Integrated school garden, nutrition, water, sanitation and hygiene interventions for improving nutritional and health status of schoolchildren in Nepal

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Summary

Background: Malnutrition occurs at all stages of the lifecycle. However, there is little information on malnutrition during school age. The concentration of malnutrition in Asia is greater than anywhere else on Earth. It is reported that 156 million children are stunted, 50 million children are wasted and more than 50% of the 146 million underweight children in the world are living in the South Asia.

Malnutrition is a major underlying cause of child mortality within Nepal and anaemia among infants and children is high. In Nepal, 41% percent of children were suffering from chronic malnutrition in 2011. Similarly, iron-deficiency anaemia is one of the top ten leading causes of years of life with disability among all age groups. The dietary risks, malnutrition, unsafe water, sanitation and hygiene (WASH) are among the top ten causes of disability-adjusted life years (DALYs). Intestinal parasitic infections are among common public health problems of children in Nepal and the infection rate has primarily been attributed to the appalling unhygienic environmental conditions. The intestinal parasitic infection and amoebic dysentery stand second among the top ten causes of hospital visits within the country. According to the Global Burden of Diesease Study (GBD) and the World Health Organisation (WHO)/United Nations Chidren's Fund (UNICEF) "Joint Monitoring Programme for Water Supply and Sanitation", 8% of deaths in children aged 8-14 years in Nepal, were caused by diarrhoeal diseases and attributed to inadequate WASH condition as a primary risk factor.

Nutrition as a cross-cutting theme is closely interlinked with multifactorial determinants. Malnutrition is an outcome of poor nourishment (i.e. inadequate, unbalanced or excessive nourishment), while other factors, such as illness and poor sanitation also contribute to malnutrition. Three interacting groups of underlying factors contribute, in turn, to inadequate dietary intake and infectious diseases: household food insecurity; inadequate maternal and child care; and poor health and environmental services. Hence to address these challenges, the more recent strategic frameworks call for a combination of nutrition-sensitive and nutrition-specific interventions, including synergies between agriculture, nutrition and WASH. However, there is lack of evidence about the contribution of integrated agriculture, nutrition and WASH interventions in minimising malnutrition and anaemia.

A project entitled "Vegetables go to School: improving nutrition through agricultural diversification" (VgtS) has been developed to improve schoolchildren's nutrition, through introducing school vegetable gardens and additional complementary school-based health interventions. The VgtS project is funded by the Swiss Agency for Development and Cooperation (SDC) and was implemented in five countries (i.e. Burkina Faso, Bhutan, Indonesia, Nepal and the Philippines). This PhD thesis was embedded in the VgtS project in Nepal as an operational research study to contribute to the outcome 3 of the project;

generating increased knowledge on how school vegetable gardens contribute to improved nutrition and health of schoolchildren, as well as the interaction with WASH.

Goal and objectives: This PhD thesis aims at assessing the effects of complementary school garden, nutrition and WASH interventions on nutrition and health status of schoolchildren in Nepal. In order to achieve this aim, the following four specific objectives were pursued:

(i) to investigate the WASH conditions at the unit of selected schools, households and community in the districts of Dolakha and Ramechhap in Nepal;

(ii) to determine the local epidemiology of malnutrition and intestinal parasitic infection among schoolchildren;

(iii) to assess the knowledge, attitude and practices (KAP) of schoolchildren and caregivers regarding nutrition and WASH conditions; and

(iv) to evaluate the effects of supplemented complementary school gardens, nutrition and WASH interventions on children nutritional and health status.

Methods: The study was designed as a cluster-randomised controlled trial (RCT). The trial included 12 schools randomised into three arms: arm 1 implementing a school garden (SG); arm 2 with additional WASH and nutrition complementary interventions (SG+WASH); and arm 3 without any interventions (control) in the districts of Dolakha and Ramechhap of Nepal. The baseline cross-sectional survey was conducted between March and May 2015 among 705 children aged 8-16 years. The pack of complementary interventions to the school garden has been implemented after the baseline survey. A follow-up survey was conducted within the same cohort of children one year after the baseline survey, in June 2016.

In both surveys, questionnaires were administered to evaluate WASH conditions at the level of schools, households and communities. Dietary intake was assessed using a food frequency questionnaire and 24-hours (24-h) recall. Haemoglobin (Hb) levels were measured using a HaemoCue digital photometer. Stool samples were subjected to wet-mount, Kato-Katz and formalin-ether concentration methods for the diagnosis of intestinal parasitic infections. Water quality was assessed using the Delagua testing kit and flame atomic absorption method.

Results: A total of 75% of school drinking water source samples and 77% point-of-use samples at schools, 40% water source samples in the community, and 27% point-of-use samples at household levels were contaminated with thermo-tolerant coliforms (TTC). The values of water samples for pH (6.8–7.6), free and total residual chlorine (0.1–0.5 mg/L), mean lead concentration (0.01 mg/L), and mean arsenic concentration (0.05 mg/L) were within national drinking water quality standards. The presence of domestic animals roaming

inside schoolchildren's homes was significantly associated with drinking water contamination (adjusted odds ratio (aOR): 1.64; 95% confidence interval (CI): 1.08–2.50; *p*=0.02).

Overall, 27.0% of the participating children were stunted and 11.3% were wasted. We observed a significant difference of stunting and wasting between boys and girls (stunting: 31.6% for boys *versus* 22.8% for girls, *p*=0.01; wasting: 15.9% for boys *versus* 7.1% for girls, *p*=0.01). We also found a significant difference in stunting between the two districts where Dolakha had a higher stunting rate than Ramechhap (30.1% in Dolakha *versus* 15.7% in Ramechhap; *p*=0.01).

The overall prevalence of anaemia was 23.9% at baseline. The lack of meals prepared in the households (aOR=2.36, 95% CI: 1.14-4.92; *p*=0.01) and not having supper (aOR=3.46, 95% CI: 1.09-11.03; *p*=0.04) were significantly associated with anaemia. The dietary diversity scores were lower among anaemic compared to non-anaemic children. Consumption of vitamin A-rich fruits and vegetables were negatively associated with anaemia, but not significantly so. More than half (55.0%) of the children had at least one sign (e.g., loss of hair pigment) of nutritional deficiency.

The overall prevalence of intestinal parasite infections was 39.7%. *Trichuris trichiura* and *Giardia intestinalis* were the predominant helminth and intestinal protozoa species, with a prevalence of 31.0% each. Children from households lacking soap for hand washing were at higher odds of intestinal parasite infections (aOR=1.81; 95% CI: 1.13-2.89; *p*=0.01), while children from households without freely roaming domestic animals showed lower odds of *G. intestinalis* compared to those households with such animals (aOR 0.52; 95% CI: 0.33-0.83; *p*=0.01). We found considerable morbidity among the surveyed children, including fever (31%) and watery diarrhoea (22%). Water contamination with TTC did not emerge as significant risk factor for intestinal parasitic infections.

This study shows that the diet of surveyed schoolchildren mainly comprised of starchy staples and legumes. The mean consumption of animal product per week was low (1.96 for poultry, 1.18 for red meat, 0.81 for fish and 0.91 for milk products). Five dietary patterns were derived: mixed food, vegetables and lentils, milk products, salty snacks, and processed food pattern scores. The vegetables and lentils pattern scores were negatively associated with stunting (aOR 0.84; 95% CI: 0.66-1.08, *p*=0.17) after adjusting for regional differences, demographic and behaviour risk factors, however not significant.

At the follow-up, stunting was slightly reduced in complementary intervention arm (SG+WASH) (20% to 18%; *p*=0.92, compared to control) contrary to a slight increase in the school garden arm (SG) (18% to 20%; *p*=0.54, compared to control) and control (20% to 19%). Anaemia slightly decreased in SG+WASH (33% to 32%; *p*<0.01, compared to control)

and markedly increased in the control arm (23% to 42%) and the SG (21% to 44%; $p=0.56$, compared to control). Handwashing with soap (i) before eating and (ii) after defecation strongly increased in SG+WASH arm (i) 74% to 97%; $p=0.01$ compared to control with 78% to 84%; (ii) 77% to 99%; $p=0.36$ compared to control with 78% to 92%. While the prevalence of parasite infections significantly declined in SG+WASH arm (37% to 9%; $p<0.01$, compared to control) and a minor decline in SG (34% to 27%; $p=0.42$, compared to control) and stable in the control arm (44% to 42%).

Conclusions: Malnutrition, anaemia and intestinal parasitic infections, particularly soil-transmitted helminths, are of an important public health concern among schoolchildren in the districts of Dolakha and Ramechhap, Nepal. Our complementary interventions implemented in schools and households, increased children's awareness on fruits and vegetables intake, reduced anaemia, stunting and intestinal parasitic infections among schoolchildren within one year. Hence, this study showed that a combination of agricultural, nutritional and WASH-based interventions, readily delivered through the school platform, could improve schoolchildren's health and nutritional status. Our findings call for a sustained joint national effort for integrating agriculture, nutrition and WASH interventions at schools, households and communities levels.

List of Abbreviations

ALU	Albert-Ludwigs-Universität Freiburg
AVRDC	World Vegetable Center
BMI	Body mass index
DALY	Disability-adjusted life years
DHS	Demographic and Health Survey
FAO	Food and Agricultural Organisation
FEC	Formalin-ether concentration
GBD	Global burden of diseases
GDP	Gross domestic products
HAZ	Height-for-age z-score
JMP	Joint Monitoring Programme for Water Supply and Sanitation
KAP	Knowledge, attitude, and practices
LMIC	Low- and middle-income country
MDA	Mass drug administration
MDG	Millennium Development Goal
MND	Micronutrient deficiency
MOE	Ministry of Education
MOHP	Ministry of Health and Population
NTD	Neglected tropical disease
PCR	Polymerase chain reaction
PEM	Protein-energy malnutrition
PhD	Doctor of Philosophy
PRECEDE	Predisposing, reinforcing and enabling constructs in educational diagnosis and evaluation
PROCEED	Policy, regulatory, and organizational constructs in educational and environmental development
RCT	Randomised controlled trial
SD	Standard deviation
SDC	Swiss Agency for Development and Cooperation
SDG	Sustainable Development Goal
STH	Soil-transmitted helminth
Swiss TPH	Swiss Tropical and Public Health Institute
USAID	United States Agency for International Development
UNICEF	United Nations Children's Fund
VgtS	Vegetables Go to School: improving nutrition through agricultural diversification
WASH	Water, sanitation and hygiene
WAZ	Weight-for-age z-score
WHO	World Health Organization
YLD	Years lived with disability
YLL	Years of life lost

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1. Thesis outline

The thesis starts with an introduction (Chapter 2) presenting details of the causes, burden of malnutrition, including the low consumption of fruits and vegetables, anaemia, intestinal parasitic infections and best practices for mitigating these problems. The importance of health, with regard to the post-Millennium Development Goals (MDGs) agenda and the Sustainable Development Goals (SDGs), describing more explicitly on existing goals of ending hunger, achieving food security and improved nutrition, promoting sustainable agriculture, and to ensure healthy lives and wellbeing for all at all ages, were put forward.

Chapter 3 presents the goals and objectives of the study. Chapter 3 outlines the background of the thesis starting with the background of the study country (i.e. Nepal), identified research needs, outline of “Vegetables go to School: improving nutrition through agricultural diversification” (VgtS) project, overall project research design, and the complementary intervention packages, designed and implemented in the intervention schools.

Chapter 4 presents the study protocol, “Complementary school garden, nutrition, water, sanitation and hygiene interventions to improve children’s nutrition and health status in Burkina Faso and Nepal: a study protocol” describing the study framework, design and assessment approaches used in Burkina Faso and Nepal. Chapter 5, entitled “Water quality, sanitation, and hygiene conditions in schools and households in Dolakha and Ramechhap districts, Nepal: results from a cross-sectional survey” presents the results of environmental assessment of WASH conditions in the schools, households and communities indicating microbial contamination of water samples. In Chapter 6, entitled “Prevalence of anaemia and risk factors in schoolchildren in Dolakha and Ramechhap districts, Nepal” and Chapter 7, entitled “Prevalence of intestinal parasitic infections and associated risk factors among schoolchildren in Dolakha and Ramechhap discticts, Nepal,” is presented. The results of epidemiological cross-sectional studies related to the prevalence of malnutrition, anaemia and intestinal parasitic infection and its associated risk factors among schoolchildren were put forward. Chapter 8, entitled “Dietary pattern measured by principal component analysis and its association with stunting among Nepalese schoolchildren” presents the results of dietary patterns at the unit of the household. Chapter 9, entitled “Nutritional and health status of schoolchildren 15 months after integrated school garden, nutrition and water, sanitation and hygiene interventions: a cluster-randomised controlled trial in Nepal” presents the main results of the one year randomised controlled trial (RCT) for the intervention schools in comparison to the control schools. Chapter 10 presents an overall discussion of the key findings of this research, highlighting the contribution to new knowledge in the field, practical recommendations for policy work, limitations of the studies, directions for future research and conclusion. Subsequently, an attempt was made to discuss and compare the effects of the implemented package of intervention, highlighting the lessons learned and limitations of the study in the context of Nepal. Finally, conclusions and specific recommendations to authorities in Nepal and the broader international community (concerning the post-MDGs and SDGs) are provided.

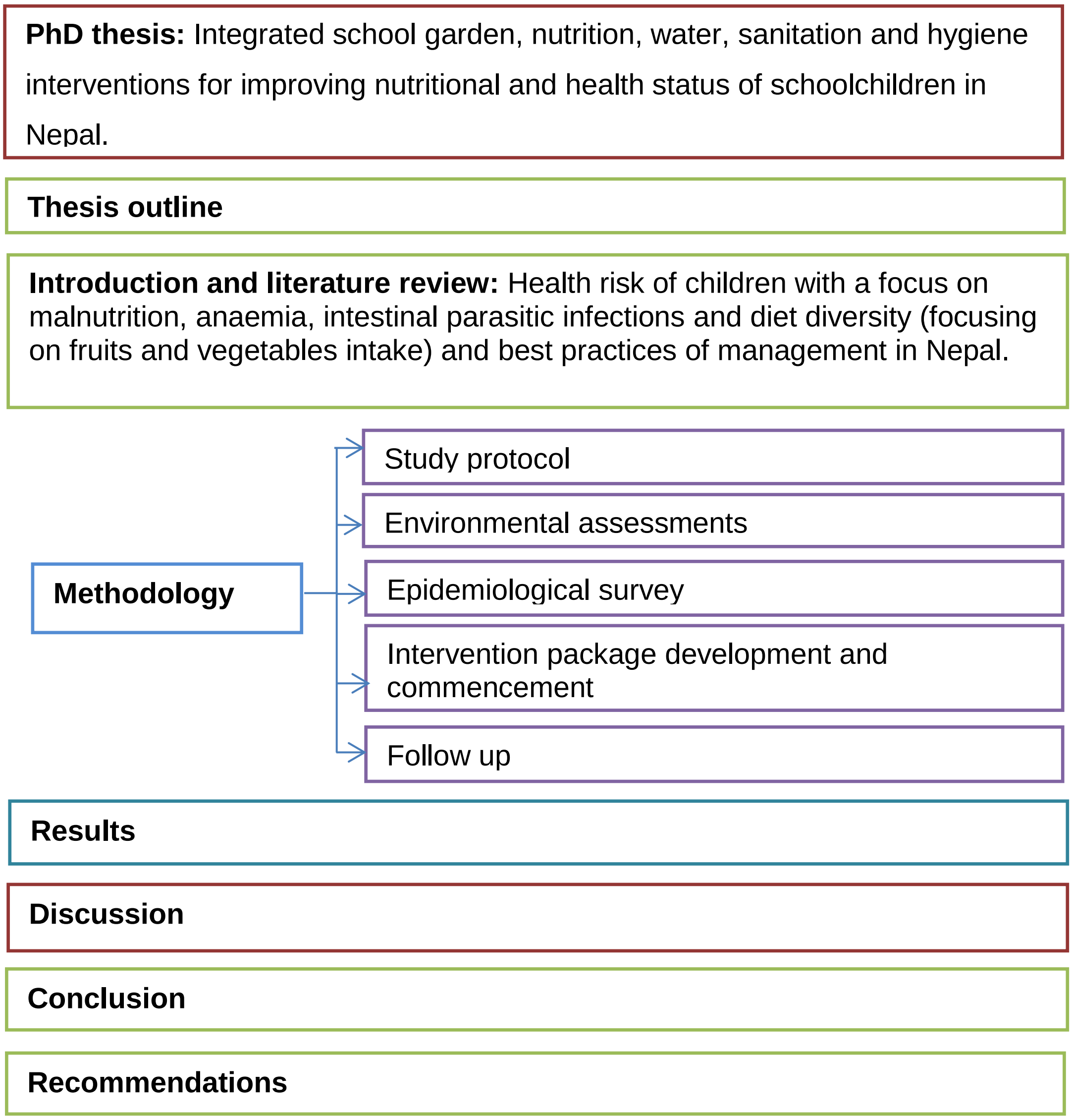

Figure 1.1: Schematic PhD thesis structure

2. Introduction

2.1. Description of malnutrition

Malnutrition is a term commonly used as an alternative to undernutrition but technically, it also refers to overnutrition. People gets malnourished if the diet does not possess adequate requirements for growth and maintenance of body or are unable to fully utilise the food consumed due to illness (undernutrition). It is also because of the consumption of too many calories (overnutrition). Other factors, such as illness and poor sanitation also contribute to malnutrition. An immediate level malnutrition results from interactions between inadequate dietary intake, including less consumption of fruit and vegetable and diseases. Three interacting groups of underlying factor contribute in turn to inadequate dietary intake and infectious diseases: household food insecurity, inadequate maternal and child care, and poor health and environmental services (Gillespie and Haddad, 2003).

2.1.1. Global burden of malnutrition

Malnutrition occurs at all stages of the lifecycle (Gillespie and Haddad, 2003). However, there is a dearth of information on malnutrition among schoolchildren (WHO, 2012a; Bhutta et al., 2013). Among children under-five years old, malnutrition causes nearly half of all deaths, and together with poor diets, it is the number one driver of global burden of disease (WHO, 2012a). Many faces of malnutrition continue to feature prominently in the Global Burden of Diseases (WHO, 2012a). The concentration of malnutrition in Asia is greater than anywhere else on Earth (Gillespie and Haddad, 2003). The study reports that globally, 156 million children are stunted (too short for age), 50 million children are wasted (too thin for height) and more than 50% of 146 million underweight and are living in South Asia (PMNCH and WHO, 2016). One in every three preschool children in Asia is stunted, in a country of South Asia, such as India, Bangladesh, and Nepal (Gillespie and Haddad, 2003, PMNCH and WHO, 2016, Reddy, 2016). Over 50% of all deaths of young children are due to infectious diseases such as diarrhoea, malaria, measles, pneumonia have undernutrition as an underlying cause (Caulfield et al., 2004). This translates to that between 5 to 6 million children are dying each year from infections that would not have been fatal if they had been properly nourished (Reddy, 2016). Approximately, 1.7 million (2.8%) of deaths worldwide are attributable to low fruit and vegetable consumptions (WHO, 2003).

2.1.2. Aetiology of malnutition

Malnutrition can exist in populations even where food supply is adequate, in terms of meeting energy requirement. People’s diets may be grossly deficient in one or more micronutrients although they might not be considered hungry. The resulting malnutrition is a pathological problem that steams principally from a lack of essential nutrients, which can cause growth to falter and increases susceptibility to common diseases. Poor nutrition then causes mental impairment in children, leading to reduced potential to succeed in education (Brown and Pollitt, 1996). Furthermore, malnourished adolescent girls give birth to stunted and thin babies; in this way malnutrition could be handed down as a terrifying inheritance from one generation to another. They are more likely to get sick, they do not learn well, and they are less productive as adults. Analysis included in the report shows a USD 70 billion gap globally to meet milestones of severe acute malnutrition, stunting and anaemia (Gillespie and Haddad, 2003).

One additional fact cannot be ignored about malnutrition that of its association with chronic diseases. Nutrition at various life stages had been associated with a risk of chronic diseases later in life such as obesity and heart diseases (WHO and FAO., 2003; Hutchinson et al., 2015). Therefore, it is important that healthy eating patterns, including consumption of adequate levels of fruits and vegetables, be established in childhood, since dietary habits developed in childhood persist throughout life (Lytle et al., 2000; Mikkilä et al., 2004; Craigie et al., 2011; Taylor et al., 2013; Christian et al., 2014; Hutchinson et al., 2015).

2.1.3. Importance of nutrition sensitive programs and way forward

Nurturing care targeted to the children's development should be generally supported by a large array of social contexts i.e. from home to parental work, childcare, schooling, the wider community, and policy influences (WHO, 2001a). This is because for children, schools are the site of most important relationships outside the family i.e. with teachers and peers without limiting the school-based nurturing care to the provision of health education (Patton et al., 2016). Hence, a core set of inter-related components, including knowledge of, attitude and behaviours, such as of health, hygiene and feeding practices (WHO and Global Targets, 2014) together with engagement of families and communities, must be covered (Harden et al., 2009). Positive associations between nurturing care and children's health, growth, and development have been demonstrated worldwide (WHO, 2001; Molyneux et al., 2016). Meanwhile, the interventions on specific environments and on diversity in the diet play a vital role in the nurturing care for child development (Stoltzfus and Dreyfuss, 1999; WHO and Global Targets, 2014). According to the World Health Organisations (WHO) nutrition recommendation, an intake of ≥5 serving of fruits and vegetables per day (≥400 g with 80 g per serving) is considered as an adequate consumption for combating malnutrition (Esteghamati et al., 2012). Diets associated with low non-communicable diseases risks are generally high in fibre-rich fruits and vegetables, legumes pulses, nuts, whole grains, and cis-unsaturated vegetable oils (Bradley and Putnick, 2012).

In September 2015, the United Nations General Assembly established the SDGs specifying 17 universal goals, 169 targets, and 230 indicators leading up to the year 2030. Health is a core dimension of the SDGs. Goal 2 is aiming to “end hunger, achieve food security and improved nutrition; and promote sustainable agriculture” and goal 3 aiming to “ensure healthy lives and promote wellbeing for all at all ages” (Lim et al., 2016). The WHO (2012) concluded that “improvements in areas like agriculture, health, education, social protection and sanitation could help address a large percentage of malnutrition” (WHO, 2012a).

Agricultural growth hence plays a vital role in the reduction of hunger and malnutrition. Furthermore, “nutrition-sensitive” interventions should result in better nutritional outcomes through: (i) diversified diets; (ii) improved access to safe WASH; (iii) sustainable access to health services; (iv) great awareness regarding adequate nutrition; (v) good child care practices; and (vi) wide distribution of supplements in a situation of acute micronutrient deficiencies (FAO, 2012). Hence, the promotion of greater vegetable consumption; including widely consumed local vegetables; should be promoted. Nutrition and health sectors should help promote food-based approaches that lead to diversification of crops, balanced diets, and ultimately a better health (Yang and Hanson, 2009).

Schools are the obvious place to promote healthy eating habits such as the 5-a-day fruits and vegetables recommended by the WHO (WHO, 2003; Hutchinson et al., 2015). Moreover, involvement of schoolchildren in gardening could be a type of intervention that has potential to increase the fruit and vegetable intake by providing an opportunity for fruit and vegetable tasting, learning in an interactive manner; teaching how fruits and vegetables are grown; and its benefits to their health (Ozer, 2007, Hutchinson et al., 2015). The repeated exposure to fruits and vegetables could also have a positive impact on liking and an intake leading to the consumption of the recommended ≥5 portion of vegetables per day (Taylor et al., 2013). This could be done through establishing school gardens.

The FAO defines school gardens as “cultivated areas around or near to schools, tended at least partly by learners producing mainly fruits and vegetable and activities including small-scale animal husbandry and fishery, beekeeping, ornamental plants and shading, and small-scale staple food production” (FAO, 2010). The school is usually the centre of a community as nearly everybody in a community has a connection to the school. Hence, school vegetable gardens as an educational and promotional tool to enhance healthy food choices, good nutrition and health practices. Furthermore, school gardens, in addition to a school feeding programme and educational tools, can enhance healthy eating and improve the nutritional status of schoolchildren. Bundy et al. (2009) emphasised the importance of school-based approaches for health and nutrition programmes by arguing that (i) the promotion of good health and nutrition is essential to the effective growth and development in children as well as for effective learning; and (ii) the pre-existing infrastructure of the educational system can be a more cost-effective route for delivering simple health interventions and health promotion to the health system (Bundy et al., 2009). Introducing school gardens encourages the production and consumption of a wide diversity of vegetables, which is particularly important when persuading children to favour a balanced and nutritious diet as part of a healthy lifestyle (Keatinge et al., 2013).

Given this growing understanding of the need of dietary approaches to health, which can be implemented through the education systems, and recognising this potential of nutrition, agriculture and health promotion in schools, many countries have started national programs for promoting gardening in schools. The program tries to influence and encourage children to develop a preference for a diverse and healthy diet including adequate quantities of vegetables. Such countries are the Philippines and Indonesia and have initiated a large-scale programme to promote school gardens. The aim was to influence children to develop a preference for a diverse and healthy diet, including adequate quantities of fruits and vegetables (AVRDC, 2014). This can lead children to be able to grow their own food, and improve their awareness about nutrition and the environment (FAO, 2010). Although, this strategy is compelling, there is little scientific evidence that school garden programs contribute to a balanced diet among the children and thus improve nutritional status. In the same way, iron deficiency is the primary cause of anaemia. Iron-fortified foods, including vegetables have demonstrated to reduce the prevalence of anaemia in pre-school children from 40 to 100% in less than a year (Penny et al., 2005; PMNCH and WHO, 2016).

2.1.4. Impact of malnutrition and infections interactions

The interaction between malnutrition and infection creates a potentially vicious cycle of worsening illness and deteriorating the nutritional status at least with three direct pathways via diarrhoeal diseases, intestinal parasitic infections and environmental enteropathy (Fenn et al., 2012; WHO, 2015). Diarrhoea can impair the nutritional status through loss of appetite, malabsorption of nutrients and increased metabolism whereas undernourished children have a weakened immune system, which makes them more susceptible to enteric infections leading to severe and prolonged episodes of diarrhoea (Caulfield et al., 2004; Petri et al., 2008; Dewey and Mayers, 2011; WHO, 2015). The RCTs have been employed to measure the effect of WASH on nutritional outcomes. These studies, conducted in low-income settings, found evidence for a small, but statistically significant effect of WASH interventions on stunting (WHO, 2015a). The interventions were focused on mostly water quality and hygiene behaviour which were implemented for short duration (Du Preez et al., 2010; du Preez et al., 2011).

However, given the complexity of factors that cause malnutrition, especially the lack of access to safe and adequate WASH, no single intervention will achieve effective or lasting results. Hence, the effective and sustainably improving nutrition outcomes require a coordinated, multi-sectorial approach among the agriculture, nutrition and WASH sectors with strong community engagement (USAID, 2015).

2.2. Description of anaemia

Anaemia stems from ancient Greek "ἀναιμία", which means "without blood." It is defined as "a condition in which the number and size of red blood cells, or the haemoglobin (Hb) concentration, falls below an established cut-off value, consequently impairing the capacity of the blood to transport oxygen around the body which is an indicator of poor nutrition and poor health" (Black et al., 2003; Allen et al., 2006). In clinical terms, anaemia is considered as a Hb concentration that is insufficient to meet the oxygen needs of the tissues and distinct cut-offs are available in guidelines put forward by WHO for different age groups, males and females (WHO, 2011). Anaemia is interlinked with stunting, childhood overweight and wasting among schoolchildren.

2.2.1. Global burden of anaemia

Anaemia is a global public health problem which affects all population groups in low- and middle-income countries (LMICs), with an estimated 800 million children and women. In 2011, the highest prevalence of anaemia was in children (42.6%) (WHO, 2015b). The prevalence of anaemia was the highest in the South Asia, Central and West Africa (Allen et al., 2006). Children with anaemia resided in the South-East Asia region, including 96.7 million children (WHO, 2015b). The global burden of anaemia, in terms of disability adjusted life index (DALYs) is hard to quantify, as anaemia can result from various diseases and other conditions of ill-health (Murphy and Breman, 2001; Mathers et al., 2008).

2.2.2. Aetiology of anaemia

Anaemia is multifactorial, resulting from micronutrient deficiencies such as riboflavin, folate, vitamin B12 or vitamin A and iron deficiency (Suharno et al., 1993; Savage et al., 1994; Stoltzfus, 2001; Stabler and Allen, 2004); or from intestinal parasitic infections such as helminthiases; infectious disease such as malaria; as a consequence from chronic inflammatory diseases; or from genetic disorders (Yip and Dallman, 1988; Menendez et al., 2000; Brooker et al., 2004; Stuart and Nagel, 2004; Rund and Rachmilewitz, 2005; Cappellini and Fiorelli, 2008; Gimnig et al., 2016). While the causes of anaemia are variable, it is estimated that half of the cases are due to iron deficiency (Black et al., 2003). Iron deficiency is not clinically apparent until anaemia is severe and generally develops slowly (Haselow et al., 2016). Globally, iron deficiency is the most common form of malnutrition affecting more than 2 billion population and is highly prevalent in LMICs (Haselow et al., 2016).

2.2.3. Consequences of anaemia

The consequences of anaemia are manifold depending on the individual factors and its severity. The main consequences includes reduced oxygen transport and energy metabolism leading to tiredness, weakness and may decrease working capacity (Haas and Brownlie, 2001). Anaemia reduce the individuals well-being, causes fatigue and lethargy, and impairs the physical capacity and work performance (Kirchhoff et al., 1985; UNICEF and Organización Mundial de la Salud, 2009; Korpe and Petri, 2012; Boisson et al., 2013; Clasen et al., 2015; Haselow et al., 2016). The failure of reduction of anaemia worldwide affect children resulting to an impaired development, and nations to impaired economic productivity and hence development (Black et al., 2003). However, even the effect of iron deficiency anaemia, on learning and educational attainment in schoolchildren is largely unknown (Patton et al., 2016).

