \sup L(t) \leq \frac{\lambda_L}{(\mu_2+\alpha)}$$

Therefore the solution is bounded for $0 \leq L(t) \leq \frac{\lambda_L}{\mu_2+\alpha}$. Therefore by the same approach for all equations it can be shown that all solutions of (4.2.1) are bounded in the region $\in \mathbb{R}^8_+$:

$$\begin{cases} \Phi = (V_H(t), P(t), L(t), T(t), G_J(t), M(t), E(t), W_H) \mid 0 \leq V_H \leq \frac{\kappa}{\rho}(\rho - \mu_V), \\ 0 \leq P(t) \leq \frac{\lambda_P}{\mu_1}, 0 \leq L(t) \leq \frac{\lambda_L}{(\mu_2+\alpha)}, 0 \leq T \leq \frac{\beta}{\mu_T}, 0 \leq G_J \leq \frac{G_0}{\mu_J}, 0 \leq M \leq \frac{\alpha\lambda_L}{\mu_M(\mu_2+\alpha)} \end{cases}$$

□

4.5 The Disease Free Equilibrium and its Stability

The system of equations (4.2.1) have the equilibrium point, where there is no disease in the population and the endemic equilibrium. To obtain the disease-equilibrium point we set the right-hand side of equations of the model to zero and $V_H = T = 0$, Then the disease free-equilibrium is given by

$$E_0 = [V_H, P, L, T, G_J, M, E, W_H] \tag{4.5.22}$$

$$= \left[0, \frac{\lambda_P}{\mu_1}, \frac{\lambda_L}{(\mu_2+\alpha)}, 0, \frac{G_0}{\mu_J}, 0, \frac{\Lambda_E}{\mu_E}, \frac{\Lambda_W}{\mu_W}\right] \tag{4.5.23}$$

Theorem 4.3. *If $R_0 < 1$ then the disease-free equilibrium point is locally asymptotically stable and if $R_0 > 1$ the disease-free equilibrium is unstable.*

4.5.1 The Reproductive Number (R_0)

The next generation operator will be used to calculate the basic reproduction number of the system of equations (4.2.1). The system can then be written as follows:

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$$\begin{cases} \dfrac{dX}{dt} &= f(X,Y,Z), \\ \dfrac{dY}{dt} &= g(X,Y,Z), \\ \dfrac{dZ}{dt} &= h(X,Y,Z). \end{cases} \tag{4.5.24}$$

Where

- $X = (P, L, G_J, M, E, W_H)$ represent all compartment which are not infected.
- $Y = (T)$ represent compartment which is not capable of infecting.
- $Z = (V_H)$ represent compartment which is infected and capable of infecting.

Now let

$$\bar{U}_0 = \quad = \left[0, \frac{\lambda_P}{\mu_1}, \frac{\lambda_L}{(\mu_2+\alpha)}, 0, \frac{G_0}{\mu_J}, 0, \frac{\Lambda_E}{\mu_E}, \frac{\Lambda_W}{\mu_W}\right] \tag{4.5.25}$$

In this case

$$\tilde{g}(X^*, Z) = (\tilde{g}_1(X^*, Z)) \tag{4.5.26}$$

With

$$\tilde{g}(X^*, Z) \quad = \quad \frac{\beta V_H(\mu_2+\alpha)}{\rho_T \lambda_L + (\mu_2+\alpha)} \tag{4.5.27}$$

Now

$$h(X,Y,Z) = (h_1(X,Y,Z)) \tag{4.5.28}$$

With

$$(h_1(X,Y,Z)) = \rho - \frac{2\rho V_H}{k} - (\phi_I G_J + \phi_I P + \phi_I L + \mu_V) \tag{4.5.29}$$

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Because at DFE, $V_H = 0$ then the above expression can be represented by:

$$A = D_Z h(X^*, \tilde{g}(X^*, 0), 0) = \rho - (\phi_I G_J + \phi_I P + \phi_I L + \mu_V) \tag{4.5.30}$$

which can be written as in the form $A = M - D$, where

$$M = \rho \tag{4.5.31}$$

and

$$D = \phi_I(G_J + P + L) - \mu_V \tag{4.5.32}$$

since the basic reproductive number is given by

$$R_0 = MD^{-1} \tag{4.5.33}$$

In our case

$$R_0 = \frac{\rho\mu_J\mu_1(\mu_2 + \alpha)}{\phi_I\mu_1(\mu_2 + \alpha)G_0 + \phi_I\lambda_P\mu_J(\mu_2 + \alpha) + \phi_I\lambda_L\mu_J\mu_I - \mu_V\mu_J\mu_1(\mu_2 + \alpha)} \tag{4.5.34}$$

