ASSOCIATION OF INTESTINAL PARASITIC INFECTION IN DEVELOPMENT OF REACTION IN LEPROSY PATIENTS

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have specifically acknowledged by reference to the author(s) or institution(s).

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RECOMMENDATIONS

This is to recommend that the thesis entitled "**Association of intestinal parasitic infection in development of reaction in leprosy patients"** has been carried out by Saurav Kumar Singh for the partial fulfillment of Master's Degree of science in Zoology with special paper Parasitology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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ABSTRACT

A hospital based study was conducted on intestinal parasitic co-infection in reaction and non reaction leprosy patients visiting at Lalgadh Leprosy Services Centre, Dhanusha, Nepal, during 2011/2012. The objective of the present study was aimed to determine the prevalence of intestinal parasites in leprosy reaction and non reaction patients and to analyze the association between leprosy reaction and gastrointestinal parasitic infection. Patients with leprosy reaction and non reaction were grouped according to their clinical form and as per the guidelines of the resident Dermatologist of LLSC. The total of 200 stool samples were collected from each patient and examined to identify the presence of intestinal parasites. Result of stool examination revealed that, the intestinal parasitic infection was significant ($\chi 2$ =16.324, $p<0.05$) in 51% of reaction leprosy patients when compared to 26% in non reaction leprosy patients. The most frequently observed intestinal protozoan parasites includes *E. histolytica* (25%) in leprosy reaction and (16%) in non reaction patients as well as *Giardia lamblia* (8%) in leprosy reaction and (9%) in non reaction patients. Among the protozoan parasites, *T. hominis* was observed only (4%) in reaction leprosy patients. The frequency of intestinal helminths observed in the study includes Nematode and Cestode group. The nematode group includes *S. stercoralis* and hookworm which were found only in leprosy reaction patients (4.5%). Similarly least infection with *H. nana,* only species belonging to Cestode found (0.5%) in both reaction and non reaction leprosy patients. Overall result indicated that there is a positive association in development of reaction in leprosy patients to that of hookworm and *S. stercoralis* (helminths) and *T. hominis* (protozoa) infection. KAP survey among leprosy reaction and non reaction patients in relation to parasitic infection showed that, there was no significant difference ($p<0.05$) in the prevalence of intestinal parasites with respect to literacy, knowledge of mode of transmission of parasites, sanitary condition, occupation, food or water consumption by both groups of leprosy reaction and non reaction patients.

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
ENL	Erythema Nodosum Leprosum
IFAT	Immunoflorescent Antibody Test
IL	Interleukin
LLSC	Lalgadh Leprosy Services Centre
LR	Leprosy Reaction
MB	Multibacillary Leprosy
MDT	Multidrug Therapy
NPC	National Planning Commission
NTD	Neglected Tropical Disease
PB	Paucibacillary Leprosy
PGL	Phenolic glycolipid
RE	Reactional Episodes
T1R	Type 1 Reaction
T2R	Type 2 Reaction
Th1	T helper type 1
Th2	T helper type 2
TNF	Tumor Necrosis Factor
WBC	White Blood Cells
WHO	World Health Organization

1. INTRODUCTION

1.1 Background

Leprosy is a chronic infectious disease caused by the bacteria *Mycobacterium leprae* and *Mycobacterium lepromatosis*. *Mycobacterium leprae* was discovered by Dr. Gerhard Armauer Hansen in Norway in 1873. It was the first bacterium to be identified as causing disease in human beings. So leprosy is known as Hansen's disease (WHO 1997). The *Mycobacterium* usually affects peripheral tissues, such as the skin, peripheral nerves, the mucosa of respiratory tract and other tissues such as bone and some viscera. Leprosy, if left untreated, can be progressive, causing permanent damage to the skin, nerves, limbs and eyes.

The primary external signs of the disease are skin lesions with sensory and motor impairment followed by the disease progression in the later stage. The loss of sensation (anesthesia) in the extremities leads to infections and progressive damage to the fingers and feet caused by injuries and ulcers. The loss of body function results in disabilities and disfigurements.

Individuals who suffer from leprosy, particularly those with multi-bacillary leprosy (high bacterial load), are sources for the spread of infection. The mode of transmission of bacteria is through respiratory system, mainly the nose. Its dissemination through skin lesions seems to be less important (Visschedijk et al. 2000). Patients' household contacts, neighbours and social contacts are at particular risk of contracting the disease (Visschedijk et al. 2000, Remme 2006). The other routes of transmission could be congenital transmission (Duncan 1983), dermal inoculation via tattoo needles (Porrit and Olsen 1947), and exposure to insect or arthropod vectors (Gelber 2008).

Leprosy has affected humanity since time immemorial. It once affected human population had left behind a terrifying image in history and human memory of mutilation, rejection and exclusion from the society (WHO 1997).

Effective treatment became available only in late 1940's with the introduction of dapsone and its derivatives. This revolutionized the approach to leprosy control, since patients could be treated in outpatient clinics, making the highly stigmatizing isolation no longer necessary (WHO 1997).

Leprosy can be cured easily with a simple and highly effective course of three drugs i.e. multi drug therapy (MDT) consisting of Clofazimine, Rifampicin and Dapsone. Currently the regimen lasts for 12 months for both paucibacillary and multi bacillary leprosy (WHO 2010). Oral corticosteroids and thalidomide are helpful in preventing nerve damage by reduce swelling. Long courses are necessary to decrease severity of deformities and disabilities (Legendre et al. 2012). Surgery may sometimes used to drain abscesses to restore nerve function or to improve function of collapsed part.

1.2 *Mycobacterium leprae*: The etiologic agent of leprosy

Mycobacterium leprae and *Mycobacterium lepromatosis* are the causative agents of leprosy. *M. lepromatosis* is a newly identified *Mycobacterium* which was isolated from a fatal case of diffuse lepromatous leprosy in 2008 (Kenneth et al. 2004).

M. leprae is a non-motile, non-spore forming, microaerophilic, acid-fast staining bacterium that usually forms slightly curved or straight rods (Daffe et al. 1993, Vissa et al. 2001). Its cell wall is typical of *Mycobacterium* and has been shown to contain mycolic acids of the Mycobacterial type (Etemadi and Convit 1974). It also contains tuberculostearic acid, which is present only in Mycobacteria (Andersen et al. 1982). The peptidoglycan present on cell wall contains glycine instead of alanine which is a unique feature of *M. leprae*. The examination of tissue infected with *M. leprae* by electron microscope revealed that the bacilli are surrounded by an electron transport zone thought to represent capsule protecting the bacillus against mechanical and chemical degradation (Draper and Rees 1970).

More recently it has been suggested that capsule is composed of phenolic glycolipid (PGL) which is antigenic and species specific (Hunter and Brennan 1981). The antigenic composition of *M. leprae* has so far been studied mainly by gel immunodiffusion and

crossed immunoelectrophoresis. The water soluble extract of organism has been shown to contain a number of antigenic component although less than in cultivable mycobacteria (Closs et al. 1979). One of the water soluble components has been shown to contain *M. leprae* specific epitopes, namely the ML4 antigen (Kronvall et al. 1976). A fluorescent antibody test, specific for *M. leprae* further indicating the presence of species specific epitopes on the surface of the bacterium (Abe et al. 1976). Recent studies suggest that PGL-1 (phosphoglycolipid-1) is involved in the interaction of *M. leprae* with the laminin of Schwann cells, suggesting a role for PGL-1 in peripheral nerve-bacillus interactions (Scollard et al. 2006).

The genome of *M. leprae* was found to be largely inactive, with only 49.5% being protein coding genes compare to *Mycobacterium tuberculosis* genome being 91% protein coding genes. TN genome of *M. leprae* had 50 genes that encode 50 stable ribonucleic acids (RNAs). It is the smallest genome and the most adenine and thymine (A+T) rich genome of any known Mycobacterium. 50% of the genome of *M. leprae* is composed of pseudogenes (inactive and non coding) may account for its slow division time and lack of viability *in vitro*. *M. leprae* specifically lacks a key signaling gene that induces a promoter sequence to form heat shock response proteins (Porrit and Olsen 1947).

1.3 Classification of leprosy

According to Ridley and Jopling system of classification, leprosy is classified into five groups. The Ridley and Jopling classification of leprosy was based on immunological response of the host to *M. leprae* (Ridley and Jopling1966, Dharmendra 1985).

Polar tuberculoid (TT): It is the initial stage of leprosy, characterized by one or more hypopigmented skin macules and anesthetic patches, where skin sensations are lost because of damaged peripheral nerves that have been attacked by human host's immune cells. This stage of leprosy is known as polar tuberculoid or indeterminate leprosy. They may be subsiding in few years or may progress to borderline or lepromatous leprosy.

Borderline tuberculoid (BT): As the disease progress to subsequent stage, which is similar to polar tuberculoid type except that lesions are smaller and more numerous, such type of leprosy is known as borderline leprosy. Disease may stay in this stage or revert back to tuberculoid form or may it will progress to more severe forms.

Borderline borderline leprosy (BB): It is of intermediate severity and is the most common form present in leprosy patient. In this stage, numerous, red, irregularly shaped lesion are present in a patient. Sensory loss is moderate and peripheral nerve involvement is common. This type of leprosy is unstable, may improve or get worst to further severe form.

Borderline lepromatous (BL) and polar lepromatous (LL) are lepromatous form of disease. It is associated with symmetric skin lesions, nodules, plaques, thickened dermis and frequent involvement of the nasal mucosa resulting in nasal congestion and epitasis (nose bleeding). However lepromatous leprosy is the most severe form of disease having optimum bacterial load than any other type.

In 1982, the WHO study group for chemotherapy for control programs recommended the classifications of all patients be based on the Ridley-Jopling classification and the estimated bacterial load in the slit-skin smears. The TT and BT patients who had bacillary index (BI) <2+ were classified as paucibacillary leprosy (PB) and BB, BL and LL who had BI>2+ were classified as multi bacillary leprosy (MB) (Ridley and Jopling 1965, Dharmendra 1985).

1.4 Leprosy reactions

During the course of leprosy, immunologically mediated episodes of acute or sub-acute inflammation may occur known as leprosy reaction. Leprosy reactions (LR) are characterized by an intense and sudden activation/reactivation of host immune responses that frequently affect the peripheral nerves. The studies indicated that during the course of study 16-56% of patient develop irreversible nerve function impairment (Britton and Lockwood 2004) that is mainly caused by leprosy reaction. In 2010, approximately 5.8% of newly detected leprosy cases worldwide presented grade-2 disabilities at diagnosis

(WHO 2011). Substantial fractions of leprosy reaction (30-40%) are diagnosed concurrently with leprosy (Scollard et al. 1994, Ranque et al. 2007), which could partially explain the persistent detection of severe disability at the leprosy diagnosis. One current goal of leprosy control is a 35% global reduction in grade-2 disabilities by the end of 2015 (Pannikar 2009).

There are two major types of leprosy reactions: Type-1 reaction (T1R) or reversal reaction and Type-2 reaction (T2R) or erythema nodosum leprosum. However patients may also develop Lucio's phenomenon and diffuse lepromatous leprosy, but these episodes represents a small proportion of all leprosy reaction cases. Though reactional episodes are characterized as acute outcomes but patient may also present a chronic reactional state with one reaction type. Patients present with one reaction type rarely suffer from different types of leprosy reaction (Rea and Sielings 1998, Benard et al. 2009).