2.2.4. Way forward for prevention of anaemia

The WHO published adequate guidelines and evidences for interventions, targeting schoolchildren, that support policies for the prevention and control of anaemia (WHO and FAO, 2003; UNICEF and Organización Mundial de la Salud, 2009; Clasen et al., 2015). To mention a few, leveraging diet containing adequate amounts of bioavailable iron; regular deworming and education on safe and adequate WASH and food production should underpin for the prevention and control of anaemia (Black et al., 2003; UNICEF and Organización Mundial de la Salud, 2009; WHO, 2013; Clasen et al., 2015). Using schoolchildren as a distribution point for household food packages decreased anaemia

(Patton et al., 2016). Furthermore, community-led total sanitation and improved access to clean water are important complementary interventions for reducing anaemia (Bartram and Cairncross, 2010; Dongre et al., 2011). Health and nutritional education at different levels should be considered in order to achieve a sustainable impact on the burden of anaemia (Righetti, 2014).

2.3. Description of intestinal parasitic infections

Intestinal parasitic infections consist of a group of soil-transmitted helminth and intestinal protozoa. Soil- transmitted helminth infects humans through contact with parasite eggs or larvae that live predominantly in the moist soil. Adult soil-transmitted helminth resides in the human intestinal tract. The soil-transmitted helminth infections infecting human are hookworms (*Ancylostoma duodenale* and *Necator americanus*), roundworm *(Ascaris lumbricoides)*, and whipworm *(Trichuris trichiura)* (Bethony et al., 2006a)*.* The protozoan infections are mostly caused by consumption of contaminated water. The most common protozoan infection is caused by *Giardia intestinalis* (Haque, 2007). They are transmitted by eggs present in human faeces, contaminating soil in areas where sanitation is poor. Hence, soil-transmitted helminth belongs to the group of neglected tropical diseases (NTDs); one of 17 infectious diseases currently targeted for prevention and research by the United Nations Development Programme (UNDP), World Bank and the WHO (Bieri, 2013).

2.3.1. Biology and lifecycle

Factors influencing intestinal parasitic infections transmission can be divided into environmental and behavioural risk factors. The two important environmental factors are climate and soil conditions, as soil-transmitted helminth larvae predominantly develop with moist soils, warm temperatures, high humidity and land surface temperature (Pullan and Brooker, 2012). An intestinal parasitic infection, especially soil-transmitted helminth is caused by oral ingestion of eggs from contaminated soil, food and dirty hands (*A. lumbricoides and T. trichiura*), or by penetration of the skin by larvae in the soil (hookworms) (Bieri, 2013). Inadequate sanitation and water supply, the use of untreated night soil fertilizer are man-made environmental factors favouring soil-transmitted helminth transmission (Bethony et al., 2006a; Ohta and Waikagul, 2007). Behavioural risk factors include personal hygiene and habits such as handwashing and eating raw food and not wearing shoe. Occupations with high soil contacts, such as farming also increase the risk of soil-transmitted helminth infection (Balen et al., 2011). Both environmental (e.g. tropical climate, high humidity, unhygienic sanitation, land surface temperature, night soil fertilizer) and behavioural risk factors commonly occur in poor socio-economic conditions, making poverty and limited education, one of the key risk factors for soil-transmitted helminth transmission (Ohta and Waikagul, 2007; Bieri, 2013).

2.3.2. Clinical manifestations of intestinal parasitic infections

Intestinal parasite generally produces clinical manifestations from moderate to high intensity infections or from infections with multiple parasites, whereas low intensity infections may remain unnoticed (Ezeamama et al., 2005). The highest intensity of infections most commonly occur in children. The clinical features of soil-transmitted helminth infections can be either due to acute manifestations associated with larval migration through skin, viscera; or acute and chronic manifestations due to parasitism of the gastrointestinal tract by the adult worm (Bethony et al., 2006a). The most common clinical manifestations of intestinal parasitic infections includes abdominal pain, diarrhoea, anaemia, intestinal obstruction and malnutrition (Bieri, 2013). In general, a soil-transmitted helminth infection most severely affects children where the childrens are more prone to harbouring heavy infections resulting in malnutrition that can dramatically affect both physical and mental development (Bethony et al., 2006a). The soil-transmitted helminth infection also impairs the nutritional status of the humans they infect in multiple ways: (i) worms feed on host tissue, including blood, leading to iron and protein deficiency, (ii) worms increase the mal-absorption of nutrients, roundworm may possibly compete for vitamin A in the intestine, (iii) soil-transmitted helminth may also cause loss of appetite and therefore, a reduction of nutritional intake and physical fitness (Bieri, 2013).

Ascaris larval antigens can cause an inflammatory response and results in verminous pneumonia, which is commonly accompanied by wheezing, dyspnoea, cough, fever and even blood-tinged sputum during heavy infections. The reinfection may cause a severe disease making it susceptible to pneumonitis (Bethony et al., 2006a). It can also cause lactose intolerance and mal-absorption of vitamin A and other nutrients, partly causing malnutrition and stunted growth (Taren et al., 1987). Large numbers of *Ascaris* worms in small intestine causes abdominal distension and pain leading to partial or complete obstruction; intestinal perforation; and peritonitis which is fatal (Farrar et al., 2013). The study reported that the average height of children with *Ascaris* infection is 2.5 cm lower than that of healthy children and their body weight is 2.3 kg lower after expelling the worms (Ying-Dan et al., 2008).

Hookworm causes chronic intestinal blood loss, resulting in anaemia (Bethony et al., 2006a). The third stage larvae of hookworm can cause pneumonitis. They penetrate the intact skin, causing several cutaneous syndromes, such as a ground itch and rash on the hands and feet. Oral ingestion of hookworm larvae can result in wakana syndrome, which is accompanied by nausea, vomiting, cough, pharyngeal irritation and hoarseness (Bethony et al., 2006a).

T. trichiura causes diarrhoea, dysentery leading to inflammation of the intestinal mucosa at the site of the attachment of large numbers of whipworm, resulting in colitis. The longstanding colitis can cause signs and symptoms similar to inflammatory bowel disease, including chronic abdominal pain and diarrhoea as well as squeal of impaired growth, anaemia and clubbed fingers (Bieri, 2013). Furthermore, the serious condition is *Trichuris* dysentery syndrome, a manifestation of a heavy whipworm infection, resulting in dysentery and rectal prolapse (Farrar et al., 2013).

Various epidemiological studies have shown that infections with multiple parasites, can lead to clinically significant morbidity, even at low infection intensities (Bieri, 2013). A study showed that the chances of having anaemia for children, with low-intensity poly-parasite infections, were nearly five times higher than for children without infection or with one parasite species at low intensity (Ezeamama et al., 2005). A study conducted in Brazil found that children, hosting both *Ascaris* and *Trichuria* suffered more severely from malnutrition than children hosting these parasites in isolation (Silva, 1999).

The research conducted in the Philippines showed that the effect of concomitant high infection, by hookworm or *T. trichiura* had a synergistic negative impact on the Hb level (Ezeamama et al., 2008). Moderate-to-heavy infections with hookworm (≥2000 eggs/g of stool, EPG) are strongly associated with an insufficient Hb level and the risk of anaemia is correlated with the infection intensity (Stephenson et al., 1985). The amount of blood loss in hookworm infections is strongly and linearly correlated with the worm load and faecal egg count, and even light infections contribute significantly to anaemia (Hotez et al., 2006b; Jonker et al., 2012). *T. trichiura* also causes intestinal blood loss, and schoolchildren with heavy *Trichiura* infections have a higher prevalence of anaemia than non-infected counterpart (Ramdath et al., 1995). A matched case-control study reported the correlation between intestinal protozoa and diarrhoea, including other clinical symptoms of nausea/vomiting and abdominal pain (Hawash et al., 2015).

2.3.3. Global burden of intestinal parasitic infections

The estimates of the worldwide burden of soil-transmitted helminth have been shown to be variable ranging from 4.7-39.0 million DALYs (Bethony et al., 2006a; Murray et al., 2012). According to the World Bank, soil-transmitted helminth accounts for almost half of the global burden of diseases among children aged 5-14 years (Musgrove, 1993). More than 1.5 billion people are infected with soil-transmitted helminth infections worldwide. Infections are widely distributed in the sub Saharan Africa, East Asia, China and America

(Bieri, 2013). Over 270 million preschool children and over 600 million schoolchildren live in areas where these worms are intensively transmitted, and are in need of treatment and preventive interventions (Penny et al., 2005).

2.3.4. Laboratory diagnosis of intestinal parasitic infections

Different methods have been developed to detect helminth eggs in stool. The Kato-Katz thick smear technique is the most common in the field studies, because of its simplicity and low cost. It is used to examine a calibrated amount (40-50 mg) of faeces and is a simple, rapid way of quantitatively detecting infections (Katz et al., 1972b). To maximise the sensitivity of the method, the standard procedure was to examine three slides each from two stool samples (Ross et al., 1998). The limitation of Kato-Katz includes its low sensitivity for hookworm diagnosis and its inability to detect light and recently acquired infections (Utzinger et al., 2001). The formalin-ether concentration method is another widely used method (Ridley and Hawgood, 1956). It has the advantages of fixing the parasite, thus making samples non-infectious as well as preserving many types of cysts (Dacombe et al., 2007).

2.3.5. Adverse effect of intestinal parasitic infection on nutritional status

Parasitic infections have been shown to have deleterious effects on the host nutritional status contributing to undernutrition resulting into subtle reduction in digestion, absorption leading to chronic inflammation and nutrient loss (Amare et al., 2013). In turn, undernutrition can make a person more susceptible to parasitic diseases, which causes a vicious cycle (Walker and Walker, 1997; Savioli et al., 2002). There are documented reports implicating intestinal parasitic infections with a poor nutritional status in schoolchildren (Silva et al., 2009; Valverde et al., 2011; Opara et al., 2012).

2.3.6. Way forward for prevention of intestinal parasitic infections

School-based helminthiasis control, in the form of mass drug administration is promoted by the WHO and is a cost-effective way in low- and middle- income countries (LMICs). The major strategy recommended by the WHO for soil-transmitted helminth infections is to control morbidity through the periodic deworming of at-risk population such as children living in endemic areas (Bieri, 2013). The WHO recommends periodic deworming once a year in the communities where soil-transmitted helminth prevalence is over 20%, twice a year when the prevalence is over 50% to all at-risk population in endemic areas, for reducing the morbidity by reducing the worm burden. Albendazole (400 mg) and mebendazole (500 mg) are the recommended drugs of choice for treating the soil-transmitted helminth infection (Molyneux et al., 2016; WHO, 2016). However, long-term health benefits of mass drug administration are limited due to rapid re-infection and need for preventative measures, such as behaviour change facilitated through health education (Tomono et al., 2003).

As an additional intervention to health and hygiene education, provision of adequate sanitation is recommended for reducing transmission, reinfection and intensity of infection (Black et al., 2003). Several studies have shown that integrated approaches, chemotherapy, health education, improved sanitation leads to more significant reduction in helminth infections and better long-term benefits (Esrey et al., 1991; Narain et al., 2000; Asaolu et al., 2002; Asaolu and Ofoezie, 2003). For long-term sustainability, the WHO recommends an improved access to safe water, adequate sanitation and improved hygiene (WASH) behaviour through health education, in addition to mass drug administration (WHO, 2005). The regular deworming has been found to reduce the total burden of soil-transmitted helminth by 70% (PMNCH and WHO, 2016).

2.4. Description of water, sanitation and hygiene (WASH)

The United Nations Human Rights Council presented a resolution, in September 2013, stating that "the human right to safe drinking water and sanitation entitles everyone, without discrimination, to have access to sufficient, safe, acceptable, physically accessible and affordable water for personal and domestic use and to have physical and affordable access to sanitation, in all spheres of life, i.e. safe, hygienic, secure, socially and culturally acceptable and that provides privacy and ensures dignity" (Meier et al., 2013; Cronk et al., 2015). The Millennium Development Goals (MDGs), which were established in the aftermath of the United Nations Millennium Summit in the year 2000 targeted to eradicate extreme poverty by 2015. Water and sanitation issues were associated with and defined as a sub-target of goal seven (i.e. environmental sustainability). In order to assess the global trends and progress made towards this goal, a Joint Monitoring Programme for Water Supply and Sanitation (JMP) was put in place (Bieri, 2013). Substantial improvements were made in the last decades with more than 2.1 billion people who gained access to safe drinking water since 1990s. Despite of this progress, there are still about 770 million people without access to safe water. In the field of sanitation, the progress made is more uneven. Worldwide, there were still 2.5 billion people who lacked access to adequate sanitation facilities (WHO and UNICEF, 2013).

The latest assessment by the JMP suggests that again there are strong regional disparities that have to be highlighted especially in less developed countries in South East Asia. Regarding open defecation, yet, in absolute numbers there were still more than one billion people doing it in 2011. While the percentage was declining in most parts of the world and the progress was impressive in South East Asia (Kümmerle, 2014). The provided numbers make it obviously clear that there still is a pressing need to enhance the promotion and improvement of WASH conditions on a global scale, particularly in LMICs (WHO and UNICEF, 2013).

2.4.1. WASH in schools

WASH in schools can broadly be defined as a “combination of technical (hardware) and human development (software) components that are necessary to produce a healthy school environment and to develop or support appropriate health and hygiene behaviours” (Mooijman et al., 2010). There are a number of reasons why WASH in schools is increasingly considered. First, improving WASH conditions in schools aims at preventing the spread of diseases among children, which is particularly important since in many LMICs children suffer a disproportional share of the diseases that can be related to inadequate WASH conditions (UNICEF, 2012). Secondly, inadequate WASH conditions in schools are also supposed to have effects on educational outcomes. According to UNICEF (2012), every year about 272 million school days are lost due to diarrhoea only (Young et al., 2012). Thirdly, improvement of WASH in schools might lower the financial burden among household, due to saving in costs for treatment of illness among children increasing productivity (Kümmerle, 2014). Finally, schools might be suitable institutional entry points allowing it more easily to scale up health promotion activities, since lot of children can be reached (Danida, 2010).

2.4.2. Impact of inadequate WASH among schoolchildren

Diarrhoea is the most important WASH-related disease and especially for children in low- and middle- income countries (LMICs). Since, 90% of all diarrhoeal diseases can be attributed to poor WASH conditions, it is estimated that around 1.4 million child deaths would be preventable (WHO, 2008a). Importantly, diarrhoea can lead to severe health problems such as malnutrition, poor growth and an impaired immune function (Selendy, 2011). Diarrhoea could be prevented, to a large extent, by the disruption of the faecal-oral transmission route by sanitary interventions, including improving personal hygiene, disposal of waste, and appropriate preparation of food (Kümmerle, 2014).

2.4.3. Way forward for prevention of WASH related problems among schoolchildren

Provision of access to WASH, a safe water supply, appropriately constructed sanitation infrastructure that ensures safe disposal of human excreta, and the promotion of hygiene is critical for soil-transmitted infections (Campbell et al., 2014). There are a number of possible WASH interventions that were and still are commonly implemented with the goal to improve health outcomes. The four classical interventions are improvements in (i) water quantity; (ii) water quality; (iii) sanitation; and (iv) hygiene.

(i) ***Water quantity***

Intervention of water quantity should have an effect on the prevalence of disease categorised as water-washed (i.e. diarrhoeal disease, worm infections and trachoma). Fewtrell (2005) showed a risk reduction of 25% of diarrhoea cases with an increased supply of water (Fewtrell et al., 2005).

(ii) ***Water quality***

The interventions aiming to improve water quality usually focus on water treatment in order to remove pathogens and microbial contaminants. Thus, these interventions should have the greatest impact on diseases that are categorised under water-borne diseases. Common methods of water treatment are chemical treatment such as chlorination as well as solar disinfection, boiling or pasteurizing (Kümmerle, 2014). There are also, basically, two major points of intervention i.e. treatment of the water sources; and treatment at the point of use. There are numerous ways of contamination that can occur at the unit of households (e.g., unsafe storage), which might make treatment at the water source less efficient, hence most studies were focused on the treatment at the point-of-use (Waddington et al., 2009).

(iii) ***Sanitation***

Regarding the role of different sanitation technologies, there is empirical evidence that improvements in sanitation could lead to better health. Several meta-analysis reviews confirmed that improvements in sanitation can lead to relative diarrhoea morbidity reductions ranging from 22 to 37% (Fewtrell et al., 2005; Waddington et al., 2009). Regarding the effects of sanitation on the prevalence of infections with intestinal worms, Ziegelbauer (2012) found that the availability of sanitary facilities was strongly associated with protection against infections with soil-transmitted helminth (Ziegelbauer et al., 2012a).

(iv) ***Hygiene***

The hygiene behaviour, e.g., handwashing is an important barrier in pathogen transmission since it can prevent direct hand-to-mouth transmission but also indirect transmission via water or food. Regarding parasitic infections with soil-transmitted helminth there is substantial evidence that hygiene can play an important role in prevention (Bieri, 2013; Kümmerle, 2014). Strunz (2014) showed that both handwashing after defecation and use of soap were associated with lower infections with soil-transmitted helminth (Strunz et al., 2014a). Curtis and Cairncross (2003), Fewtrell (2005) and Waddington

(2009) showed that handwashing with soap could reduce the risk of diarrhoeal diseases up to 47% (Curtis and Cairncross, 2003; Fewtrell et al., 2005; Waddington et al., 2009).

2.5. Background of the PhD thesis

2.5.2. Country background

<u>Geography and population</u>

Nepal is a landlocked country borders which with India and China with total area 147,181 sq.km. The country has three geographical regions: mountain, hill and terai (MOHP et al., 2012). Nepal has a population of 29,033,914 people with the population growth rate at 1.24% per annum (Wikipedia, 2017). The adolescent population in Nepal is approximately 6 million (i.e., 24% of the total populations) (UN, 2017). The elevation in Nepal ranges from <100 meters (300ft) to >8,000 meters (26,000 ft.) and precipitation ranges from 160 mm (6 in) to over 5,000 mm (16 ft.). The country has eight climate zones (tropical, subtropical, temperate, subalpine, alpine and nival) from tropical to perpetual snow (Barnekow Lillesø et al., 2005).

<u>Socio-culture and economy</u>

Nepal has a variety of religions, cultures, languages and castes. The majority (81%) of the population follows Hinduism with and official language “Nepali”. There are 103 recognised ethnic groups some of which speak regional dialects. The gross domestic per capita of country in 2009 was 435.9. Agriculture is the main occupation for the majority of Nepalese and almost 65.7% are employed in the agricultural sector (UN, 2009).

<u>Health system and health situation</u>

Nepal has a comprehensive framework in regard to health policies, strategies and plans (e.g., national health policies and second long term health plan). After signing the Alma Ata Declaration in 1978, Nepal committed to the provision of basic health care adopting the primary health care approach for achieving health for all. The major aim of the national health policies is extension of primary health care system to the rural population focusing on the health infrastructure, local resource mobilization, multi-sectorial coordination, and decentralized planning and management. Similarly, the target of the second long term health plan is the improvement of the health status of the poor, rural, unprivileged and marginalised population through the provision of essential health services (WHO, 2007).

The Department of Health Services under the Ministry of Health and Population (MOHP) is responsible for delivering preventive, promotive and curative health services. The network of health services then goes from health post to primary health care centres to district, zone, regional and finally to tertiary hospitals (MOHP et al., 2012). The total expenditure on health per capita (PPP US$) is $69. The expenditure of the government on health as percentage of total expenditure is 5.5%. Expenditure on health remains 5.3% of GDP and per capita health expenditure is US$ 18.09 (World Bank, 2011). More than 55% of total health expenditure is financed by out of pocket payments (MOHP, 2010).

Nutritional situation in Nepal

In Nepal, the nutritional status of mothers (especially adolescent girls) and children under-five is extremely poor. The prevailing high rate of child undernutrition in the country is one of the major contributing factors of under-five mortality. Anthropometric deficits among children vary with ecological zone; stunting and underweight are more prevalent in the mountains and hills and wasting in the terai (Janwal, 2014). Around 41% of children under-five years of age are suffering from chronic undernutrition (stunting) while more than 10% are acutely undernourished (MOHP et al., 2012). Almost 50% of children under-five years are anaemic which at least partially reflects the low intake of iron-rich food as well as the low dietary diversity and absence of regular iron supplementation. Non-nutritional causes such as infection, including parasites and malaria, are also likely contributors to anaemia in Nepal (USAID et al., 2015).

The low consumption of fruits and vegetables among children in Nepal is highly dependent on the availabilitiy of local and seasonal vegetables. It is also contributing to severe nutritional disorders such as iron and vitamin A deficiencies (Janwal, 2014). High (72%) percentage of dietary energy supply is from cereals, roots and tubers. Unfortunately, since no recent nation-wide food consumption surveys are available, information on the adequacy of food consumption cannot be reported (Janwal, 2014).

Addressing malnutrition in Nepal requires a life cycle approach that focuses on addressing nutrition in adolescents, delaying the age of first marriage and pregnancy, appropriate infant and young child feeding practices, and community management of acute malnutrition, particularly among infants (USAID et al., 2015).

Water and sanitation status in Nepal

In Nepal, the Ministry of Water Supply and Sanitation (MOWSS) has the responsibility of planning, implementation, operation and maintenance of water supply and sanitation systems. Nepal has already met the MDGs targets, set in WASH sector, with 89% of the population with access to improved drinking water sources (USAID et al., 2015). The MOWSS is now striving toward achieving the national target of "basic water supply and sanitation facilities by all by 2017." Similarly, the MOWSS is also engaged in finalizing a "15 year development plan of Nepal WASH sector" aligning it with the SDGs. The recent studies revealed that there has been a significant acceleration in the sanitation (e.g., access to improved sanitation and decrease in open defecation). The percentage of population with access to sanitation facilities reached 40% (USAID et al., 2015). The safety of drinking water is another issue that must be addressed (MOWSS, 2017).

2.5.3. Identified research needs

Nepal is a developing country with 34,000 schools in 75 districts; however, does not have an official school garden program. Agriculture has been removed from the school curriculum. Only 17 districts have school feeding programmes, including deworming and nutritional supplements, supported by the World Food Program (WFP). This scheme is presently being transferred to the government (AVRDC, 2013). While past Nepalese government nutritional initiatives have focused mainly on supplementation, school garden is implicitly supported in Nepal's three year interim plan (July 2010-June 2013). The working policy for agricultural states that (i) preference will be given to production and consumption of locally available commodities in food and nutrition insecure areas, and (ii) farmers shall be encouraged to grow nutrient rich vegetables in urban and peri-urban areas. The working policy for health states that (i) school health education will be promoted in collaboration among government, private and non-government organisations; (ii) short and long term strategic plans for the improvement of nutrition will be formulated and gradually executed with necessary capacity building and institutional strengthening; and (iii) local communities will be mobilised to implement nutrition programmes effectively and also be involved in multi-stakeholder monitoring and supervision at the local level (AVRDC, 2013). No policy related to the integrated application of agriculture, nutrition and WASH targeting schoolchildren is found. Furthermore, the current availability of fruits and vegetables in Nepal is far below 400 g / day which is the rate recommended by the WHO and FAO. For instance daily fruit and vegetable availability is 352 g in Nepal (WHO, 2003b).

Malnutrition is the major underlying cause of child mortality in the country and anaemia among the infants and children is high (Gaurav et al., 2014). Apart from an insufficient intake of safe and quality food, the consumption of unbalanced food is also a problem, which together is responsible for the chronic nutritional problem among children (Singh et al., 2014). Forty one percent of children's are suffering from chronic malnutrition (GON, 2012). Additional factors such as poor hygiene and sanitation, lack of healthy environment are compromising early childhood development (Gyawali et al., 2009; Shakya et al., 2012a).

The intestinal parasitic infections are common public health problems of children and the infection rate has primarily been attributed to the appalling unhygienic environmental conditions (Sah et al., 2013; Tandukar et al., 2013; Pradhan et al., 2014). Children are dying each year due to preventable and treatable diseases such as diarrhoea, dysentery, and intestinal parasites (Acharya et al., 2013). For instance, diarrheal diseases caused each year a minimum of 30,000 deaths and each child suffers from at least 3.3 episodes of diarrhoea per year (Pokhrel and Viraraghavan, 2004). *A. lumbricoids*, the most common helminth parasite, is stable in its prevalence since last decade (Sharma et al., 2004; Dib et al., 2008). Among protozoans, *G. intestinalis* and the *E. histolytica* are most common (Mukhopadhyay et al., 2007; Shakya et al., 2012a; Shrestha et al., 2012a). *G. intestinalis* mostly infects children from an age group of preschool to 19 years old (Dib et al., 2008). Outbreaks of these infectious diseases occur frequently and are attributable to the faecal contamination of drinking water. The intestinal parasitic infection and amoebic dysentery stands second among the top ten causes of hospital visits within the country (Shrestha et al., 2012a). The number of children who died of diarrheal disease annually due to poor hygiene and sanitation is 12,700 (GON, 2011a).

Similarly, the iron-deficiency anaemia is one of the top ten leading causes of years of life with disability among all age groups and the dietary risks, malnutrition, unsafe WASH are the top ten causes of disability adjusted life years (DALYs). The diarrhoea and nutritional deficiencies are the leading cause of DALYs per 100,000 among the age group 5 to 14 years old in Nepal.

In Nepal, the school gardens and nutrition programs have so far not been implemented. Chronic malnutrition is one critical area where past development efforts did not make a satisfactory impact and poor diet diversity among children has been a serious problem across the country (GON, 2012). Many governmental projects involve improving WASH with sanitation facilities constructions but are still inadequate. Effective government policy and guidelines for the promotion of sanitation and hygiene in school is lacking and most teachers are not trained on WASH (GON, 2011a). Yet, infections related to inadequate WASH might have long term impacts on the children, often affecting their attitude, behaviour, intelligence quotient levels, performance, health and productivity in later life (Niehaus et al., 2002; Shakya et al., 2012; Acharya et al., 2013). A joint project comprising of a school garden, nutrition, and WASH has not been implemented in the past.

2.5.4. Project background: “Vegetables go to School: improving nutrition through agricultural diversification” (VgtS) project

The project “Vegetables Go to Schools: improving nutrition through agricultural diversification” (VGtS) was implemented in five countries of Africa and Asia (i.e. Burkina Faso, Bhutan, Indonesia, Nepal, and the Philippines). The project was funded by the Swiss Agency for Development and Cooperation (SDC). A school garden is implemented and adapted to the local conditions using country specific vegetables with targeted cropping schedules, adequate vegetable management technologies and protocols for ensuring seed supply. The agricultural demonstration plots with a variety of vegetables were constructed. In each participating country, 30 school vegetable gardens were established. The agricultural messages were provided during the agricultural training to the teachers. Analysis of the diversity of iron rich vegetables were explored and planted. Nutrition and hygiene education were promoted among the schoolchildren and their caregivers. The main outcome sought was the change in knowledge and child’s dietary intake of nutrient rich fruits and vegetables.

3. Goals and objectives of the thesis

3.1. Goal

This PhD thesis aims to assess the effects of complementary school garden, nutrition and WASH interventions on nutrition and health status of schoolchildren in the districts of Dolakha and Ramechhap, Nepal.

3.2. Specific objectives

In order to achieve this aim, the following four specific objectives were pursued:

i. to investigate the water, sanitation and hygiene (WASH) conditions at the unit of selected schools, households and community;
ii. to determine the local epidemiology of malnutrition and intestinal parasitic infection among schoolchildren;
iii. to assess the knowledge, attitude and practices (KAP) of schoolchildren and caregivers regarding nutrition and WASH conditions; and
iv. to evaluate the effects of implemented complementary complementary and integrated school gardens, nutrition and WASH interventions on childrens nutritional and health status.

3.3. Hypotheses

The following hypotheses were made:

i. the WASH conditions will improve significantly with well-tailored package of WASH interventions at the implemented schools and households;
ii. the KAP of schoolchildren and caregivers, regarding nutrition and WASH conditions in intervention schools will be better than that of the control schools after intervention;
iii. the incidence of intestinal parasitic infection among children from schools having school gardens will decrease if they are linked with an improved WASH at school; and
iv. the nutritional status of the children from schools, having school gardens, nutrition and WASH interventions will be better than from the control schools.

Therefore, the main research question was whether the contribution of school vegetable gardens (agriculture), nutrition and WASH interventions could reduce malnutrition and anaemia, and decrease the incidence of intestinal parasitic infections among schoolchildren of Nepal.

4. Complementary school garden, nutrition, water, sanitation and hygiene interventions to improve children's nutrition and health status in Burkina Faso and Nepal: a study protocol

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4.1. Abstract

Background: Malnutrition and intestinal parasitic infections are common among children in Burkina Faso and Nepal. However, specific health-related data in school-aged children in these two countries are scarce. In the frame of a larger multi-stakeholder project entitled "Vegetables go to School: Improving Nutrition through Agricultural Diversification" (VgtS), a study has been designed with the objectives to: (i) describe schoolchildren's health status in Burkina Faso and Nepal; and to (ii) provide an evidence-base for programme decisions on the relevance of complementary school garden, nutrition, water, sanitation and hygiene (WASH) interventions.

Methods/design: The studies will be conducted in the Centre Ouest and the Plateau Central regions of Burkina Faso and the Dolakha and Ramechhap districts of Nepal. Data will be collected and combined at the level of schools, children and their households. A range of indicators will be used to examine nutritional status, intestinal parasitic infections and WASH conditions in 24 schools among 1,144 children aged 8-14 years at baseline and a 1-year follow-up. The studies are designed as cluster randomised trials and the schools will be assigned to two core study arms: (i) the 'complementary school garden, nutrition and WASH intervention' arm; and the (ii) 'control' arm with no interventions. Children will be subjected to parasitological examinations using stool and urine samples and to quality-controlled anthropometric and haemoglobin measurements. Drinking water will be assessed for contamination with coliform bacteria and faecal streptococci. A questionnaire survey on nutritional and health knowledge, attitudes and practices (KAP) will be administered to children and their caregivers, also assessing socioeconomic, food-security and WASH conditions at household level. Focus group and key-informant interviews on children's nutrition and hygiene perceptions and behaviours will be conducted with their caregivers and school personnel.