4.5.2 Local stability of the Disease Free Equilibrium

The stability of DFE can be obtained by eigenvalues of the Jacobian matrix of the linearized system. In order to determine the local stability of the DFE of our system we linearise equations of the system so that we determine the Jacobian matrix. To determine the local stability we evaluate the Jacobian matrix at disease-free equilibrium point

$$E_0 = \left[0, \frac{\lambda_P}{\mu_1}, \frac{\lambda_L}{(\mu_2 + \alpha)}, 0, \frac{G_0}{\mu_J}, 0, \frac{\Lambda_E}{\mu_E}, \frac{\Lambda_W}{\mu_W}\right] \tag{4.5.35}$$

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thus the Jacobian matrix is as follows ,

$$J(E_0) = \begin{bmatrix} -a_1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{(\gamma_1-\phi_P)\lambda_P}{\mu_1} & -\mu_1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{(\gamma_1-\phi_L)\lambda_L}{(\mu_2+\alpha)} & \frac{\lambda_P}{\mu_1} & \mu_2 & 0 & 0 & 0 & 0 & 0 \\ \beta & 0 & 0 & -a_2 & 0 & 0 & 0 & 0 \\ \frac{\delta G_0}{\mu_J} & 0 & 0 & 0 & -\mu_J & 0 & 0 & 0 \\ \gamma_L & 0 & 0 & 0 & 0 & -\mu_m & 0 & 0 \\ 0 & 0 & 0 & -\frac{\rho_E \Lambda_E}{\mu_E} & 0 & 0 & -\mu_E & 0 \\ 0 & 0 & 0 & \frac{\rho_E N_E \Lambda_E}{\mu_E} & 0 & 0 & 0 & -\mu_W \end{bmatrix} \tag{4.5.36}$$

where

$$\begin{cases} a_1 = [\phi_I(\frac{G_0}{\mu_J} + \frac{\lambda_P}{\mu_1} + \frac{\lambda_L}{\mu_2+\alpha}) + \mu_V - \rho] \\ a_2 = \frac{\rho_T \lambda_L}{(\mu_2+\alpha)} + \mu_T \end{cases} \tag{4.5.37}$$

Local asymptotic stability is confirmed if all the eigenvalues of the Jacobian matrix are negative. In our case the characteristic equation is given by,

$$(-a_1-\lambda)(-\mu_1-\lambda)(-(\mu_2+\alpha)-\lambda)(-a_2-\lambda)(-\mu_J-\lambda)(-\mu_M-\lambda)(-\mu_E-\lambda)(-\mu_W-\lambda) \tag{4.5.38}$$

The characteristic equation has eight eigenvalues. Now we look at the eighth eigenvalue to confirm its negativity. Thus

$$\left[\phi_I(\frac{G_0}{\mu_J} + \frac{\lambda_P}{\mu_1} + \frac{\lambda_L}{\mu_2+\alpha}) + \mu_V - \rho\right] - \lambda = 0 \tag{4.5.39}$$

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This is equal to

$$\left[\frac{\phi_I}{\rho}\left(\frac{G_0}{\mu_J}+\frac{\lambda_P}{\mu_1}+\frac{\lambda_L}{\mu_2+\alpha}\right)+\frac{\mu_V}{\rho}-1\right]-\lambda = 0. \tag{4.5.40}$$

Therefore

$$\lambda = R_0 - 1 \tag{4.5.41}$$

If $R_0 < 1$, all the eigenvalues have negative real parts and hence the equilibrium is locally asymptotically stable.

4.5.3 Global Stability Analysis of the Disease-Free Equilibrium.

For the global stability analysis of the disease-free equilibrium we apply the method used by (Castillo-Chavez *et al*, 2002), that guarantees the global asymptotic stability of DFE. We rewrite the system of equations (4.2.1) as follows,

$$\begin{cases} \dfrac{dX}{dt} = F(X,Z), \\ \dfrac{dY}{dt} = G(X,Z) \end{cases} \tag{4.5.42}$$

where

- $X = (P, L, G_J, M, E, W_H)$ represents all uninfected components, and
- $Z = (V_H, T)$ represents infectious components.