The clinical presentation of T1R and T2R are distinct. However, both reaction types might share certain molecular control mechanism, which is shown by similar cytokine profiles presented during T1R and T2R (Scollard et al. 2006). When patients with either LR type were compared with non-reactional leprosy patients, the cell - mediated immune responses were observed at significantly higher levels in both systemic and local cutaneous lesions (Modlin et al. 1985, Moraes et al. 2001). The elevations of tumor necrosis factor (TNF), which is a pro-inflammatory cytokine, have been observed in the serum and cutaneous lesions of T1R and T2R and in the nerve biopsies of T1R patients (Scollard et al. 2006).

Type-1 reactions are characterized by a delayed hypersensitivity to *Mycobacterium leprae* antigens (Gell & Coombs type-IV reaction) and a sudden, abrupt increase in the cell-immune responsiveness in the lesions (Ridley and Radia 1981, Little et al. 2001). Type-1 reaction affects 20-30% of leprosy patients (Roche et al. 1991, Scollard et al. 1994, Saunderson et al. 2000, Ranque et al. 2007). The majority of these patients are classified in the borderline spectrum of the Ridley and Jopling (1966). T1R occurs more frequently than T2R (Van Brakel et al. 2005). The clinical manifestations of T1R are an acute inflammation of pre-existing lesions, which can become erythematous, oedematous and

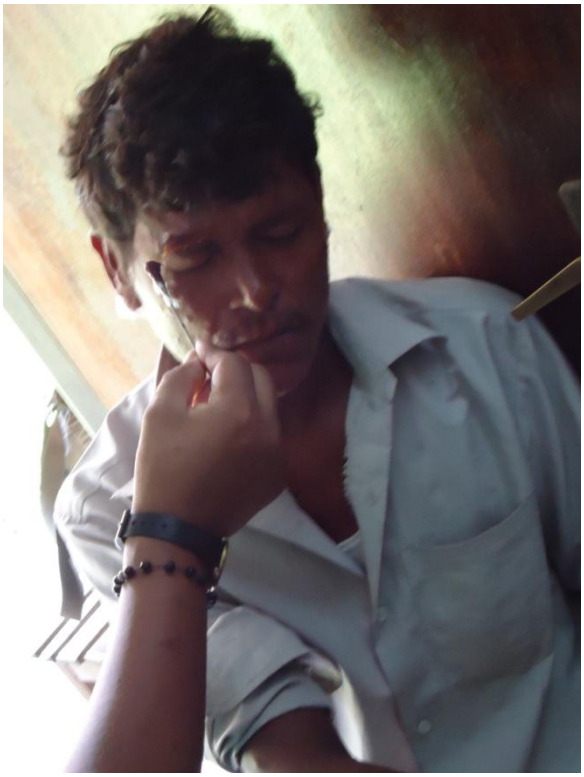


Photo 1: **Patient with Type 2 reaction (ENL)**

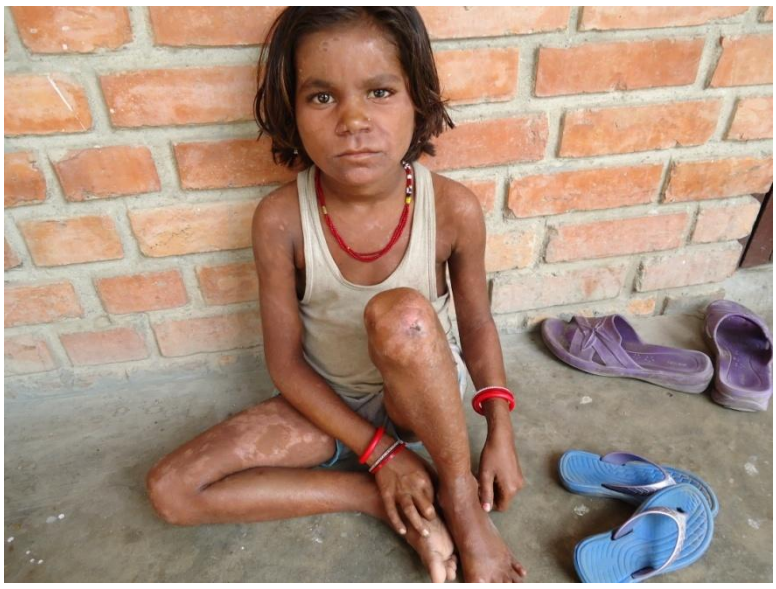


Photo 2: **Patient with Type-1 reaction**

infiltrated (Saunderson et al. 2000). New leprosy lesions may become apparent, most likely caused by an inflammatory response to previously undetected bacilli in the dermis (Rose and Waters 1991). Oedema of the extremities may be present, but systemic complications are unusual (Walken and Lockwood 2008). Risk factors for T1R include age of the individuals (Ranque et al. 2007, Sousa et al. 2007), a bacilloscopic index greater then 4+ (Saunderson et al. 2000), an increased number of lesions at the leprosy diagnosis (Van Brakel and Khawas 1996, Kumar et al. 2004) and *M. leprae* DNA detection by polymerase chain reaction in the lesion biopsies (Sousa et al. 2007).

Type- 2 reaction has been considered an immune complex-mediated disorder (Gell & Coombs type-III reaction) that resembles serum sickness (Wemambu et al. 1969, Naafs 2006). T2R has been characterized as the consequence of both a transistory shift in the CD4/CD8 T-cell ratio towards T-helper (Th) lymphocytes (Modlin et al. 1985) and the increased levels of pro-inflammatory cytokines, such as interferon-γ (INF-γ), IL-1β, TNF, Il-6 and IL-12, in patients, who initially display a predominantly humoral immune response (Sarno et al. 1991, Moraes et al. 1999, Kahawita and Lockwood 2008, Stefani et al. 2009). The histopathological finding in the skin biopsies of acute lesions of T2R patients presents a predominance of neutrophils, eosinophils and mast cells. In chronic lesions, there is a reduction of neutrophils and an increase in the number of lymphocytes and plasmocytes (Mabaley et al. 1965).

The clinical presentation of T2R includes generalized erythematous lesions, nodules and papules that may be superficial or deep, which may become ulcerative or necrotic. Some nodules reach chronicity, become painful and fibrotic and lead to scars. The systemic effects of T2R are notorious and include high fever, oedema and a variety of complications such as nephritis, arthritis and iridiocytes (Mabaley et al. 1965, Kahawita & Lockwood 2008).

T2R mainly affects patients with multibacillary (MB) leprosy which are classified as the borderline lepromatous (BL)/LL pole of the disease spectrum. Patients presenting bacterial index higher than 4+ in skin smears are at higher risk for T2R (Becx-Bleumink and Berhe 1992, Manandhar et al. 1999).There is a wide variation of T2R prevalence across distinct geographical settings and ethnic boundaries. In Brazil, approximately 37%

of BL and LL cases develop T2R, while in India, Nepal and Thailand; the proportion is between 19-26 % (Kahawita and Lockwood 2008).

1.5 Host response to *M. leprae*

In very early infection, *M. leprae* is readily phagocytosed by host cells, usually macrophages. The ability to kill parasites in macrophages is dependent upon fusion of phagosomes and lysosomes and effective activation of the macrophages. Defective fusion has been described in macrophages infected with *M. tuberculosis* (Hart et al. 1972) and *M. leprae* has been found free in the cytoplasm of macrophages (Levy and Evans 1973).

An alternative way for the immune apparatus to cope with *M. leprae* is by means of cytotoxic T lymphocytes. The bacilli seem to be very dependent up on an intracellular environment and so defense mechanisms might depend to a considerable harboured cell such as Schwann cells.

In the massive infection of lepromatous patients immunosuppressive mechanisms probably play an important role in counter- acting the immune responses to *M. leprae* antigens. Immune suppressive plasma factors, antiidiotype antibodies, immune complexes and mycobacterial products could be involved.

The cell wall of *Mycobacteria* is difficult for macrophages to digest and eliminate. Cell wall antigens will thus persist in the infected cells long after the bacilli are dead (Krieg and Meyers 1974). Delayed type hypersensitivity cause only harm to host and have no effect on the infection. Suppression of this delayed hypersensitivity could be accomplished through specific suppressor cells, non-specific plasma factors, or anti-idiotype antibodies.

Non-specific immune suppression may come into action when high levels of antigen are present due to multiplication on privileged sites or when the host's protection has failed. Plasma factors which have a suppressive effect on mitogen responses and mixed lymphocyte cultures is a common finding in lepromatous leprosy (Nelson et al. 1975) and to a lesser extent also in borderline leprosy.

Immune complexes in antibody excess also have an inhibitory effect on lymphocytes responses which is common in lepromatous leprosy (Bjorvatn et al. 1976). Macrophages can suppress lymphocyte responses when loaded with mycobacterial products (Rook 1975) and the disease in mycobacterial load by effective anti-leprosy treatment could result in a working of immune suppression and thus the high incidence of reversal reactions during the first year of treatment. Non-specific suppressor T lymphocytes have been described in lepromatous leprosy (Mehra et al. 1974), in these patients erythema nodosum leprosum (ENL) commonly occurs, which is considered to be a typical example of immune complex disease. An imbalance between helper and suppressor T lymphocytes has recently been demonstrated in patients with ENL with a temporary predominance of helper cells over suppressor cells (Msana et al. 1982). This illustration that T-lymphocytes are important in modulating the course of the disease even on patients at the lepromatous pole

1.6 Intestinal parasites

Intestinal parasites are microorganisms that live in the intestine. Some are pathogenic which cause problems while some are non-pathogenic can live for long periods in the bowel without causing symptoms or requiring treatment. Infection by intestinal parasitic worms is wide spread throughout the world, affecting hundreds of millions of people (WHO 2002).

Parasites are the organism that lives in another organism (host), consumed host nutrients and leave toxic waste inside the host. There are different types of parasites but the important one fall under 4 different groups. They are Protozoa, Trematoda (flukes), Cestoda (tapeworms) and Nematodes. Intestinal parasitic infections such as amoebiasis, ascariasis, hookworm infection and trichiuriasis are among the 10 most common infections in the world (WHO 1987).

Parasitic infections are governed by behavioral, biological, environmental, socioeconomic and health system factors. Local conditions such as quality of domestic and village infrastructure, economic factors such as monthly income, employment and social factors such as education influence the risk of infection disease transmission and associated morbidity and mortality (Yakubu et al. 2003).

Apart from causing morbidity and mortality, infections with intestinal parasites have been associated with stunning, physical weakness and commonly slow mental progress especially in the case of children (Nokes and Bundy 1994).

1.7 Intestinal parasites in reaction and non-reaction leprosy patient

Leprosy reactional episodes (REs) are serious complications of leprosy because those reactions are most likely the predominant cause of permanent nerve damage, leading to disability and deformities. There is an urgent need to understand the pathogenesis of these alterations to determine which patients may be considered to be at risk, as these episodes can occur before during and after treatment for leprosy (Shegal and Sharma 1988, Rea and Modlin 1991). Chronic intestinal parasitic infections have become the subject of speculation and investigation in relation to the spreading and severity of infectious diseases such as leprosy and HIV/AIDS (Bentwich et al. 1995, Diniz et al. 2001).