Discussion: The studies will contribute to fill a data gap on school-aged children in Burkina Faso and Nepal. The data collected will also serve to inform the design of school-based interventions and will contribute to deepen the understanding of potential effects of these interventions to improve schoolchildren's health in resource-constrained settings. Key findings will be used to provide guidance for the implementation of health policies at the school level in Burkina Faso and Nepal.

Trial registration: ISRCTN17968589 (date assigned: 17 July 2015)

Keywords: Burkina Faso; Malnutrition; Nepal; Parasitic infections; School-aged children; Study protocol; Water, sanitation, and hygiene (WASH)

4.2. Background

Malnutrition, intestinal parasitic infections and diarrhoeal diseases are common public health problems in children in low- and middle-income countries (LMIC) (De Onis et al., 2004; Best et al., 2010; Prüss-Üstün et al., 2008; WHO, 2013b, 2015c; Prüss-Ustün et al., 2014; Pullan et al., 2014a; IHME, 2015). In many countries, Demographic and Health Surveys (DHS) and national nutrition surveillance systems have been measuring height and weight of children below the age of 5 years, starting in the early 1990s. However, there is a paucity of anthropometric data for school-aged children (5-14 years) (WHO, 2010; Friedman, 2014; Haddad et al., 2015). Additionally, there are currently no estimates neither for school-aged children, nor the entire population, on the global burden of diseases from polyparasitism of intestinal parasitic infections caused by helminths and intestinal protozoa (Pullan et al., 2014a). Data on the burden of disease caused by intestinal protozoa is scarce, partially explained by the lack of diagnosis at the periphery (Ouattara et al., 2010; Becker et al., 2013; Polman et al., 2015). Although no estimates on the burden of diseases caused by helminth infections for school-aged children exist, an estimate for the burden of disease of sub-groups of helminth infections is available (e.g., schistosomiasis and soil-transmitted helminthiasis) (Murray et al., 2013; Pullan et al., 2014a; IHME, 2015). Estimates from the Global Atlas of Helminth Infection (GAHI; http://www.thiswormyworld.org/) showed that, in 2010, 1.01 billion school-aged children lived in areas where prevalence of any soil-transmitted helminth (STH) was above 20% (Pullan et al., 2014a). Furthermore, in 2013, diarrhoeal diseases were responsible for an estimated 7% of deaths in school-aged children in LMICs, with more than 96% attributable to unsafe water, inadequate sanitation and hygiene (WASH)(WHO, 2013b; IHME, 2015).

Burkina Faso and Nepal are both low-income countries that face an array of similar health challenges. Whilst health data among under 5-year-old children, such as nutritional indicators, anaemia or *Plasmodium* prevalence, are collected during national surveys, statistics on school-aged children in these two countries are typically unavailable (Nepal, 2011; INSD, 2012). Malnutrition, anaemia and diarrhoeal diseases are highly prevalent in under 5-year-old children. Indeed, according to the 2010 and 2011 DHS in Burkina Faso and Nepal, respectively, 35% and 41% of children were stunted; almost 15% of children in both countries reported diarrhoea 2 weeks prior to a DHS; and 88% of the children in Burkina Faso and 46% in Nepal were anaemic (Nepal, 2011; INSD, 2012). Both countries also face considerable ill-health due to inadequate WASH conditions. For example, according to data from the 2013 Global Burden of Disease Study (GBD) and the World Health Organization (WHO)/United Nations Children's Fund (UNICEF) 'Joint Monitoring Programme for Water Supply and Sanitation', 7% and 8% of deaths in children aged 5-14 years in Burkina Faso and Nepal, respectively, were caused by diarrhoeal diseases, with over 96% in both countries attributed to inadequate WASH conditions as primary risk factor (WHO, 2012b; IHME, 2015). Table 4.1 provides an overview of selected health and WASH indicators in Burkina Faso and Nepal for the years 2010 to 2013.

Table 4.1: Overview of health and WASH indicators of Burkina Faso and Nepal

Indicator	**Burkina Faso**	**Nepal**
Health	*DHS 2010*	*DHS 2011*
Stunting (<5 years)	35%	41%
Wasting (<5 years)	16%	11%
Underweight (<5 years)	26%	29%
Diarrhoea (<5 years)	15%	14%
Anaemia (<5 years)	88%	46%
Mortality (a) and morbidity [DALYs] (b)	*GBD 2013*	*GBD 2013*
Diarrhoeal diseases (5 to 14 years old)	7% (a), 5% (b)	8% (a), 4% (b)
Iron-deficiency anaemia (5 to 14 years old)	1% (a), 6% (b)	1% (a), 15% (b)
Intestinal infectious diseases (5 to 14 years old)	4% (a), 2% (b)	10% (a), 4% (b)
Water, sanitation and hygiene (WASH)	*DHS 2010 (a) and WHO Progress Report on Drinking-Water and Sanitation 2012 (b)*	*DHS 2011 (a) and WHO Progress Report on Drinking-Water and Sanitation 2012 (b)*
Improved latrines	15% (a), 19% (b)	38% (a), 37% (b)
Non-improved latrines	6% (a), 17% (b)	43% (a), 6% (b)
Open defecation (bush/field, no latrines)	62% (a), 57% (b)	36% (a), 40% (b)
Soap and water for hand washing available	14% (a)	48% (a)

(a) Mortality rate among children aged 5 to 14 years old
(b) Disability-adjusted life year (DALYs) as indicator of morbidity among children aged 5 to 14 years

Malnutrition, intestinal parasitic infections and inadequate WASH conditions are intricately linked. First, inadequate WASH conditions are important risk factors for both, malnutrition and intestinal parasitic infections (Annette Prüss-Üstün et al., 2008; Ziegelbauer et al., 2012b; Strunz et al., 2014b; IHME, 2015; Speich et al., 2016a;). The pathogenic agents associated with poor WASH conditions are viral pathogens, bacterial pathogens, protozoan cysts or oocysts and helminth eggs found in faeces and transmitted through the faecal-oral pathway and can lead to diarrhoea and under-nutrition, whereby exposure to one increases vulnerability to the other (Feachem et al., 1983; Cissé, 1997; Blössner et al., 2005; Schaible and Stefan, 2007; Acharya et al., 2008; Victora et al., 2008b). Second, malnutrition can render a child more susceptible to infection. An inadequate dietary intake leads to weight loss, weakened immunity, invasion by pathogens and mucosal damage, and impaired growth and development in children (Stephenson and Holland, 2001; Katona and Katona-Apte, 2008; Alum et al., 2010). Third, parasitic infections also contribute to growth stunting by causing a decline in food intake (loss of appetite), diarrhoea, mal-absorption and/or an increase in nutrient wastage for the immune response, all of which lead to nutrient losses and further damage to the defence mechanisms, causing a vicious cycle (Stephenson and Holland, 2001; Katona and Katona-Apte, 2008; Alum et al., 2010). Moreover, it is well documented that infections with intestinal parasites may cause internal bleeding, leading to a loss of iron and anaemia (Hotez et al., 2006a; Hall et al., 2008a). Intestinal parasitic infections can go unnoticed for years due to delayed onset of symptoms, which can exacerbate the effects on malnutrition, and hence compromise the development of their cognitive abilities in their formative years (Alum et al., 2010). It is therefore crucial to consider the strong interlinkages of malnutrition, parasitic infections, diarrhoeal diseases and WASH for preventive actions and sustainable programmes.

“Vegetables go to School: improving nutrition through agricultural diversification”

A multi-country and multi-stakeholder project entitled “Vegetables go to School: Improving Nutrition through Agricultural Diversification” (VgtS in short) was developed and is funded by the Swiss Agency for Development and Cooperation (SDC) to address schoolchildren’s nutrition in an interdisciplinary approach through the implementation of school vegetable gardens and other school-based health, nutrition and environmental interventions. The VgtS project was launched in 2012 in six target countries (Bhutan, Burkina Faso, Indonesia, Nepal, the Philippines and Tanzania) and is implemented by country teams composed of members of different ministries, (i.e. education, agriculture and health), in collaboration with the World Vegetable Center (AVRDC; headquartered in Taiwan), the University of Freiburg in Germany and the Swiss Tropical and Public Health Institute (Swiss TPH) in Switzerland as academic partners.

The objectives of the VgtS project are threefold: (i) to encourage agricultural production at the unit of the school and to increase the availability and access to a wide diversity of vegetables in order to favour a balanced and nutritious diet; (ii) to link the school garden to an educational programme that covers basic topics of agriculture, nutrition and WASH (overall project approach in all the countries); and (iii) to link the school garden programme to complementary nutrition and WASH interventions. In this context, the VgtS project embeds two intervention studies in Burkina Faso and Nepal, which include intervention schools benefitting from a complementary school garden, nutrition and WASH intervention package and control schools without any intervention. Here, we present the research protocol for the studies in Burkina Faso and Nepal.

4.3. Methods/design

Goal

The overarching goal of the studies within the frame of the VgtS project in Burkina Faso and Nepal is to address the current data gap on schoolchildren (aged 8-14 years) and to assess the effects of complementary school garden, nutrition and WASH interventions on schoolchildren's health status, as assessed by a baseline and a 1-year follow-up survey through a range of previously identified nutrition, WASH and health indicators (Table 4.2).

Table 4.2: Selected indicators for the two studies in Burkina Faso and Nepal

Objective	Indicator	Methods and tools	Survey module
Individual level of child			
To assess schoolchildren's nutritional status at baseline and follow-up	Nutritional status (BMIZ, HAZ, WAZ and clinical signs of malnutrition)	Digital scale, height measuring board and clinical examination	Nutritional survey (module 1)
To assess the prevalence of anaemia in schoolchildren at baseline and follow-up	Anaemia based on haemoglobin levels < 11.5 g/dl for children aged 7 to 11 years and < 12 g/dl for those aged 12 to 14 years	HemoCue Hb 201^{+}	Nutritional survey (module 1)
To assess the prevalence of intestinal parasitic infections in schoolchildren at baseline and follow-up	Presence and intensity of intestinal and urinary parasitic infections	Kato-Katz and formalin-ether concentration method for stool samples and centrifugation method for urine samples	Parasitological survey (module 2)
To assess schoolchildren's nutrition and health knowledge, attitudes and practices (KAP) at baseline and follow-up	KAP related to nutrition and health	Questionnaire survey with schoolchildren Focus group discussions with schoolchildren In-depth interviews with school directors and teachers	Children's health KAP (module 3)
Environmental indicators			
To assess drinking water quality of children's drinking water recipients at baseline and follow-up	Presence of thermotolerant coliform bacteria and faecal streptococci	Portable DelAgua field kit and RAPID E. COLI 2 AGAR (coliform bacteria, *Escherichia coli*) and Bile Esculine Azide AGAR (faecal streptococci) tests	Water quality testing (module 4)
Household level			
Demographic and socio-economic data			
To assess basic household socio-demographic and economic characteristics at baseline and follow-up	Caregiver's age, educational level, occupational status, assets, food security	Household questionnaire	Household questionnaire survey (module 5)
Household nutrition and health -related knowledge, attitudes and practices data			
To assess caregivers' nutrition and health knowledge, attitudes and practices at baseline and follow-up	Caregiver's knowledge, attitudes and practices related to nutrition and health	Household questionnaire Focus group discussions with schoolchildren's caregivers	Household questionnaire survey (module 5) Caregivers' health KAP (module 6)
Socio-environmental conditions data			
To assess household WASH conditions at baseline and follow-up	Drinking water source and distance to it, water storage, improved/non-improved latrine, location of kitchen, available hand washing facilities and soap, presence of domestic animals	Household living condition and information related to hygiene Direct observation	Household questionnaire survey (module 5)
Environmental indicators			
To assess drinking water quality at schoolchildren's households at baseline and follow-up	Presence of thermotolerant coliform bacteria and faecal streptococci	Portable DelAgua field kit and RAPID E. COLI 2 AGAR (coliform bacteria, *Escherichia coli*) and Bile Esculine Azide AGAR (faecal streptococci) tests	Water quality testing (module 4)
School and community level			
Socio-environmental conditions data			
To assess the WASH conditions at schools at	Available drinking water, available improved/non-	In-depth interviews with school directors and teachers	WASH survey (module 7)

baseline and follow-up	improved toilet/latrine, available hand washing facilities and soap	Direct observation	
Environmental indicators			
To assess drinking water quality at schools and community sources at baseline and follow-up	Presence of thermotolerant coliform bacteria and faecal streptococci	Portable DelAgua field kit and RAPID E. COLI 2 AGAR (coliform bacteria, *Escherichia coli*) and Bile Esculine Azide AGAR (faecal streptococci) tests	Water quality testing (module 4)

Study sites and school selection

The studies will be conducted in Burkina Faso and Nepal. The study sites are located within the VgtS project sites, which were selected by the local VgtS country teams, following a set of criteria: (i) accessibility from the capital; (ii) availability of land for the school garden and continuous access to water at schools; (iii) coeducation of boys and girls in public schools; and (iv) willingness of the school principals and the community to participate.

In both countries, the study will be implemented in two different regions. In Burkina Faso, these are the Centre Ouest and the Plateau Central regions, both located in proximity to the capital Ouagadougou (30-180 km). The study sites in Nepal are the Dolakha and Ramechhap districts in the eastern part of the country, both located in proximity of the district headquarters Charikot (133 km) and Manthali (131 km), respectively.

The selection of the schools participating in the two studies is based on a three-stage sampling procedure of schools within the overall VgtS project sites. In a first step, about 100 schools fulfilling the aforementioned eligibility criteria were selected. In a second step, from these 100 schools, a sample of 30 schools were randomly chosen to be included in the VgtS school garden implementation and were randomly allocated to three groups, which receive the school vegetable garden interventions in 2014, 2015 and 2016, respectively. In a third step, out of the 30 VgtS project schools, a total of eight schools in Burkina Faso and 16 schools in Nepal were randomly selected to accommodate the sampling needs of the two complementary and slightly different study designs of Burkina Faso and Nepal.

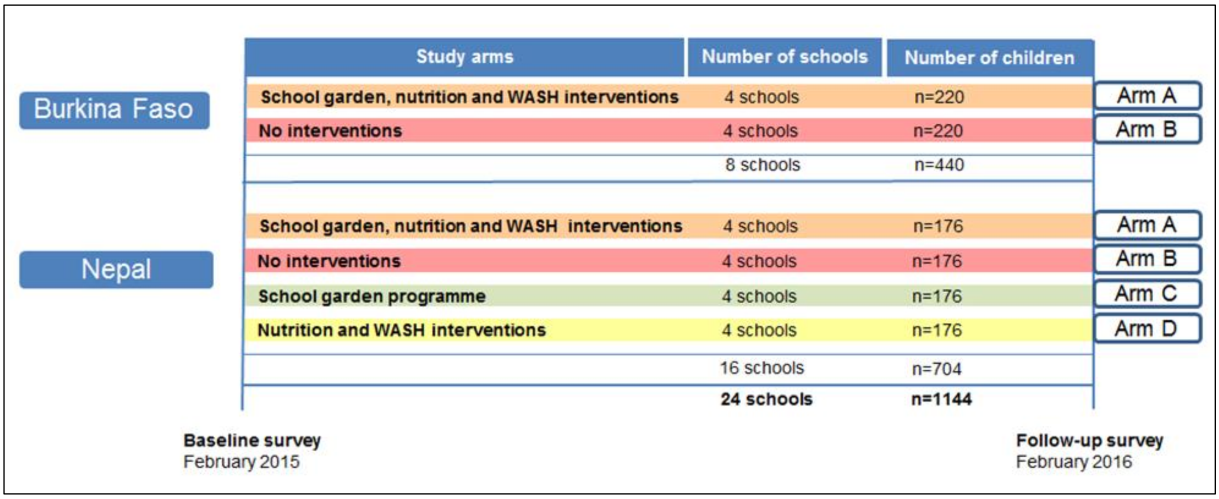

Figure 4.1: Study design for Burkina Faso and Nepal

Study design

The two studies in Burkina Faso and Nepal are designed as cluster randomised trials with an equal number of schools randomly allocated to two core study arms (A, B) and with a cohort of children followed in two consecutive surveys, at baseline and 1-year follow-up. Two additional study arms are included in Nepal (C, D). The four study arms are designed as follows:

arm A: school garden programme and complementary nutrition and WASH interventions;

arm B: no interventions, i.e. controls;

arm C: school garden programme without nutrition and WASH interventions; and

arm D: nutrition and WASH interventions without the school garden programme.

Each arm comprises four schools. Figure 19 shows the study design with the different study arms for Burkina Faso and Nepal. In both countries, schools of arm A will receive the complementary school garden, nutrition and WASH intervention package starting in March 2015. In Nepal, the interventions from arms C and D will be implemented over the same period. In both countries, the control schools of arm B will benefit from the school garden intervention in the year following the interventions.

Sample size

Two separate sample size calculations were conducted for the two study designs of Burkina Faso and Nepal. For the sample size calculation of the study in Burkina Faso, the prevalence of intestinal parasitic infection in children aged 8-14 years was selected as the primary outcome in the comparison between high- and low-risk of intestinal parasitic infection in children. The power calculation was based on the assumption of:

- an average intestinal protozoa and helminth infection rate across schools of 40% (Touré et al., 2008);

- a coefficient of variation of 10% across schools; and
- a proportion of high risk children of 25%.

A Monte Carlo simulation with 5,000 iterations shows that a total of 400 children from eight schools (i.e. 50 children per school) would provide 85% power for detecting a significant difference in infection rates between high- and low-risk children at the usual level of 5% under these assumptions and for a true odds ratio of 2. Recruitment will be increased by 10% to account for drop-outs or non-participation, which leads to an optimal sample size of at least 440 children.

The sample size calculation for the study in Nepal was also based on the prevalence of intestinal parasitic infections in children aged 8-17 years as the primary outcome. The power calculation was based on the assumption of:

- the prevalence rate of intestinal protozoa and helminth infection is 30% (Pradhan et al., 2014b) and this rate will remain in steady state in the absence of any intervention;
- probability of new intestinal protozoa and helminth infection at the 1-year follow-up will be reduced by at least 10% through the implementation of the complementary nutrition and WASH intervention package; and
- a coefficient of variation of 10% across schools.

A Monte Carlo simulation with 5,000 iterations shows that a total of 640 schoolchildren from 16 schools (i.e. 40 children per school) would provide 80% of power for detecting a significant difference in infection rates between the four study arms. Recruitment will be increased by 10% accounting for drop-outs and non-participants, which leads to an optimal sample size of at least 704 children.

<u>Eligibility and selection criteria of study participants</u>

In both Burkina Faso and Nepal, children enrolled in school are eligible to participate in the baseline survey if they are between 8 and 14 years old in Burkina Faso and 8 and 17 years in Nepal, have signed a written informed consent by their parents, guardians or teachers, and themselves assented orally.

<u>Data collection procedures</u>

Four weeks prior to the study, district and village authorities, school directors and children's parents/guardians will be informed about the forthcoming survey activities by the local survey team. They will be re-informed about the purpose and procedures of the study shortly before the start of the survey activities. Written informed consent (signed or fingerprint) will be obtained from children's parents or legal guardians, whilst children will assent orally. It will be emphasised that participation is voluntary and that children and parents/guardians can withdraw anytime without further obligation.

In each school, a random selection of children aged 8-14 years will be enrolled until at least 55 in Burkina Faso and 44 in Nepal are reached. Moreover, at the follow-up survey, the same children will be re-assessed, who by then will be 9-15 years old. Each child will be attributed a unique identification code (ID) for the different assessments at the onset of the study. A separate household ID connected to the schoolchild's personal ID will be given to children's households in order to link children's clinical data and nutritional and health knowledge, attitudes and practices (KAP) with the household characteristics. Children will thereafter be invited to provide stool and urine samples, to take anthropometric and haemoglobin (Hb) measurements and to participate in the KAP survey. In Burkina Faso, stool and urine samples will be collected on two consecutive days. In Nepal, a single stool sample will be collected, while urine samples will not be collected as urogenital schistosomiasis is not endemic. Infected, anaemic or undernourished children in all schools will be subjected to clinical, parasitological and nutritional examinations, and will be treated according to national policies.

After these assessments with children at the schools, the same enumerators in Burkina Faso will visit children's households and will invite children's caregivers to respond to a household questionnaire during the two survey days. In Nepal, due to the scattered locations and geographical constraints, additional enumerators will visit the children's households during the same survey period. In both countries, trained and experienced *enumerators will conduct the questionnaire surveys in local languages.*

Collection of stool and urine samples

The sampled children at the schools will be asked to provide a fresh mid-morning, post-exercise stool sample. The stool samples will be processed and analysed each day (at mid-day the latest) by experienced laboratory technicians and medical microbiologists as follows: first, stool samples will be visually examined for macroscopic appearance of adult worms, also checking the stool consistency and the presence of blood and mucus. Second, a single Kato-Katz thick smear, using 41.7 mg templates, will be prepared on a slide and examined under a microscope for the presence of eggs of *Schistosoma mansoni*, hookworm, *Ascaris lumbricoides, Trichuris trichiura* and *Hymenolepis nana*, adhering to standard operating procedures (Katz et al., 1972c; Yap et al., 2012a).

Third, a formalin-ether concentration technique will be used to enhance sensitivity for the diagnosis of helminths and to detect intestinal protozoa (*Blastocystis hominis*, *Chilomastix* *mesnili*, *Endolimax nana*, *Entamoeba coli*, *Entamoeba histolytica*/*Entamoeba dispar*, *Entamoeba hartmanni*, *Giardia intestinalis* and *Iodamoeba bütschlii*) (Marti and Escher, 1990). Approximately 1-2 g of stool will be placed in 15 ml Falcon tubes filled with 10 ml of 5% formalin and will be examined by experienced laboratory technicians for the presence of helminths and intestinal protozoa, using an ether-concentration technique, adhering to an SOP (Utzinger et al., 2010b). Additionally, in Nepal, 20 mg of stool will be prepared on a single slide with the saline wet mount concentration for the microscopic detection of the same intestinal protozoa and helminths, according to SOPs (Endris et al., 2012; Koltas et al., 2014). Furthermore, the intensity of infection will be calculated as the number of eggs per 1 g of stool (EPG) and categorised according to the WHO standard classification (WHO, 2002).

In Burkina Faso, children will also be asked to provide fresh, mid-morning and post-exercise urine samples, collected at the same time as the stool samples. Urine samples will be analysed for microhaematuria (biochemical marker and proxy for *Schistosoma haematobium*), using reagent strips (Hemastix; Siemens Healthcare Diagnostics GmbH; Eschborn, Germany) (Bogoch et al., 2012), and for the presence and number of *S. haematobium* eggs in 10 ml of urine using a urine centrifugation technique and microscopy (Hodges et al., 2012). *S. haematobium* infection will be grouped into light (< 50 eggs/10 ml of urine) and heavy (≥ 50 eggs/10 ml of urine) (Stephenson et al., 2000).

In order to achieve a higher sensitivity in diagnostics, in selected schoolchildren stool and urine samples will be obtained on two consecutive days in Burkina Faso (Booth et al., 2003; Speich et al., 2014). For quality control, 10% of all processed stool samples will be re-read under a microscope by independent laboratories (Speich et al., 2015). Slides identified with discrepant results will be re-examined by the Institut de Recherches en Sciences de la Santé (IRSS) laboratory and Kirnetar Health Centre team until agreement has been reached.

<u>Collection of anthropometric indicators and measuring Hb levels</u>

Selected schoolchildren will be subjected to anthropometric measurements according to SOPs, as described by WHO, using a digital scale and a height measuring board with a precision of 0.1 kg and 0.1 cm, respectively (WHO, 2008b). Individual z-score will be computed using the new WHO growth reference values for children and adolescents (Onis et al., 2007). The nutritional status of schoolchildren will be classified as follows: a z-score within the interval of -3 standard deviation (SD) < z < -2 SD will be used to classify body-mass-index-for-age (BMIZ, thinness), height-for-age (HAZ, stunting) and weight-for-age (WAZ, underweight) as moderate undernutrition, and a z-score <-3 SD to define severe undernutrition. WAZ will only be used for children aged 8-10 years as reference data are not available beyond the age of 10 years. Overweight will be classified as BMIZ >1.0 SD and obesity as BMIZ >2.0 SD (WHO, 2009).

The Hb level will be measured to determine anaemia prevalence. A finger-prick capillary blood sample will be taken, and Hb concentration measured using a HemoCue® 201+ testing device (HemoCue Hb 201+ System; HemoCue AB, Ängelholm, Sweden). Age-specific criteria will be used to identify anaemic children: Hb <11.5 g/dl for children aged 8-11 years and Hb <12 g/dl for children aged 12-14 years (WHO, 2001b).

Additionally in Nepal, trained health care professionals will conduct clinical examinations for detecting clinical signs of nutritional deficiencies (e.g., dermatitis, bitot's spot, dry and infected cornea, oedema, enlargement of liver, loss of peripheral sensation, angular stomatitis, pale conjunctiva, red inflamed tongue, swelling of the thyroid gland and bowed legs) (Webb, 2012).

<u>Drinking water quality assessment</u>

In Burkina Faso, drinking water samples will be collected in sterile 250 ml bottles at the selected schools and community sources, children's households and from their drinking water recipients to assess drinking water quality at source and point of use. Water samples will be randomly taken in 20% of participating children's households and in five community sources per study site (always including the school source). Water samples from children's drinking water recipients brought to school will be randomly collected in 30% of the children. Before analysis, the water samples collected will be preserved in cool boxes at 4^{o}C, and transferred to a nearby laboratory. The water samples will be analysed by membrane filtration for the presence/absence (PA) of thermotolerant faecal coliforms (TTC) as colony forming units per 100 ml of water (CFU/100 ml). Furthermore, *E. coli* and faecal streptococci as indicators for faecal contamination will be assessed by the use of the RAPID E. COLI 2 AGAR (coliform bacteria and *E. coli*; Bio-Rad Laboratories, Hercules, USA) and the Bile Esculine Azide AGAR (faecal streptococci; Bio-Rad Laboratories, Hercules, USA) tests according to WHO drinking water standards (WHO, 2004).

In Nepal, drinking water samples will be collected in 250 ml sterile bottles from the school drinking water source and children's drinking water recipients, household and community water sources. Water samples will be collected at every school (n=16) and every child's household (n=440). For the community sources, one water sample per study site will be taken from the principal water distribution channel of the community source (n=16). The water samples will be analysed *in situ* at the schools and households for turbidity, pH and chlorine residual using the *DelAgua* kit (Oxfam-DelAgua; Guildford, UK) using readily available SOPs (Delagua, 2009). If the concentration of free chlorine residual is greater than 0.2 mg/l (0.2 ppm) and the turbidity less than 5 turbidity units, the water samples will not be analysed for TTC (Delagua, 2009). If the results do not meet these criteria, water samples will be transported in cool boxes to the laboratory in Kirnetar Health Centre and stored in a refrigerator at 4°C before analysis using the *DelAgua* kit. The water samples will be tested for CFU/100 ml according to WHO drinking water standards (WHO, 2004). Quality control will be conducted with 10% of all water samples collected by independent laboratories.

Questionnaire survey with schoolchildren and their caregivers

The KAP survey was established with the guidelines and KAP manual provided by FAO, using standardised questions and amendments by the Swiss TPH research team (Marías et al., 2014). Children's caregivers will also be invited to respond to a questionnaire investigating socio-demographic, economic, health and food security issues. The questionnaire surveys for children and their caregivers will be tablet-based using the Open Data Kit software (ODK, 2015).

Focus group discussions and in-depth interviews

Focus group discussions (FGDs) will be conducted with 6 to 10 randomly selected caregivers from sampled children in each school to better understand the caregivers' perceptions on nutrition and health. The school director and teachers will be interviewed with a semi-structured questionnaire to record characteristics of children's health challenges, and to discuss children's nutrition and health education in the curricula, school health activities, school water and sanitary installations and, if existing, the school feeding programmes.

Data management and analysis

Quantitative data from stool and urine examinations, anthropometrics and Hb measurements will be entered in Microsoft Excel 2010 (Microsoft; Redmond, USA). A double data entry system will be used to ensure data quality. Data will be evaluated for discrepancies and validated after removing inconsistencies. The z-score values for height-, weight- and BMI-for-age relative to the WHO 2007 reference will be calculated using WHO AnthroPlus (WHO; Geneva, Switzerland). Statistical analyses will be carried out with Stata version 13 (StataCorp; College Station, USA). Analysis of baseline data will be conducted to describe the prevalence of malnutrition, intestinal parasitic infections, WASH conditions, KAP and basic socioeconomic characteristics. Logistic regression models will be employed to estimate associations of malnutrition, intestinal parasitic infections and anaemia with risk factors.

FGDs and in-depth interviews (IDIs) will be transcribed, translated into English by bilingual research assistants and entered as Microsoft Word documents into MAXQDA version 11 (VERBI GmbH 2012; Berlin, Germany) for data coding and analysis. Main themes will be identified and coded in order to categorise explanations and descriptions of nutritional and health related perceptions and issues.

Longitudinal analysis will be conducted to evaluate the intervention effects of the complementary interventions under study. The results from the different study arms will be compared at the end of the 1-year intervention period.

Data storage and handling

All data files will be stored in a secure server at Swiss TPH. ID codes and name-linked information on participants will remain confidential and will be used only to communicate clinical results to participants for their respective treatments.

Ethical considerations

The two research protocols for Burkina Faso and Nepal were reviewed by (i) the Institutional Research Commission of Swiss TPH (reference number FK 116; date of approval 30 October 2014); (ii) the "Ethikkommission Nordwest- und Zentralschweiz" (EKNZ) in Switzerland for the Nepal study protocol (reference no. UBE-15/02; date of approval 12 January 2015); (iii) the EKNZ in Switzerland for the Burkina Faso study protocol (reference no. 2014-161; date of approval 19 January 2015); (iv) the "Comité d'Ethique pour la Recherche en Santé, Ministère de la Recherche Scientifique et de l'Innovation, et Ministère de la Santé" in Burkina Faso (reference no. 2014-5-058; date of approval 20 May 2014); (v) the "Institutional Review Committee of Kathmandu University School of Medical Sciences, Dhulikhel Hospital", Nepal (reference no. 86/14, date of approval 24 August 2014); and the (vi) Institutional Review Committee, Health Research Council, Nepal (reference no. 565; date of approval 11 November 2014).