The following represent the disease-free equilibrium of the system,

$$U_0\,(X^*, 0) = \left(\frac{\lambda_P}{\mu_1}, \frac{\lambda_L}{(\mu_2+\alpha)}, \frac{G_0}{\mu_J}, 0, \frac{\Lambda_E}{\mu_E}, \frac{\Lambda_W}{\mu_W}\right) \tag{4.5.43}$$

To guarantee local asymptotical stability the conditions below must be satisfied:
The following conditions should be met X^* to be globally asymptotic stable (g.a.s).

H1. for $\dfrac{dX}{dt} = F(X, 0)$ is globally asymptotically stable ,

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H2. $G(X,Z) = AZ - \hat{G}(X,Z)$, $\hat{G}((X,Z) \geq 0$ for $(X,Z) \in \mathbb{R}^7_+$ where $A = D_Z G(X^*, 0)$ is an M-matrix and $\mathbb{R}^7_+$ and is the region where the model makes a biological sense.

$$F(X,0) = \begin{bmatrix} \lambda_P - \mu_1 P \\ \lambda_L - \mu_1 L \\ G_0 - \mu_J G_J \\ 0 \\ \Lambda_E - \mu_E E \\ \Lambda_W - \mu_W W \end{bmatrix}, \tag{4.5.44}$$

and

$$A = \begin{bmatrix} \rho - \dfrac{2\rho V_H}{\kappa} - \phi_I (G_J + P + L) - \mu_V & 0 \\ \beta & -\rho_T L - \mu_T \end{bmatrix} \tag{4.5.45}$$

At DFE,

$$AZ = \begin{pmatrix} \rho - \phi_I \left(\dfrac{G_0}{\mu_J} + \dfrac{\lambda_P}{\mu_1} + \dfrac{\lambda_L}{(\mu_2 + \alpha)} \right) - \mu_V & 0 \\ \beta & -\rho_T \dfrac{\lambda_L}{\mu_1} - \mu_T \end{pmatrix} \begin{pmatrix} V_H \\ T \end{pmatrix} \tag{4.5.46}$$

From H_2, we have

$$\hat{G}(X,Z) = AZ - G(X,Z) \tag{4.5.47}$$

$$= \begin{pmatrix} \dfrac{\rho V_H^2}{\kappa} \\ 0 \end{pmatrix} \tag{4.5.48}$$

It can be seen that $\hat{G}(X,Z)$ is positive for all$(X,Z) \in \mathbb{R}^8_+$, as in the first model, it is also clear that matrix A is an M-matrix. We therefore state the following theorem which summarizes the results found.

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Theorem 4.4. *: The fixed point*

$$E_0 = \left[0, \frac{\lambda_P}{\mu_1}, \frac{\lambda_L}{(\mu_2+\alpha)}, 0, \frac{G_0}{\mu_J}, 0, \frac{\Lambda_E}{\mu_E}, \frac{\Lambda_W}{\mu_W}\right] \tag{4.5.49}$$

is a g.a.s equilibrium of system (4.2.1) if $R_0 < 1$ and the assumptions (H_1) and (H_2) are satisfied.

4.5.4 The endemic equilibrium point

Similar to the endemic point found in the previous chapter, at endemic equilibrium state , *V. cholerae* are ingested in large volume, they suppress the immune system and eventually cause diarrhoea in the human host and are shed to the water again. We equate the derivatives of the equation systems (4.2.1) to zero and solve the system algebraically. At endemic V_H and T are not equal to zero. At endemic state the Cholera toxin is given by,

$$T^* = \frac{\beta V_H}{\rho_T L - \mu_T} \tag{4.5.50}$$

This expression tells us that cholera toxin during endemic times is proportional to the amount of *V. cholerae* ingested, and inversely proportional to the amount of cholera toxin which runs ineffective because of the reaction of the lymphocytes when $\mu_T < \rho_T L$. The following equation gives the expression of the lymphocytes at endemic point

$$L^* = \frac{\lambda_L}{(\mu_2+\alpha) + (\phi_L - \gamma_2)V_H*} \tag{4.5.51}$$

The above expression gives lymphocytes at endemic point. At endemic point the lymphocytes are inversely proportional to the exhaustion of the lymphocytes through natural decay and exhaustion through binding,this requires that $\gamma_2 < \phi_L$. Below is the expression for the phagocytes at endemic point.