Resistance to intracellular pathogens such as *M. leprae* is dependent upon an effective T helper type1 (Th1) immune response. On contrary, intestinal helminth are known to subvert the host's immune response towards to Th2-type immune response, which may affect the host's ability to mount an effective response to *Mycobacteria*. The study suggests that a pre-existing infection by intestinal helminth may facilitate the establishment of *M. leprae* infection or its progression to more severe form of leprosy (Diniz et al. 2010).

A non- reaction leprosy patient if continuously exposed to intestinal parasite infection may acquire reactional episodes and with serious complications and severity of disease (Diniz et al. 2010). It means that intestinal parasites are high in reactional leprosy patient than in non-reactional leprosy patient (Diniz et al. 2010).

1.8 Objectives of the study

The objectives of the study are categorized as general objectives and specific objectives.

1.8.1 General Objective

To study the prevalence of intestinal parasitic infection among reaction leprosy patient and non reaction leprosy patient visiting at Lalgadh leprosy service centre, Dhanusha.

1.8.2 Specific Objectives

(i) To determine the prevalence of intestinal parasites in the reaction and non–reaction leprosy patient.
(ii) To analyze the association between leprosy reaction and gastrointestinal parasitic infection.

1.9. Significance of the Study

A significant association between intestinal parasites infections and different bacterial infections such as multibacillary leprosy, pulmonary tuberculosis (Diniz et al. 2010) and Staphylococcal Pyomiositis (Moreira-Silva et al. 2002) was reported. A recent research suggests that multi-bacillary leprosy prevalence is higher among patients with parasites infections when compared to patients without worms (Diniz et al. 2010).

Nepal declared elimination of leprosy as public health problem (defined as reducing the prevalence < 1case/10000populations) in January 2010. However fiscal year 2011/2012 saw an increase in the number of new cases as well as registered prevalent cases and thereby an increase in the overall prevalence rate. This FY, 17 districts were observed with PR more than one case per 10000 populations. Banke reported the highest PR of 2/10000 populations followed by Dhanusha, Mahottari and others districts (DoHS 2012).

So, data from these previous studies helped to design a prospective study to investigate the "prevalence of intestinal parasitic infections in leprosy reaction and non-reaction leprosy patients". As very few work had been conducted in past to see the co-infection of intestinal parasites in leprosy patients, an attempt has been done to carry out the above mentioned comparatives study (Gupta 2002, Diniz et al. 2010).

2. LITERATURE REVIEW

2.1. Epidemiological aspects of leprosy: International overview

Most of the neglected tropical diseases (NTD) burden in Latin America and the Caribbean occurs in Brazil, where about 40 million people living under, poverty, are infected with one or more NTD. In the year 2013, Brazilian Ministry of Health aims to focus on two NTDs. They are leprosy and intestinal parasites. Leprosy and intestinal parasites both are curable, therefore early detection and treatment are essential in improving lives and reducing disease burden in endemic area (Corona 2013).

In Africa, the leprosy prevalence dropped from 57,516 cases in 2000 to 33,690 in 2010 which represents a decrease of 42%. About 500,000 leprosy cases were successfully treated in Africa during the last decade. However the regional average detection of new leprosy cases in the African region has been about 30,000 per year with 66% of new multibacillary cases (WHO 2012). Apart from leprosy, Soil Transmitted Helminthiasis (STH) is next big challenge in this region with greatest number of infection in Sub-Saharan Africa.

Besides Brazil and Africa, the pockets of high endemicity of leprosy cases can be found in Indonesia, Philippines, India, Madagascar, Mozambique, Nepal, Democratic republic of Congo and United Republic of Tanzania (WHO 2012).

2.2 Immunological aspects of leprosy co-infected with intestinal parasites: International overview

Helminth are the group of parasites which predominantly infects the intestines of humans and are known to elicit an immune modulation in the host characterized by an up-regulation of T-helper type 2 (Th2) responses, which results into eosinophilia and high immunoglobulin E titers, often associated with the patients(Allen and Maizels 1996).

Besides up-regulating Th2 responses, helminthic antigens also down-regulate Th1 responses, which affects the ability of the hosts immune system to mount an effective response to other infections (Pearlman 1993, Macedo 1998). In case of leprosy patients, an effective T-helper type 1(Th1) responses is responsible for providing a resistance to *M. leprae* particularly in paucibacillary tuberculoid form. On the other hand, T- helper type 2 (Th2) immune responses favours the mycobacterial antigens to produces the severe multibacillary lepromatous form (Abulafia and Vignale 1999).

Hence it is assumed that an up-regulation of Th2 cytokines, elicited by an infection with intestinal helminth, may affect the immune response against *M. leprae,* favouring the establishment of this bacterial infection or multibacillary forms of leprosy(Abulafia and Vignale 1999) suggesting one previous study as an evidence that, by investigating individuals for leprosy co-infected with filariasis in different geographic regions with the similar leprosy prevalence were compared, it was reported that frequency of lepromatous leprosy was higher in areas where filariasis was hyperendemic, than on those where it was either of low endemicity or absent (Prost et al. 1979).

A study on immunological consequences of leprosy patients co-infected with intestinal parasites particularly the helminths revealed that, intestinal helminths may disturb the immune regulation through the onset of a hypoergic/anergic state (Bradford 1976, Baran et al. 2001), in which interferon (IFN)-γ were decreased significantly in tuberculoid and lepromatous leprosy patients co-infected with intestinal helminths ($p<0.005$) when compared to leprosy patients without worms. Conversely, an intracellular interleukin (IL) - 4 increased significantly ($p<0.005$) in both tuberculoid and lepromatous leprosy patients co-infected with intestinal parasites when compared to leprosy patients without worms. Similarly intracellular IL-10 levels increased ($p<0.003$) in lepromatous leprosy patients co-infected with intestinal worms when compared to leprosy patients without worms (Diniz et al. 2010), which in turn could facilitate subsequent infections to severe form of the disease (Bradford 1976, Baran et al. 2001).

The result from the above study further provide an evidence that Th1 immunity is down modulated during intestinal helminthic infection and consequently an up-regulation of Th2 response mediated by intestinal worms(Bentwich et al. 1995, Kalinkovich et al.

1998, Borkow et al. 2004). Therefore it is possible that an existing infection with intestinal helminths may facilitate a subsequent infection by *M. leprae* or its progression to more severe form of leprosy (Abulafia and Vignale 1999, Goulart et al. 2002).

Studies with rodents infected with gastrointestinal nematodes in U.S.A in 1997 have provided information about immune mechanisms that how the immune system of the host protect against these nematodes. The study suggests that immune mechanism includes CD4+T cells which are responsible for the host protection against intestinal parasites (Finkelman et al. 1997). In the case of tuberculoid leprosy patients co-infected with intestinal parasites show reduced CD4+T cell frequency (Diniz et al. 2010).

Other most important immune cells include IL-4 which is involved in host protection and limits severity of infection. As the IL-4 has multiple effects on immune system, more than one of which may protect against particular parasite (Finkelman et al. 1997). However the hosts do not have the ability to identify individual parasites as stimuli for specific protective cytokine responses. As a result hosts display a set of defense mechanisms against these parasites but the specific defense mechanism may not be required to defend against a particular parasite and may even further damage a host infected with that parasite (Finkelman et al. 1997), as in case of tuberculoid and lepromatous leprosy patients co-infected with intestinal worms, has higher frequency of cells expressing Th2 cytokines with IL- 4 and IL- 10 in WBC were observed than from those patients without intestinal worms (Diniz et al. 2010).

A down-regulation of the cellular immune response to *M. leprae* and *M. tuberculosis* antigens and an up-regulation of Th2 cytokines were reported in children infected with *Onchocerca volvulus* in the Republic of Cameroon. This study suggests that infection with intestinal helminths intervene with the normal immune response to mycobacterial infection (Stewart et al. 1999). Cell mediated immunity is not invariably induced in the natural infection by certain slow growing parasites, as in the case of leprosy and antibody can be induced that is exclusive of a strong cell mediated response (Bretscher 1992). It is proposed that certain events in such cases subvert the normal regulatory process that control the class of immunity induced (Diniz et al. 2010). In this case, parasite infected cells (bearing low parasite antigen) induce antibody but are not susceptible to antibody-

dependent mechanisms, so they are not eliminated. As a result chronic infection and uncontrolled growth of the parasites occurs, often with fatal consequences (Bretscher 1992).

Apart from the above study, there was a comparative study done in Ethiopians and Israeli (non-Ethiopians) regarding their immune profile to fight against infections (Protozoan, helminthes, bacterial or viral) diseases. After examined those people, it was found that immune cells from highly immune-activated individuals (harbouring intestinal parasites) were defective in several signaling responses, and all of which are restored gradually following antihelminthic treatment. These findings support that chronic helminthes infections cause persistent immune activation that results in hypo-responsiveness and impaired immune functions and become susceptible to different bacterial, viral, protozoan or helminths infections or co- infections (Borkow et al. 2000).

This result add further evidence of impaired T cell activation, through the observed CD69 down-regulation in WBC from both tuberculoid leprosy patients and lepromatous leprosy patients co-infected with intestinal helminths, which may be due to a Th2 effect resulting from exposure to intestinal helminth antigens (Diniz et al. 2010). Supporting the above facts, reports from laboratory department of tropical medicine (Egypt) suggests that the interaction between helminths and the host's immune system provokes particularly immune-modulatory mechanisms that ensures their survival in the host for years and these changes might impair the immunological responses to different infectious diseases (Kamal and EL Sayed 2006).

2.3. Leprosy co-infected with intestinal parasites

In the further study to investigate the association of intestinal nematodes and leprosy, a prospective case control study was carried out, which reports that overall leprosy cases contain 147 nematode with most prevalent nematodes were *Ascaris lumbricoides* and *Trichuris trichiura.* Similarly in protozoan parasites, there is no significant difference found in case and control group. However the most frequent protozoan identified in this study were *Entamoeba spp.* and *Giardia lambia* (Diniz et al. 2001). This study was further elaborated to elucidate the immunological consequences of leprosy patients co-

infected with intestinal parasites. The result shows that infection with intestinal helminth was similar to previous study (Diniz et al. 2001), except for *Strongyloides stercoralis* infection in leprosy cases and *Enterobius vermicularis* in control group (Diniz et al. 2010). This study further supports the evidence that intestinal helminth deviate the host immune response towards Th2 immune response which established the mycobacterial infections to more severe form of the disease (Abulafia and Vignale 1999).

Leprosy reaction (LR) patients who presents with type 1 reaction (T1R) or type 2 reaction (T2R) were treated with long term immunosuppressive doses of steroids like Prednisone (prednisolone) (Legendre et al. 2012) is associated with numerous metabolic side effects and a reported case of fatal *Strongyloides* (Kahawita et al. 2008). Study suggested that female *Strongyloides stercoralis* possesses receptors for these drugs (corticosteroids) that induce its multiplication and development contributing to its dissemination to other organs and resulting in severe complications (Genta 1986).