The two studies have been registered under a single trial registration number at the International Standard Randomised Controlled Trial Number Register ISRCTN17968589 (date assigned: 17 July 2015; http://www.isrctn.com/ISRCTN17968589).

4.4. Discussion

Malnutrition and intestinal parasitic infections are a major burden on children's health globally and particularly in LMIC, including Burkina Faso and Nepal. Inadequate WASH conditions play an important role in the high burden of communicable diseases (Grimes et al., 2014; Strunz et al., 2014b). The morbidity due to malnutrition, intestinal parasitic infections and diarrhoeal diseases in Burkina Faso and Nepal continue to be considerable (IHME, 2015). Given the global persistence of malnutrition and ill-health, the research and international development communities are increasingly paying attention to enhancing nutrition and health as the primary goals and outcomes of food production and delivery systems (Thompson and Amoroso, 2011; Waage et al., 2013; Webb, 2013b). Agriculture as a source of nutritious food and well-being has recently been recognised and is addressed in the new Sustainable Development Goals (SDGs), particularly in SDG 2: "End hunger, achieve food security and improved nutrition and promote sustainable agriculture" (Kates et al., 2005). There is, however, an insufficient evidence-base which supports these agriculture, nutrition and health linkages (Thompson and Amoroso, 2011; Waage et al., 2013; Webb, 2013b). Indeed, according to Masset et al. (2011), who undertook to date the largest systematic review on agricultural intervention to improve the nutritional status of children, there is "no evidence of the impact [of agricultural interventions] on prevalence rates of stunting, wasting and underweight among children under five" (Masset, 2011). Even though agriculture interventions were beneficial in promoting consumption of nutritious foods, evidence of improved nutritional indicators was not consistent (Allen and Gillespie, 2001; Ruel, 2001; Masset, 2011). However, according to Webb (2013), the lack of evidence on the impact of agricultural interventions on nutrition and health outcomes should not be attributed to the inefficacy of these interventions, but rather to insufficient statistical power (small simple size), lack of rigorous counterfactual analysis, inadequate selection of outcome indicators for the kind of interventions considered, and few accounted for the heterogeneity of impacts even when they were positive (Ruel, 2001; Masset, 2011; Turner et al., 2013; Webb, 2013b). Furthermore, school-aged children are moving into the focus of recent initiatives by governments, bilateral and multilateral organisations, and other development actors; which have recognised the benefits of good health and nutrition of schoolchildren to contribute to educational achievement, growth and development (Jukes et al., 2007; Black et al., 2008b, 2013a; Humphrey, 2009; Best et al., 2010; Smith et al., 2013).

The two studies in Burkina Faso and Nepal within the frame of the overall VgtS project that we describe here will support the reinforcement of this recent attention on schoolchildren's nutrition and health by focusing on schools as an entry point for health promotion, infection control and life-skills education. Moreover, the studies will contribute to fill existing data gaps on schoolchildren in these two countries, concurrently identifying their nutritional and health challenges and needs. The data collected will serve to inform the design of appropriate and tailored school-based interventions with close participation of the local community, school teachers and directors, as well as with the local research and VgtS project team. The precise set of interventions will be developed after the baseline survey in Burkina Faso and Nepal. The interventions will be designed with a multidisciplinary team of educators, epidemiologist, nutritionist, parasitologists and WASH experts in order to improve water quality, sanitary and hygiene environments and to translate the nutritional and health risk factors into effective educational messages, thereby encouraging schoolchildren to change their behaviour.

The two studies also aim to address the scientific research gap by conducting rigorous intervention studies and quantifying the possible effects of complementary school garden, nutrition and WASH interventions. With the two particular study designs as suggested in Burkina Faso and Nepal, we will be able to analyse the different types of school garden, nutrition and WASH intervention packages. While in Burkina Faso the focus will be on the comparison of integrated and complementary school garden, nutrition and WASH interventions (arm A) as compared to the control schools with no interventions (arm B); in Nepal, we will additionally be able to conduct comparisons between these two study arms to the school garden intervention schools (arm C) and the nutrition and WASH intervention schools (arm D).

Several considerations underscore the relevance for the two concerted and complementary study designs. First, with the same research methods and questionnaire tools applied, data collected from Burkina Faso and Nepal will be used for comparative analysis. Second, the two similar study designs will offer strategies for comparing different public health approaches with an emphasis on schoolchildren's health and will provide opportunities for discussing the long-term sustainability of these programmes, especially in areas where the targeted diseases are highly prevalent.

Taken together, the overarching goal of the two studies is to assess the potential of suitable interventions to improve health of schoolchildren in resource-constrained settings. The insights gained will contribute to estimate the burden of malnutrition and intestinal parasitic infections in schoolchildren and may provide guidance for future research activities, for the implementation of health policies at the school level, as well as for future public health recommendations and health policy planning.

Competing interests

The authors declare no conflict of interest.

Authors' contributions

All listed authors contributed to the development of the study design, essential study documents and standard operating procedures to be employed for the two intervention studies. According to their different areas of expertise, the authors critically revised specific parts of this manuscript (clinical aspects: SD, PO, JU, GC; data management: SE, AK, JG, CS; diagnostic techniques: SD, PO, JU, GC; methodology: SE, AS, AK, PO, JU, GC; study country-specific issues: AS, SD, SS). SE, in collaboration with AS and RH, wrote the first draft of the manuscript. All authors read and approved the final version of the paper prior to submission.

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5. Water quality, sanitation, and hygiene conditions in schools and households in Dolakha and Ramechhap districts, Nepal: results from a cross-sectional survey

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5.1. Abstract

This study assessed drinking water quality, sanitation, and hygiene (WASH) conditions among 708 schoolchildren and 562 households in Dolakha and Ramechhap districts of Nepal. Cross-sectional surveys were carried out in March and June 2015. A Delagua water quality testing kit was employed on 634 water samples obtained from 16 purposively selected schools, 40 community water sources, and 562 households to examine water quality. A flame atomic absorption spectrophotometer was used to test lead and arsenic content of the same samples. Additionally, a questionnaire survey was conducted to obtain WASH predictors. A total of 75% of school drinking water source samples and 76.9% point-of-use samples (water bottles) at schools, 39.5% water source samples in the community, and 27.4% point-of-use samples at household levels were contaminated with thermo-tolerant coliforms. The values of water samples for pH (6.8–7.6), free and total residual chlorine (0.1–0.5 mg/L), mean lead concentration (0.01 mg/L), and mean arsenic concentration (0.05 mg/L) were within national drinking water quality standards. The presence of domestic animals roaming inside schoolchildren's homes was significantly associated with drinking water contamination (adjusted odds ratio: 1.64; 95% confidence interval: 1.08–2.50; p = 0.02). Our findings call for an improvement of WASH conditions at the unit of school, households, and communities.

Keywords: cross-sectional survey; drinking water quality; hygiene; Nepal; sanitation; schoolchildren.

5.2. Introduction

Water, sanitation, and hygiene (WASH) are fundamental to human development and wellbeing. The World Health Organization /United Nations Children's Fund (WHO/UNICEF) Joint Monitoring Program (JMP) for water supply and sanitation estimates that, in 2015, 663 million people lacked improved drinking water sources and 2.4 billion lacked improved sanitation facilities (WHO/UNICEF, 2015). Unsafe and insufficient quantity of drinking water, inadequate sanitation, and unimproved hygiene account for 7% of the global burden of disease and 19% of child mortality worldwide (Prüss-Üstün et al., 2008; Cairncross et al., 2010). The era of the Millennium Development Goals (MDGs) from 2000–2015 had specific targets for "improved" access to drinking water supply and "basic sanitation"; however, coverage fell short of the sanitation target (WHO/UNICEF, 2015; Alexander et al., 2016). Still today, many schools and households in low- and middle-income countries (LMICs) lack adequate and safe WASH services, compromising people's health and wellbeing (Jasper et al., 2012). For example, in 2012, UNICEF reported that only 51% of schools in LMICs, had access to adequate water and 45% had access to sanitation facilities (UNICEF, 2012). The lack of reliable access to safe and sustainable WASH infrastructure, in conjugation with related hygiene and sanitation behaviours, remains a major public health problem (Bowen et al., 2007; Prüss-Ustün et al., 2008; Freeman et al., 2012; Garn et al., 2013; Wolf et al., 2014). In LMICs, each year, 1.5–2 million children die from WASH-related diseases and many more are debilitated by illness, pain, and discomfort (Tarrass and Benjelloun, 2012). While the majority of deaths occur in children below the age of five years, the burden of disease among schoolchildren is considerable (Freeman et al., 2014). Approximately 74% of the health burden in schoolchildren in LMICs is due to intestinal helminth infections and 60% of the mortality is linked to infectious diseases such as schistosomiasis, soil-transmitted helminthiasis, and trachoma (Bartram et al., 2005). Approximately 88% of diarrhoeal diseases and 47% of soil-transmitted helminthiasis are due to WASH issues in LMICs, which in turn cause malnutrition and impair food intake and nutrient absorption (A Prüss-Üstün et al., 2008; Hall et al., 2008b; Tarrass and Benjelloun, 2012; Dangour et al., 2013) .

The heavy metals such as arsenic and lead introduced into drinking water primarily by dissolution of naturally occurring ores, minerals, and industrial effluents are public health problems. Arsenic is one of the most dangerous trace elements and is predominantly found in rocks, soils, and natural water. The studies reported that arsenic affects the organs and systems in the body, including skin, heart, respiratory organs, and kidney consequently leading to cancer of the lung, kidney, and bladder (J. Aryal et al., 2012). Similarly, lead, another heavy metal, acts as an anti-essential trace element, highly toxic cumulative element in the human body and is widely distributed in soil and groundwater (Lalor et al., 2006; Mandour et al., 2013). For neurological, metabolic, and behavioural reasons, children are more vulnerable to the effects of lead compared to adults (Graff et al., 2006).

Nepal faces a plethora of problems related to WASH issues (Warner et al., 2007; Prüss-Ustün et al., 2014a). In 2015, the World Health WHO/UNICEF JMP reported that 92% of the Nepalese population had access to improved water, and hence, met this specific MDG target (WHO/UNICEF, 2015). However, it remains to be determined whether the water classified as improved is safe for consumption. Sanitation coverage was 46%, while 37% of the population were still practicing open defecation, causing serious risks of environmental contamination, such as to open water sources (GON, 2011b; WHO/UNICEF, 2015). At the unit of the school, 61.9% had at least one toilet facility. Water supply facilities are not adequate to meet and maintain sanitation requirements in most of the schools (GON and Water Aid, 2014). According to data from the Department of Health Service in Nepal, about 3500 children die each year due to water-borne diseases (Aryal et al., 2012a). Intestinal parasitic infections and diarrhoeal diseases due to inadequate WASH are the principal causes (Sah et al., 2013a; Tandukar et al., 2013b). The most common intestinal helminths among Nepalese children reported are *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*, with manifestations that include malnutrition, iron deficiency anaemia, malabsorption syndrome, intestinal obstruction, and impaired physical growth (Tandukar et al., 2013b). There is a large body of evidence indicating that WASH interventions improve health and lead to significant reductions in both the severity and prevalence of diarrhoea and helminthiases (Jasper et al., 2012; Ziegelbauer et al., 2012c; Grimes et al., 2014). Several studies investigated heavy metals, such as lead and arsenic. With regard to lead, a study reported high concentrations (15–35 µg/L) in drinking water samples collected from different parts of Nepal (Shrestha and Kafle, 2009) . Meanwhile, a study investigating the quality of groundwater, especially in the Terai region, revealed high arsenic content (Pokhrel et al., 2009). Furthermore, some studies have revealed high concentrations of arsenic in shallow tube wells (<50 m depth) with reported arsenic concentrations of up to 10 µg/L (Mahat and Shrestha, 2008; Yadav et al., 2012).

The project entitled "Vegetables go to School: improving nutrition through agricultural diversification" (VgtS) is a multi-country study that seeks to deepen the understanding of whether school vegetable gardens, nutrition, and WASH interventions might lower the incidence of intestinal parasitic infections among schoolchildren and reduce malnutrition. Five countries are involved: Bhutan, Burkina Faso, Indonesia, Nepal, and the Philippines. The study protocol for Burkina Faso and Nepal has been published elsewhere (Erismann et al., 2016c). The specific objectives of the research presented here were: (i) to assess WASH conditions at the units of the school, households, and community; (ii) to conduct a baseline appraisal and identify gaps from which to identify priority needs and required interventions; and (iii) to analyse the association between water contamination and WASH predictors at the household level. We examined the water quality (physiochemical characteristics, microbiological contamination by thermo-tolerant coliforms (TTC), and heavy metals content), and sanitation and hygiene conditions at schools, households, and communities of the sampled children.

5.3. Materials and methods

<u>Study sites</u>

This study was conducted at the VgtS project sites in Dolakha and Ramechhap districts in Nepal. Dolakha is located approximately 180 km and Ramechhap approximately 150 km from Kathmandu, the capital of Nepal (Figure 1). Most of Dolakha district lies in the temperate zone (28.5%), followed by subtropical (26.2%), nival (17.4%), subalpine (16.6%), alpine (9.4%), and tropical (1.9%) zones. Similarly, Ramechhap district lies mostly in the subtropical zone (42.1%), followed by temperate (21.0%), tropical (18.0%), nival (7.3%), subalpine (6.7%), and alpine (3.6%) zones (Barnekow Lillesø et al., 2005). Dolakha and Ramechhap districts have 54 village development committees (VDCs) and one municipality. We collected water samples from 32 VDCs in Dolakha and eight in Ramechhap.

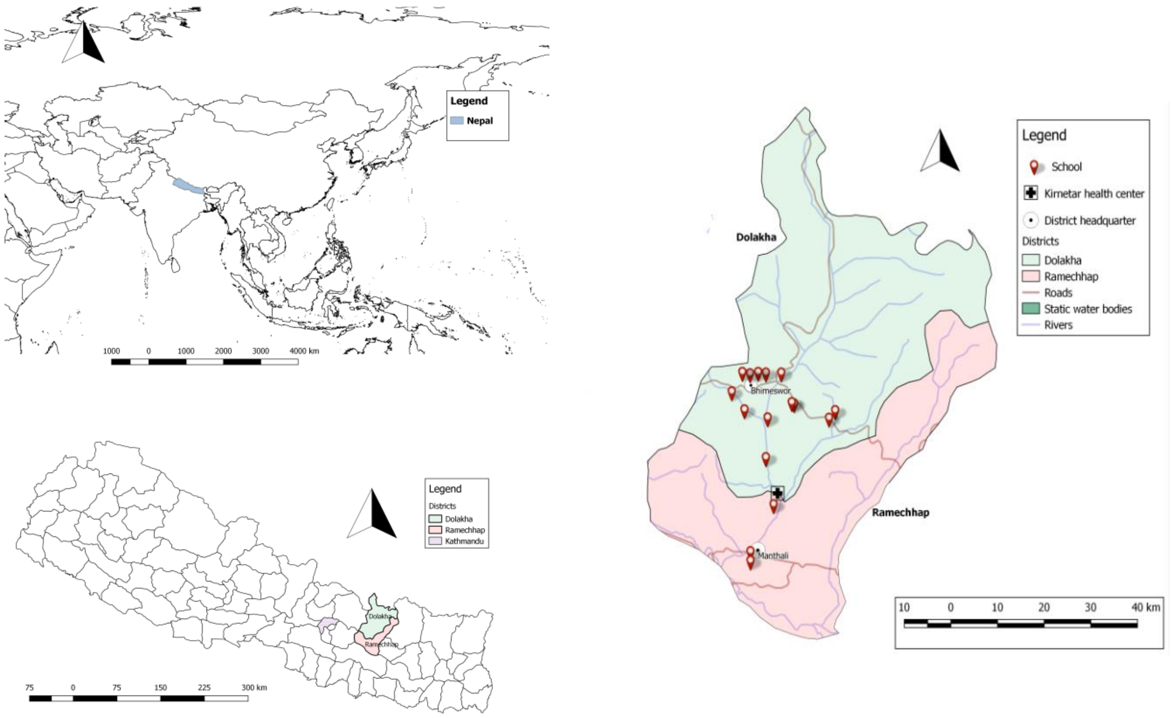

Figure 5.1: Study Sites, Dolakha and Ramechhap Districts

School selection

In mid-2015, a cross-sectional survey was conducted in 16 schools (13 in Dolakha and three in Ramechhap) of the 30 purposively selected schools of the National Agriculture Research Council and the Department of Education, within the frame of the VgtS project. Selection criteria were as follows: (i) schools located within 1-h walking distance from a main street; and (ii) water available at school for vegetable cultivation. In the Ramechhap district, only three schools were selected, as the aforementioned criteria were hard to meet. The 16 schools were randomised to one of four interventions, as follows:

- schools benefiting from a school garden intervention only;
- schools allocated a nutrition and health education programme, including school garden implementation;
- schools benefiting from nutrition and WASH intervention; and
- schools without any interventions (considered as control schools).

Study population and sample size

The study population consisted of children in grades 6 and 7, aged 8–16 years. A Monte Carlo simulation showed that 800 children, with 50 children per school and four schools per intervention arm would provide at least 75% power for finding simultaneous significant effects of the implemented type of intervention under the following assumptions:

- the prevalence of intestinal protozoan and helminth infections is about 30% (Sah et al., 2013b) and remains constant in the absence of any intervention;
- the probability of new intestinal protozoa and helminth infections at the end of follow-up is 15%;
- the same effect odds ratios (ORs) apply to incidence and persistence of intestinal protozoa and helminth infection; and
- each of the two interventions reduces the odds of infection by 50%, and their effects are additive on the logit-scale.

Sample collection and treatment

Sterilised polyethylene bottles (250 mL) were used for water sampling. Membrane filter and membrane lauryl sulphate broth were used in the estimation of TTC. Water samples were collected from:

- schools, between 30 May and 6 June 2015, from one main functioning drinking water point in each of 16 schools and 13 point-of-use (water container, cups);
- households, between 6 and 30 June 2015, at point-of-use in every 562 surveyed schoolchildren’s households; and

- communities, from 1 to 10 June 2015, in approximately 10% of the water sources in the community. Of note, 43 drinking water sources were collected from 40 communities selected at random (at least one sample per community). Water was collected from stand pipes (n = 37), protected springs (n = 3), protected wells (n = 2), and ponds (n = 1).

The sample collections were done from the stand pipes and springs, ponds, wells, and reservoirs according to the standard guidelines of the Delagua water testing kit. To collect water samples from stand pipes, the tap was opened for 1 min before taking a sample. This ensured that any deposits in the pipes were washed out and the water sample was representative of the water in the supply pipes. To collect water from ponds, reservoirs, open wells, or other surface water sources, the sterilized cups were rinsed twice with the specific water source before taking the sample (Delagua, 2015a).

Physical, chemical, and microbiological parameters

Physical parameters of the water sample were measured, including temperature (°C), pH, and turbidity (nephelometric turbidity unit (NTU)). Similarly, chemical parameters were measured, such as residual chlorine (free and total), lead, and arsenic contents. Measured microbiological parameters included TTC. The standards of each parameter are 5 NTU for turbidity, 6.5–8.5 for pH, 0.01 mg/L for lead, 0.05 mg/L for arsenic, 0.1–0.2 mg/L for residual chlorine, and <1 for TTC as per the national drinking water quality standard guideline (NDWQS) of the Government of Nepal (GON, 2005).

Drinking water quality analysis

Drinking water samples were collected according to the standard guidelines of the Delagua water testing kit (Delagua, 2015a). The 250 mL polyethylene bottles were sterilised in an autoclave at 121 °C for 15 min. These sample bottles were then rinsed three times by the water collected for analysis, made watertight by air tightening and marked with a unique code and date of sampling. The water samples were stored in a portable cool box, transferred to the laboratory within 3 h of collection, and stored at 4 °C in a refrigerator preceding analysis done within a maximum of 30 h. The water samples were brought to room temperature before analysis. We filtered 100 mL of each sample using sterile filter paper with a 0.45 μm pore size, applied vacuum suction, and incubated at 44 °C for 18 h. After incubation, bacteria were enumerated by colony count (Delagua, 2015a).

Heavy metal analysis

Lead and arsenic contents were analysed in all 16 samples from the schools. The samples were subjected to a flame atomic absorption spectrophotometer (AAS, model 2380, Perkin-Elmer GmbH; Überlingen, Germany); in combination with high-pressure liquid chromatography (HPLC, Akvilon, Moscow, Russia) for arsenic. Standardisation of the instrument was carried out before laboratory procedures to verify consistency in instrument response. In each water sample, lead and arsenic contents were determined in triplicate for quality control.

Questionnaire survey

A semi-structured questionnaire was used to determine WASH conditions for schools, surveyed schoolchildren, and their households. School WASH information was obtained from the school principals. Observational measures were used to collect information related to the cleanliness of the latrine and availability of water around-the-clock. Knowledge, attitude, and practices (KAP) related to WASH were collected from schoolchildren. Household-related WASH information was collected from caregivers. Questions included topics such as availability of improved water in households, water treatment, livestock, and disease prevalence in the preceding two weeks and socio-demographic information. Data were collected using tablets (Samsung Galaxy note 10.1 N8010; Seoul, Korea) and open data kit (ODK) software (University of Washington, Seattle WA, USA). To ensure the reliability of the information, schoolchildren and their caregivers were interviewed in their mother tongue by enumerators familiar with the study area and fluent in local languages. The data collection device was password-protected and automatic deletion of data after synchronising with the server was activated to maintain confidentiality. The data were thereafter transferred and stored electronically in a password-protected server at the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland). Analysis was done using STATA version 14 (Stata Corporation; College Station, TX, USA).

Statistical analysis

Water quality data were entered into an Excel 2010 spread sheet (Microsoft; Redmond, WA, USA). A new variable for socioeconomic status was created using factor analysis of 13 asset indicator variables and retaining the first factor. The households were then classified into one of three categories: high, middle, and poor socioeconomic status, using the k-means procedure. New variables for sanitation and hygiene in school were also created using factor analysis of respective indicator variables, and we retained the first factor of each analysis. Both factor scores were classified into two categories-adequate and inadequate, using the k-means procedure. Similarly, a new variable for hygiene for the schoolchildren and their caregivers was created using factor analysis separately with two conceptually similar binary variables of mode of hand-washing (with water only, ash, mud/soil, water and soap, no hand washing); and its occasions (for schoolchildren: before eating, after eating, after playing, after toilet; and for their caregivers: before preparing food, before eating, after eating, after defecation, after child's defecation, before breastfeeding, after breastfeeding, no hand-washing). The score of the first factor was then classified into three categories: high, middle, and low using the k-means procedure. Mixed logistic regression adjusted for the clustering of data within schools was applied to investigate the association between the dependent variable; namely, TTC and 14 independent variables (e.g., household drinking water and water treatment) based on a literature review. The outcome variable (i.e., TTC) was treated as binary outcome representing absence (TTC =0) or presence (TTC =1) of TTC in the water sample, the latter category also applying if TTC were too numerous to be counted (i.e.,>100). ORs were calculated, including 95% confidence intervals (CI) and Wald test *p*-values were obtained. Explanatory variables in the final mixed logistic regression model included household drinking water source (i.e., private tap, shared tap, public tap, and other sources), container for fetching water (i.e., clay pot, plastic container, and metal container), and livestock kept inside the house. Adjustments were made for potential confounder variables, such as regional differences, educational attainment of the caregivers, and socioeconomic status. All variables were assessed one-by-one and retained for the maximal model if their *p*-value was <0.2. Backward stepwise elimination was used in the multivariable logistic regression with school as a random effect and removing non-predicting covariates up to a significance level of 0.2. Associations were considered statistically significant if *p*-values were <0.05. The results of physicochemical and microbiological analyses were compared with the NDWQG by the Government of Nepal (GON, 2005).

Ethics statement

The study was approved by the research commission of Swiss TPH (FK no. 116; approval date: 30 October 2014). Ethical approval was obtained from the "Ethikkommission Nordwest- und Zentralschweiz" (EKNZ) in Switzerland (reference number UBE-15/02; approval date: 12 January 2015), the institutional review board of Kathmandu University, School of Medical Sciences, Dhulikhel Hospital, Nepal (reference no. 86/14; approval date: 24 August 2014), and the institutional review board, Nepal Health Research Council (reference no 565; approval date: 11 November 2014). The study is registered at International Standard Randomised Controlled Trial Number register (identifier: ISRCTN30840; date assigned: 17 July 2015). The schools and households with TTC were provided with chlorine solution and health promotion programmes. Community stakeholders were informed about the status of water sources in their communities.

5.4. Results

Study compliance and population characteristics

A total of 708 children were included in the study. However, due to an earthquake that hit in the midst of the study period and damaged most of the houses, 146 caregivers were not accessible. Complete data were available from 708 schoolchildren and 562 households, and were used for final analysis.

The socio-demographic characteristics of the schoolchildren and their caregivers are summarised in Table 5.1. There were similar numbers of boys and girls participating in the 16 schools. The median age of the children was 13 years with an interquartile range of 2 years. Over one-third of caregivers did not have any formal education. Three-quarters of the houses of schoolchildren were made up of iron sheets for walls and roofs and mud for floors. Domestic animals were kept by over 90% of the households, while over one-third of caregivers reported to have animals freely roaming inside their houses.

Table 5.1: Characteristics of study population in Dolakha and Ramechhap districts, Nepal

Characteristics		Overall *N* (%)	Dolakha *n* (%)	Ramechhap *n* (%)
Sex of the schoolchildren (*n* = 708)				
Girls		339 (47.9)	261 (47.0)	78 (51.0)
Boys		369 (52.1)	294 (53.0)	75 (49.0)
Age of children (*n* = 708)				
Age group 1 (8–12 years)		108 (15.2)	86 (15.5)	22 (14.4)
Age group 2 (13–16 years)		600 (84.8)	469 (84.5)	131 (85.6)
Education level of children (*n* = 708)				
Grade 6		333 (47.0)	258 (46.5)	75 (49.0)
Grade 7		375 (53.0)	297 (53.5)	78 (51.0)
Educational attainment of caregivers (*n* = 562)				
No formal schooling		210 (37.4)	174 (39.2)	36 (30.5)
Primary education		144 (25.6)	130 (29.3)	14 (11.9)
Secondary education		143 (25.4)	82 (18.5)	61 (51.7)
Higher education		65 (11.6)	58 (13.0)	7 (5.9)
Age of caregivers (*n* = 562)				
18–24 years		2 (0.4)	1 (0.2)	1 (0.9)
24–40 years		239 (42.5)	184 (41.4)	55 (46.6)
>40 years		321 (57.1)	259 (58.3)	62 (52.5)
Ethnicity of caregivers (*n* = 562)				
Brahmin		101 (17.9)	97 (21.9)	4 (3.4)
Chhetri		210 (37.4)	154 (34.7)	56 (47.5)
Newar		33 (5.9)	22 (4.9)	11 (9.3)
Tamang		213 (37.9)	166 (37.4)	47 (39.8)
Janajati		5 (0.9)	5 (1.1)	0 (0.0)
Socioeconomic status of caregivers (*n* = 562)				
Roof material	Corrugated iron roof	415 (73.8)	325 (73.2)	90 (76.3)
	Wood and tiles	147 (26.2)	119 (26.8)	28 (23.7)
Wall material	Wood	66 (11.7)	61 (13.7)	5 (4.2)
	Corrugated iron	407 (72.4)	331 (74.6)	76 (64.4)
	Bricks	89 (15.9)	52 (11.7)	37 (31.4)
Floor material	Mud	524 (93.2)	430 (96.9)	94 (79.7)
	Cement	38 (6.8)	14 (3.1)	24 (20.3)
Socioeconomic status * (*n* = 562)	High	49 (8.7)	39 (8.8)	10 (8.5)
	Middle	215 (38.3)	163 (36.7)	52 (44.1)
	Poor	298 (53.0)	242 (54.5)	56 (47.5)
Owning agricultural land (*n* = 562)		511 (90.9)	412 (92.8)	99 (83.9)

Possession of domestic animals (*n* = 562)	507 (90.2)	401 (90.3)	106 (89.8)

*The mean (± SD) age of schoolchildren was 12.8 (± 1.2) years. The median age of caregivers was 39.5 with an interquartile range of 11 years. * A new variable for socioeconomic status was created using factor analysis of 13 binary variables indicating the possession of household assets such as a radio, television, mobile phone, table, stove, petroleum lamp, gas lamp, etc. The score of the first factor was then classified into three wealth categories (high, middle, or poor) using the k-means procedure.*

School and community WASH characteristics

Table 5.2 summarises school condition and WASH characteristics at the unit of the school. About one-third of schools were constructed more than 20 years ago with three-quarters of the schools having at least 500 pupils. About three-quarters of the schools were built with iron sheets (walls and roofs) and mud (floor). Fourteen out of 16 schools had some type of water infrastructure (standpipe and piped water into the dwelling); however, several were broken at the time of the survey. None of the schools had round-the-clock availability of water. Only around one-third of the schools reported having drinking water available throughout the year. None of the school principals reported that water at the school was treated prior to consumption. Drinking water quality testing by a health inspection team within two months prior to the survey occurred in only one of the 16 schools surveyed.