$$P^* = \frac{\lambda_P}{\mu_1 + (\phi_P - \gamma_1)V_H^*} \tag{4.5.52}$$

The above expression gives lymphocytes at endemic point. At endemic point the phagocytes are inversely proportional to the exhaustion through natural decay and exhaustion through binding, but it requires that $\gamma_1 < \phi_P$. Below is the expression for the the gastric juice at endemic point.

$$G_J = \frac{G_0}{\mu_J - \delta V_H} \tag{4.5.53}$$

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The expression for gastric acid is similar to the expression described for gastric acid in the first model. The following is an equation of memory cells during endemic times.

$$M^* = \frac{\alpha\lambda_L}{\mu_M[(\mu_2+\alpha)-(\gamma_2-\phi_L)V_H^*]} \tag{4.5.54}$$

Memory cells at endemic state are inversely proportional to the amount of lymphocytes activated upon arrival of ingested vibrios, supply of activated lymphocytes and natural decay of lymphocytes provided that $\gamma_2 < \phi_L$. At endemic the following gives the expression for the epithelial cells

$$E^* = \frac{\Lambda_E[\rho_T\lambda_L - \mu_T[(\mu_2+\lambda)(\phi_L-\gamma_2)V_H^*]]}{\rho_E[\beta V_H^*(\mu_2+\alpha)+(\phi_L-\gamma_2)V_H^* + \mu_E[\rho_T\lambda_L - \mu_T(\mu_2+\alpha)+(\phi_L-\gamma_2)V_H^*]]} \tag{4.5.55}$$

Epithelial cells are inversely proportional to supply of lymphocytes, the amount of toxin released,decay of lymphocytes and the amount of memory cells produced when $\gamma_2 < \phi_L$. Water within an infected individual during endemic times is given by

$$W_H^* = \frac{\Lambda_W\Lambda_E\beta V_H^*[(\mu_2+\alpha)+(\phi_L-\gamma_2)V_H^*]}{\beta V_H^*[(\mu_2+\alpha)-(\phi_L-\gamma_2)V_H^* - \mu_E[\rho_T\lambda_L - \mu_T(\mu_2+\alpha)+(\phi_L-\gamma_2)V_H^*]} \tag{4.5.56}$$

At endemic times, water is inversely proportional to the epithelial cells, the amount of released toxin, natural decay of lymphocytes, decay of lymphocytes due to vibrios, natural decay of toxin and also the activation rate of lymphocytes, this is true for $\gamma_2 < \phi_L$ and $\mu_E\rho_T\lambda_L < \mu_E\mu_T(\mu_2+\alpha)$.

$$V_H^* = \kappa + \frac{(\phi G_J + \phi_I P + \phi_I L + \mu_V)\kappa}{\rho} \tag{4.5.57}$$

The expression for *V. cholerae* at endemic point shows the existence of a unique endemic point by the positive value. This is indicative of the progression of the disease. Thus there is at least one endemic equilibrium point at which lymphocytes, phagocytes, toxin, gastric acid, memory cells, epithelial cells and water in the host respectively are positive since are all expressed in terms of V_H. This leads to a conclusion of the existence of unique endemic point.

4.6 Sensitivity Analysis

We perform sensitivity analysis to evaluate the relative change in basic reproduction number when each of the parameters of our model system changes. We used the normalized forward sensitivity index of the

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R_0 to each of the model parameters of model system (4.2.1). We then let R_0 be a differentiable function on the parameter say u, so that the normalized forward sensitivity index of R_0 at u is defined as

$$\Upsilon_u^{R_0} = \frac{\partial R_0}{\partial u} \times \frac{u}{R_0} \tag{4.6.58}$$

The index of V. cholerae death rate given by,

$$\Upsilon_{\phi_I}^{R_0} = \frac{\phi_I(\mu_1(\mu_2+\alpha)G_0 + \mu_J(\lambda_P + \mu_J\mu_1\lambda_L)}{\phi_I[\mu_1(\mu_2+\alpha)G_0 + \mu_J\mu_1\lambda_L] + \mu_J\mu_1(\mu_2+\alpha)\mu_V} = 0.9 \tag{4.6.59}$$

(4.6.60)

Similar expressions can be derived for the remaining parameters. The table below gives sensitivity indices of R_0 to the different model parameters.