Sometimes during the management of reaction in leprosy patients particularly with type 2 reactions treated with prolonged steroids, if infected with *S. stercoralis* acquired hyper-infection due to it and can cause death of the patients (Leang et al. 2004). Diagnosis of *S.stercoralis* in the early stage *is* very important, if the individual is found to be infected and the prevention is of utmost importance because this parasite has the huge mortality rate due to its hyper- infection syndrome (Hagelskjaer 1994, Leang et al. 2004, Corti et al. 2011). These studies suggest that possibility of *S. stercoralis* infection would be higher in leprosy patients due to its hyper infection syndrome (Diniz et al. 2010). A similar study regarding infection due to *Strongyloides stercoralis* was carried out in Argentina and the result demonstrates that 57% patients infected with diarrhea and were classified as chronic intestinal strongyloidiasis, 20% remains asymptomatic and 20% developed hyperinfection syndrome (Corti et al. 2011).

Leprosy can be cured easily with a simple and highly effective course of three drugs i.e. multi-drug therapy (MDT) consisting of Clofazimine, Rifampicin and Dapsone. The regimen lasts for 12 months for both paucibacillary and multibacillary leprosy (WHO 2010). A recent report suggests that about half of leprosy patients experience acute episodes of inflammatory reactions caused by their immune response. Reactions may

occur before, during and even after the completion of MDT (Worobec 2012). To manage the reactional states in patients an oral dose of corticosteroids (prednisone) is given to the patients (Kahawita et al. 2008, Legendre et al. 2012). Based on this treatment approach, an hypothesis was generated which suggests that immunosuppression due to treatment of multi-drugs therapy induced adverse reactions with glucocorticoid and the change in host immune response due to leprosy itself, might increase the risk of parasitic infections (Dolo et al. 2002).

To test this hypothesis, a case control study was carried out in Mali (Africa) based on the systematic search for parasites among leprosy patients. The results of the study reveals that among protozoan parasites prevalence of *Entamoeba histolytica* and *Entamoeba coli* is higher in leprosy patients (Dolo et al. 2002) which resembles the previously discussed study (Diniz et al. 2001, 2010). Among the helminth infection hookworms were higher in the case than in controls ($p=0.02$). The above results suggest that despite the corticotherapy and immunosuppression due to leprosy, there was no difference in prevalence of pathogenic parasites and treatment with glucocorticoid did not suggest any impact on parasite infection (Dolo et al. 2002). However this study did not show any presence of *S. stercoralis,* which can cause hyperinfection syndrome due to prolonged immunosuppressive therapy, and therefore supports the evidence that it is the most pathogenic intestinal worms which should be prevented (Leang et al. 2004, Corti et al. 2011)

As far as above, several studies had been put forwarded regarding intestinal parasites associated with leprosy and its impact on host's immune system. Apart from these, a new dimension of research work had been under taken in Cairo (Egypt) where a study was based on parasitic infections associated with malignancy and leprosy. It was a case control study among patients with different malignant diseases, leprosy patient and control group.

The result of parasitic infections, as revealed by urine and stool examination was significant ($p<0.05$) in 43.3% of patients suffering from different malignant diseases and non- significant ($p>0.05$) in 29.3% of leprosy patients compared to 22% in control groups (Azab et al. 1992).

The most prevalent protozoan parasites were *E. histolytica* and *G. lambia* (Azab et al. 1992) which is similar in leprosy patients co-infected with intestinal parasites (Diniz et al. 2001, 2010). By immunoflorescent antibody test (IFAT), strongyloidiasis gave significant higher positivity in malignancy group than in leprosy (Azab et al. 1992). This result suggest that patients with different malignant diseases were subjected to prolonged immunosuppressive therapy like corticosteroids (Leang et al. 2004, Corti et al. 2011) due to which they were more prone to intestinal parasitic manifestations than leprosy and control groups.

Apart from America, Africa and Mediterranean region, few other countries had attempted to see the impact of intestinal parasites in leprosy patient. In 1983, patients in a leprosarium in Korea were under taken for the study in January 1983. The result shown that total egg positive rate of any kind of helminths was 78.2% (Hong et al. 1983) which resembles the study of leprosy co-infected with intestinal helminths (Diniz et al. 2010).The egg positive rate for each helminthes was *Taenia* spp (3.4%), *Ascaris lumbricoides* (4.5%), *Trichuris trichiura* (72%), *Clonorchis sinensis (*2.8%) and other 0.05%. The results revealed significantly high egg positivity rate of *T. trichiura* (Hong et al. 1983).

In Asia, a case of systemic strongyloidiasis was described in a patient in 1994 who received systemic steroid treatment in district Nilphamari of Bangladesh. The increased use of immunosuppressive and cytotoxic treatment increases the risk of hyperinfection syndrome, if the patient is suffering from *S. stercoralis* (Leang et al. 2004, Corti et al. 2011). Systemic strongyloidiasis is a rare but serious complication of intestinal strongyloidiasis. The condition occurs mainly in immunosuppressed patients and had a significant mortality rate (Leang et al. 2004). Hence awareness of the positivity of systemic strongyloidiasis is essential if such patient develops gastrointestinal or pulmonary symptoms or has repeated episodes of unexplained gram-negative infections while undergoing immunosuppressive treatment (Hagelskjaer 1994).

2.4. Intestinal parasitic infections in leprosy patient in context of Nepal.

About 70% of all health problems and deaths in Nepal are attributed to infectious diseases (NPC, 1998). Many children die from easily preventable and treatable diseases such as diarrhea and dysentery and acute respiratory infection. Recently Ono et al (2001) reported various types of organisms (bacteria, viruses and parasites) associated with diarrhea in Nepal. Of the various infectious diseases, intestinal parasitosis alone constitutes one of the major public health problems in Nepal. Roughly, over 60% of Nepalese are infected with one or more than one species of parasites (Estevez et al. 1983, Ishiyama et al. 2001).

In some rural areas, infection rate can be over 90%. Soil transmitted helminthes are most common (Rai et al. 2000) and the soil even in the capital city is contaminated with helminthes eggs (Rai et al. 2000).Prevalence of leprosy in certain areas is as high as 3.61 Per 1,000 (Bhatt 1991).

It has been observed that food consumption and average energy intake of the Nepalese in some area adequate (Ohno et al. 1998). However a significant loss of nutrient is associated with infection particularly intestinal parasitic infection. Intestinal parasites, even in low or moderate number, cause persistent and poor nutritional status, persistent in children, by causing subtle reduction in appetite, digestion, absorption and acute phase status and increasing intestinal nutrient losses (Lun and Northrop-Clewes, 1993). In the case of vitamin A deficiency, it is caused either by chronic low intake of vitamin A rich foods (Shankar et al. 1996) and or parasite infections (Lun& Northrop-Clewes 1993, Friis et al. 1997, Atukorala and Lanerolle 1999).

In the context of Nepal, leprosy had been eliminated as the public health problem in January 2010 and since has successfully sustained the elimination at national level. However in the fiscal year 2011/2012, 17 districts reported highest prevalence rate of more than 2/10000 population. In the new cases 52.20% were multi-bacillary cases and sex wise 31.6% were female and 6.26% were children. This figure is an increase from 5.19% of previous reporting year which shows the new cases appearing significantly (DoHS 2012).

Regarding leprosy co-infection in leprosy patients there was only a single study conducted in the year 2001. A hospital based study was done in intestinal parasites in leprosy patients and non-leprosy people of Aanadaban leprosy Hospital (ALH) Lalitpur, Kathmandu, to determine the intestinal parasitic infections. The overall prevalence of intestinal (protozoal and helminth) parasites were (47.5%). The prevalence of hookworm was (26.78%), *A. lumbricoides* (23%), *E. histolytica* (12.5%), *Taenia* spp (11%), *Giardia* spp (2.5 %), *H.nana* (2.5%), *T. trichiura* (4%) and *E .coli* (1%). Out of 280 stool samples examined the intestinal parasitic infection was observed higher in leprosy patients (57%) than in a non-leprosy person (36%) (Gupta 2002).

3. MATERIALS AND METHODS

3.1 Study area:

Lalgadh Leprosy Services Centre (LLSC) is located in the Dhanusha district of Nepal. This service centre was established in 1991 and is now one of the largest leprosy regional referral centers in the area. Patients from all over central development region (CDR) of Nepal come to receive treatment at LLSC. In addition of this, Indian patients also make a significant percentage in receiving LLSC's Services. In the year 2068/69, LLSC has treated approximately 2,80,700 people directly and indirectly through its various programmes which includes Rekh-dekhchoutari (RC), Releasing the energy and capabilities of Leprosy affected Individuals and marginalized people – central Dev-Region (RECC-AIM-CDR) Project, village alive project (VAP), Acute Leprosy Complication management Project (ALCOMP) and community awareness and IEC programmes (Annual Report 2012).

3.2. Study design.

The study was designed to assess the association between leprosy reaction and parasite prevalence. Hence purposive sampling method was used. The identification of leprosy reaction and non reaction was done by the leprosy expert (Dermatologist) of LLSC.

3.2.1 Stool sample collection

Out of the 200 stool samples, 100 samples belong to reaction leprosy Patients and 100 belonged to non-reaction leprosy patients visiting at Lalgadh leprosy service centre, Dhanusha. The Laboratory work was conducted in Lalgadh leprosy services centre.

To ensure better condition during sample collection the following precaution were taken;

a) The sampling vials were properly washed, dried and filled with 2-5 ml of 5% formalin solution.
b) Each stool container was distributed after interviewing individually and the stool samples were collected for the examination.

Photo 3: **Stool sample collection**

Photo 4: **Stool smear preparation**

Photo 5: **Microscopic examination**

c) Immediately after collecting the vials, they were brought to the nearest laboratory for further processing such as slide preparation and identification of ova and cysts of the parasites.

3.2.2 Questionnaire survey

Prepared questionnaire was administered in study population to assess the knowledge, attitudes and practices of leprosy reaction and non reaction patients.

3.3. Stool examination

The stool samples collected were undergone macroscopic and microscopic examination. Macroscopic examination of stool samples includes the observation of consistency of the stool (watery, loose, semi-solid), and identification of adult worm or segment of intestinal helminths by naked eyes. Before the microscopic examination of stool samples, thick smear of stool is prepared on the glass slide. Smear was prepared by two methods.

3.3.1 Saline preparation

One drop of normal saline (0.9%) was placed on a clean glass slide using an applicator stick, a small amount of fecal specimen about 2 mg was mixed with saline. Smooth thin preparation was made and covered with fine cover slip by the help of a needle, so as to spread out the emulsion into a thin, fairly uniform and transparent layer. The entire saline preparation was examined systematically under an electric microscope.

3.3.2 Iodine stain preparation

To prepare an iodine mount one drop of Iodine solution (2%) was put on clean glass slide and with an applicator stick a small portion of the faecal specimen was picked and mixed with the drop of iodine. A fine cover slip was put over it by the help of a needle. The microscopic examination was performed.

3.3.3 Microscopical examination of the stool

The smear prepared slides were first examined under the low power of microscope starting from one end of the cover slip to another end and vice-versa after changing field. If any suspicious object was seen it was centered and focused under the high power objective for a detailed diagnosing. The collected stool samples were examined in microscope under 10x and 40x objectives. Specific attention was given to the characters of the helminthes eggs such as shape, size, colour and cysts of protozoan parasites.

3.4. Data analysis

The data obtained from questionnaire survey and result of stool examination were analyzed using SPSS (Statistical Package for the social sciences), version 16.0 for window .A P-value equal to or smaller than 0.05 was considered statistically significantly in the analysis. As well as Ms- Excel 2007 was also used for significant analysis of data.