Table 5.2: School characteristics and WASH (water quality, sanitation, and hygiene) conditions in Dolakha and Ramechhap districts, Nepal, March–May 2015

Variables		Overall *N* (%)	Dolakha *n* (%)	Ramechhap *n* (%)
School level				
Secondary		10 (62.5)	9 (69.2)	1 (33.3)
Above secondary		6 (37.5)	4 (30.8)	2 (66.7)
School building age				
0–10 years		7 (43.7)	6 (46.2)	1 (33.3)
11–20 years		4 (25.0)	3 (23.1)	1 (33.3)
>20 years		5 (31.2)	4 (30.8)	1 (33.4)
School size				
<500 students		4 (25.0)	3 (23.1)	1 (33.3)
>500 students		12 (75.0)	10 (76.9)	2 (66.7)
School conditions				
Electricity at the school		11 (68.7)	9 (69.2)	2 (18.2)
Roof material	Iron sheet	15 (93.7)	13 (100.0)	2 (66.7)
	Bamboo	1 (6.3)	0 (0.0)	1 (33.3)
Floor material	Cement	2 (12.5)	0 (0.0)	1 (33.3)
	Soil	14 (87.5)	12 (92.3)	2 (66.7)
Wall material	Brick	2 (12.5)	1 (7.7)	1 (33.3)
	Bamboo	2 (12.5)	0 (0.0)	2 (66.7)
	Iron sheet	12 (75.0)	12 (92.3)	0 (0.0)
Water source *				
Surface water		2 (12.5)	0 (0.0)	2 (66.7)
Standpipe		6 (37.5)	5 (38.5)	1 (33.3)
Piped water		8 (50.0)	8 (61.5)	0 (0.0)
Drinking water *				
Drinking water available throughout year		7 (43.8)	7 (100.0)	0 (0.0)
Treatment of drinking water by school		0 (0.0)	0 (0.0)	0 (0.0)
Water in school #	Inadequate	14 (87.5)	13 (100.0)	1 (33.3)
	Adequate	2 (12.5)	0 (0.0)	2 (66.7)
Hand washing facility in any area of school		0 (0.0)	0 (0.0)	0 (0.0)
Latrine				
Flush toilet		3 (18.8)	2 (15.4)	1 (33.3)
Pit latrine with cement floor and composting latrine		4 (25.0)	4 (30.8)	0 (0.0)
Pit latrine without cement floors, hanging latrine		9 (56.2)	7 (53.9)	2 (66.8)

Latrine condition				
Presence of door		16 (100.0)	13 (100.0)	3 (100.0)
Sharing with opposite gender		14 (87.5)	12 (92.3)	2 (66.7)
Damaged floor (i)		9 (56.3)	8 (61.5)	1 (33.3)
No privacy (ii)		8 (50.0)	7 (53.9)	1 (33.3)
Clean floor (iii)		5 (31.3)	4 (30.8)	1 (33.3)
Clean wall (iv)		7 (43.8)	6 (46.2)	1 (33.3)
Flies present		8 (50.0)	5 (38.5)	3 (100.0)
Odour present		15 (93.8)	12 (92.3)	3 (100.0)
Regular water for anal cleansing		2 (12.5)	2 (15.4)	0 (0.0)
Washbasin for handwashing		0 (16.0)	0 (0.0)	0 (0.0)
Water for hand washing		6 (37.5)	4 (30.8)	2 (66.4)
Soap for hand washing		0 (0.0)	0 (0.0)	0 (0.0)
Sanitation in school (v)	Inadequate	8 (50.0)	7 (53.9)	1 (33.3)
	Adequate	8 (50.0)	6 (46.1)	2 (66.7)
Hygiene in school (vi)	Inadequate	8 (50.0)	6 (46.2)	2 (66.7)
	Adequate	8 (50.0)	7 (53.9)	1 (33.3)
Safe solid waste disposal (vii)		0 (0.0)	0 (0.0)	0 (0.0)

** Multiple responses were possible for the variables. # A new variable for water adequacy/inadequacy was created using factor analysis with conceptually similar categorical variables of: types of water source in school (surface water, borehole/tube well/protected dug well, standpipe, rainwater, protected spring, unprotected dug well, piped water); number of these sources available (1–2, >2), drinking water availability during the day of survey (yes/no). Water quality was not considered for calculating water adequacy. (i) Floor is cracked, broken into separate pieces, fallen into the pit; (ii) walls with holes or no walls; (iii) presence of faeces, urine, dirt, (iv) presence of faeces on the wall; (v) a new variable for sanitation was created using factor analysis of variables characterising types of latrine (flush/pit latrine with cement floor and composting latrine and pit latrine without cement floors, hanging latrines, door, sharing by both sexes, damaged latrine floor, privacy, clean floor, clean wall, roof, flies, and odour); (vi) a new variable for hygiene was created using factor analysis of conceptually similar binary variables of: hygiene such as broom, regular water for anal cleansing, sanitary bins, water and soap, and washbasins for hand washing, solid waste disposal; and (vii) schools having reported to dispose their solid waste were expected through burial in safe place, collection at a safe place, or disposal. The score of the first factor was then classified into two categories adequate and inadequate, using the k-means procedure.*

Table 5.3 summarises the physicochemical and microbiological parameters of the water samples taken from schools and communities, including turbidity, pH, chlorine, and TTC. The average temperature of water samples obtained in schools of Dolakha and Ramechhap districts was 13.2 °C and 16.0 °C, respectively. The median turbidity was 2.5 NTU (range: 0–5 NTU). The median turbidity of school point-of-use water samples was 10.1 NTU (range: 5–20 NTU). The average turbidity of school point-of-use water samples was 6.15 NTU. In the communities, four out of 43 water sources had a turbidity >5 NTU, while turbidity of the remaining 39 sources were between 2 NTU and 5 NTU. The pH level of functioning school water points had an average of 6.9 (range: 6.8–7.6). The average pH of point-of-use water samples was 7.0 with a range between 6.8 and 7.4. Similarly, for the community water points, the average pH was 6.8 with a range between 6.8 and 7.2.

The median of free and total chlorine of the main drinking water points and the point-of-use water samples from schools were 0.1 mg/L and 0.5 mg/L, respectively. The free residual chlorine of the water sources in the communities were within the acceptable national limits (0.1–0.2 mg/L). All the water samples from the communities had total residual chlorine

below 1 mg/L. The lead and arsenic levels in school drinking water sources were, on average, 0.01 mg/L and 0.05 mg/L, respectively, hence below the national standards.
Of the 16 school drinking water sources, 6.2% had TTC levels >100 colony forming unit (CFU) per 100 mL. The median value of TTC was 6 CFU/100 mL. Of the 13 water samples obtained from school points-of-use, 30.8% had >100 CFU/100 mL. Of the 43 community water source samples, 18.6% ranged between 11 and 100 CFU/100 mL and 13.9% had values of 100 CFU/100 mL and above.

In the study area, more than half of the schools had a pit latrine without cement floor. Although water seal latrines on the premises were seen, 41.6% of the latrines were not used as they were full. None of the schools had toilet paper and only 12.5% of the schools had regular water supply for anal cleansing. In all schools where latrines were present, the use was separated by gender. In schools with at least one toilet, the median student-to-toilet ratio was 65 per toilet. Approximately 4% of children reported that they did not use the latrine at school. Only 6.3% of schools reported that they cleaned the latrine each week; the rest reported less frequent cleaning. None of the schools had a dedicated budget for purchasing cleaning supplies for the latrines or soap for handwashing. The survey also investigated the implementation of any hygiene training programme at school during the past two months. More than half of the schools (62.5%) had never implemented a hygiene programme, including a hygiene component and school action plan. Furthermore, handwashing stations and soap were not available at any of the surveyed schools.

Table 5.3: Physicochemical and microbiological parameters of water samples in school and community in Dolakha and Ramechhap districts, Nepal (sampling period: May and June 2015)

Category	Parameter	Unit	Range	School						Community		
				Overall		Dolakha		Ramechhap		Overall	Dolakha	Ramechhap
				Main Source * (*N* = 16)	Point-of-Use ** (*N* = 13)	Main Source * (*n* = 13)	Point-of-Use (*n* = 10)	Main Source * (*n* = 3)	Point-of-Use (*n* = 3)	(*n*= 43)	(*n* = 33)	(*n* = 10)
				n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Physical characteristics	Turbidity	NTU	>5	0 (0.0)	1 (7.7)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	4 (9.3)	3 (9.1)	1 (10.0)
			2–5	16 (100.0)	12 (92.3)	13 (100.0)	9 (90.0)	3 (100.0)	3 (100.0)	39 (90.7)	30 (90.9)	9 (90.9)
	pH		6.5–8.5	0 (0.0)	13 (100.0)	13 (100.0)	10 (100.0)	3 (100.0)	3 (100.0)	43 (100.0)	33 (100.0)	10 (100.0)
Chemical characteristics	Free residual chlorine ***		0.1–0.2	16 (100.0)	13 (100.0)	13 (100.0)	10 (100.0)	3 (100.0)	3 (100.0)	43 (100.0)	33 (100.0)	10 (100.0)
	Total residual chlorine	mg/L	0.2–0.5	16 (100.0)	13 (100.0)	13 (100.0)	10 (100.0)	3 (100.0)	3 (100.0)	41 (95.3)	31 (93.9)	10 (100.0)
			0–0.199	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.7)	2 (15.1)	0 (0.0)
	Lead	mg/L	<0.01	16 (100.0)	0 (0.0)	13 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)			
	Arsenic	mg/L	<0.05	16 (100.0)	0 (0.0)	13 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)			
Microbiological characteristics	Thermo-tolerant coliforms (TTC) ***	CFU/100 mL	<1	4 (25.0)	3 (23.1)	3 (23.1)	3 (30.0)	1 (33.3)	0 (0.0)	26 (60.5)	21 (63.6)	5 (50.0)
			1–10	5 (31.3)	4 (30.8)	5 (38.5)	2 (20.0)	0 (0.0)	2 (66.7)	3 (7.0)	2 (6.0)	1 (10.0)
			11–100	6 (37.5)	2 (15.4)	4 (30.8)	2 (20.0)	2 (66.7)	0 (0.0)	8 (18.6)	6 (18.2)	2 (20.0)
			>100	1 (6.2)	4 (30.7)	1 (7.6)	3 (30.0)	0 (0.0)	1 (33.3)	6 (13.9)	4 (12.1)	2 (20.0)

** Main sources are the main drinking water sources, such as stand pipes, piped water, and spring water, which are available at the school. ** Point-of-use is the drinking water cups used for drinking water by the surveyed school-aged children; *** The presence of TTC despite the residual chlorine at acceptable range depends on the "contact time" and the bacterial type. CFU, Colony forming unit; NTU, Nephelometric turbidity unit.*

KAP of schoolchildren on WASH at schools and households

The findings from the KAP survey pertaining to WASH among schoolchildren are shown in Table 5.4. More than half of the schoolchildren reported washing their hands with soap and water before eating and after defecation. However, 11.7% reported that they did not wash their hands at any of these occasions. Over 90% of schoolchildren reported that they regularly drank water at the school. Around 90% of schoolchildren reported that they had heard about dirty water causing illness, however, they were not aware about specific types of water-borne diseases and modes of transmission.

Table 5.4: Questionnaire findings on KAP (i) on WASH among schoolchildren and caregivers in Dolakha and Ramechhap districts, Nepal, March–May, 2015

Variables (*n* = 708)	Overall *N* (%)	Dolakha *n* (%)	Ramechhap *n* (%)
KAP indicators: schoolchildren			
Hand washing			
Before eating	525 (74.2)	427 (76.9)	98 (64.1)
After eating	434 (61.3)	357 (64.3)	77 (50.3)
After playing	422 (59.6)	345 (62.2)	77 (50.3)
After defecation	534 (75.4)	427 (76.9)	107 (69.9)
Do not wash hands	66 (11.7)	45 (10.1)	21 (17.8)
With water only	687 (97.0)	540 (97.3)	147 (96.1)
With ash	17 (2.4)	12 (2.2)	5 (3.3)
With mud/soil	4 (0.6)	4 (0.7)	0 (0.0)
With water and soap	689 (97.3)	539 (97.1)	150 (98.0)
Hygiene (ii)			
Higher category	261 (36.9)	225 (40.5)	36 (23.5)
Middle category	211 (29.8)	165 (29.7)	46 (30.1)
Lower category	236 (33.3)	165 (29.7)	71 (46.4)
Sanitary practices at school			
Using latrine at school	679 (95.9)	543 (97.8)	136 (88.9)
No latrine use	29 (4.1)	12 (2.2)	17 (11.1)
Drinking water of children at school*			
Drinking water from school	637 (90.0)	535 (96.4)	102 (66.7)
Bringing water from home	102 (14.4)	67 (12.1)	35 (22.9)
Households (*n* = 562)			
Use of toilet at home			
Latrine in the household	394 (70.1)	320 (72.1)	74 (62.7)
Shared latrine	68 (12.1)	57 (12.8)	11 (9.3)
Bush	73 (13.0)	57 (12.8)	16 (13.5)
River, swamp, lake	27 (4.8)	10 (2.2)	17 (14.4)
Type of latrine at home			
Water seal latrine	283 (50.4)	233 (52.5)	50 (42.4)
Open pit latrine with slab	97 (17.3)	77 (17.3)	20 (16.9)
Open pit latrine without slab	14 (2.5)	12 (2.7)	2 (1.7)
Soap in household for hand-washing	417 (74.2)	319 (71.9)	98 (83.0)
Hygiene of caregivers (iii) (*n* = 252)			
Lower category	72 (28.7)	60 (27.0)	12 (41.4)
Middle category	26 (10.4)	23 (10.4)	3 (10.3)
Better category	153 (60.9)	139 (62.6)	14 (48.3)
Drinking water at home*			
Drinking water source during dry season			
Private tap	287 (51.1)	257 (57.9)	30 (25.4)
Spring	13 (2.3)	3 (0.7)	10 (8.5)

Public tap	36 (6.4)	36 (8.1)	0 (0.0)
Other [(iv)]	226 (40.2)	148 (33.3)	78 (66.1)
Drinking water source during rainy season			
Private tap	285 (50.7)	258 (58.1)	27 (22.9)
Spring	1 (0.18)	1 (0.2)	0 (0.0)
Public tap	44 (7.8)	40 (9.0)	4 (3.4)
Other [(v)]	232 (41.3)	145 (32.7)	87 (73.7)
Container to fetch water at the principle source			
Clay	40 (7.1)	16 (3.6)	24 (20.3)
Plastic	258 (45.9)	205 (46.2)	53 (44.9)
Metal	264 (47.0)	223 (50.2)	41 (34.8)
Frequency of washing drinking water storage container with soap			
Never	40 (7.1)	20 (4.5)	20 (17.0)
Daily	347 (61.8)	277 (62.4)	70 (59.3)
Weekly	175 (31.1)	147 (33.1)	28 (23.7)
Status of drinking water container			
Covered	417 (74.2)	322 (72.5)	95 (80.5)
Uncovered	145 (25.8)	122 (27.5)	23 (19.5)
Drinking water container used for other activities	112 (19.9)	89 (20.5)	23 (19.5)
Regular water treatment	76 (13.5)	50 (11.3)	26 (22.0)
Aware of boiling	203 (36.1)	181 (40.8)	22 (18.6)
Aware of chlorination	32 (5.7)	28 (6.3)	4 (3.4)
Aware of filtration	70 (12.5)	28 (6.3)	42 (35.6)
Water sufficiency	439 (78.1)	333 (75.0)	106 (89.8)
Safe solid waste disposal*	273 (48.6)	237 (53.4)	36 (30.5)

** Multiple answers were possible for several questions. [(i)] Knowledge, attitude, and practices; [(ii)] and [(iii)] a new variable for hygiene for the schoolchildren and their caregivers was created using factor analysis separately with two conceptually similar categorical variables of: mode of hand-washing (with water only, ash, mud/soil, water and soap, no hand washing); and its occasions (for schoolchildren: before eating, after eating, after playing, after toilet, and for their caregivers: before preparing food, before eating, after eating, after defecation, after child's defecation, before breastfeeding, after breastfeeding, no hand-washing; the score of the first factor was then classified into three categories - high, middle, and low using the k-means procedure; [(iv)] and [(v)] others included hand-pump, river, swamp, and ponds.*

WASH characteristics of households

WASH information for schoolchildren's households is also summarised in Table 7.4. Water sources were categorised as improved (i.e., private tap, shared tap, public tap, hand pump, protected deep well, bore hole, and protected springs) and unimproved (i.e., non-improved source, including surface water such as river, lake swamp, and ponds). While 44.7% of households did not have a piped water distribution network, 78.1% reported having insufficient drinking water throughout the year. A total of 22.6% households did not cover their water container and 86.4% of households did not treat drinking water. Among those households that treated water, boiling was the most commonly known means of purification (36.1%).

Table 5.5 summarises the physicochemical and microbiological parameters of the water samples taken from the households. The average turbidity recorded in the household point-of-use water samples was 6.4 NTU (range: 5–14 NTU); average pH was 6.9 (range: 6.5–7.6); the average free chlorine was 0.14 mg/L (range: 0.1–0.3 mg/L), and the average total chlorine was

0.5 mg/L (range: 0.1–1.0 mg/L). Drinking water was contaminated with TTC in 27.4% of the household point-of-use water samples. Out of 562 water samples examined, 12.5% had >100 CFU/100 mL.

Almost half (45.7%) of the caregivers reported that children had suffered from water-borne diseases within the two weeks preceding the survey. A total of 38.2% of caregivers complained of dysentery, 30.9% of fever, 22.4% of watery diarrhoea, while 8.5% reported other conditions of ill-health.

Table 5.5: Physicochemical and bacteriological parameters of point-of-use water samples in households in Dolakha and Ramechhap districts, Nepal (sampling period: June 2015)

Category	Parameter	Unit	Range	Overall *N* (%)	Dolakha *n* (%)	Ramechhap *n* (%)
Physical characteristics	Turbidity	NTU *	>5	131 (23.3)	115 (25.9)	16 (13.6)
			2–5	431 (76.7)	329 (74.1)	102 (86.4)
	pH		6.5–8.5	562 (100.0)	444 (100.0)	118 (100.0)
Chemical characteristics	Free residual chlorine	mg/L	0.3–0.5	121 (21.5)	105 (23.6)	16 (13.6)
			0.1–0.2	441 (78.5)	339 (76.4)	102 (86.4)
	Total residual chlorine	mg/L	>0.5	2 (0.4)	1 (0.2)	1 (0.8)
			0.2–0.5	548 (97.5)	439 (98.9)	109 (92.4)
			0–0.199	12 (2.1)	4 (0.9)	8 (6.8)
Microbiological characteristics	Thermo-tolerant coliforms (TTC)	CFU/100 mL **	<1	408 (72.6)	333 (75.0)	75 (63.6)
			1–10	42 (7.5)	24 (5.4)	18 (15.3)
			11–100	42 (7.5)	36 (81.1)	6 (5.1)
			>100	70 (12.5)	51 (11.5)	19 (16.1)

** Nephelometric turbidity unit; ** Colony forming unit.*

Association of TTC with household WASH predictors

Table 5.6 shows the association of water contaminated with TTC with household WASH predictors. Significant differences in TTC were observed between the two districts, with Ramechhap having higher odds of TTC compared to Dolakha district (adjusted odds ratio (aOR) 2.25, 95% CI: 1.16–4.34). We found a significant association between domestic animals freely roaming in households and contamination of water with TTC compared to household without freely roaming domestic animals (aOR 1.64, 95% CI: 1.08–2.50). Households using a protected spring water source for drinking were more likely to experience TTC contamination, but the association lacked statistical significance (aOR 2.48, 95% CI: 0.64–9.66).

Table 5.6: Results from univariate and multivariate logistic regression analysis for thermo-tolerant coliforms (TTC) from water samples from households of Dolakha and Ramechhap districts, Nepal (sampling period: March–May 2015)

Risk factor	*N* (%)	Univariate Analysis			Multivariate Analysis		
		OR	95% CI	*p*-Value	aOR	95% CI	*p*-Value
District							
Dolakha	444 (79.0)	1.00			1.00		
Ramechhap	118 (21.0)	1.79	1.02–3.13	0.04	2.25	1.16–4.34	0.02
Education of the respondent							
No formal education	210 (37.4)	1.00					

Primary education	144 (25.6)	1.21	0.74–1.99	0.44	1.26	0.76–2.07	0.37
Secondary education	143 (25.4)	0.75	0.43–1.32	0.34	0.73	0.41–1.30	0.29
Superior	65 (11.6)	0.76	0.36–1.61	0.47	0.92	0.43–1.95	0.82
Socioeconomic status							
Low	298 (53.0)	1.00					
Medium	215 (38.3)	1.10	0.73–1.64	0.65	1.07	0.71–1.61	0.75
High	49 (8.7)	1.06	0.53–2.11	0.87	1.02	0.50–2.06	0.97
Household drinking water during the dry season							
Private tap	287 (51.1)	1.00			1.00		
Spring	13 (2.3)	3.98	1.14–13.97	0.03	2.48	0.64–9.66	0.19
Public tap	36 (6.4)	1.67	0.70–3.95	0.24	1.68	0.71–3.96	0.23
Other	226 (40.2)	0.93	0.59–1.44	0.73	0.87	0.55–1.37	0.55
Container for fetching water							
Metal container	264 (47.0)	1.00					
Plastic container	258 (45.9)	1.11	0.73–1.69	0.62	0.96	0.60–1.52	0.85
Clay pot	40 (7.1)	1.86	0.86–4.02	0.12	0.82	0.34–1.99	0.67
Status of container during storage							
Covered	417 (74.2)	1.00					
Uncovered	127 (22.6)	0.97	0.60–1.57	0.89			
Not seen	18 (3.2)	0.90	0.30–2.70	0.86			
Container washing frequency							
Daily	347 (61.7)	1.00					
Never	40 (7.1)	0.82	0.35–1.93	0.65			
Weekly	175 (31.1)	0.95	0.60–1.51	0.82			
Drinking water container used for other activities							
Yes	451 (80.3)	1.00					
No	111 (19.7)	0.76	0.46–1.27	0.30			
Water treatment *							
No	486 (86.5)	1.00					
Yes	76 (13.5)	0.74	0.42–1.31	0.30			
Latrine in household							
Yes	395 (70.3)	1.00					
No	167 (29.7)	1.20	0.76–1.87	0.43			
Latrine type							
Water seal latrine	283 (50.4)	1.00					
No latrine	168 (29.9)	1.30	0.81–2.11	0.28			
Open pit latrine with slab	97 (17.3)	1.21	0.69–2.11	0.51			
Flushed toilet	14 (2.5)	0.91	0.24–3.49	0.89			
Solid waste disposal							
Yes	273 (48.6)	1.00					
No	289 (51.4)	0.99	0.67–1.48	0.99			
Possession of livestock							
Yes	507 (90.2)	1.00					
No	55 (9.8)	0.92	0.47–1.80	0.80			
Livestock kept inside household							
No	307 (54.6)	1.00					
Yes	255 (45.4)	0.63	0.41–0.95	0.03	1.64	1.08–2.50	0.02

** Households reported to treat their drinking water through boiling, chlorination, and filtration. The multivariate global model included a random intercept at the level of school where all the variables were assessed one by one and retained for the global model if their p-value was <0.2. The final model was obtained by using backward selection with the same level of <0.2.*

5.5. Discussion

Our study revealed several WASH challenges at the unit of the school, household, and community in the districts of Dolakha and Ramechhap in Nepal. Indeed, our data provide evidence of inadequate drinking water availability at the main water sources in the schools surveyed. Moreover, water samples subjected to chemical and microbial tests revealed considerable faecal contamination. The access to “safe” water coverage from improved water sources in 12 schools was, in fact, not safe for consumption. Contamination of water samples with >100 TTC CFU/100 mL was detected in about one-third of the water samples obtained from schools. Furthermore, due to inadequate availability of drinking water at 14 schools, children obtain drinking water from other locations where safe drinking water consumption is not guaranteed.

Linking observational WASH assessment, out of all the surveyed schools, more than a quarter of schools had no sanitation infrastructures with a regular water supply available for anal cleansing. The conditions of latrines were poor and lacked essential hygiene materials (e.g., soap). Moreover, none of the schools had separate handwashing stations in close proximity to the sanitation infrastructure for handwashing. Additionally, none of the schools had any allocated budget for purchasing toilet-cleansing supplies. Another challenge identified by our study is insufficient coverage of improved/sanitary latrines and handwashing stations within the schools. The majority of schools did not meet the national student-to-toilet standard set by the Government of Nepal where one latrine per 50 students and at least one set of handwashing stations for a set of latrines (one for boys, one for girls) were recommended (GON and Water Aid, 2014). To improve this ratio, the school committee or parents' associations might focus their efforts also on building an adequate number of toilets for girls and boys. In addition to latrines, building more urinals for boys (which have considerably lower costs than latrines) could also be beneficial for schoolchildren. We found that the surveyed schools had usually one or two water taps available at a school for handwashing, and these were located at central places, far away from latrines. The findings of this study regarding WASH in schools are consistent with evidence on WASH in schools in Nicaragua where schools were without adequate sanitation infrastructures and handwashing facilities, highlighting several WASH challenges (Jordanova et al., 2015). Similar observations have been made in South Africa where the majority of the schools had access to unhygienic pit latrine and had one water tap, which was mostly located at a central point on the school premises (Sibiya and Gumbo, 2013).

In terms of WASH at the unit of household, more than half of the households had access to an improved water source and sanitary infrastructure. However, water quality was typically not suitable for drinking in 112 households (20.0%). The water qualities from stored household samples were found to be worse than the water samples from the community source. This might be due to further contamination during transportation, storage, and point-of-use at households. This finding was consistent with the evidence from meta-analysis that reported the association of supply type with faecal contamination of source of water and household stored drinking water in LMICs (Ziegelbauer et al., 2012c; Shields et al., 2015).

In the case of water quality of the samples obtained from the community, more than 30% were contaminated by TTC with maximum coliform count of >100 CFU/100 mL, with drinking water quality standards exceeded in 14 (32.5%) water sources. This finding is consistent with a prior study conducted in the communities of Kathmandu valley and Myagdi district of Nepal, where a maximum TTC of 267 CFU/100 mL had been reported (Pant, 2011) and where 27.3% water sources were contaminated with TTC, respectively (Aryal et al., 2012).

Our survey included the examination of the physicochemical quality of water samples. Importantly, most of the physicochemical parameters were within national thresholds, except for turbidity. Some of the water samples showed high turbidity (>10 NTU), which might be due to the discharge of domestic effluents and runoff from agricultural activities. In turn, this might call for adequate and proper treatment of water before consumption (Shields et al., 2015). The pH was within the national standard (6.5–8.5). The schools, households, and communities mostly had a natural water source, and hence, pH levels were expected to be in this range. Similar observations have been reported from studies conducted in Myagdi district and Dharan, where pH levels of 7.6 were reported (Aryal et al., 2012; Pant et al., 2016).

When drinking water leaves a water point (e.g., tap), a residual free chlorine of about 1 mg/L is recommended, and similar levels are recommended for points-of-use during consumption (Tabor et al., 2011). In our study, none of the stored water samples from schools and households had detectable residual free chlorine of 1 mg/L, even though chlorine solution had been distributed free of charge by various relief organisations after the April 2015 earthquake. The possible explanation for the low levels of detectable residual free chlorine might be that the aftershocks due to the earthquake were still quite frequent during the survey period, and hence, the chlorine promotion programme might have received only little attention, or people may dislike the odour of chlorine or they might regard chlorination as being an extra form of work during an emergency period.

Regarding heavy metals, fortunately, our investigation revealed acceptable levels of arsenic contents in all 16 water samples from school drinking water sources, indicating no significant threat to people's health. This finding is in line with a study conducted in hilly parts of the Myagdi district, where values of arsenic are reported to be within the NDWQS (Aryal et al., 2012). Other studies conducted in Asia (e.g., Cambodia) showed higher levels of arsenic (0.13–0.2 µg/L) and lead (0.1–0.3 µg/L) in drinking water (Luu et al., 2009).

The high values of TTC are indicative of polluted drinking water sources or drinking water vessels, and of inadequate sanitary integrity of the water source and vessels (Shields et al., 2015). Such contamination may be due to construction defects, poor sanitation, poor hand hygiene, and open defecation by freely roaming animals and humans in close proximity to open water sources (Shields et al., 2015). In our study, the microbiological analyses of water samples revealed the presence of TTC in 193 water samples with 81(42.0%) of these samples having a TTC >100 CFU/100 mL, which calls for urgent treatment. Of note, despite households reporting that they obtain water from improved sources, faecal contamination was still observed in some of these. Yet, this water was being consumed by schoolchildren. Additionally, some improved water sources in the community were also not free from faecal contamination. This observation highlights that "improved" drinking water sources, considered safe by the global monitoring framework and burden of disease analyses, may entail health risks at some sources (Bartram et al., 2005; Shaheed et al., 2014; Prüss-Ustün et al., 2014a; SDG, 2015). Cross-contamination at leakage points in old pipes, back siphoning, and drainage systems had been reported by a study conducted in Myagdi district and mountainous parts of Nepal (Rai et al., 2009; Aryal et al., 2012). Our findings of water contamination with TTC might be also linked to garbage discarded in open spaces in close proximity to drinking water points, open defecation practices, or cross-contamination between water supply and sewage systems.

The practice of open defecation was still common in the study region. Indeed, 17% of the households surveyed reported open defecation. However, this percentage of households practicing open defecation is considerably lower than what has been reported by WHO/UNICEF JMP in Nepal, where 37% of the rural population reported to practice open defecation (WHO/UNICEF, 2015; McMichael and Robinson, 2016a). This difference might be explained by the fact that temporary latrines were constructed immediately after the April 2015 earthquake with Dolakha district being the epicentre. It should also be noted that some VDCs of the Dolakha district had declared the states of "open defecation-free".

Re-growth of TTC in drinking water sources occurs at temperature above 15 °C, in the presence of sufficient bacterial nutrients and the absence of free residual chlorine in the water. In our study area, some sites were located in settings where the average temperature is above 15 °C. Of note, we found that more than 85% of the households where TTC was present reported no treatment of drinking water, while only 13.5% reported treatment. Boiling was the most frequently known water treatment procedure; however, boiling alone might not confer full protection from TTC. The finding of boiling as the main known water treatment procedure is in line with a previous study conducted in rural Nepal, where 15% of households consistently boiled water before consumption (Kovalsky et al., 2008; Shrestha et al., 2013). Additionally, poor maintenance of sanitation facilities and inefficient disinfection are other likely reasons for the observed TTC contamination in our study.

In terms of KAP on WASH among schoolchildren, results indicated that 97% of the students reported washing their hands solely with water when soap was not available and 97.3%

reported using soap and water for handwashing if soap was available. Of note, the presence of soap and water is crucial for schoolchildren for handwashing in that it might form a sustained habit of proper handwashing. Similar findings of the importance of the availability of soap and water were reported in studies conducted elsewhere such as in Nepal, Bangladesh, Nicaragua, and Kenya (Cairncross and Shordt, 2004; Luby et al., 2011; Shrestha et al., 2013; Jordanova et al., 2015).