Parameter	Description	Sensitivity index
ϕ_I	Vibrios death rate	+0.9
μ_J	natural decay rate of gastric acid	+0.06
ρ	growth rate of *V. cholerae*	+ 1.0538
Λ_P	supply of phogocytes cells	+ 0.02
λ_L	supply of lymphocytes cells	+ 0.01
μ_1	natural decay rate of phagocytes	- 0.6
μ_2	natural decay rate of lymphocytes	- 0.01
μ_V	Natural death rate of vibrios	-0.5
G_0	initial gastric acid	0.2

Table 4.2: Sensitivity Indices of model reproduction number R_0 to parameter for the model system (4.2.1)

Based on the above table we can conclude that R_0 is most sensitive to changes in parameters ρ and ϕ_I. This means that an increase in this parameters will lead to increase in R_0 and also a decrease in ρ and ϕ_I will mean that R_0 decreases proportionally. An increase in ρ means there will be more than enough of vibrios to release cholera toxin to cause diarrhoea. We can see that the increase in vibrios also affect the immune system as we can see that the natural decay immune system is high, and this will lead to a large

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volume of water out of the body through diarrhoea. We can also realise that parameters μ_1 and μ_2 will affect R_0. These two parameters represent natural decay of lymphocytes and phagocytes respectively. This implies that the parameter ρ should be given much attention. We learn that if there will not be a growth rate of the vibrios in the stomach, the decay rate of immune system will not happen and there will not be a cholera epidemic.

4.7 Numerical Simulations

In this section we provide some numerical simulations to show the dynamics of model (4.2.1). We solved the equations of the basic model numerically. Graphs of the numerical solution are used to understand the effect of some parameters of our model(fig 4.1-4.4). In order to perform this task we used the estimated parameter values given in table(4.3) for sensitivity analysis. The model system (4.2.1) was solved numerically using a Python programme version V 2.6 on the linux operation system (Ubuntu 14.04). The programme uses a package odeint function in the scipy.integrate for solving any system of differentiated equations. The initial conditions used for simulation are given by $V_H(0) = 10000,\ P(0) = 0, L(0) = 0\ T(0) = 0,\ G_J(0) = 0,\ M(0) = 0,\ E(0) = 0$, and $W_H(0) = 0$.

Parameter	Description	Initial values	Units	Source
ϕ_L	Vibrios death rate due to gastric	0.1	day^{-1}	Estimated
ϕ_I	Death rate of vibrios killed by immune cells	0.003	day^{-1}	Estimated
λ_P	Supply rate of phagocytes	0.008	day^{-1}	Estimated
λ_L	Supply rate of lymphocytes	0.009	day^{-1}	Estimated
γ_1	Phagocytes activation rate	0.02	day^{-1}	Estimated
γ_2	Lymphocytes activation rate	0.02	day^{-1}	Estimated
μ_1	Decay rate of activated phagocytes	0.000001	day^{-1}	Estimated
μ_2	Decay rate of activated lymphocytes	0.002	day^{-1}	Estimated
ϕ_P	Exhaustion rate phagocytes due *V. cholerae*	0.001	day^{-1}	Estimated
ϕ_L	Exhaustion rate of lymphocytes due to *V. cholerae*	0.001	day^{-1}	Estimated
γ	Activation rate of lymphocytes	0.00001	day^{-1}	Estimated
ρ_I	Multiplication rate of immune cells	0.003	day^{-1}	Estimated

Table 4.3: Estimated values of some parameters used for numerical simulation to show the dynamics of the model.