4. RESULTS

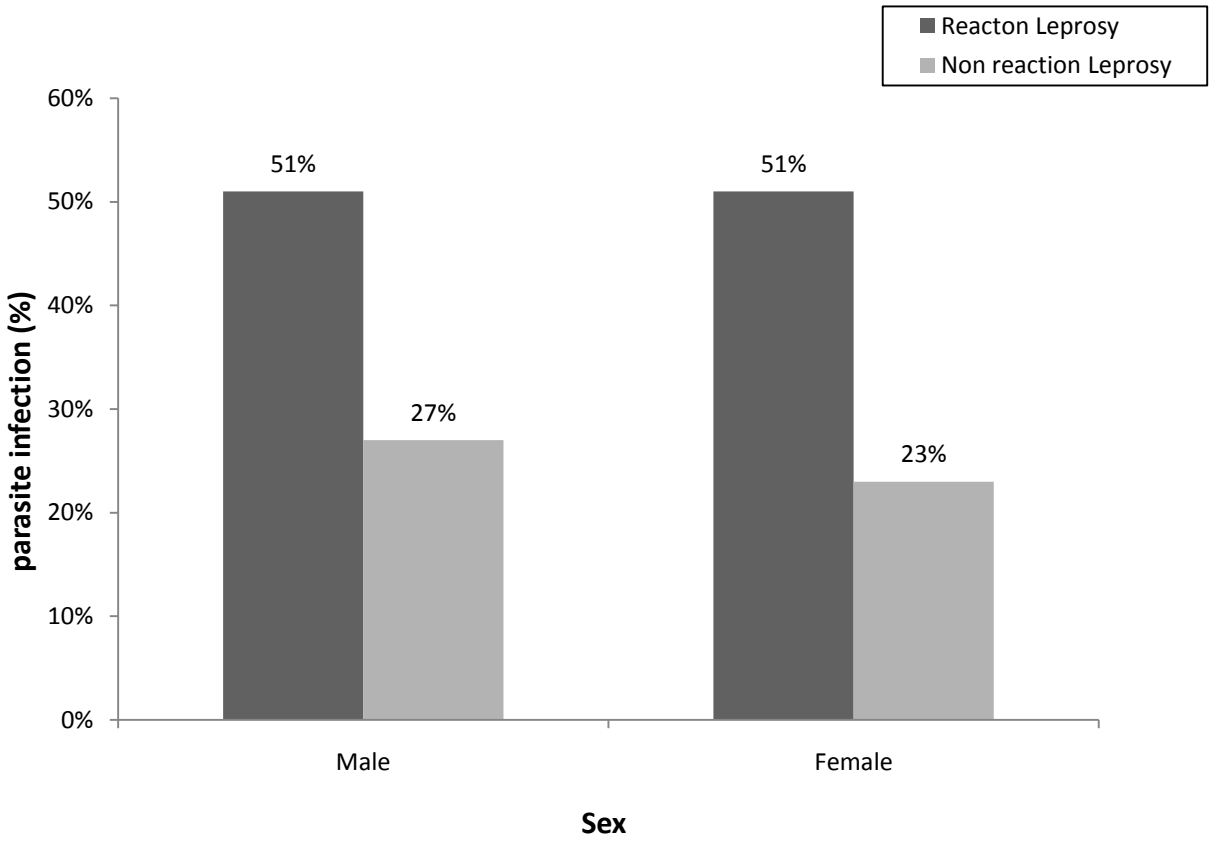
The study was carried out in 100 reaction leprosy patient and 100 non- reaction leprosy patients visiting at Lalgadh leprosy service centre during 2011-2012. The result obtained from stool examination and data obtained from surveillance study through questionnaire were analyzed.

4.1 Association of Intestinal parasites in reaction and non reaction leprosy patients.

A total of 200 stool samples were collected and examined to assess the general prevalence of intestinal parasites. The result indicated that parasitic infection in male (39%) and female (37%) was almost same. Statistically there was no significant difference ($p<0.05$) between sex with respect to parasitic infection. The similar results were obtained while comparing parasitic infection in between sexes among reaction and non reaction leprosy patients (Table 1, Graph 1).

Table 1: Sex wise prevalence of intestinal parasites in reaction and non reaction leprosy patients

Group	**Reaction Leprosy Patient (N=100)**			**Non- Reaction leprosy patient (N=100)**			**Total (N=200)**
Sex	N	Protozoa	Helminth	N	Protozoa	Helminth	No of Parasites
Male	56	23 (41.07%)	6 (10.71%)	58	15 (25.86%)	1 (1.72%)	45 (39.47%)
Female	44	18 (41.00%)	4 (10.00%)	42	10 (23.08%)	0 (0%)	32 (37.00%)



Graph 1: Representation of sex-wise prevalence of intestinal parasites in reaction and non- reaction leprosy patient

Age-wise prevalence of intestinal parasites in reaction and non reaction leprosy patients showed almost similar results. However the rate of infection (30%) was comparatively less in (21-40) years of age group than the other age groups. Statistically there was no significant difference ($p<0.05$) among age groups with respect to intestinal parasitic infection. But the prevalence of protozoan parasitic infection was significantly high in all age groups compared to helminth infection (Graph 2).

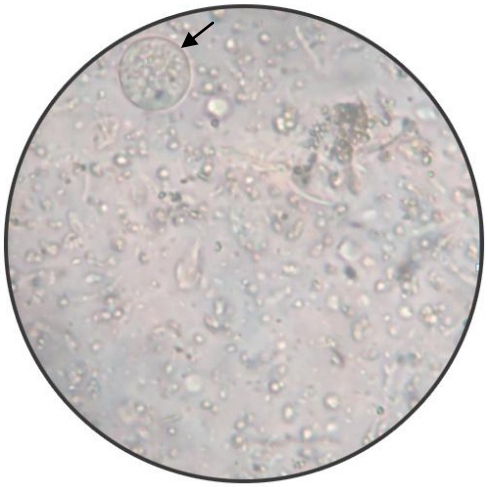


Photo 6: **Cyst of *E. histolytica* (10X x 40X)**

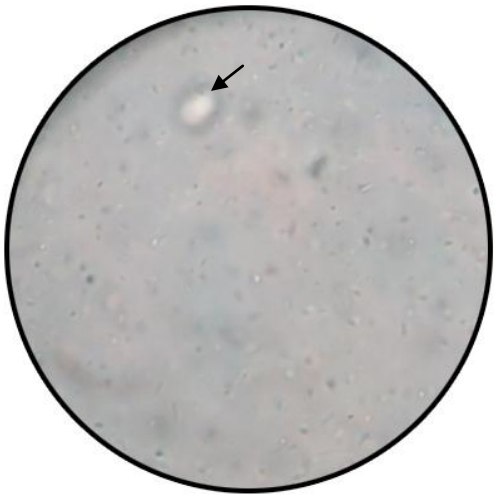


Photo 7: **Cyst of *Giardia lamblia* (10X x 40X)**

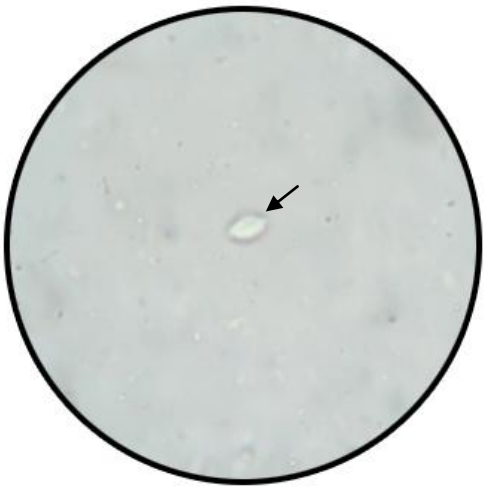


Photo 8: **Trophozoit of *Trichomonas hominis* (10X x 40X)**

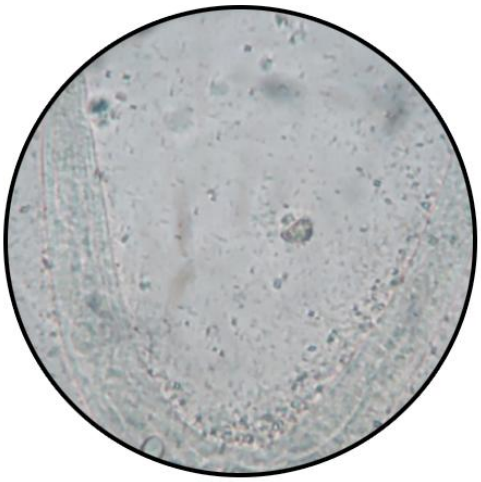


Photo 9: **Larva of *Strongyloides stercoralis* (10X x 40X)**

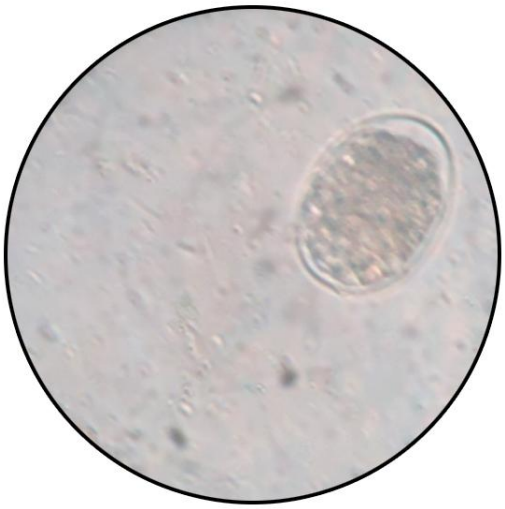
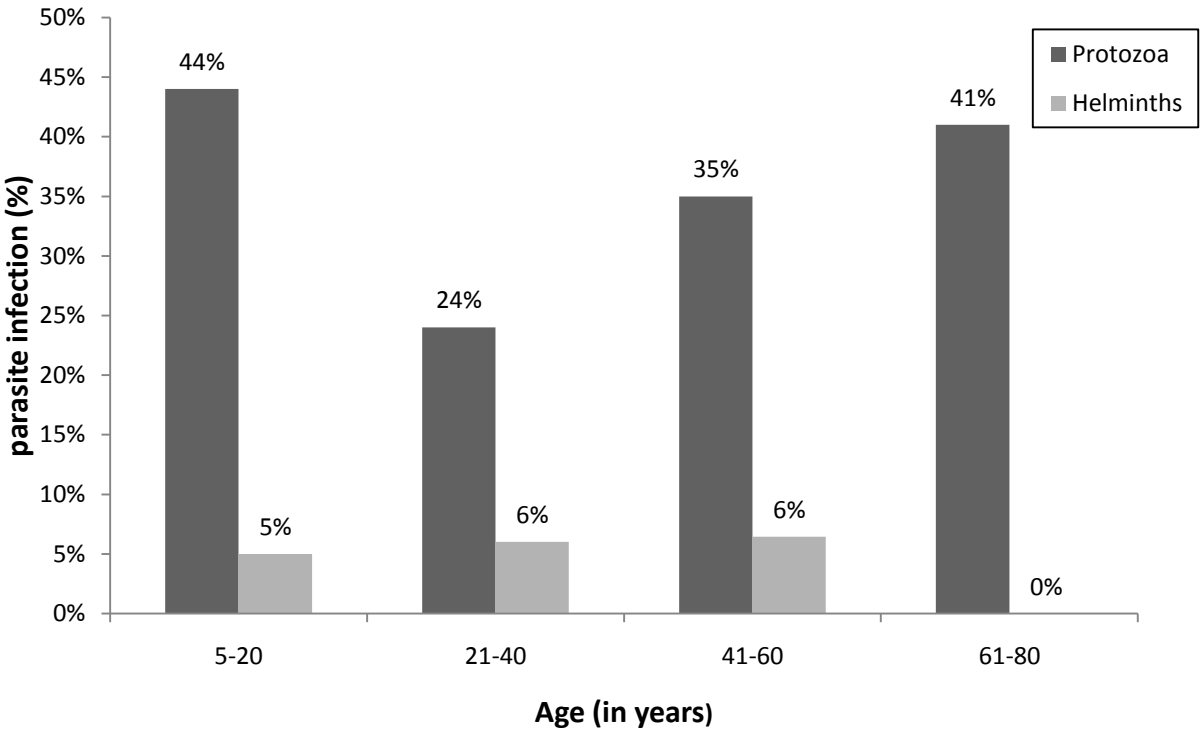