In terms of knowledge of water-borne diseases, children had a general awareness that dirty water can cause ill-health. Yet, the exact types of water-borne diseases and transmission pathways were poorly understood, thus confirming previous observations made in South Africa where the schoolchildren from rural schools were reported to have a disparity of knowledge on water-borne diseases (Sibiya and Gumbo, 2013). It follows that the provision of adequate resources and long-term behaviour change in children to form a sustained habit of hygienic behaviours such as washing hands with soap, including awareness regarding water-borne disease with its mode of transmission, should be initiated in the VgtS study site of Nepal. There was a lack of access to sufficient quantities of water and soap at the unit of both school and households that impedes personal hygiene (Blanton et al., 2010; Luby et al., 2011; Lim et al., 2012). WHO recommends minimum availability of 100 L of water per capita per day for all purposes (WHO, 2011; WHO/UNICEF, 2015).

We found a significant association between the presence of TTC contamination of drinking water and domestic animals freely roaming within the households compared to households where domestic animals were kept outside. This might be due to faecal contamination of water sources by domestic animals. Such faecal contamination of drinking water and possession of different types of livestock was also reported in studies conducted in Burkina Faso and Rwanda (Erismann et al., 2016a; Kirby et al., 2016). We observed inadequate washing of drinking water storage containers, containers having no lids, or lids not fitting properly, and drinking water cups left on dirty grounds, as well as kitchens in close proximity to animal sheds. Similar observations have been made in a study conducted in Botswana, where the drinking water containers were kept without lids (Tubatsi et al., 2015).

Our study has several limitations. First, there was a huge challenge posed by the April 2015 earthquake. Around 20% of households could not be visited due to frequent aftershocks and post-earthquake emergency crisis. A number of villages had been severely destroyed, and hence, it was not possible to obtain water samples from all the schoolchildren’s households. Second, water quality analysis was carried out during the spring season only, thus the observed results might not represent the drinking water quality over the whole year. Third, although having found standard residual free chlorine in some samples, TTC was high. This might be explained by the time required to destroy bacteria, which depends on the type of bacteria, but as we did not further isolate bacteria, we are unable to investigate this issue further. Fourth, the results from selected school, household, and community water sources in the two districts of Dolakha and Ramechhap may not be considered to be representative for other parts of Nepal. Fifth, the self-reporting of diarrhoeal episodes among children's caregivers may not be accurate. However, this is the standard procedure.

Despite these limitations, our study provides a baseline for the status of WASH indicators at selected schools, households, and communities in two districts of Nepal. We rigorously assessed water quality, including physicochemical, microbiological, and heavy metal contents, using Delagua kit and flame atomic absorption spectrophotometer. Information about self-reported morbidities helped for timely referral of children to health care delivery centres. Meanwhile, the analytical approach taken (i.e., multivariate analysis) allowed for adjustments of potential confounders, such as educational attainment of caregivers, socioeconomic status, and regional differences.

5.6. Conclusion

We found that about one-third of water samples obtained from selected schools and households in two districts of Nepal were unsafe for drinking. The microbiological characteristics were critical for some samples, which indicate a public health risk. Although the physicochemical parameters of the water samples collected were within permissible limits, disinfection with chlorine prior to supply, as recommended by the NDWQS, is required to maintain water quality at the source. Regarding point-of-use, contamination of drinking vessels by domestic animals freely roaming inside the houses is a concern. Households' drinking water was mostly from improved sources; however, regular monitoring of water quality in different seasons is recommended to generate evidence regarding water quality throughout the year.

Water source protection strategies (e.g., proper fencing of domestic animals, maintenance and proper disposal of human and animal faeces) should be promoted. When school budgets do not allow for WASH improvements at schools, parents and community organisations might provide resources to ensure healthy school environments. Regular inspections are required to identify causes of contamination and to determine the risk of future contamination. In turn, mitigation measures can be implemented, such as maintenance and operation of water supply systems by the school administration, household caregivers, and other community stakeholders. Additionally, engaging the communities to take responsibility for management of water sources may also be an appropriate strategy to improve the quality of community water sources. Promotion of hand washing with soap and safe disposal of faeces must be encouraged at the unit of the school. More emphasis should be placed on water treatment. Hygiene training programmes at schools should be incorporated into the school curriculum.

Our study gathered helpful baseline WASH information at school, household, and community levels. In order to create better conditions for the VgtS project, specifically nutritional and health-related objectives, the results will be useful to design and implement a complementary WASH intervention package for targeted schools. As the VgtS project in Nepal has involved the Ministry of Education as a key stakeholder, the model of interventions implemented in these pilot schools could be readily replicated nationwide. There is a need for ensuring safe WASH in schools, households, and the communities to improve children's health and wellbeing. The study has therefore a potential to impact on the public health in the surveyed districts and schools, and also beyond.

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Author contributions

All listed authors contributed to the study design. Akina Shrestha coordinated the field and laboratory work, collected data, supervised research assistants, performed the statistical analysis under the supervision of Christian Schindler, and drafted the manuscript. Subodh Sharma, Jana Gerold, Séverine Erismann, Sanjay Sagar, Rajendra Koju, Christian Schindler, Peter Odermatt, Jürg Utzinger, and Guéladio Cissé contributed to the interpretation of the data, manuscript writing, and revisions. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

6. Prevalence of anaemia and risk factors in schoolchildren in Dolakha and Ramechhap districts, Nepal

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6.1. Abstract

Background: Anaemia is a public health problem in many low- and middle-income countries, yet its importance among school-aged children is poorly understood. This study investigated the prevalence of childhood anaemia and its risk factors in remote setting in Nepal.

Methods: We assessed the prevalence of anaemia and its risk factors in school-aged children in two districts of Nepal; Dolakha and Ramechhap. We conducted a cross-sectional survey among 708 children aged 8-16 years from 16 purposively selected schools. Socio-demographic and dietary data were collected using a pretested questionnaire. Capillary blood was taken from the fingertip of each child and hemoglobin was measured using a HemoCue digital photometer. Stool samples were subjected to a suite of standardized, quality-controlled methods. The association between predictors and health outcomes were measured by mixed logistic regression analysis.

Results: The overall prevalence of anaemia and soil-transmitted helminth infection was 23.9% and 35.3%, respectively. Lack of meals prepared in the household [odds ratio (OR) = 2.36, 95% confidence interval (CI): 1.14-4.92; *P*=0.01] and not having had supper on the day before the interview (OR=3.46, 95% CI: 1.09-11.0; *P*=0.04) were significantly associated with anaemia. Consumption of vitamin A-rich fruits and vegetables were negatively associated with anaemia, but not significantly so. More than half (55.2%) of the children had at least one sign of nutritional deficiency (e.g., loss of hair pigment).

Conclusions: Our findings call for interventions to protect school-aged children from anaemia, such as dietary improvements, health promotion programs, early detection, and prompt treatment of intestinal parasites.

Key words: Anaemia, Cross-sectional survey, malnutrition, nutritional deficiency, school-aged children

Word count abstract: 247

6.2. Background

Anaemia is defined as a low concentration of haemoglobin (Hb) in the blood. It is a common public health problem in many parts of the world [1, 2]. Anaemia is particularly prevalent in low- and middle-income countries (LMICs) and mainly affects children below the age of 5 years and women of reproductive age [3]. The prevalence of anaemia ranges from 9% in high-income countries to as much as 43% in LMICs [2, 4]. It is estimated that anaemia affects 305 million (25.4%) school-aged children worldwide [4]. The prevalence of anaemia among school-aged children in LMICs is 40%, and is classified as a major public health issue [5]. Because of its impact on cognitive development and physical growth, studies on the extent of anaemia among school-aged children are of considerable public health relevance. Of note, anaemia is pathophysiologically diverse and multifactorial; causes include congenital haemolytic diseases, genetic factors, heavy blood loss, parasitic infections (e.g., malaria and hookworm infections), poor nutritional practices, deficiency of one of more essential nutrients, low bioavailability of the diet, inflammation, low educational status of mothers and other caregivers, and lack of awareness of anaemia [2, 4–7]. Most of the burden is concentrated in children under the age of five and women of reproductive age [5, 8]. Young children from low-income families are particularly prone of developing anaemia due to iron deficiency, which occurs as a result of not only high iron demand during periods of rapid growth [9], but also due to deficiencies of micronutrients such as folate and vitamin B12, and infections with intestinal parasites, malaria, and tuberculosis [10, 11]. The major consequences of anaemia among young children include tiredness, impaired cognitive and physical development, reduced educational achievement, increased morbidity and mortality, and increased susceptibility to infection [9, 12–15]. Although anaemia has a variety of causes, it is generally assumed that 50% of cases are caused by iron deficiency, whilst the main risk factor for iron deficiency is malnutrition [16].

The prevention and control of anaemia is complex and depends on the setting and causes, it might require a package of interventions such as iron fortification, regular provision of iron-rich fruits and vegetables, periodic administration of anthelmintic drugs and, in malaria-endemic settings, sleeping under an insecticide-treated net. Implementing such integrated strategies holds promise for decreasing the prevalence of anaemia in LMICs [15, 17–19]. Nepal faces a plethora of problems related to nutritional and health issues among school-aged children, anaemia being one of them. Indeed, studies of anaemia among school-aged children in Nepal have reported prevalence rates of 34.5%, 37.9%, and 65.6% in different districts of the country [20–22]. To date, however, there are no specific studies focusing on school-aged children from rural areas in Nepal, where children usually suffer from a lack of food diversity leading to micronutrient deficiencies. Yet, it is crucial to characterize the baseline situation of anaemia among school-aged children, in order to provide a benchmark for planning and implementing control measures targeting this vulnerable population group.

To address the issue of anaemia and nutritional problems, a multi-country, multi-sectorial project entitled "Vegetables go to School: improving nutrition through agricultural diversification (VgtS)", was developed to determine school-aged children's nutritional and health status along with the implementation of school vegetable gardens, and other complementary interventions [23]. The goal of the present study, which was designed as a baseline cross-sectional survey among school-aged children in two primarily rural districts of Nepal within the frame of the VgtS project, was to determine the prevalence of anaemia in this population group and to identify risk factors. The study results were used to design complementary school-based interventions to improve the health and nutritional status of school-aged children as part of the larger VgtS project.

6.3. Methods

<u>Study setting and sample size calculation</u>

This study was carried out within the frame of the VgtS project in the districts of Dolakha and Ramechhap, located approximately 180 km and 150 km, respectively, from Kathmandu, the capital of Nepal. The two districts cover 2,191 km^2 and 1,546 km^2, respectively, and are located in the eastern part of Nepal. According to the 2011 census, there were 186,557 people in Dolakha and 202,646 in Ramechhap districts. Of note, both districts are malaria-free. Dolakha has one district hospital, two primary health care centres, nine health posts, and 43 sub-health posts. Ramechhap has one district hospital, two primary health care centres, 11 health posts, and 41 sub-health posts.

In mid-2015, a cross-sectional survey was conducted among 16 (13 in Dolakha and three in Ramechhap) purposively selected schools, representative of the main ecologic and socioeconomic contexts of the two districts, as part of the wider trans-national VgtS project [23]. The criteria adopted for the selection of schools were as follows: (i) schools located within a maximum of one-hour walking distance from a main highway; and (ii) water available at school for vegetable gardening. In the Ramechhap district, only three schools were selected, as few schools met the aforementioned criteria. The 16 schools were randomly allocated to one of four study arms defined by varying combinations of the presence/absence of interventions that were implemented after the current cross-sectional baseline survey: (i) schools benefiting from a school garden intervention only; (ii) schools additionally receiving nutrition, and water, sanitation and hygiene (WASH) interventions with school garden; (iii) schools where nutrition and WASH interventions will be implemented; and (iv) schools without any interventions (control schools). A sample size of 800 children was determined using a Monte Carlo simulation. The study population consisted of school-aged children attending grades 6 and 7, aged 8-16 years. The detailed methodology of sample selection and study methodology is described elsewhere [23].

Data collection and processing

One week before the cross-sectional survey, the selected schools were visited to explain the purpose, procedures, and potential risks and benefits of the planned study to the school principals and pupils. A semi-structured, pre-tested questionnaire was employed to determine the socio-demographic characteristics, socioeconomic status, and knowledge, attitude, and practices (KAP) regarding health and nutrition among school-aged children and their caregivers (e.g., mother, father, or other legal guardian). Dietary variables were collected using a 24-hour food recall dietary history among the surveyed children, using specific probes to help participants remember all the food items they had consumed the previous day. Data were collected using tablets (Samsung Galaxy note 10.1 N8010; Seoul, South Korea) with open data kit (ODK) software as the data entry mask. To ensure the reliability of the information, school-aged children and their caregivers were interviewed in their mother tongue, by trained enumerators familiar with the study area and the local languages. Capillary blood samples were collected from each participant to determine Hb levels, using a portable hemoglobinometer (HemoCue Hb 201^+ analyser; HemoCue AB, Angelholm, Sweden). Blood was collected by finger pricking after rubbing the fingertip with cotton immersed in 70% ethanol, and pricking with a sterile disposable lancet. The first drop of blood was discharged and then a small amount of blood was allowed to enter the optical window of the microcuvette through capillary action. The microcuvette was placed into the cuvette holder for photometric determinants of Hb level. Daily calibration of the device was done with reference blood.

The two study districts are comparable in terms of altitude; both are mountainous, and hence, no adjustment to Hb for altitude was done. Anaemia was determined according to age-specific Hb cut-offs, as specified by the World Health Organization (WHO) [5]: Hb level 110-114 g/dL for children aged 5-11 years, 110-119 g/dL for children aged 12-14 years, and 110-129 g/dL for children aged 15 years and older. Severe anaemia was defined as Hb below 80 g/dL for all children regardless of age, while moderate anaemia was defined as Hb between 80 and 109 g/dL [24]. Anthropometric measurements were taken by trained health workers adhering to standard guidelines [25]. Height was recorded to the nearest 0.1 cm using a standard scale with a length of 2 m and a precision of 0.1 cm. Weight was measured to the nearest 0.1 kg using a digital portable calibrated weighing scale (SECA; Hamburg, Germany) with a sensitivity of 0.1 kg and a capacity of 130 kg. The weighing scale was calibrated to zero before taking any measurement. The children were weighted wearing a light uniform, with empty pockets and without shoes. The growth and development status of children were evaluated by (i) weight-for-age ratio (for underweight), (ii) height-for-age ratio (for stunting), and (iii) weight-for-height ratio (for wasting), as per WHO's child growth standards [26]. School-aged children were considered as undernourished when any of the z-score values were below -2 [26, 27]. An experienced physician conducted clinical examinations to detect any signs of nutritional deficiencies, such as oedema, dermatitis, etc.

Study participants provided a single stool sample. Within 2 hours of stool collection, 2 g of stool were prepared on a single slide using the saline wet mount technique, to microscopically detect intestinal parasites, adhering to standard procedures [28]. Duplicate Kato-Katz thick smears, using 41.7 mg templates, were prepared, adhering to standard protocols [29]. The Kato-Katz thick smears were allowed to clear for 30 min before experienced laboratory technicians examined them under a microscope. Helminth eggs were counted and recorded for each species separately [30]. A formalin-ether concentration technique was used, following standard procedures [31]. For quality control, 10% of the samples were re-examined by a senior technician. Helminth species-specific egg counts were compared with the original readings and, whenever discrepancies occurred, the slides were read a third time and the results discussed until agreement was reached. To ensure high data quality, a 2-day training course was provided for data collectors, who were constantly supervised during data collection. Standard operating procedures and manufacturer's instructions were strictly followed for all laboratory activities. Laboratory results were recorded on standard report formats, using participant's identification codes.

Statistical analysis

To maintain confidentiality, the data collection device was password-protected and data were automatically deleted after synchronizing with the server at the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland). Quantitative variables were described by their mean and standard deviation (SD) if the underlying distribution was close to normal and by their median and interquartile range otherwise. We used χ^2 statistics to assess the differences in distributions for categorical variables. A new variable for socioeconomic status was created using factor analysis of asset and building variables and retaining the first factor. The households were classified into one of three wealth categories; poor, moderate, or better-off, using k-means procedure.

Mixed logistic regression models with random intercepts at the level of schools were used to determine variables associated with anaemia. We used the categorical variables sex, age (8-12 years and 13-16 years), district, and household socioeconomic status as core variables.

All other variables were added to the core model, one by one, and those with a *p*-value <0.2 were included in an initial multivariable model, along with the core variables. Backward selection retaining variables with *P*-value <0.2 was used to obtain the final model. Adjusted odds ratios (aOR) with 95% confidence intervals (CI) were calculated and Wald test *P*-values <0.05 were considered statistically significant.

Dietary data were analysed based on Food and Agriculture Organization (FAO)/Food and Nutrition Technical Assistance (FANTA) guidelines for measuring dietary diversity [32]. We used factor analysis with varimax rotation to obtain food patterns reflecting the specific food items consumed [33]. The food data were summarized into nine food groups as per FAO guideline [32]. The three factors with the highest eigenvalues were retained and their scores included as potential predictors of anaemia. All analyses were done using STATA version 14 (Stata Corporation; College Station, TX, USA).

6.4. Results

<u>Socio-demographic characteristics of study participants</u>

A total of 708 school-aged children (52.1% females) participated in the study. The mean age was 12.8 years with a range from 8 to 16 years. Children were either in grade 6 (n=333, 47.0%) or grade 7 (n=375, 53.0%). The median age of caregivers was 39.5 years, with an interquartile range of 11 years. Of the caregivers, 37.4% had no schooling (39.2% in Dolakha district and 30.5% in Ramechhap district), 25.6% had completed primary education, 25.4% completed secondary education and 11.6% obtained higher education. Most household heads were farmers (88.1% in Dolakha district and 56.8% in Ramechhap district). Of the surveyed children, 55.0% and 45.0% were of poor socioeconomic status in Dolakha and Ramechhap districts, respectively. About one of five (22%) of the caregivers in Dolakha district were Brahmin and thus had taboos on consumption of animal products (Table 6.1).

Table 6.1. Characteristics of study population in Dolakha and Ramechhap districts, Nepal, March-May 2015

Characteristics	Overall [N=708] [N (%)]	Dolakha [n=555] [n (%)]	Ramechhap [n=153] [n (%)]	*P*-value
Sex				
Male	339 (47.9)	261 (47.0)	78 (51.0)	0.39
Female	369 (52.1)	294 (53.0)	75 (49.0)	
Age of children				
Age group 1 (8-12 years)	108 (15.2)	86 (15.5)	22 (14.4)	0.73
Age group 2 (13-16 years)	600 (84.8)	469 (84.5)	131 (85.6)	
Grade				
Class 6	333 (47.0)	258 (46.5)	75 (49.0)	0.58
Class 7	375 (53.0)	297 (53.5)	78 (51.0)	

Caregivers demographic characteristics (n=562)					
Age of caregivers					
18-24 years		2 (0.4)	1 (0.2)	1 (0.9)	0.34
24-40 years		239 (42.5)	184 (41.4)	55 (46.6)	
>40 years		321 (57.1)	259 (58.3)	62 (52.5)	
Education level of caregivers					
No formal schooling		210 (37.4)	174 (39.2)	36 (30.5)	<0.001
Primary education		144 (25.6)	130 (29.3)	14 (11.9)	
Secondary education		143 (25.4)	82 (18.5)	61 (51.7)	
Higher education		65 (11.6)	58 (13.0)	7 (5.9)	
Ethnicity of caregivers					
Brahmin		101 (17.9)	97 (21.9)	4 (3.4)	<0.001
Chhetri		210 (37.4)	154 (34.7)	56 (47.5)	
Newar		33 (5.9)	22 (4.9)	11 (9.3)	
Tamang		213 (37.9)	166 (37.4)	47 (39.8)	
Janajati		5 (0.9)	5 (1.1)	0 (0.0)	
Main occupation of caregivers					
No occupation		25 (4.5)	10 (2.2)	15 (12.7)	<0.001
Farmer		458 (81.5)	391 (88.1)	67 (56.8)	
Public service		39 (6.9)	29 (6.5)	10 (8.5)	
Business owner		40 (7.1)	14 (3.2)	26 (22.0)	
Socioeconomic characteristics of caregivers [n=562]					
Roof material	Corrugated iron roof	415 (73.8)	325 (73.2)	90 (76.3)	0.50
	Wood and tiles	147 (26.2)	119 (26.8)	28 (23.7)	
Wall material	Wood	66 (11.7)	61 (13.7)	5 (4.2)	<0.001
	Corrugated iron	407 (72.4)	331 (74.6)	76 (64.4)	
	Bricks	89 (15.9)	52 (11.7)	37 (31.4)	
Floor material	Mud	524 (93.2)	430 (96.9)	94 (79.7)	<0.001
	Cement	38 (6.8)	14 (3.1)	24 (20.3)	
Energy for cooking	Charcoal/wood	473 (84.2)	390 (87.8)	83 (70.3)	<0.001
	Electricity	89 (15.8)	54 (12.2)	35 (29.7)	
Socioeconomic status *	High	49 (8.7)	39 (8.8)	10 (8.5)	0.33
	Middle	215 (38.3)	163 (36.7)	52 (44.1)	
	Poor	298 (53.0)	242 (54.5)	56 (47.4)	
Owing agricultural land		511 (90.9)	412 (92.8)	99 (83.9)	0.003
Total production in household	≤ 10%	44 (7.8)	30 (6.8)	14 (11.9)	<0.001
	10-13%	20 (3.6)	6 (1.4)	14 (11,9)	
	>30%	498 (88.6)	408 (91.9)	90 (76.3)	
Possession of domestic animals		507 (90.2)	401 (90.3)	106 (89.8)	0.86

Knowledge, attitude and practice (KAP) on health and nutrition among school-aged children

Table 6.2 summarizes the KAP findings on health and nutrition among school-aged children in the two districts of Nepal. Less than one third (31.2%) of the participants had heard about malnutrition and 1.1% could identify lack of healthy food as a cause. About 75% of the school-aged children thought that two portions of fruits and vegetables per day are required.

Table 6.2. Questionnaire findings on nutrition among school children in two districts of Nepal, March-May 2015

Children	Overall [N=708] [n (%)]	Dolakha [n=555] [n (%)]	Ramechhap [n=153] [n, (%)]	*P*-value
Nutrition practices and habits				
Breakfast	645 (91.1)	504 (90.8)	141 (92.2)	0.61
Lunch	698 (98.6)	549 (98.9)	149 (97.4)	0.16
Snack	332 (46.9)	249 (44.9)	83 (54.3)	0.04
Supper	691 (97.6)	543 (97.8)	148 (96.7)	0.43
Nutrition: knowledge and attitude				
Heard about malnutrition	221 (31.2)	171 (30.8)	50 (32.7)	0.66
Perception of malnutrition as problem**	178 (80.5)	144 (84.2)	34 (68.0)	0.01
Causes of malnutrition*				
Diseases	2 (0.3)	2 (0.4)	0 (0.0)	0.46
Lack of food	41 (5.8)	28 (5.1)	13 (8.5)	0.12
Unbalanced food intake	65 (9.2)	56 (10.1)	9 (5.9)	0.11
Poorly prepared food	6 (0.9)	4 (0.7)	2 (1.3)	0.48
Lack of means to afford good foods	8 (1.1)	7 (1.3)	1 (0.7)	0.53
Opinion on requirement of consumption of fruit and vegetables per day				
5 per day	43 (6.1)	38 (6.9)	5 (3.3)	0.03
2 per day	528 (74.6)	426 (76.8)	102 (66.7)	
1 per day	54 (7.6)	35 (6.3)	19 (12.4)	
None per day	83 (11.7)	56 (10.1)	27 (17.7)	
Opinion about eating variety of vegetables and fruits				
It is not good	2 (0.3)	2 (0.4)	0 (0.0)	0.01
I am not sure	164 (23.4)	116 (21.2)	48 (31.4)	
It is good	485 (69.3)	395 (72.2)	90 (58.8)	
Others	49 (7.0)	43 (6.2)	6 (9.8)	

Table 6.3 summarizes the KAP about health and nutrition at the unit of the household. About a quarter were aware of malnutrition (28.3%) and anaemia (24.7%), while only 22.2% of caregivers could identify the causes of anaemia, specifically lack of iron. Most of the caregivers reported preparing vegetables (83.6%) and giving fruits (79.4%) to their children. While 95.3% of caregivers reported breastfeeding their child immediately after birth, 4.7% reported of no breastfeeding. A total of 24.9% reported giving fortified food supplements, in addition to the usual meal before complementary feeding period. Around 40% of caregivers from Ramechhap reported about anaemia being caused by lack of iron in the consumed foodstuff.

Table 6.3: Questionnaire findings about health and nutrition among caregivers in two districts of Nepal, March-May 2015

Characteristics	Overall [N=562] [n (%)]	Dolakha [n=444] [n (%)]	Ramechhap [n=118] [n, (%)]	*P*-value
Health				
Children suffered from diseases in the past two weeks				
Fever	174 (31.0)	146 (32.9)	28 (23.7)	0.06
Cough	132 (23.5)	105 (23.7)	27 (22.9)	0.86
Respiratory difficulties	28 (5.0)	22 (5.0)	6 (5.1)	0.95
Watery diarrhoea	126 (22.4)	104 (23.4)	22 (18.6)	0.27
Bloody diarrhoea	8 (1.4)	4 (0.9)	4 (3.4)	0.04

Mucus in stool	16 (2.9)	15 (3.4)	1 (0.9)	0.14
Heard about intestinal parasites	397 (70.6)	305 (68.7)	92 (78.0)	0.05
Knowledge on protection against intestinal parasites				
By washing hands with soap	417 (74.2)	319 (71.9)	98 (83.1)	0.01
By cutting finger nails	210 (37.4)	146 (32.9)	64 (54.2)	<0.001
By washing fruits and vegetables	94 (16.7)	59 (13.3)	35 (29.7)	<0.001
By wearing shoe	52 (9.3)	42 (9.5)	10 (8.5)	0.74
By drinking clean water	184 (32.7)	133 (30.0)	51 (43.2)	0.001
Nutrition				
Heard about malnutrition	159 (28.3)	121 (27.3)	38 (32.2)	0.29
Knowledge about causes of malnutrition				
Lack of food	29 (5.2)	21 (4.7)	8 (6.8)	0.37
Irregular meals	136 (24.2)	100 (22.5)	36 (30.5)	0.07
Diseases	2 (0.4)	2 (0.6)	0 (0.0)	0.47
Early weaning	34 (6.1)	26 (5.9)	8 (6.8)	0.71
Negligence when breastfeeding	93 (16.6)	62 (14.0)	31 (26.3)	0.001
Lack of food diversity	102 (18.2)	67 (15.1)	35 (29.7)	<0.001
Breastfeeding to the child*	489 (87.0)	390 (87.8)	99 (83.9)	0.26
Breastfeeding to the child after birth				
Immediately after birth	237 (42.2)	159 (35.8)	78 (66.1)	<0.001
Hours after birth	215 (38.3)	198 (44.6)	17 (14.4	
Days after birth	15 (2.7)	14 (3.2)	1 (0.9)	
No breastfeeding	95 (16.9)	73 (16.4)	22 (18.6)	
Food given except breastfeeding*				
Nothing	176 (31.3)	148 (33.3)	28 (23.7)	<0.001
Ordinary water	42 (7.5)	26 (5.9)	16 (13.6)	
Oral rehydration solution	274 (48.7)	228 (51.4)	46 (39.0)	
Rice water	43 (7.7)	28 (6.3)	15 (12.7)	
Milk powdered	37 (6.6)	28 (6.3)	9 (7.6)	
Glucose	75 (13.4)	56 (12.6)	19 (16.1)	
Herbs	37 (6.6)	27 (6.1)	10 (8.5)	
Rice pap	61 (10.9)	38 (8.6)	23 (19.5)	
Heard about anaemia	139 (24.7)	102 (23.0)	37 (31.4)	<0.001
Causes of anaemia				
Lack of iron in the consumed foodstuff	125 (22.2)	78 (17.6)	47 (39.8)	<0.001
Heavy bleeding during menstruation	52 (9.3)	28 (6.3)	24 (20.3)	<0.001
Meals schoolchildren eat per day				
One	61 (10.9)	35 (7.9)	26 (22.0)	<0.001
Two	195 (34.7)	163 (36.7)	32 (27.1)	
Three	306 (54.5)	246 (55.4)	60 (50.9)	
Sweet given to schoolchildren besides usual meal	171 (30.4)	132 (29.7)	39 (33.1)	0.49
Prepare vegetables to school children to eat	470 (83.6)	374 (84.2)	96 (81.4)	0.45
Factor considering for preparing vegetable				
Affordability	75 (13.4)	59 (13.3)	16 (13.6)	0.94
Availability	213 (37.9)	168 (37.8)	45 (38.1)	0.95
Freshness	244 (43.4)	190 (42.8)	54 (45.8)	0.56
Taste	93 (16.6)	67 (15.1)	26 (22.0)	0.07
Texture	17 (3.0)	12 (2.7)	5 (4.2)	0.39
Seasonality	129 (23.0)	116 (26.1)	13 (11.0)	0.001
Reasons for not preparing vegetables				
Schoolchildren does not like vegetables	15 (2.7)	5 (1.1)	10 (8.5)	n/a
Vegetables are expensive	31 (5.5)	30 (6.8)	1 (0.9)	
Vegetables are not available	43 (7.7)	43 (9.7)	0 (0.0)	
Fruits to the children	446 (79.4)	350 (78.8)	96 (81.4)	0.55
Factors considering for giving fruits				
Affordability	119 (21.2)	99 (22.3)	20 (17.0)	0.21
Availability	240 (42.7)	204 (46.0)	36 (30.5)	0.003
Freshness	266 (47.3)	189 (42.6)	77 (65.3)	<0.001
Taste	8 (1.4)	7 (1.3)	1 (0.9)	0.55
Texture	28 (5.0)	23 (5.2)	5 (4.2)	0.68
Seasonality	165 (29.4)	135 (30.4)	30 (25.4)	0.29

Anthropometry and clinical outcome of study participants

Table 6.4 summarizes children’s anthropometry, anaemia, and clinical signs related to nutritional deficiency, stratified by sex, age group, and district. Overall, 27.0% of the participating children were stunted and 11.3% were diagnosed with wasting. We observed a significant difference in stunting and wasting between males and females (stunting: 31.6% for males *versus* 22.8% for females, *P*=0.01; wasting: 15.9% for males *versus* 7.1% for females, *P*=0.01). We also found a significant difference in stunting between the two districts; a higher stunting rate was observed in Dolakha compared to Ramechhap (30.1% *versus* 15.7%; *P*=0.01).