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4.8 Results

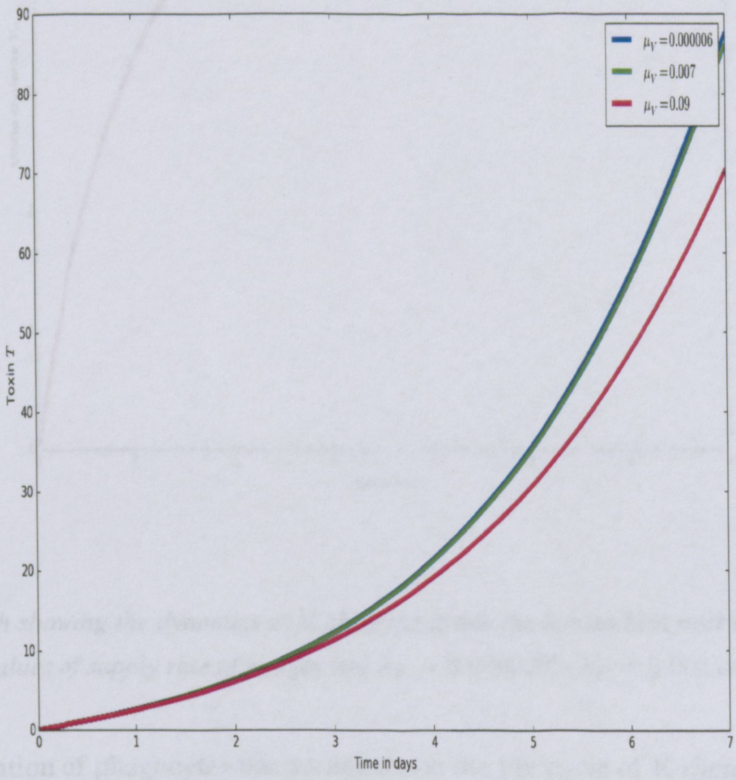


Figure 4.1: *Graph showing the dynamics of cholera toxin inside the human host over a period of time for a different values of decay rate of V. cholerae* $\mu_V = 0.000006$, $\mu_V = 0.007$ *and* $\mu_V = 0.09$

Figure 4.1 above shows cholera toxin released by *V. cholerae*. The graph shows that natural decay of *V. cholerae* influence the amount of toxin produced. A low decay rate of *V. cholerae* results in high amounts of toxin produced, which will have a negative impact on humans.

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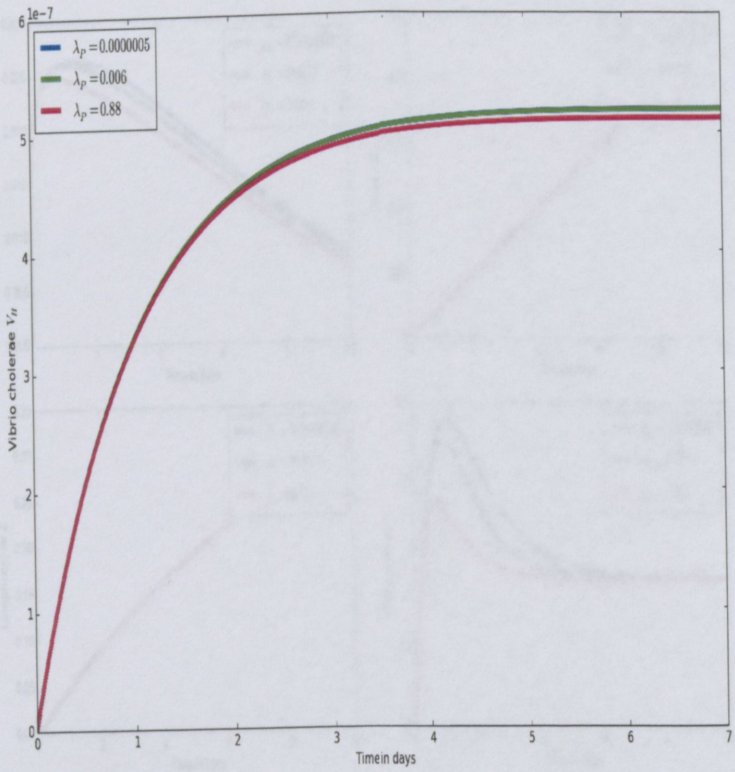


Figure 4.2: *Graph showing the dynamics of V. cholerae inside the human host over a period of time for a different values of supply rate of phagocytes* $\lambda_P = 0.0000005$, $\lambda_P = 0.006$ *and* $\lambda_P = 0.88$

Figure 4.2 The activation of phagocytes has an impact on the life cycle of *V. cholerae* inside the human host. We noted that when phagocytes are activated in small amounts, the amount of *V. cholerae* inside the human host remains high. However as the activated phagocytes increase we find that *V. cholerae* decreases, this means that toxin released will be too little to cause the disease.