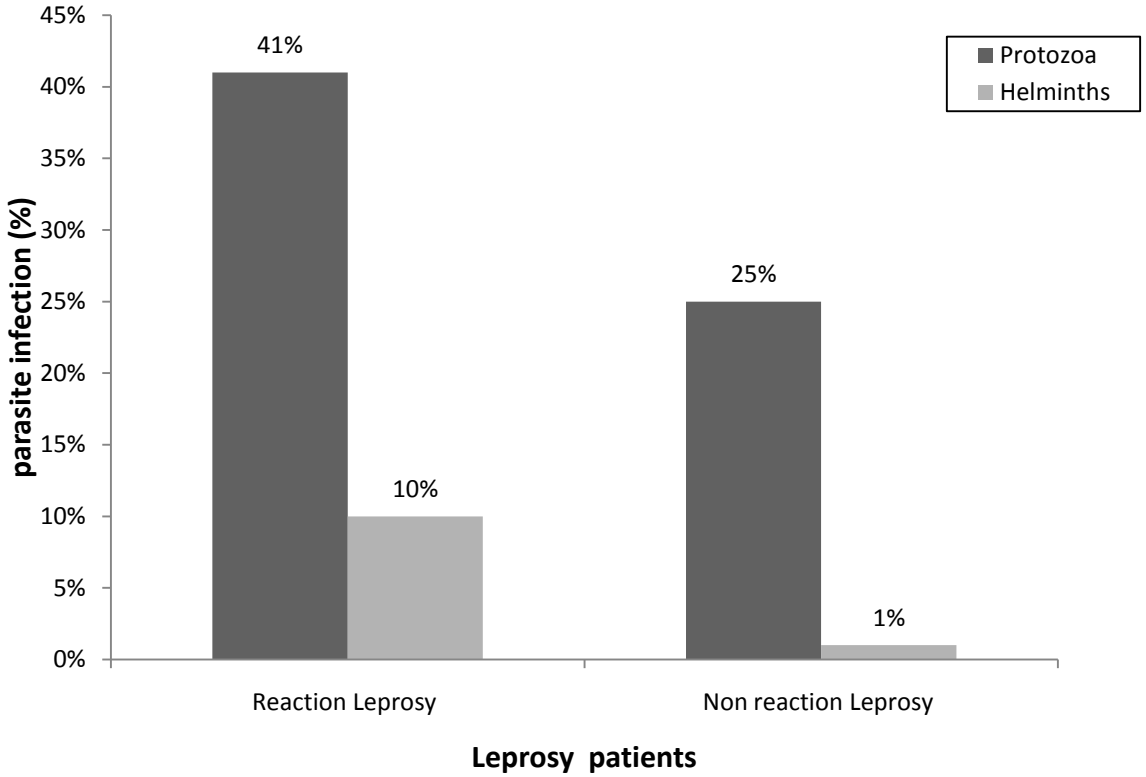
Photo 10: **Egg of hookworm (10X x 40X)**

Photo 11: **Egg of *H. nana.* (10X x 40X)**



Graph 2: Representation of age wise prevalence of intestinal parasites in reaction and non- reaction leprosy patients.

The previous study showed the association between intestinal parasites and leprosy (Diniz et al. 2001& 2010). In the present study, the result revealed that, the infection with at least one specific intestinal parasites was significantly high (51%) in reaction leprosy patients when compared to (26%) in non reaction leprosy patients. Statistically there was significant difference ($p<0.05$) between reaction and non reaction group of leprosy patients with respect to intestinal parasitic infection. However the protozoan parasitic infection was observed more frequent in both reaction and non reaction leprosy patients compared to the helminths infection (Graph 3).

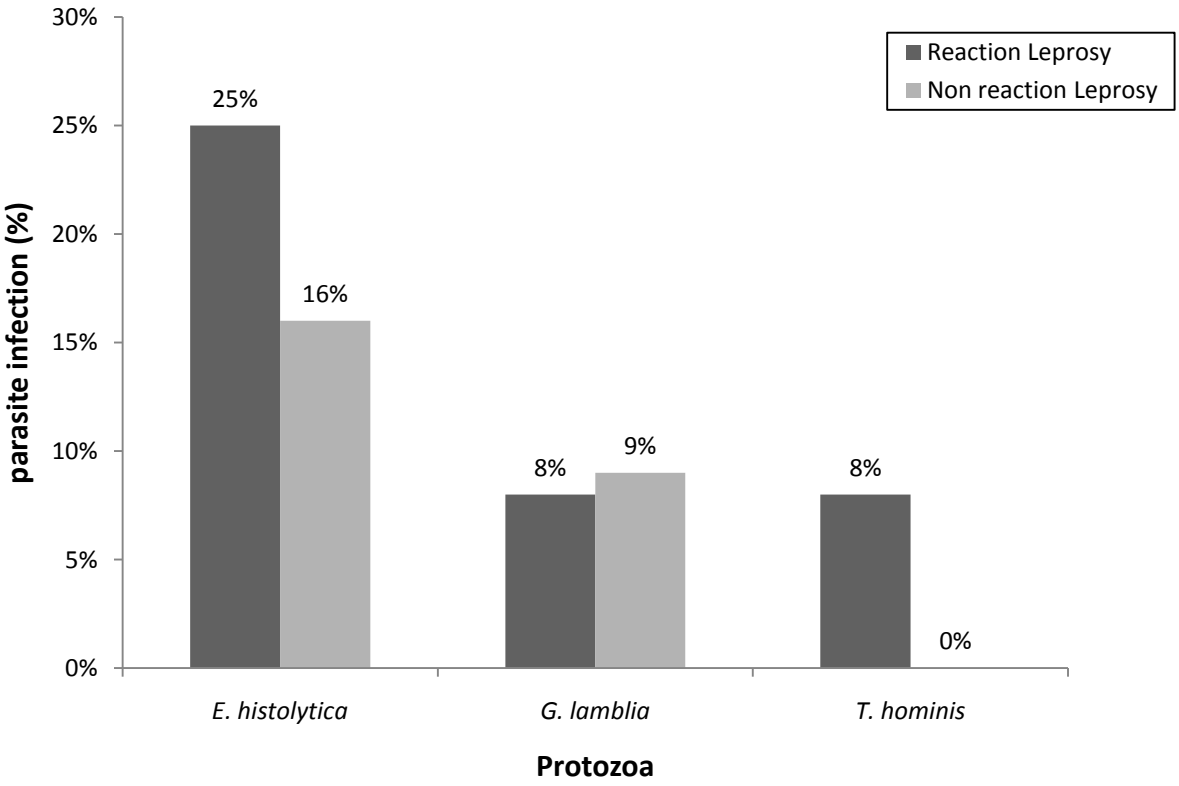


Graph 3: Prevalence of intestinal parasites in reaction and non reaction leprosy patients

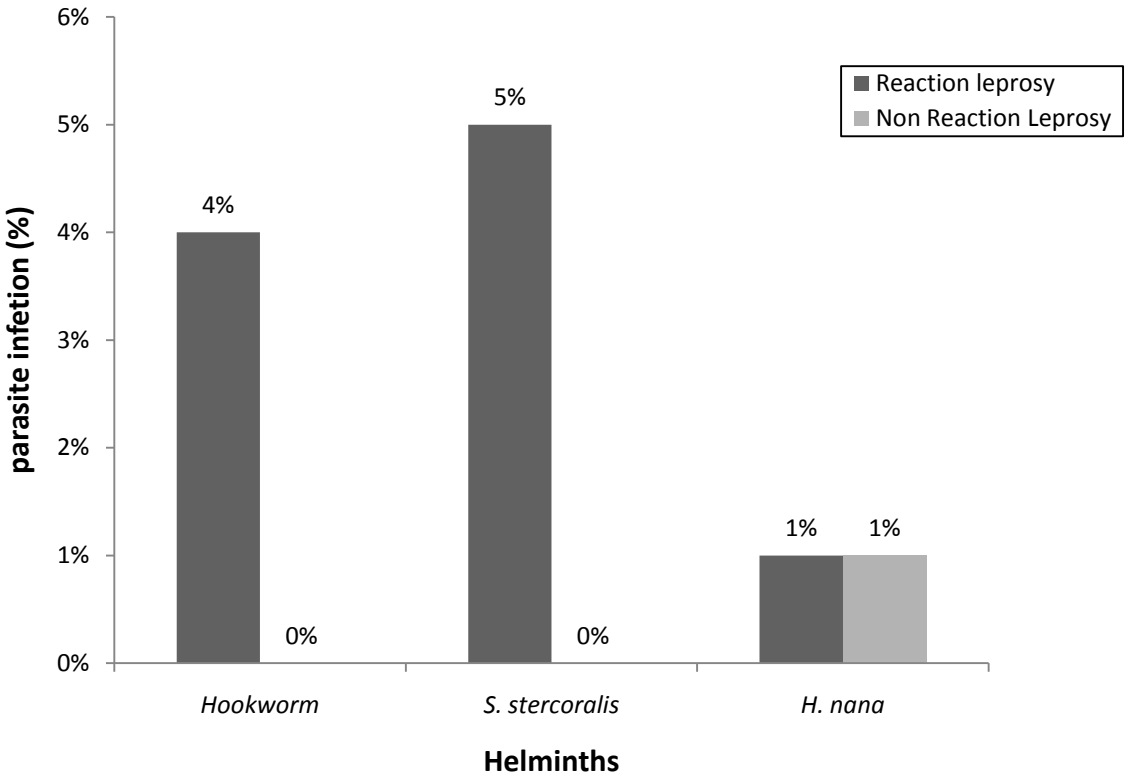
Microscopic examination of stool samples from both reaction and non reaction leprosy patients revealed three different groups of parasites i.e. Protozoa, Nematoda and Cestoda. Trematode parasites were not observed during the study. In general, highest prevalence of *E. histolytica* (20%) (Photo 6) was observed compare to others. While least prevalent parasite was *H. nana* (Photo 11) only one species belonging to group Cestoda. Protozoans and Cestodes parasite were observed in both reaction and non reaction leprosy patients. Statistically there was no significant difference ($p<0.05$) (Table 2). Interestingly *T. hominis* (Photo 8) which is a commensal protozoan parasite was found only in reaction leprosy patients (Graph 4). Similarly Hookworm and *S. stercoralis* (Photo 9) were also found in leprosy reaction patients only (Graph 5). The result indicated the positive co-relation between these parasites and development of reaction in leprosy patients.