Table 6.4: Prevalence of malnutrition, anaemia, and frequency of clinical outcomes obtained from physical examination of school children in two districts of Nepal, March-May 2016, stratified by sex, age group and district

Nutritional indicators and clinical outcomes	Overall [N=708] [n (%)]	Sex				Age group				District			
		Boys, [n (%)]	Girls, [n (%)]	χ^2	*p*-value	8-12 years [n (%)]	13-16 years [n (%)]	χ^2	*p*-value	Dolakha [n (%)]	Ramechhap [n (%)]	χ^2	*p*-value
Thinness	80 (11.3)	54 (15.9)	26 (7.1)	13.90	0.00	11 (10.2)	69 (11.5)	0.16	0.69	60 (10.8)	20 (13.1)	0.61	0.43
Sever thinness	14 (2.0)	7 (2.1)	7 (1.9)	0.03	0.87	4 (3.7)	10 (1.7)	1.96	0.16	14 (2.5)	0 (0.0)	3.94	0.05
Stunting	191 (27.0)	107 (31.6)	84 (22.8)	6.94	0.01	23 (21.3)	168 (28.0)	2.09	0.15	167 (30.1)	24 (15.7)	12.63	<0.01
Overweight	21 (3.0)	11 (3.2)	10 (2.7)	0.18	0.68	3 (2.8)	18 (3.0)	0.02	0.90	12 (2.2)	9 (5.9)	5.77	0.02
Obesity	3 (0.4)	1 (0.3)	2 (0.5)	0.26	0.61	1 (0.9)	2 (0.3)	0.76	0.38	3 (0.5)	0 (0.0)	0.83	0.36
Anaemia	167 (23.6)	93 (27.4)	74 (20.1)	5.34	0.02	19 (17.6)	148 (24.7)	2.54	0.11	142 (25.6)	25 (16.3)	5.69	0.02
Presence at least one sign and symptom of nutritional deficiency	391 (55.2)	206 (60.8)	185 (50.1)	8.08	0.00	64 (59.3)	327 (54.5)	0.84	0.36	280 (50.5)	111 (72.5)	23.7	<0.01
Wasted appearance (a)	65 (9.2)	37 (10.9)	28 (7.6)	2.34	0.13	13 (12.0)	52 (8.7)	1.25	0.26	65 (11.7)	0 (0.0)	19.7	<0.01
Loss of hair pigment (b)	258 (36.4)	138 (40.7)	120 (32.5)	5.11	0.02	40 (37.0)	218 (36.3)	0.02	0.09	170 (30.6)	88 (57.5)	37.3	<0.01
White foamy spotting cornea (c)	1 (0.1)	0 (0.0)	1 (0.3)	0.92	0.34	0 (0.0)	1 (0.2)	0.18	0.67	1 (0.28)	0 (0.0)	0.28	0.60
Dry and infected cornea (d)	11 (1.6)	6 (1.8)	5 (1.4)	0.20	0.66	1 (0.9)	10 (1.7)	0.33	0.57	10 (1.8)	1 (0.7)	1.03	0.31
Oedema (e)	2 (0.3)	2 (0.6)	0 (0.0)	2.18	0.14	1 (0.9)	1 (0.2)	1.87	0.17	0 (0.0)	2 (1.3)	7.28	0.01
Dermatitis (f)	16 (2.3)	8 (2.4)	8 (2.2)	0.03	0.86	0 (0.0)	16 (2.7)	2.95	0.09	9 (1.6)	7 (4.6)	4.74	0.03
Enlargement of liver (g)	1 (0.1)	1 (0.3)	0 (0.0)	1.09	0.30	1 (0.9)	0 (0.0)	5.56	0.02	1 (0.2)	0 (0.0)	0.28	0.60
Spongy bleeding gums (h)	2 (0.3)	2 (0.6)	0 (0.0)	2.18	0.14	0 (0.0)	2 (0.33)	0.36	0.55	2 (0.4)	0 (0.0)	0.55	0.46
Angular stomatis (i)	1 (0.1)	0 (0.0)	1 (0.3)	0.92	0.34	0 (0.0)	1 (0.2)	0.18	0.67	0 (0.0)	1 (0.7)	3.63	0.05
Pale conjunctiva (j)	59 (8.3)	24 (7.1)	35 (9.5)	1.34	0.25	10 (9.3)	49 (8.2)	0.14	0.71	43 (7.8)	16 (10.5)	1.15	0.28
Spontaneous bruising (k)	1 (0.1)	0 (0.0)	1 (0.3)	0.92	0.34	0 (0.0)	1 (0.2)	0.18	0.67	1 (0.2)	0 (0.0)	0.28	0.60
Sub dermal hemorrhage (l)	2 (0.3)	0 (0.0)	2 (0.5)	1.84	0.18	0 (0.0)	2 (0.3)	0.36	0.55	2 (0.4)	0 (0.0)	0.55	0.46

(a) Wasted appearance: protein energy deficit
(b) Loss of hair pigment : protein energy deficit
(c) White foamy spotting cornea: vitamin A deficit
(d) Dry and infected cornea : vitamin A deficit
(e) Oedema : thiamine, B1 deficit
(f) Dermatitis: niacin deficit
(g) Enlargement of liver: protein energy deficit
(h) Spongy bleeding gums: vitamin C deficit
(i) Angular stomatitis : riboflavin deficit
(j) Pale conjunctiva: iron deficiency anaemia
(k) Spontaneous bruising: vitamin C deficiency
(l) Sub-dermal haemorrhage : Vitamin C deficiency
(m) Wasting: low weight for length
(n) Stunting: short height for age
(o) Overweight: high weight for age

The overall presence of any one clinical sign indicating nutritional deficiency was 55.2%. We observed significant differences in the presence of at least one sign of nutritional deficiency between males (60.8%) and females (50.1%) (*P*=0.01). Most school children suffered loss of hair pigment (36.4%), followed by wasted appearance (9.2%), pale conjunctiva (8.3%), and dermatitis (2.3%). We observed a significant difference in loss of hair pigment between males and females (40.7% *versus* 32.5%; *P*=0.02), and between the two districts (30.6% in Dolakha *versus* 57.5% in Ramechhap; *P*=0.01). A significant difference in dermatitis prevalence was observed between the two districts (1.6% in Dolakha *versus* 4.6% in Ramechhap; *P*=0.03).

Of the children surveyed, 11.5% reported being ill in the past 2 weeks. The most frequently reported symptom was fever (30.9%). Watery diarrhoea, mucus in stool, and bloody diarrhoea were reported by 22.4%, 2.9%, and 1.4% of the children, respectively. Diarrhoea was significantly more frequent in children aged 8-12 years compared to their older counterparts (*P*=0.01).

Prevalence and severity of anaemia

Prevalence of anaemia among school children was 23.6%, with statistically significant differences by sex (males 27.4% *versus* females 22.8%; *P*=0.02) and between districts (Dolakha 25.6% *versus* Ramechhap 16.3%; *P*=0.02). The mean Hb concentration was 12.6 g/dL (SD=1.29 g/dL), ranging from 7.7 g/dL to 17.6 g/dL. Of the children with anaemia, 15.8% were severely anaemic, 60.6% were moderately anaemic, and 23.6% had mild anaemia. Anaemia prevalence was 17.6% among children aged 8-12 years and 24.7% among 13 to 16 year old children. The prevalence of anaemia among stunted study participants was 28.7%.

Prevalence of soil-transmitted helminth and intestinal protozoal infection

Table 6.5 summarizes the children's soil-transmitted helminth and intestinal protozoa infection status, stratified by sex, age group, and district. The overall prevalence of soil-transmitted helminth and intestinal protozoal infection was 35.3% and 30.5%, respectively. *Trichuris trichiura* was the predominant helminth species (30.9%), followed by hookworm (30.2%), *Hymenolepis nana* (27.5%), *Ascaris lumbricoides* (26.1%), *Enterobius vermicularis* (0.4%), and *Strongyloides stercoralis* (0.3%). Overall, 250 school children (35.3%) had a helminth infection that is transmitted through the faecal-oral route. Co-infections with up to five helminth species concurrently infecting a single individual were observed. All helminth infections were of light intensity. Significant differences in the prevalence of hookworm and *H. nana* were seen between the two districts (hookworm 32.8% in Dolakha *versus* 20.9% in

Ramehhap; *P*=0.01; *H. nana* 29.6% in Dolakha *versus* 20.3% in Ramechhap; *P*=0.02). Of those children infected with soil-transmitted helminths, 37.5% were anaemic. *Giardia intestinalis* was the predominant intestinal protozoa species with an overall prevalence of 30.5%.

Table 6.5. Intestinal protozoa and soil-transmitted helminth infections among school children in Dolakha and Ramechhap districts of Nepal, in March-May 2015

Parasites	Prevalence	Sex				Age group				District			
	[N=708] [n (%)]	Male [n=339]	Female [n=369]	χ^2	*P*-value	8-12 [n=108]	13-16 [n=600]	χ^2	*P*-value	Dolakha [n=555]	Ramechhap [n=153]	χ^2	*P*-value
Intestinal protozoan													
Giardia intestinalis	216 (30.5)	101 (29.8)	115 (31.2)	0.16	0.69	22 (20.4)	194 (32.3)	6.18	0.01	176 (31.7)	40 (26.1)	1.75	0.19
Nematodes													
Ascaris lumbricoides	185 (26.1)	87 (25.7)	98 (26.6)	0.07	0.79	21 (19.4)	164 (27.3)	2.95	0.09	154 (27.8)	31 (20.3)	3.48	0.06
Trichuris trichiura	219 (30.9)	109 (32.2)	110 (29.8)	0.45	0.50	27 (25.0)	192 (32.0)	2.10	0.15	176(31.7)	43 (28.1)	0.73	0.39
Hookworm	214 (30.2)	99 (29.2)	115 (31.2)	0.32	0.57	27 (25.0)	187 (31.2)	1.65	0.12	182 (32.8)	32 (20.9)	8.02	0.01
Strongyloides stercoralis	2 (0.3)	1 (0.3)	1 (0.3)	0.01	0.95	0 (0.0)	2 (0.3)	0.36	0.55	2 (0.36)	0 (0.0)	0.55	0.46
Enterobius vermicularis	2 (0.4)	1 (0.4)	1 (0.4)	0.01	0.96	0 (0.0)	2 (0.5)	0.39	0.53	2 (0.5)	0 (0.0)	0.62	0.43
Cestodes													
Hymenolepis nana	195 (27.5)	90 (26.6)	105 (28.5)	0.32	0.57	24 (22.2)	171 (28.5)	1.81	0.18	164 (29.6)	31 (20.3)	5.18	0.02
Total fecal-oral transmitted helminths*	250 (35.3)	120 (35.4)	130 (35.2)	0.01	0.96	31 (28.7)	219 (36.5)	2.44	0.12	203 (36.6)	47 (30.7)	1.80	0.18

**The category of total faecal-oral transmitted helminths includes infections with nematodes and cestodes.*

Dietary pattern

Table 6.6 shows consumption from the different food groups and the distribution of diet diversity score (DDS). The children's diet mainly comprised of starchy staples (60.0%), followed by legumes (58.6%), vitamin A-rich fruits and vegetables (46.6%), and other fruits and vegetables (45.5%). The consumption of animal products such as meat and fish, eggs and milk/milk products was low with 25.3%, 4.5%, and 8.9%, respectively. Anaemic children tended to have lower consumption levels in all food groups.

Table 6.6: Food consumption patterns and diet diversity score among school children in two districts of Nepal, March-May 2015, stratified by sex, age group, and district

Characteristics	Overall	Sex		Age		District		Anaemia	
	[N=708] [n (%)]	Males [n, (%)]	Females [n, (%)]	8-12 years [n, (%)]	13-16 years [n, (%)]	Dolakha [n, (%)]	Ramechhap [n, (%)]	Non-anaemic [n, (%)]	Anaemic [n, (%)]
Food groups									
Group 1: starchy staples [a)]	425 (60.0)	207 (61.1)	218 (59.1)	61 (56.5)	364 (60.7)	379 (68.3)	46 (30.1)	321 (59.3)	104 (62.3)
Group 2: dark green leafy vegetables[(b)]	301 (42.5)	142 (41.9)	159 (43.1)	45 (41.7)	256 (42.7)	272 (49.0)	29 (18.9)	226 (41.8)	75 (44.9)
Group 3: other vitamin A rich fruits and vegetables[(c)]	330 (46.6)	159 (46.9)	171 (46.3)	50 (46.3)	280 (46.7)	280 (50.1)	50 (32.7)	250 (46.2)	80 (47.9)
Group 4: other fruits and vegetables[(d)]	322 (45.5)	154 (45.4)	168 (45.5)	46 (42.6)	276 (46.0)	237 (42.7)	85 (55.6)	251 (46.4)	71 (42.5)
Group 5: organ meat[(e)]	0 (0.0)								
Group 6: meat and fish[(f)]	179 (25.3)	83 (24.5)	96 (26.0)	30 (27.8)	149 (24.8)	138 (24.9)	41 (26.8)	148 (27.4)	31 (18.6)
Group 7: eggs	32 (4.5)	11 (3.2)	21 (5.7)	8 (7.4)	24 (4.0)	24 (4.3)	8 (5.2)	23 (4.3)	9 (5.4)
Group 8: legumes, nuts and seeds[(g)]	415 (58.6)	204 (60.2)	211 (57.2)	67 (62.0)	348 (58.0)	320 (57.7)	95 (62.1)	320 (59.2)	95 (56.9)
Group 9: milk and milk products[(h)]	63 (8.9)	28 (8.3)	35 (9.5)	14 (13.0)	49 (8.2)	48 (8.7)	15 (9.8)	46 (8.5)	17 (10.2)
Diet diversity scores									
1	63 (8.9)	28 (8.3)	35 (9.5)	10 (9.3)	53 (8.8)	29 (5.2)	34 (22.2)	53 (9.8)	10 (6.0)
2	91 (12.9)	41 (12.1)	50 (13.6)	16 (14.8)	75 (12.5)	53 (9.6)	38 (24.8)	76 (14.1)	15 (9.0)
3	77 (10.9)	47 (13.9)	30 (8.1)	11 (10.2)	66 (11.0)	56 (10.1)	21 (13.7)	57 (10.5)	20 (12.0)
4	78 (11.0)	35 (10.3)	43 (11.7)	11 (10.2)	67 (11.2)	63 (11.4)	15 (9.8)	50 (9.2)	28 (16.8)
5	69 (9.8)	34 (10.0)	35 (9.5)	9 (8.3)	60 (10.0)	55 (9.9)	14 (9.2)	51 (9.4)	18 (10.8)
6	92 (13.0)	44 (13.0)	48 (13.0)	14 (13.0)	78 (13.0)	80 (14.4)	12 (7.8)	72 (13.3)	20 (12.0)
7	73 (10.3)	34 (10.0)	39 (10.6)	14 (13.0)	59 (9.8)	64 (11.5)	9 (5.9)	62 (11.5)	11 (6.6)
8	82 (11.6)	37 (10.0)	45 (12.2)	13 (12.0)	69 (11.5)	75 (13.5)	7 (4.6)	64 (11.8)	18 (10.8)
9	83 (11.7)	39 (11.5)	44 (11.9)	10 (9.3)	73 (12.2)	80 (14.4)	3 (2.0)	56 (10.4)	27 (16.2)

(a) Starchy staples: rice, maize, wheat grains, millet, bread, noodles, white potatoes, white yam
(b) Dark green leafy vegetables: spinach, mustard, broad leaf spinach
(c) Other vitamin A-rich fruits and vegetables: pumpkin, carrot, squash, mango, papaya
(d) Other fruits and vegetables: tomato, onion, eggplant, cabbage, cauliflower, orange, guava, apple
(e) Organ meat: liver, kidney
(f) Meat and fish: goat, chicken, fish, pork, buff
(g) Legumes, nuts and seeds: dried beans, lentils, dried peas, nuts
(h) Milk and milk products: milk, yogurt, cheese

The factor analysis revealed three major dietary patterns (the factor loadings of each pattern after orthogonal rotation are shown in Table 6.7). The three patterns were labelled based on the food items that loaded highly as follows: (i) pattern 1: dark green leafy vegetables (spinach and locally available green vegetables); (ii) pattern 2: milk and milk products (milk and yogurt); and (iii) pattern 3: vitamin A-rich fruits and vegetables (papaya, pumpkin, carrot, squash, and mango). The dietary patterns were not associated with anaemia (Table 6.8).

Table 6.7: Description of the three main factors derived from the food groups consumed by school children participating in the "Vegetables go to School" project in two districts of Nepal, March-May 2015

Food groups	Factor 1	2	3
Starchy staples	0.394	-0.048	-0.127
Dark green leafy vegetables	0.605	0.173	-0.024
Vitamin A-rich fruits and vegetables	0.153	0.066	0.574
Other fruits and vegetables	-0.335	0.329	-0.156
Meat and fish	-0.124	-0.725	-0.093
Eggs	0.170	0.149	-0.438
Legumes, nuts and seeds	-0.162	0.165	0.527
Milk and milk products	-0.106	0.379	-0.157

Extraction method: factor analysis, rotation method: varimax with Kaiser normalization
Score coefficients with highest values in each component are shown in bold
The organ meat is not included into factor analysis as none of the children had consumed it.
The eigenvalues of factor 1, factor 2 and factor 3 are 1.45, 1.16 and 1.10 respectively. The first factor is dominated by dark green leafy vegetables; the second one by meat and fish consumption and the third one by vitamin A rich fruits and vegetables.

Table 6.8. Association of anaemia with the three main food patterns** among 708 school children in two districts of Nepal, March-May 2015

	Model 1 Univariate analysis N=708			Model 2 Multivariate analysis adjusted for demographic variables * N=708		
	OR	95% CI	*P*-value	aOR	95% CI	*P*-value
Outcome: anaemia						
Dark green leafy vegetables pattern	1.13	0.94-1.35	0.18	1.07	0.88-1.29	0.50
Meat and fish consumption pattern	1.18	0.98-1.41	0.08	1.18	0.98-1.42	0.08
Vitamin A-rich fruits and vegetables pattern	0.98	0.83-1.17	0.86	0.96	0.80-1.15	0.65

**The results are from the mixed logistic regression models with random intercepts for schools and adjusted for age, sex, educational status, districts*.
***Food patterns are characterized by scores which were derived through principal component analysis from nine food group variables*

Factors associated with anaemia

Table 6.9 summarizes the associations between anaemia and behavioural and household risk factors, adjusted for potential confounders, such as sex, district, and socioeconomic status. The odds of anaemia among females was 1.48 that of males with a borderline significant association (aOR 1.48, 95% CI: 0.98-2.25; *P*=0.06). Children in Dolakha district had a significantly higher prevalence of anaemia compared to those in Ramechhap district (aOR 2.05, 95% CI: 1.02-3.98; *P*=0.04). Children who reported not having had supper the day before the interview had a higher odds of anaemia compared to those who had a supper (aOR 3.46, 95% CI: 1.09-11.03; *P*=0.04). Similarly, children from households where no daily meal was cooked had a 2.36 times higher odds of anaemia (aOR 2.36, 95% CI: 1.14-4.92; *P*=0.02). Children having suffered from any disease in the 2 weeks preceding the survey had a higher odd of being anaemic. The same was true for children infected with soil-transmitted helminths. However, both associations were not statistically significant.

Table 6.9. Association of anaemia with child, household and caregiver characteristics in two district, Nepal, March-May 2015

Attribute	[n (%)]	Univariate analysis			Multivariate analysis[#]		
	[N=562]	OR	95% CI	*P*-value*	aOR	95% CI	*P*-value*
Sex							
Male	280 (49.8)	1.00					
Female	282 (50.2)	1.45	0.97-2.17	0.07	1.48	0.98-2.25	0.06
Age							
13-16 years	440 (78.3)	1.00					
8-12 years	122 (21.7)	1.15	0.72-1.86	0.56	1.08	0.66-1.77	0.75
District							
Dolakha	444 (79.0)	1.90	1.04-3.47	0.04	2.05	1.02-3.98	0.04
Ramechhap	118 (21.0)	1.00					
Socioeconomic status							
High	49 (8.7)	1.00					
Medium	215 (38.3)	1.39	0.62-3.10	0.42			
Poor	298 (53.0)	1.33	0.61-2.92	0.48			
Breakfast prior to the day of survey							
Yes	514 (91.5)	1.00					
No	48 (8.5)	0.65	0.29-1.44	0.28			
Snacks prior to the day of survey							
Yes	257 (45.7)	1.00					
No	305 (54.3)	1.22	0.81-1.83	0.34			
Supper prior to the day of survey							
Yes	548 (97.5)	1.00					
No	14 (2.5)	2.61	0.86-7.95	0.09	3.46	1.09-11.03	0.04
Total food production in household							
>30%	498 (88.6)	1.00					
≤10%	44 (7.8)	1.35	0.64-2.84	0.43			
10-30%	20 (3.6)	1.51	0.56-4.09	0.42			
Preparation of vegetables for the children							
Yes	470 (83.6)	1.00					
No	92 (16.4)	0.56	0.29-1.08	0.09	0.74	0.36-1.49	0.40
Giving fruits to the children (usually)							
Yes	446 (79.4)	1.00					
No	116 (20.6)	0.99	0.56-1.74	0.97			
Giving sweets to child regularly							
No	391 (69. 6)	1.00					
Yes	171 (30.4)	1.50	0.94-2.38	0.09	1.33	0.83-2.14	0.40
Meals prepared in household per day							
More than once	482 (85.8)	1.00					
None	35 (6.2)	2.34	1.13-4.85	0.02	2.36	1.14-4.92	0.02
Once	45 (8.0)	0.39	0.13-1.18	0.10	0.42	0.13-1.37	0.15
Caregiver heard about anaemia							
Yes	248 (44.1)	1.00					
No	314 (55.9)	1.41	0.90-2.19	0.13	1.05	0.65-1.71	0.83
Soil-transmitted helminth infection							

No	342 (60.9)	1.00		
Yes	220 (39.2)	1.27	0.83-1.92	0.27
Disease suffered in past two weeks				
No	305 (54.3)	1.00		
Yes	257 (45.7)	1.15	0.75-1.77	0.51
Breastfed				
Yes	489 (87.0)	1.00		
No	73 (13.0)	0.89	0.46-1.70	0.72

Results from mixed logistic regression models with random intercepts for schools
**p values were obtained using the likelihood ratio test.*
 # the multivariable model included random intercepts at the level of schools and the categorical variables sex, age group and district as potential confounders. All the variables were assessed one by one and retained into the final maximal model if their P-value was <0.2.

6.5. Discussion

According to WHO, anaemia is classified as public health problem when the prevalence is above 20% in a population [1, 34, 35]. Data from the current cross-sectional study in two primarily rural districts of Nepal revealed that 23.6% of school-aged children were anaemic, indicating a moderate public health problem. Our findings thus confirm prior results from children of the same age group in Dolakha and Ramechhap districts [20, 21, 36]. Yet, in the previous studies, the level of anaemia was even higher, with 37.9% and 34.5%, respectively [21, 36]. The difference might be due to geographic variation, food consumption patterns including breastfeeding among new-borns, hygiene behaviours, and infection with soil-transmitted helminths. However, the differences might also be explained by measurement techniques. For instance, it is well known that the HemoCue method tends to underestimate prevalence of anaemia when compared to automated haematology analysers.

The school principals in some of the surveyed schools emphasized that mass deworming of school-aged children is provided twice a year, but this intervention is not being implemented consistently in all of the study schools. This policy intends to decrease soil-transmitted helminth infections, which, in turn, might result in increased Hb concentration. Some of the surveyed schools were targeted by deworming after our baseline cross-sectional survey. The effect of deworming campaigns against anaemia will hopefully be reflected in our subsequent follow-up surveys, as has been revealed in another similar study [15]. We found a mean Hb concentration of 12.6 g/dL (SD=1.3 g/dL) in both sexes. This finding is in line with studies conducted in eastern Nepal that reported a mean Hb level of 12.2±1.8 g/dL [21]. Our study revealed a higher prevalence of anaemia in children aged 13-16 years, compared to those aged 8-12 years (24.7% *versus* 17.6%). This observation is in line with increased vulnerability during adolescence due to starting puberty and growth spurt. However, the sample size was considerably higher in the older age group (600 *versus* 108). The different anaemia rates in the two districts might be explained by differences in exposure to soil-transmitted helminth infection.

More than half of the surveyed children had at least one clinical sign of nutritional deficiency and the presence of at least one sign of nutritional deficiency was higher in Ramechhap district. This might be due to higher availability and affordability of food in Dolakha compared to Ramechhap district. Additionally, anaemia could be caused by a lack of iron-rich vegetables and animal source foods. Overall, our study found a low consumption of vitamin A-rich fruits, vegetables, and animal source foods, but a high consumption of cereals-based foods. Consistent with the typical Nepalese diet, school-aged children reported a high intake of tea and legumes, which are not favourable for iron absorption [3]. In addition, heat exposure during food preparation largely reduces the bioavailable hemopoietic nutrients, such as folic acid and vitamin C (iron absorption enhancer) from the food [3]. This situation might be compounded by an increased intake of other dietary factors that reduce the bioavailability of non-heme iron, such as polyphenols (e.g., tea and spices), phytates (e.g., whole grains and legumes), and calcium (e.g., dairy products) [6]. Similar findings were reported by a study conducted in India, where cereals and legumes were the predominant component in children's diets and consumption of vegetables and fruits was low [37]. The diet diversity was low among school-aged children in our study. The DDS was higher in the farming community (Dolakha district) compared to the more business-oriented community (Ramechhap district). The food consumption pattern was different between the two districts with children from Dolakha districts consuming considerably more starchy foods, green leafy vegetables, and other vitamin A-rich foods in comparison to children from Ramechhap district. This observation might be explained by an easily production and better availability of healthier food in Dolakha district, which is richer in water availability for irrigation. In the present study, only 18.6% of the children with anaemia reported intake of animal products (i.e. meat, eggs and milk) and only 44.9% had plant source food. Similar observations were made in Ethiopia, where irregular consumption of meat and vegetables were found to be important correlates of anaemia among school-aged children [3].Factor analysis has been used to describe the patterns of dietary intake and to relate these patterns with the outcome variable anaemia. In our study, none of the three dietary factor scores derived was significantly associated with anaemia. Low levels of education among caregivers may have negatively affected the children's nutritional and breastfeeding status, while low socioeconomic status might have limited the type of food available. The prevalence of anaemia was higher among school-aged children whose socioeconomic status was low (53.8%) and whose caregivers had no or only little education. These findings are in line with a study conducted in India [38]. Families with low socioeconomic status may not get enough iron-rich diets and food might be monotonous with fewer iron-rich green leafy vegetables and more carbohydrates. Similar observations have been made in Ethiopia and in rural parts of the People's Republic of China [39–42]. School-aged children who did not consume meals and supper prior to the day of the household survey had higher odds of being anaemic compared to those who ate one or more meal per day in the household. This could be a chance finding, although our research suggests that regular meals and having supper might be crucial for preventing anaemia. One might expect that those children who consumed more meals and supper also consumed more nutrients, thereby increasing their odds of meeting micronutrients needs, including iron, which is needed for Hb production.

Severe lack of awareness related to nutrition and anaemia was found among caregivers. Only one-fourth were aware that lack of iron in the diet is a cause of anaemia. Furthermore, less than one-third of the caregivers reported having provided fortified food to their children. These findings call for strategies to prevent nutritional deficiencies and anaemia by improving dietary intake and food diversification, providing food fortification/supplementation with iron and other micronutrients, going hand-in-hand with education and training.

Other studies highlight that stunted children are more likely to be anaemic than non-stunted children due to long-term effects of low iron intake and other micronutrient deficiencies [18, 42]. We found a significant difference in stunting between the two surveyed districts in Nepal, which might again be due to the geographic variation in food consumption patterns including patterns of exclusive breastfeeding, hygiene behaviours, socioeconomic status, awareness level of caregivers, and concurrent infection with soil-transmitted helminths. Indeed, soil-transmitted helminths, particularly hookworm, have been reported as a major cause of anaemia, and these infections are highly clustered in poor neighbourhoods, governed by lack of WASH, improper solid and liquid waste management, and unhealthy food hygiene and safety measures [43]. Hookworm can cause chronic blood loss, with severity depending on the intensity of infection, the species of hookworm, host iron reserves, and other factors such as age and comorbidity [2]. In our study, we found higher odds for school-aged children to be anaemic if they were infected with any soil-transmitted helminth, but the association lacked statistical significance. The limited case numbers of the intestinal parasites and the relatively low infection intensity may be the reason. We collected only one stool sample, which was subjected to duplicate Kato-Katz thick smears, formalin-ether concentration, and wet mount examination. Hence, the reported prevalence of soil-transmitted helminths is an underestimation of the true infection prevalence [15, 30]. This issue is important, because it might have reduced the likelihood of documenting a significant relationship between helminth infection and anaemia [15]. Of note, a meta-analysis revealed that infection with hookworm is significantly associated with anaemia and up to 10% of anaemic cases could be averted by treating helminth infections [15, 43].

Our study has some limitations. First, the participation of households was affected by a major earthquake that occurred in April 2015. Second, the selected schools were not equally distributed between Dolakha and Ramechhap districts, which might limit inferences on differences between the two districts. However, we believe that the sites chosen for this study are characteristic for the main social-ecological diversity in Nepal. Third, the predominance of data from one district limits the generalizability of our results. Fourth, no specific data were available for some important risk factors of anaemia, such as deficiencies of vitamin A and of other micronutrients including riboflavin, folate, and vitamin B12. Fifth, we did not investigate the morphological appearance of red blood cells to differentiate anaemia due to vitamin B12 and folic acid deficiencies from anaemia due to iron deficiency [3].