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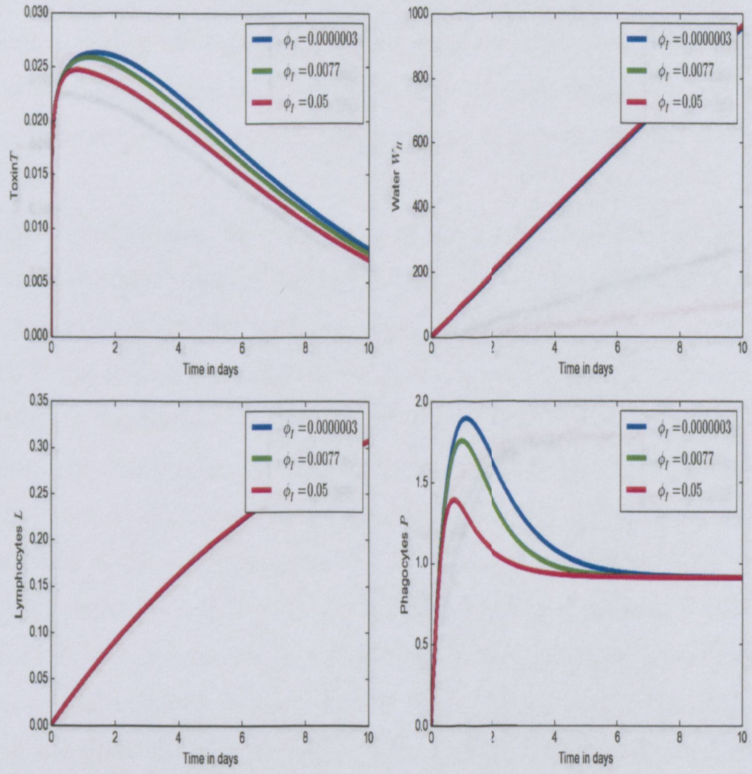


Figure 4.3: *Graph showing the dynamics of Toxin, Lymphocytes, Water, Phagocytes inside the human host in the presence of V.cholera over a period of time for a different values of death rate of vibrios* $\phi_I = 0.0000003$, $\phi_I = 0.0077$ *and* $\phi_I = 0.05$

Figure above shows that as the death rate of vibrios through gastric acid, phagocytosis and through lymphocytes increases, cholera toxin secretion decreases, and on the other hand activation of phagocytes decreases.

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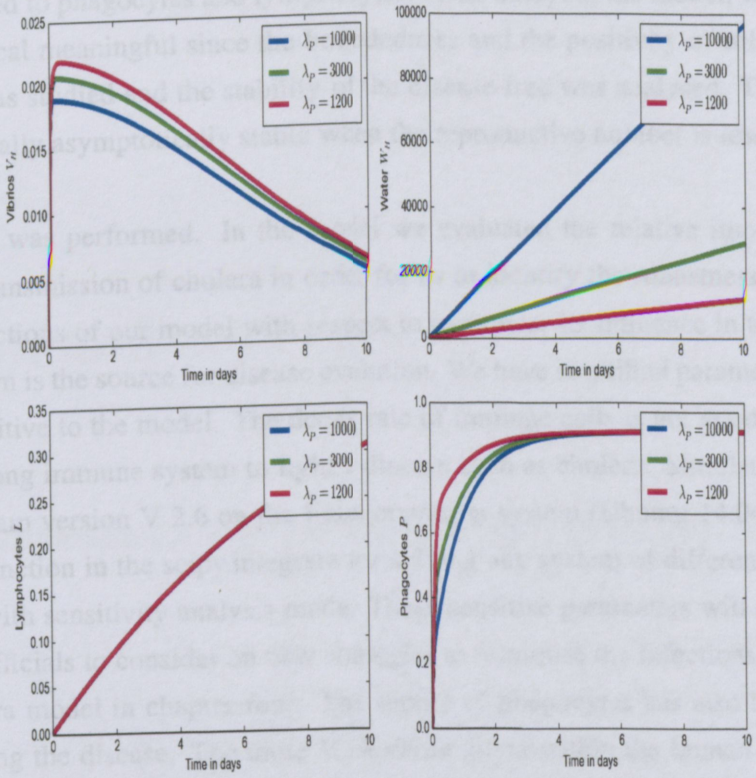


Figure 4.4: *Graph showing the dynamics of Water, Phagocytes and Lymphocytes inside the human host in the presence of V.cholera over a period of time for a different values of supply rate of phagocytes* $\lambda_P = 10000$, $\lambda_P = 3000$ *and* $\lambda_P = 1200$

From the figure above we see that as the supply of phagocytes increases the number of ingested vibrios decreases,and as the number of phagocytes decrease, water in the human body decrease, this is because as the large number of phagocytic cells means the high rate of vibrios which will lead to cholera and hence loss of water.