Table 2: Comparison of specific intestinal parasites in Reaction and Non-Reaction leprosy patients.

S.N.	Parasites	Reaction leprosy patients (N=100)			Non-reaction leprosy patients (N=100)			Total (N=200)
Protozoa		**M (%)**	**F (%)**	**Total (%)**	**M (%)**	**F (%)**	**Total (%)**	**No .of parasites**
1.	*E. histolytica*	13	12	25	11	5	16	41 (20.5%)
2.	*G. lamblia*	6	2	8	4	5	9	17 (8.5%)
3.	*T. hominis*	4	4	8	0	0	0	8 (4%)
Nematoda								
4.	Hookworms	3	1	4	0	0	0	4 (2%)
5.	*S. stercoralis*	2	3	5	0	0	0	5 (2.5%)
Cestoda								
6.	*H. nana*	1	0	1	1	0	1	2 (1%)



Graph 4: Prevalence of protozoan parasites in reaction and non reaction leprosy patients



Graph 5: Prevalence of helminths parasites in reaction and non reaction leprosy patients

4.2 Knowledge, Attitude and Practices (KAP) of leprosy reaction and non reaction patients in relation to parasitic infection.

KAP survey was conducted in all 200 leprosy reaction and non reaction patients. The obtained data were assessed along with the result obtained through microscopic examination of stool samples. Association between KAP variables to that of reaction and non reaction categories of leprosy patients were statistically analyzed.

Leprosy patients, who were obtained an education minimum upto primary level and who could read or write to some extent, those patients' name were categorized under literate group. While those leprosy patients who were unable to read and write were categorized under illiterate group. In this study, KAP survey revealed (69%) of illiterate and (30%) of literate leprosy patients. However the result of stool examination showed that, prevalence of intestinal parasites was almost same in literate (36%) and illiterate (39%) leprosy patients. Statistically there was no significant difference ($p<0.05$). However the most

frequent intestinal parasites observed in both groups belong to protozoa compared to helminth infection (Table 3).

Table 3. Intestinal parasites in relation to literacy of reaction and non-reaction leprosy patient.

Group	**Reaction Leprosy Patient (N=100)**			**Non-reaction leprosy patient (N=100)**			**Total (N=200)**
Literacy	N	Protozoa	Helminth	N	Protozoa	Helminth	No.of Parasites
Literate	27	14 (51.85%)	2 (7.41%)	34	6 (17.65%)	0 (0%)	22 (36.1%)
Illiterate	73	27 (36.98%)	8 (10.96%)	66	19 (28.79%)	1 (1.5%)	55 (39.6%)

Regarding knowledge of mode of transmission of parasite, the leprosy patients who had got either knowledge about parasites or could understand the mode of transmission to some extent, were kept under awared group. Whereas those patients who were unknown to parasites and its mode of transmission were kept under not awared group. Of the 200 patients interviewed, only (13%) were awared and (86%) were not awared. The result of stool examination showed that, there is almost similar parasitic infection rate in both awared (30%) and not awared (40%) leprosy patients. Statistically there was no significant difference ($p<0.05$). However protozoan parasites were high in both groups of leprosy patients irrespective of knowledge of mode of transmission of parasites compared to intestinal helminths (Table 4).

Table 4: Intestinal parasites in relation to knowledge of mode of transmission of parasites in reaction and non-reaction leprosy patient.

Group	**Reaction Leprosy Patient (N=100)**			**Non-reaction leprosy patient (N=100)**			**Total (N=200)**
Knowledge about mot	N	Protozoa	Helminth	N	Protozoa	Helminth	No of Parasites
Awared	9	5 (55.55%)	0 (0%)	18	3 (16.66%)	0 (0%)	8 (29.62%)
Not Awared	91	36 (39.56%)	10 (10.98%)	82	22 (26.83%)	1 (100%)	69 (39.90%)

The KAP survey indicated that, practice of sanitary disposal of faecal matter adopted by leprosy patients were of two types. Patients who were using toilet for sanitary disposal are kept under close toilet and those who used to defecate in the open field are kept under open toilet. The prevalence of intestinal parasites in reaction and non-reaction leprosy patients with respect to their sanitary condition revealed almost similar infection with (31%) in toilet user and (42%) in non toilet user. Statistically there was no significant difference ($p<0.05$) between toilet users and non user with respect to parasitic infection. However the infection rate of protozoan parasites is high among reaction and non reaction leprosy patients while comparing with helminth infection (Table 5).

Table 5: Intestinal parasites in relation to sanitary condition of reaction and Non-reaction leprosy patient.

Group	**Reaction Leprosy Patient (N=100)**			**Non-Reaction leprosy patient (N=100)**			**Total**
Sanitation	N	Protozoa	Helminth	N	Protozoa	Helminth	No of Parasites
Closed toilet	29	12 (41.37%)	3 (10.34%)	43	8 (18.60)	0 (0%)	23 (31.04%)
Open toilet	71	29 (40.84%)	7 (9.85%)	57	17 (29.82)	1 (100%)	54 (42.18%)

Leprosy patients interviewed for their occupation indicated that, maximum patients were involved in agriculture (30%) which is similar to those of housewife (30%), while least involved in service (2%). However the result of stool examination showed that intestinal parasitic infection were observed to be almost similar in both groups of leprosy patients involved in different occupation. However service holder and housewife were seemed to be least infected (25%) in both groups. Statistically there was no significant difference ($p<0.05$) in the prevalence of intestinal parasites in both groups with respect to occupation of patients. However the frequency of protozoan parasites was observed to be high in both reaction leprosy patients and non reaction leprosy patients irrespective of their occupation when compared to helminth infection (Table 6).

Table 6. Intestinal parasites in Reaction and Non- Reaction leprosy patients involved in different occupation.

Group	Reaction Leprosy Patient (N=100)			Non- reaction leprosy patient (N=100)			Total (N=200)
Occupation	N	Protozoa	Helminth	N	Protozoa	Helminth	No of Parasite
Agriculture	31	9 (29.03%)	3 (9.67%)	29	11 (37.93%)	1 (3.44%)	24 (40%)
Business	7	5 (71.43%)	0 (0%)	7	2 (28.57%)	0 (0%)	7 (50%)
Service	1	1 (100%)	0 (0%)	3	0 (0%)	0 (0%)	1 (25%)
Labor	16	6 (37.5%)	4 (25%)	15	2 (13.33%)	0 (0%)	12 (38.71%)
Housewife	31	9 (29%)	2 (6.45%)	29	4 (13.79%)	0 (0%)	15 (25%)
Unemployed	14	11 (78%)	1 (7.14%)	17	6 (35.29%)	0 (0%)	18 (58.1%)

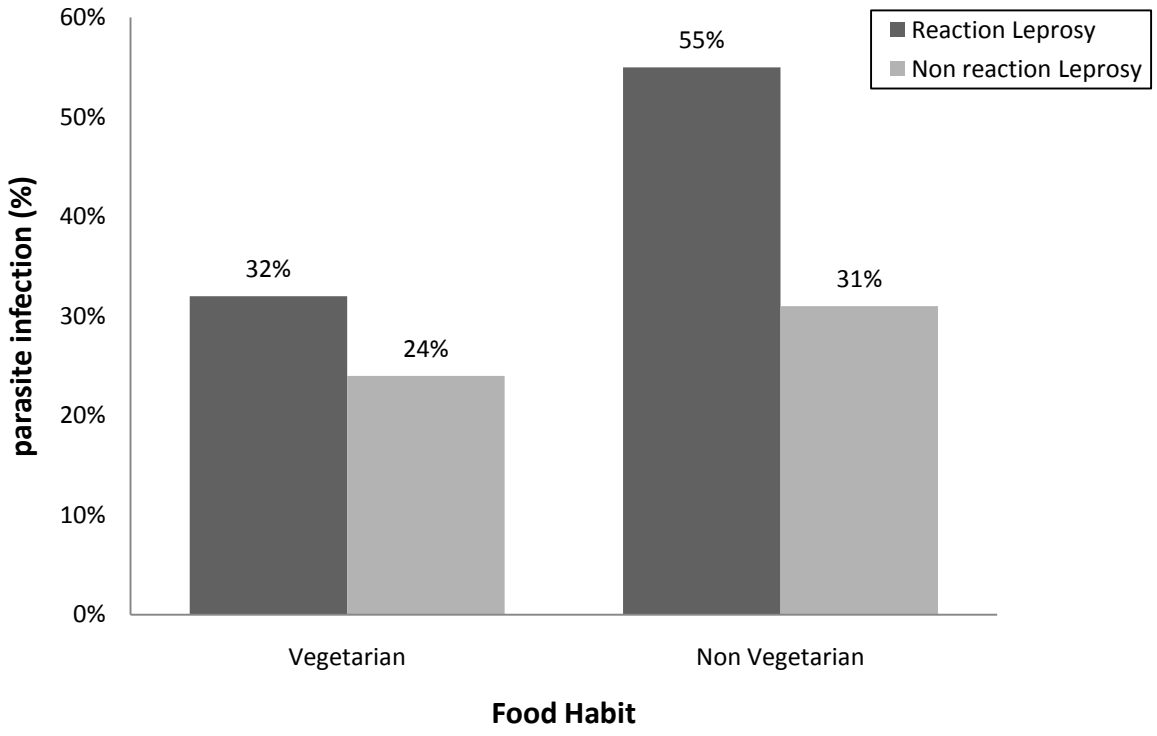
Table 7: Percentage of intestinal parasites in relation to use of water source of Reaction and Non-Reaction leprosy patient.

Group	Reaction Leprosy Patient (N=100)			Non-reaction leprosy patient (N=100)			Total (N=200)
Water supply	N	Protozoa	Helminths	N	Protozoa	Helminths	No of Parasites
Tap water	16	7 (43.75%)	1 (6.25%)	16	4 (25%)	0 (0%)	12 (37.5%)
Tube-well water	84	34(40.48%)	9 (10.71%)	84	21 (25%)	1 (1.2%)	65 (38.69%)

The result of stool examination shown that overall presence of intestinal parasites among both groups are nearly same about 38%.Statistically, there was no significant relation ($p<0.05$) between intestinal parasites and use of water resource, by reaction and non-reaction leprosy patient. This result suggests that the parasitic infection acquired by both groups, is assumed that both are having nearly same source of water consumption (Table 7).

Table 8: Percentage of intestinal parasites in relation to food habit consumed by Reaction and Non- Reaction leprosy patient.

Group	Reaction Leprosy Patient (N=100)			Non- Reaction leprosy patient (N=100)			Total (N=200)
Food habits	N	Protozoa	Helminthes	N	Protozoa	Helminthes	No of Parasites
Vegetarian	19	6 (31.8%)	0 (0%)	29	7 (24.12%)	0 (0%)	13 (27.08%)
Non-vegetarian	81	35(43.21%)	10 (12.34%)	71	18 (25.35%)	1 (5.6%)	64 (42.10%)



Graph 6: Intestinal parasites in relation to food habit of reaction and non reaction leprosy patients

The result of stool examination shows that, parasitic infection was high (42%) in non-vegetarian patients of both groups. Statistically, there was no significant relation ($p<0.05$) between intestinal parasites and food habit adopted by reaction and non- reaction leprosy patient. However presence of protozoan parasites was high in non- vegetarian in both groups i.e. in reaction leprosy patient and in non- reaction leprosy patents when compared to the helminth infection (Table 8, Graph 6).

