UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT OF A MULTIPLEX REAL-TIME POLYMERASE CHAIN REACTION ASSAY FOR DETECTION OF THREE COMMON ENTERIC PROTOZOA INFECTION

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ABSTRACT

Enteric protozoa infections remain a significant health challenge, particularly in developing countries. Conventional diagnostic methods such as microscopy or antigen test are time-consuming and have limited sensitivity and specificity, often resulting in misdiagnosis. This study aimed to develop an improved molecular method using a multiplex real-time polymerase chain reaction (qPCR) assay for the simultaneous detection of three common enteric protozoa (Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum) in a single tube. Specific primers and TaqMan probes targeting each pathogen and the internal control, labelled with different fluorophores (HEX, ROX, FAM, CY5), were designed. Analytical validation showed a limit of detection (LOD) for E. histolytica and G. lamblia of 1×10^1 pg DNA at a 10-fold serial dilution, while C. parvum was detected at 3.2×10^3 pg at a 1:5 serial dilution. The assay achieved 100% sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), with no cross-reactivity against non-target organisms in spiked stool sample tests. Compared to conventional methods, multiplex qPCR can simultaneously detect multiple targets, especially in mixed infection samples, with significantly higher sensitivity and specificity, reduces reagent consumption, and shortens turnaround time. Multiplex qPCR is a robust, accurate, and efficient method for alternate diagnosis method of enteric protozoa, allowing timely intervention to reduce the burden on healthcare workers.

Keywords: multiplex qPCR, Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica, enteric protozoal infection

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Intestinal protozoal infections represent a significant global health burden and contribute substantially to the prevalence of diarrhoeal diseases worldwide. More than 67.2 million people are affected. These infections, mainly caused by parasites such as Giardia lamblia, Cryptosporidium parvum, and Entamoeba histolytica, have serious consequences such as malnutrition, growth retardation, and cognitive impairment (Fletcher et al., 2012; Hemphill et al., 2019). Despite their devastating impact, effective detection methods are limited, especially in underdeveloped regions, leading to overlooked cases and underestimation of clinical significance. The rise in protozoan infections, due to factors such as global travel, increased immigration, and immunocompromised populations, emphasizes the urgency of accurate, efficient, and cost-effective diagnostic methods (Torgerson & Macpherson, 2011). In this study, the term "enteric protozoa" is used in the project title to highlight the clinical significance and the mode of transmission of the infections, in particular their association with diarrhoeal diseases and the faecal-oral transmission route". However, in the rest of the thesis, the protozoa living in the human gastrointestinal tract are referred to as intestinal protozoa. In the context of this study, both terms are used interchangeably to refer to the same group of organisms, namely E. histolytica, G. lamblia, and C. parvum.

The most common intestinal protozoa associated with parasitic gastroenteritis and chronic diarrheal diseases in humans are E. histolytica, G. lamblia, and C. parvum (Hemphill et al., 2019). Although these pathogens cause self-limiting diarrhoea, they can lead to serious long-term consequences such as malnutrition, growth retardation and cognitive impairment. Ongoing research is focusing on the complex cellular and molecular mechanisms underlying the pathogenesis of these diseases. Particular attention is being paid to understanding the molecules involved in the interactions between protozoal-host interactions and the complicated pathophysiological processes of these diseases (Di Genova & Tonelli, 2016). Researchers are working tirelessly to decipher the signalling pathways and immune responses triggered by these infections. This knowledge is invaluable for the development of immunomodulatory therapies that