4.9 Discussion and conclusion

The model is based on which effective prevention and intervention strategies can be possibly designed. A cholera immunological model was developed in this work . The model was developed with the view of understanding the insights of *V. cholerae* inside the human hosts and how the immune system responds

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to *V. cholerae*. This model is the extension of the basic cholera model. In this model the immune cells are divided or partitioned to phagocytes and lymphocytes. After studying the model, we have found that the systems are biological meaningful since the boundedness and the positivity of solutions of the systems hold. The model was studied and the stability of the disease-free was analysed. The disease-free states are locally and globally asymptotically stable when the reproductive number is less than one.

Sensitivity analysis was performed. In the model we evaluated the relative importance of the model parameters in the transmission of cholera in order for us to identify the robustness of the model parameters with the predictions of our model with respect to parameter its influence in the basic reproductive number which in turn is the source for disease evolution. We have identified parameters as μ_v, λ_p, ϕ_I and α are the most sensitive to the model. The decay rate of immune cells is not good for individuals, people must have a strong immune system to fight a disease such as cholera. Simulations were carried out using Python program version V 2.6 on the linux operation system (Ubuntu 14.04). The program uses a package odeint function in the scipy.integrate for solving any system of differentiated equations. The graphs are in line with sensitivity analysis made. These sensitive parameters will inform policy makers and public health officials to consider on new strategies to minimise the infections. The same applies to the extended cholera model in chapter four. The supply of phagocytes has also been found to be also helpful in eradicating the disease. The more *V. cholerae* killed within the human host, the less cholera toxin produced, which results in no cholera infection.

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Chapter 5

Discussion and conclusion

Two cholera immunological models were developed in this thesis . Both models were developed with the view of understanding the insights of *V. cholerae* inside the human hosts and how the immune system responds to *V. cholerae*. The first model is the basic cholera immunology. In this model the immune cells are represented by I. The second model is the extension of the basic cholera model. In this model the immune cells are divided or partitioned into phagocytes and lymphocytes. After studying both models, we have found that the systems are biological meaningful since the boundedness and the positivity of solutions of the systems hold. The models were studied and the stability of the disease-free were analysed. The disease-free states are locally and globally asymptotically stable when the reproductive number is less than one.

Sensitivity analysis was performed on both models. In the basic model we evaluated the relative importance of the model parameters in the transmission of cholera in order for us to identify the robustness of the model parameters with the predictions of our model with respect to parameter its influence in the basic reproductive number which in turn is the source for disease evolution. We have identified parameters as μ_I and ρ as the most sensitive to the model. The decay rate of immune cells is not good for individuals, people must have a strong immune system to fight a disease such as cholera. Simulations were carried out using Python program version V 2.6 on the linux operation system (Ubuntu 14.04). The program uses a package odeint function in the scipy.integrate for solving any system of differentiated equations. By extending existing cholera models,it was concluded that variations in water volume can have serious effects on cholera dynamics (Pascual *et al*, 2002). It was also found that there are several

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characteristics that put people living in some areas at risk for both E1 Tor and classical cholera, and also that cholera risk is high when an area is near water bodies, has a high population density and when people are less educated. The water bodies near overcrowded areas may have high fecal concentrations and the hygiene and defecation practices of people are motivated by their educational background. It was revealed that both the physical and environment and the environment created by humans are responsible for cholera endemic (Ali *et al,*, 2002). These studies have found useful information on the eradication of cholera. However up to this date there are no cholera immunological models. Through sensitivity analysis, this study has revealed parameters that need full attention in order to limit the disease. These sensitive parameters will inform policy makers and public health officials to consider on new strategies to minimise the infections. The same applies to the extended cholera model in chapter four. The supply of phagocytes has also been found to be also helpful in eradicating the disease. The more *V. cholerae* killed within the human host, the less cholera toxin produced, which results in no cholera infection. The results found are the same as those concluded by epidemiologists, this means cholera immunology must be considered also for eradicating the disease.

5.1 Recommendations for future research

In this thesis the use of mathematical modelling in infectious disease has been found to be useful. They can be used to predict the future of the disease and to decide on the method of reducing infections, as well as eliminating the disease. In view of that it is proposed that the future research should look at :

- incorporating treatment in the existing cholera immunological models in order to get the parameters which are sensitive to the treatment.
- developing immuno-epidemiology cholera models in order to develop strategies on how we can eradicate or limit cholera infection by considering both humans and environment



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