5. DISCUSSION

The present study has been carried out on intestinal parasitic infection in reaction leprosy patients and non reaction leprosy patients, of Lalgadh Leprosy Services Centre, Dhanusha. In the present work, an association ($p<0.05$) between intestinal parasitic infections in leprosy reaction and non reaction patients was reported through microscopic examination of stool samples and its co-relation with KAP variables.

In the present study, the result of stool examination revealed six different species of specific intestinal parasites belonging to protozoa and helminths, harbouring the individuals of reaction and non reaction leprosy patients. The intestinal parasites observed in the study include 3 species of protozoa i.e. *Entamoeba histolytica, Giardia lamblia* (Photo 7) and *Trichomonas hominis* and 3 species of helminths i.e. Hookworm, *Strongyloides stercoralis* and *Hymenolepis nana.* The frequently observed protozoan parasites were *E. histolytica* (25%) in reaction leprosy patients and (16%) in non reaction leprosy patients, as well as *Giardia lamblia* (8%) in reaction leprosy patients and (9%) in non reaction leprosy patients. This data further supports the study of protozoan infection in leprosy patients (Azab et al. 1992, Dolo et al. 2002, Diniz et al 2010). Further this study showed a higher prevalence of *E. histolytica* infection in both reaction and non reaction leprosy patients when compared to other intestinal parasites observed, which provides further evidence to previous study regarding leprosy co-infection with *E. histolytica* (Dolo et al. 2002).

The present work did not reveal other protozoan parasites like *Cryptosporidium* spp, *Isospora* spp, which was reported from leprosy patients (Dolo et al. 2002). Similarly Toxocariasis by IFAT (Azab et al. 1992). The present study didn't intend to study particularly these parasites which could be present among leprosy patient.

Among the protozoan parasites observed in the study, there was a presence of an intestinal flagellate i.e. *Trichomonas hominis*, which is although a commensal parasite, but interestingly it was observed only in reaction leprosy patients with each (4%) infection in male and female. However this parasite was not reported from leprosy patients so far. The presence of this parasite only in leprosy reaction patients may be

assumed that it might possess certain immuno-modulatory mechanism in the host, which favours the growth of *M. leprae* infection to more severe form of the disease. Therefore further investigation is required to understand the immunopathology of this intestinal parasite.

Helminths infection, particularly the nematodes which include hookworms (4%) and *S. stercoralis* (5%) were found only in reaction leprosy patients when compared to non reaction leprosy patients. These findings support as an evidence of the previous study of helminth co-infection in lepromatous leprosy patients (Diniz et al. 2010). Among the Cestodes only *H. nana* was found with infection of (1%) in each leprosy reaction and non reaction patients. However this parasite is not reported from other studies. Nematodes were more prevalent than Cestodes which is supported by the previous studies of nematode co-infection in leprosy patients (Diniz et al. 2001) and prevalence of hookworms in leprosy patients (Dolo et al. 2002). The present study did not reveal other nematodes like *A. lumbricoides* and *T. trichiura* in either of the leprosy reaction or non reaction patients, but reported by other studies (Hong et al. 1983, Diniz et al. 2001, 2010).

In this study, overall helminths infection was higher (5%) in reaction leprosy patients compared to non reaction leprosy patients. This study suggested that infection with intestinal helminths intervene with the normal immune response to mycobacterial infection (Stewart et al. 1999) and the possibility of an existing infection with intestinal helminths may facilitate a subsequent infection with *M. leprae* or its progression to more severe form of leprosy (Abulafia and Vignale 1999, Goulart et al. 2002). This result further provide an evidence that Th1 immunity is down modulated during intestinal helminthic infection and consequently an up-regulation of Th2 response mediated by intestinal helminths (Bentwich et al. 1996, Kalinkovich et al. 1998, Borkow et al. 2004, Diniz et al. 2010), which in turn could facilitate subsequent infections to severe form of the disease (Bradford 1976, Baran et al. 2001).

The prevalence of *S. stercoralis* infection was higher in reaction leprosy patients which further confirmed helminths co-infection in lepromatous leprosy patients (Diniz et al. 2010).Further the study indicated that, Leprosy reaction and *Strongyloides* co-infection was intimately associated, confirmed by previous observation (Leang et al. 2004 & Corti et al. 2011). In the present study, *S. stercoralis* infection were observed from those reaction patients who were subjected to prolonged immunosuppressive therapy of steroids (prednisolone), regardless of drug dosages, which further support the study regarding *S. stercoralis* hyperinfection syndrome in leprosy patients subjected to immunosuppressive therapy of steroids (Hagelskjaer 1994, Leang et al. 2004 & Corti et al. 2011). As *S. stercoralis* infection was observed higher in leprosy reaction patients, such patients should be diagnosed early because it has huge mortality rate of about 87% (Leang et al. 2004), therefore prevention is utmost important. Also hookworm infestation in reaction patients was 4% and is supported by its presence in leprosy patients through previous demonstration (Dolo et al. 2002, Diniz et al. 2010).

An independent association ($p<0.05$) between sex with respect to intestinal parasitic infection in reaction and non reaction leprosy patients was observed, which is confirmed by previous study that frequency of intestinal helminths was significantly higher in leprosy patients regardless of their sex (Diniz et al. 2001, 2010). It was assumed that both these sexes may live under the similar risk factors for acquiring intestinal parasitic infection.

Similarly, the study indicated that, there is no significant difference ($p<0.05$) among age groups with respect to intestinal parasitic infection in leprosy reaction and non reaction patients, which is confirmed by previous observation (Diniz et al. 2001, 2010). The result of the study suggested that 21-40 years age group had least infection (30%) with intestinal parasites. It may be assumed that they were awared of intestinal parasites or may subject themselves to mass drug administration against intestinal parasites (particularly helminths).

The present study showed an independent association ($p<0.05$) between literacy with respect to intestinal parasitic infection in leprosy reaction and non reaction patients. However, education helps to minimize the risk of transmission of infectious disease and

associated morbidity and mortality (Yakubu et al. 2003). It has been assumed that, the literate leprosy patients enrolled in the study did not have education enough to cope with infectious disease.

Further regarding mode of transmission of parasites, it was observed that there is no significant difference ($p<0.05$) in relation to mode of transmission of parasite with respect to intestinal parasitic infection in leprosy reaction and non reaction patients. As parasitic infection, is governed by behavioural, biological, environmental, socioeconomic and health system factors of an individual, in the case of leprosy patients, some or all of these factors plays a significant role, which influences the risk of transmission of parasites irrespective of awareness (13%) in some patients that was observed in the study.

The result indicated an independent association ($p<0.05$) among occupation adopted with respect to intestinal parasitic infection in both leprosy reaction and non reaction patients. It was observed that maximum people (30%) were involved in agriculture which suggested that majority of the patients had low socioeconomic condition, that results in lack of nutrition which in turn depressed the immunity level of an individual and make susceptible to parasitic infection.

Lack of hygiene in an individual, plays a significant role in prevalence of intestinal parasitic infection. However, it was reported that, there is no significant difference ($p<0.05$) in the prevalence of intestinal parasites in leprosy reaction and non reaction patients with respect to sanitary condition. It was assumed that patient from both groups were living under the similar circumstances to acquire the infectious disease.

From the present study, it revealed an independent association ($p<0.05$) in use of water resource in leprosy reaction and non reaction patients with respect to intestinal parasitic infection. It may be assumed that both groups of patient are having similar source of water consumption. Similarly there was no significant difference ($p<0.05$) between intestinal parasites and food habit adopted by leprosy reaction and non reaction patients. However non vegetarian were found to be more infected (42%). It was suggested that food and water contamination is one of the major sources of parasitic infection

6. CONCLUSION AND RECOMMENDATION

Data presented in the study indicated that similar parasitic infection was observed in male and female of both reaction and non reaction leprosy patients, suggested that both these sexes were under the similar risk of exposure to parasite infection. Age wise prevalence of intestinal parasites in reaction and non reaction leprosy patients showed similar results.

In the present study infection with at least one specific intestinal parasite was significantly high in reaction leprosy patients compared to non reaction leprosy patients. Microscopic examination of stool samples revealed three groups of parasites which include Protozoa, Nematoda and Cestoda. Protozoan parasites were frequently observed in both leprosy reaction and non reaction patients, which include *E. histolytica* and *Giardia lamblia. T. hominis*, the intestinal protozoan parasite which was observed only in reaction leprosy patients. Among helminths nematode group include hookworm and *S. stercoralis* which were found only in reaction leprosy patients. Similarly least infection with *H. nana*, only one species belonging to Cestodes found in both reaction and non reaction leprosy patients.

KAP survey among reaction and non reaction leprosy patients in relation to parasitic infection showed that prevalence of intestinal parasites was almost similar in literate and illiterate patients visiting at lalgadh leprosy services centre.

Similarly knowledge of mode of transmission of intestinal parasites had no significant difference in intestinal parasitic infection among leprosy reaction and non reaction patients. The prevalence of intestinal parasitic infection in leprosy reaction and non reaction patients is independent of occupation adopted by them. The present study suggested that sanitary condition did not make any difference in the prevalence of intestinal parasitic infection in leprosy reaction and non reaction patients. In addition to it, food and water consumption by leprosy reaction and non reaction patients did not have any impact on intestinal parasitic infection.

Based on the data obtained from the present study, the recommendation needed for leprosy reaction and non reaction patients co-infected with infection parasites was discussed below.

a) The people should be made aware of leprosy and intestinal parasitic infection as both of them are curable.
b) Avoid walking bare foot and any infection through the abraded skin, particularly leprosy detected people.
c) Need for educating people especially the socially marginalized group about public health, sanitary condition and infection diseases like parasitic infection and leprosy in the community.
d) The research work on the prevalence of intestinal parasitic infections in leprosy patients should be encouraged and preventive measures should be adopted.
e) If the leprosy patient is found to be infective of any intestinal parasites, deworming should be made by using anti protozoal or anti-helminthic drug.
f) Reaction leprosy patients should be given due care and put on for stool examination routinely, for identification of parasites and their deworming, if present.

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ANNEXES

QUESTIONNAIRE

Name…….. Date……

Age/Sex……..

Locality……

1) Literate: a) Literate ☐ b) Illiterate ☐

2) Occupation: a) Agriculture ☐ b) Business ☐
c) Service ☐ d) Labor ☐
e) Housewife ☐ f) Unemployed ☐

3) Water supply: a) Tap water ☐ b) Tube well water ☐
c) River water ☐

4) Food habit: a) Vegetarian ☐ b) Non vegetarian ☐

5) Knowledge of mode of transmission of parasites
a) Awared ☐ b) Not Awared ☐

6) Sanitary condition: a) Closed toilet ☐ b) Open toilet ☐

7) Type of Leprosy patient: a) PB ☐ b) MB ☐

8) Leprosy Reaction: a) Present ☐ b) Absent ☐
If present, which type of leprosy reaction?
a) Type-1 reaction ☐ b) Type-2 reaction ☐

9) Result of stool examination: a) Positive ☐ b) Negative ☐

If positive, parasite present……..

……
Signature of respondent