Despite of these limitations, our study has several strengths. A major strength is the rigorous appraisal of diet diversity, anthropometric measurements, employing a suite of laboratory methods for the diagnosis of intestinal parasitic infections, quantification of Hb level for assessment of anaemia, and examination of clinical signs of malnutrition among school-aged children. An additional strength is the analytical approach taken (i.e., multivariate analysis), which allowed for adjustments of potential confounders, such as demographics, personal and behavioural factors, and socioeconomic status. Within the frame of the VgtS project, the observed low consumption of vitamin A-rich fruits and vegetables across the different groups of children will provide the basis for teaching children to plant and consume vitamin-A rich fruits and vegetables, to provide the school-aged children with seeds of the vitamin-A rich vegetables for home gardening, and to implement an awareness program on diet diversity, avoiding malnutrition, preventing anaemia, and lowering the risk of intestinal parasitic infections by children and their caregivers.

6.6. Conclusion

We conclude that the surveyed children in the selected schools in two districts of Nepal were markedly affected by anaemia, whilst awareness of anaemia among caregivers, regarding its effects, causes, and preventive measures was limited. The observed risk factors for anaemia among school-aged children confirmed a multi-factorial aetiology. Our findings suggest that the low levels of Hb among anaemic children might be due to dietary factors, inadequate consumption of animal products, infection with soil-transmitted helminths, low bioavailability of iron in a diet with a high consumption of starchy staples and legumes, and lack of awareness among caregivers. Taken together, these findings call for an integrated approach targeting children and parents. It could involve iron supplementation, periodic administration of anthelmintic drugs, and regular health programs promoting consumption of fruits and vegetables. Intervention campaigns, including regular mass deworming, should go hand-in-hand with the provision of setting-specific information, education, and communication strategies in order to prevent anaemia. The children surveyed here are at increased risk of inadequate development. Therefore it is particularly important that public health policies in Nepal put a specific focus on improving the health of children as young as 6 months and of women of reproductive age. We also recommend interventions – in addition to the VgtS project – such as increasing dietary diversity, consumption of fruits and vegetables, hygiene promotion to address malnutrition and intestinal parasitic infections to reduce anaemia among school-aged children in Nepal.

The national program in Nepal, conducted by the Ministry of Health and the Government of Nepal, has focused on vitamin A supplementation for children under the age of 5 years and on sexual and reproductive health issues among older adolescents. However, children aged 8-16 years are also vulnerable to soil-transmitted helminths, anaemia, and nutritional deficiency. Anaemia is associated with families' socioeconomic status and the caregivers' literacy status. Children with lower consumption of animal and plant foods were more likely to develop anaemia than those who more frequently consumed these types of food. Thus, we can conclude that the problem of anaemia is directly linked to some nutritional deficiencies resulting from the lower socioeconomic status of the family. It is important to note that, in the middle of the current cross-sectional survey, a series of earthquakes hit Nepal. A month after the earthquake, several interventions have been launched. Hence, the results communicated here will serve as a benchmark for longitudinal monitoring of surveyed school-aged children.

Declarations

Ethical considerations and consent to participate

Ethical approval was obtained from the "Ethikkommission Nordwest- und Zentralschweiz" (EKNZ) in Switzerland (reference no. UBE-15/02; date of approval: January 12, 2015), the institutional review board of Kathmandu University, School of Medical Sciences, Dhulikhel Hospital, Nepal (reference no. 86/14; date of approval: August 24, 2014), and the institutional review board, Nepal Health Research Council (reference no. 565; date of approval: November 11, 2014). The study is registered at the International Standard Randomized Controlled Trial Number register (identifier ISRCTN30840; date assigned: July 17, 2015). Written informed consent was received after explaining to the teachers and parents/guardian of participating child about the purpose of the study, procedures, and samples required and any benefits involved in the study. At the end of the study, children with intestinal parasitic infections were treated free of charge. Children with parasitic worm infections were administered a single oral dose of albendazole (400 mg), while children infected with *Giardia* *intestinalis* were given metronidazole (250 mg/kg) over five consecutive days. Children with anaemia were referred to nearby health centres.

Consent for publication

Not applicable

Availability of data and material

The data analysed for this study are not publicly available, as they are part of a PhD study pursued by the first author. However, the data are available from the corresponding author upon reasonable request and signature of a mutual agreement. The questionnaire (in English) is available upon request from the corresponding author.

Competing interest

The authors declare that they have no competing interests.

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Authors contributions

All listed authors contributed to the study design. AS coordinated the field and laboratory work, assisted in data collection, supervised the research assistants, performed the statistical analysis under the supervision of CS, and drafted the manuscript. PO, JG, SE, SS, RK, CS, JU, and GC contributed to the interpretation of the data, manuscript writing and revisions. All authors read and approved the final manuscript.

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7. Intestinal parasitic infections and associated risk factors among schoolchildren in Dolakha and Ramechhap Districts, Nepal: a cross-sectional study

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7.1. Abstract

Background: Infections with soil-transmitted helminths and pathogenic intestinal protozoa pose a considerable public health burden, particularly in low- and middle-income countries, including Nepal. We assessed the extent of intestinal parasite infections among schoolchildren in two districts of Nepal and determined underlying risk factors.

Methods: A cross-sectional survey was conducted between March and May 2015 in the districts of Dolakha and Ramechhap, Nepal. A total of 708 children, aged 8–16 years from 16 purposively selected schools, were enrolled. Each child provided a single stool sample that was subjected to a suite of copro-microscopic diagnoses for intestinal protozoa and helminths. Drinking water samples from different sources at schools (n = 29), community places (n = 43) and households (n = 562) were analysed for contamination with thermotolerant coliforms (TTC). A questionnaire was administered to determine individual- and household-level risk factors of intestinal parasite infections. Self-reported symptoms were assessed and a clinical examination was undertaken by a physician. Haemoglobin was measured and used as a proxy for anaemia. Mixed logistic regression models were applied to investigate associations.

Results: The overall prevalence of intestinal parasite infections was 39.7%. *Trichuris trichiura* (30.9%), *Giardia intestinalis* (30.5%) and hookworm (30.2%) were the predominant intestinal parasite infections. Children from households lacking soap for handwashing were at higher odds of intestinal parasite infections than children who had soap [adjusted odds ratio (aOR) 1.81; 95% confidence interval (CI): 1.13–2.89; P = 0.01]. Children from households without freely roaming domestic animals showed lower odds of *G. intestinalis* compared to children from households with freely roaming animals (aOR 0.52; 95% CI: 0.33–0.83; P = 0.01). One out of three (31.0%) children suffered from fever and 22.4% had watery diarrhoea within a two-week recall period. Anaemia was diagnosed in 23.6% of the children. Water contamination with TTC showed no clear association with intestinal parasite infection.

Conclusions: Intestinal parasites are common among schoolchildren in the two surveyed districts of Nepal. An important risk factor was lack of soap for handwashing. Our findings call for efforts to control intestinal parasite infection and emphasis should be placed on improvements in water, sanitation and hygiene interventions.

Trial registration: ISRCTN17968589 (http://www.isrctn.com/ISRCTN17968589; date assigned: 17 July 2015).

Keywords: Helminths, Hygiene, Intestinal protozoa, Nepal, Sanitation, Water

7.2. Background

Intestinal parasite infections caused by soil-transmitted helminths (e.g. *Ascaris lumbricoides*, hookworm and *Trichuris trichiura*), and pathogenic intestinal protozoa (e.g. *Giardia intestinalis* and *Entamoeba histolytica*) are a major public health concern in low- and middle-income countries (LMICs) [1, 2]. More than five billion people are at risk of infection with soil-transmitted helminths and over one billion people are infected, particularly in LMICs [1, 3, 4]. Morbidities due to intestinal parasite infections vary from individual to individual and depend on the type, number and intensity of intestinal parasite and host factors (e.g. preschool- and school-aged children and women of reproductive age are at particular risk) [5, 6]. In 2012, the World Health Organization (WHO) estimated that 270 million preschool-aged children and > 600 million school-aged children lived in areas where helminths and intestinal protozoa are intensively transmitted, and thus warrant interventions [7]. The highest prevalence and intensity of infection with soil-transmitted helminths is usually observed in school-aged children [8]. Chronic helminth infections are manifested in delayed physical and mental development, anaemia and protein-energy malnutrition [3, 5, 9, 10]. Intestinal parasite infections are intimately linked to poverty and inadequate access to water, sanitation and hygiene (WASH). The impact of unsafe WASH on morbidity is particularly severe in malnourished children [11, 12]. The WHO recommends periodic deworming of preschool- and school-aged children as a public health intervention. In places where the prevalence of soil-transmitted helminth exceeds 20%, deworming should be done at least once every year [13].

In Nepal, intestinal parasite infections are widespread and polyparasitism is a concern, as infections with multiple intestinal parasite species can exacerbate morbidity [6, 14]. However, there is a paucity of data on intestinal parasite infection among school-aged children in Nepal. Indeed, only few studies investigated intestinal parasite infections and found considerable variation in the prevalence in school-aged children in different parts of Nepal. The most common helminth species infecting Nepalese children reported in the literature were hookworm, *A. lumbricoides* and *T. trichiura*, while common intestinal protozoa were *E. histolytica* and *G. intestinalis* [15–18]. Little is known about infection intensity profiles and underlying risk factors in Nepal.

To fill this gap, a cross-sectional survey was carried out focusing on school-aged children aged 8–16 years in two districts of Nepal. Our aim was to determine the prevalence of intestinal parasitic infections and to assess underlying risk factors. The study results were utilized to design complementary school-based interventions to improve the nutritional and health status of schoolchildren. Of note, the study reported here was readily embedded into a multi-country, multi-sectorial project entitled "Vegetables go to School: improving nutrition through agricultural diversification" [19].

7.3. Methods

Study design, sites and participants

The baseline cross-sectional study was conducted in the Dolakha and Ramcehhap districts, situated in the eastern part of Nepal, covering surface areas of 2191 and 1546 km^2, respectively. There are 51 village development committees (VDCs) in Dolakha District and 45 in Ramechhap District. Our cross-sectional survey was implemented from March to May 2015, in 32 VDCs in Dolakha District and 8 VDCs in Ramechhap District. The populations in Ramechhap and Dolakha districts were 202,646 and 186,557 people, respectively. Out of 43,910 households in Ramechhap District, 34,902 households had access to piped drinking water, whereas 3429 households depended on uncovered wells and 1242 households used river water for drinking. In Ramechhap District, 35,547 households had access to piped drinking water, while 1495 households depended on uncovered wells and 537 households used river water for drinking. With regard to electricity as a source of lighting, 19,970 and 37,349 households in Ramechhap and Dolakha, respectively, had access. In terms of sanitation facilities, 16,047 and 13,860 households lacked a toilet facility at home in Ramechhap and Dolakha, respectively. With regard to climate, Ramechhap District has a higher percentage of tropical (18%) and sub-tropical (42.1%) climate, whereas Dolakha District has a higher percentage of temperate climate (28.5%).

The two districts and the surveyed schools were selected as VgtS project sites by national authorities from the National Agricultural Research Councils (NARC), Ministry of Education (MoE) and Ministry of Health (MoH) of Nepal. Sixteen schools were purposely selected within the frame of the VgtS project based on the following criteria: (i) non-boarding public schools teaching at least up to grade 8 with a minimum of 150 students; (ii) schools located in rural or peri-urban areas that can be reached within a maximum of 1 h walking distance from a major road; (iii) no earlier involvement in a school garden programme; (iv) availability of at least 300 m^2 of land used for gardening and access to a source of water for irrigation; (v) not located in a commercial vegetable growing area; and (vi) the school principal being willing to participate in the project (Fig. 1). Overall, epidemiological data were obtained from 708 children aged 8–16 years. Details of the larger VgtS study and participants' characteristics have been described elsewhere [20].

Questionnaire survey

We designed, pre-tested and administered a semi-structured questionnaire to the schoolchildren, their caregivers and school principals. For schoolchildren, demographic data (age and sex) and information regarding knowledge, attitude and practices (KAP) of personal hygiene were collected. For caregivers, data on socioeconomic status, WASH behaviour and medical history of children in the preceding 2 weeks were collected. For school principals, we obtained data on school-based WASH conditions.

Our questionnaire was developed in English, translated into Nepali and back translated for validation. Pre-testing of the questionnaire was done in selected schools and households outside the study area, characterized by similar geographical and socioeconomic features. Research assistants were trained for data collection. Reliability of the information was ensured by interviewing the schoolchildren and their caregivers in their mother tongue by research assistants who grew up in the study area. For quality control, a principal researcher accompanied each research assistant to three households.

Stool examination

A pre-labelled plastic container was provided to each schoolchild, along with specific information for collection of at least 10 g of their own morning stool the following day, upon completion of the questionnaire survey [19]. Stool samples were transferred to the laboratory and stored at 4˚C pending further analysis [21]. The samples were examined following WHO standard operating procedures (SOPs) [22]. First, approximately 2 g of stool was prepared on a single slide with the saline wet mount method for microscopic detection of intestinal parasites [23]. For quality control, 10% of the slides were re-examined by a senior technician [6]. Egg counts for helminths were compared with the original readings. Whenever discrepancies were observed (e.g. negative *versus* positive results or helminth egg counts differing by over 10%), the slides were re-read and results discussed until agreement was reached [6]. Secondly, duplicate Kato-Katz thick smears using 41.7 mg templates were prepared on microscope slides [6, 24, 25]. Slides were allowed to clear for 30 min prior to examination under a light microscopy at a magnification of 400× by experienced laboratory technicians [6]. Eggs were counted and recorded for each helminth species separately [6, 26]. Infection intensity was expressed as the number of eggs per gram of stool (epg) by multiplying egg counts with a factor of 24 [27]. Thirdly, a formalin-ether concentration technique was used to detect helminth eggs and larva or cyst of intestinal protozoa [28].

For the analysis of parasitological data, only schoolchildren who provided a sufficient quantity of stool (at least 10 g) and had complete data records were included in the final analysis.

Helminth infection intensities were grouped into light, moderate and heavy, according to WHO cut-offs [22]. In brief, light, moderate and heavy classes for *A. lumbricoides* infections were 1–4999 epg, 5000–49,999 epg and ≥ 50,000 epg; for *T. trichiura* classes were 1–999 epg, 1000–9999 epg and ≥ 10,000 epg; and for hookworm, classes were 1–1999 epg, 2000–3999 epg and ≥ 4000 epg [32]. The Kato-Katz technique is characterized by a low diagnostic accuracy for *Enterobius vermicularis*, hence no attempt was made for determining infection intensity of this helminth species [33].

Clinical examination

Haemoglobin (Hb) was assessed in each child by collecting a fingerpick blood sample using a B-haemoglobin photometer (Hemocue AB, Angelholm, Sweden) [6]. Morbidity information for the 2 weeks prior to the survey was obtained from each child and their caregivers by symptoms recall (e.g. fever, watery diarrhoea, bloody diarrhoea and mucus in stool) and by clinical examination (e.g. hepatomegaly and pale conjunctiva) [6]. An experienced paediatrician conducted clinical examinations. By palpating the liver lobe (left) along the xiphoid-umblicus line (supine position), hepatomegaly was determined [34]. It was classified as present or absent when the left liver lobe was palpable/not palpable [6, 34].

Anaemia was determined according to age-specific Hb levels using WHO cut-offs. Anaemia was defined as a level of Hb < 11.0 g/dl for children aged 8–11 years, < 11.9 g/dl for children aged 12–14 years and < 12.9 g/dl for children aged ≥ 15 years [35]. Severe anaemia was defined as Hb below 8 g/dl, while moderate anaemia was considered as Hb between 8 and 10.9 g/dl [35].

Water quality analysis

Sampling of water sources and details of the assessment procedure have been described elsewhere [20].

Statistical analysis

Details of data management and statistical analyses have been described elsewhere [20]. In brief, parasitological data were entered into a MS Excel 2010 spreadsheet (Microsoft, Redmond, WA, USA). Internal consistency checks were performed, and errors removed by comparing the entries with the original laboratory sheets. Schoolchildren with complete data records were included in the final analysis [6]. Children were classified into two age groups (8–12 and 13–16 years) for summary statistics [6].

We employed Chi-square statistics to assess differences in distributions for categorical variables. Risk factors of intestinal parasite infections were assessed using mixed logistic regression models with random intercepts for schools. Children's age, sex, socioeconomic status of caregivers and district were considered *a priori* as potential confounders and hence included in multivariate regression models. A new variable for socioeconomic status was created using factor analysis to calculate a wealth index based on household assets, using a k-means procedure. Similarly, a hygiene variable was created using factor analysis with two conceptually similar categorical variables: specific types and frequency of handwashing methods, using a k-means procedure [20]. The socioeconomic and hygiene behaviour of children was categorised as low, medium or good, depending on whether the factor score was below, at or above the median value of obtained scores [20]. Results were presented as crude and adjusted odds ratios (aORs) with their corresponding 95% confidence intervals (CIs). For the analysis of the outcomes (i) any intestinal parasite infection; (ii) *T. trichiura*; and (iii) *G. intestinalis*, 24 variables were considered as potential predictors based on the extent literature [14]. For all multivariate regression models, $P < 0.2$ in univariate analysis was used for the variable entry criteria for the final model. The final model was obtained using backward selection with the same level of $P < 0.2$ [20]. The differences and associations were considered statistically significant if $P \leq 0.05$ [36]. The population attributable fraction (PAF) was estimated for significantly associated risk factors. Statistical analyses were performed using STATA version 14 (Stata Corporation, College Station, TX, USA) [20].

7.4. Results

<u>Compliance and characteristics of study population</u>

A total of 708 schoolchildren participated in the study. In the midst of our survey, a major earthquake occurred, damaging most of the houses. As a result, 146 caregivers could not be reached. Hence, only 562 households were retained for our multivariate analysis.

There were 369 female participants (52.1%). The mean age of the schoolchildren was 12.8 years [standard deviation (SD) 1.2 years] with 15.2% aged 8–12 years and the remaining 84.8% aged 13–16 years. There was no statistically significant sex-difference according to age groups ($P = 0.44$). Three-quarters of the schoolchildren belonged to the Tamang (37.9%) and Chhetri ethnicities (37.4%). Most of the Tamang lived in the Dolakha District (77.9%). Regarding caregivers' characteristics, the mean age was 40.8 years (SD 8.5 years). More than a third (37.4%) of the caregivers had no formal education and 81.5% of the caregivers were engaged in farming as their main occupation. The caregivers' houses mainly consisted of corrugated iron walls (73.8%), corrugated iron roofs (72.4%) and a mud floor (93.4%). Livestock was kept by 90.2% of the households, of which 45.4% were reported to roam freely inside the house court. Goats were the most commonly present livestock (79.4%), followed by poultry (74.9%). For further details of socio-demographic characteristics, the reader is referred elsewhere [20].

WASH characteristics of schools and households

All 16 schools had some kind of water infrastructure and 15 of them had access to water at some point during the day. Only 6.3% of school principals reported cleaning the sanitation facility at least once a week. Six out of 16 schools had not implemented a hygiene programme within the past 2 months.

About half (49.0%) of the households had no piped water connected to their house, yet 78.1% of the surveyed households reported having sufficient drinking water throughout the year. Most households (86.5%) did not treat drinking water. Almost a third (29.7%) of the households had no latrine and 25.8% said that they had no soap. Among those household with a latrine (70.1%), most reported to have a water seal latrine (50.4%). Members of 16.8% of the households were either defecating in the bush or river/swamps. Slightly more than half (51.4%) of the households reported that they do not deposit their solid waste safely. Further details of WASH-specific information and behaviour at the unit of the school and household have been reported elsewhere [20].

Drinking water quality

Contamination of water samples by TTC was observed in 76.9% of the samples collected at schoolchildren's point-of-use, in 27.4% of the samples obtained from households and in 39.5% of the samples from community water sources (e.g. spring, tap, etc.). We found significant differences in household water samples contaminated with TTC by district (36.4% in Ramechhap *versus* 25.0% in Dolakha; χ^2 = 6.13, P = 0.01). For additional details of drinking water quality information at the unit of the school and household, the reader is invited to consult our previous paper [20].

WASH KAP of schoolchildren and caregivers

Table 7.1 summarises the KAP results obtained from schoolchildren and their caregivers. On the basis of our questionnaire administered to schoolchildren, 74.7% reported washing their hands with soap and water after defecation, while 72.6% reported doing so before eating and 58.0% after playing. Approximately 4% of the children reported not using the latrine at school. One out of 100 children reported defecating either in the fields around their home or behind the latrines at home or school. The overall hygiene behavior of the children, including the occasions and materials used for hand washing, differed significantly by district (χ^2 =

19.42, P < 0.001), although no significant differences were found with regard to caregivers sanitary practices (χ^2 = 2.70, P = 0.26). The majority of surveyed schoolchildren (90.0%) reported that they drank water from the sources provided at the school. Only 10.2% of children reported that intestinal parasite infections were transmitted by dirty water. Around 8% of the children had a misconception that intestinal parasite infection may occur after eating chocolates or other sugary products.

More than 50.0% of the caregivers reported using a private tap as their main drinking water source regardless of the season. Most households reported fetching their drinking water in a metal (47.0%) or a plastic container (45.9%) and 61.8% stated that they wash these drinking water containers daily with soap. Only 19.8% reported treating drinking water before consumption. A significant difference was observed in drinking water treatment (22.0% in Ramechhap *versus* 11.3% in Dolakha; χ^2 = 9.25, P = 0.01). Most of the caregivers (70.6%) had heard about intestinal parasites. Preventive measures, as reported by caregivers, included cutting fingernails (37.7%), drinking clean water (32.7%), washing fruits and vegetables (16.7%) and wearing shoes (9.3%).

Table 7.1: Questionnaire findings on KAP among schoolchildren and water quality results in school in Dolakha and Ramechhap districts of Nepal (March and May 2015)

Children	Overall [n(%)] (N=708)	Dolakha [n (%)] (n=555)	Ramechhap [n (%)] (n=153)	*P*-value
KAP indicators				
Knowledge about handwashing				
Before eating	525 (74.2)	427 (76.9)	98 (64.1)	0.01
After eating	434 (61.3)	357 (64.3)	77 (50.3)	0.01
After playing	422 (59.6)	345 (62.2)	77 (50.3)	0.01
After the toilet	534 (75.4)	427 (76.9)	107 (69.9)	0.08
Practice about handwashing				
Before eating	0 (0.0)	0 (0.0)	0 (0.0)	-
After eating	473 (66.8)	387(69.7)	86 (56.2)	0.01
After playing something	428 (60.6)	345 (62.2)	83 (54.3)	0.08
After the toilet	641 (90.5)	505 (91.0)	136 (88.9)	0.43
Handwashing with:				
Water	687 (97.0)	540 (97.3)	147 (96.1)	0.43
Ash	17 (2.4)	12 (2.2)	5 (3.3)	0.43
Mud,soil	4 (0.6)	4 (0.7)	0 (0.0)	0.29
Soap	689 (97.3)	539 (97.1)	150 (98.0)	0.53
Why handwashing with soap				
Clean germs	196 (27.7)	137 (24.7)	59 (38.6)	-
Prevent illness	365 (51.5)	306 (55.1)	59 (38.6)	
Clean hands	94 (13.3)	61 (11.0)	33 (21.6)	
Don`t know	78 (11.0	65 (11.7)	13 (8.5)	
Hygiene				
Better category	261 (36.9)	225 (40.5)	36 (23.5)	0.01
Middle category	211 (29.8)	165 (29.7)	46 (30.1)	
Poor category	236 (33.3)	165 (29.7)	71 (46.4)	
Sanitary plactices at school				
Use latrine at school	679 (95.9)	543 (97.8)	136 (88.9)	<0.001

No latrine use	29 (4.10)	12 (2.2)	17 (11.1)	
Reasons for not using latrine (n=17)				
Dirty	12 (70.6)	1 (50.0)	11 (73.3)	0.09
No soap	1 (5.9)	0 (0.0)	1 (6.7)	
Not functional	2 (11.8)	0 (0.0)	2 (13.3)	
Other	1 (5.9)	0 (0.0)	1 (6.7)	
No response	1 (5.9)	1 (50.0)	0 (0.0)	
Defecation instead (n=17)				
Bush	13 (76.5)	2 (100.0)	11 (73.3)	0.40
Behind the latrine	4 (23.5)	0 (0.0)	4 (26.7)	
Opinion on dirty water causing illness				
Yes	694 (98.0)	544 (98.0)	150 (98.0)	0.99
No	14 (2.0)	11 (2.0)	3 (2.0)	
Knowledge on illnesses caused by dirty water				
Diarrhoea	458 (64.7)	377 (67.9)	81 (52.9)	<0.001
Cholera	138 (19.5)	108 (19.5)	30 (19.6)	0.97
Skin irritations	47 (6.6)	31 (5.6)	16 (10.5)	0.03
Icterus	12 (1.7)	11 (2.0)	1 (0.7)	0.26
Typhus	41 (5.8)	38 (6.9)	3 (2.0)	0.02
Malaria	14 (2.0)	12 (2.2)	2 (1.3)	0.50
Eye irritations/ diseases	9 (1.3)	9 (1.6)	0 (0.0)	0.11
Worms, parasites	72 (10.2)	58 (10.5)	14 (9.2)	0.64
Perception on becoming sick by not washing hands by schoolchildren				
Yes	696 (98.3)	543 (97.8)	153 (100.0)	0.50
No	5 (0.7)	5 (0.9)	0 (0.0)	
Not sure	7 (1.0)	7 (1.3)	0 (0.0)	
Drinking water				
Drinking water from school	637 (90.0)	535 (96.4)	102 (66.7)	<0.001
Bringing water from home	102 (14.4)	67 (12.1)	35 (22.9)	0.01

P-value calculated by $\chi 2$ test

Results from clinical survey

Table 7.2 presents the frequency of reported symptoms of schoolchildren, stratified by sex and age group. Of the children surveyed, 11.5% reported being sick the day before the survey; the most frequently reported symptom was fever (30.9%). Watery diarrhoea, mucus in the stool and bloody diarrhoea were reported by 22.4, 2.9 and 1.4% of the caregivers, respectively. There was a significant difference in self-reported fever by district (32.9% in Dolakha *versus* 23.7% in Ramechhap; χ^2 = 3.71, P = 0.05). The prevalence of reported diarrhoea was significantly higher in children aged 8–12 years compared to their older counterparts (P = 0.01). The most frequently diagnosed clinical sign was pale conjunctiva (9.8%), followed by hepatomegaly (0.2%).

Overall, 23.6% of the children were found to be anaemic with no significant difference by sex (56.1% in females *versus* 48.4% in males; χ^2 = 2.39, P = 0.12) nor age group (22.7% in children aged 13–16 years *versus* 26.2% in children aged 8–12 years; χ^2 = 0.65, P = 0.42). Significant differences were observed for anaemia by district (33.1% in Ramechhap *versus* 21.0% in Dolakha; χ^2 = 7.60, P = 0.006). The mean Hb concentration found was 12.6 g/dl (SD 1.2 g/dl), ranging from a minimum of 7.7 g/dl to a maximum of 16.5 g/dl.

Table 7.2: Frequency of clinical outcomes obtained from physical examination of children in Dolakha and Ramechhap districts, Nepal in 2015, stratified by sex and age group (N=562)

Clinical outcomes	Overall	Sex				Age group (years)				District			
	[N (%)] [N=708]	Males [n (%)] [n=339]	Females [n (%)] [n=369]	χ^2	*P*-value	8-12 [n (%)] [n=108]	13-16 [n (%)] [n=600]	χ^2	*P*-value	Dolakha [n (%)] [n=555]	Ramechhap [n (%)] [n=153]	χ^2	*P*-value
Symptoms in past two weeks													
Fever	174 (31)	84 (30)	90 (31.9)	0.24	0.62	37 (30.3)	137 (31.1)	0.02	0.86	146 (32.9)	28 (23.7)	3.7	0.05
Diarrhoea	126 (22.4)	66 (23.6)	60 (21.3)	0.43	0.51	38 (31.2)	88 (20)	6.82	0.01	104 (23.4)	22 (18.6)	1.2	0.27
Blood in stool	8 (1.4)	5 (1.8)	3 (1.1)	0.52	0.47	2 (1.6)	6 (1.4)	0.05	0.82	4 (0.9)	4 (3.4)	4.1	0.04
Mucus in stool	16 (2.9)	5 (1.8)	11 (3.9)	2.27	0.13	5 (4.1)	11 (2.5)	0.88	0.35	15 (3.4)	1 (0.9)	2.2	0.14
Physical examination													
Hepatomegaly	1 (0.2)	1 (0.4)	0	1.00	0.32	1 (0.8)	0	3.61	0.05	1 (0.23)	0	0.3	0.60
Pale conjunctiva	55 (9.8)	24 (8.6)	31 (11)	0.93	0.33	16 (13.1)	39 (8.9)	1.96	0.16	43 (9.7)	12 (10.2)	0.02	0.88

P- values were obtained by X^2 test

Prevalence and intensity of intestinal parasite infections

Table 7.3 summarises the overall prevalence of intestinal parasite infection, stratified by sex, age group and district. The overall prevalence of intestinal parasite infection considering both soil-transmitted helminths and intestinal protozoa was 39.7%. The predominant helminth species were *T. trichiura* (30.9%) and hookworm (30.2%), followed by *A. lumbricoides* (26.1%). *Enterobius vermicularis* and *Strongyloides stercoralis* were detected only rarely (0.4 and 0.3%, respectively) (Fig. 7.1 and 7.2). Most of the soil-transmitted helminth infections were of light intensity. The cumulative prevalence of intestinal protozoa infection was 30.5% with *G. intestinalis* (30.5%) identified as the predominant species. Occurrence of double or triple infections was frequent. For example, 13.8% of the study participants had a triple infection with hookworm, *T. trichiura* and *G. intestinalis*.

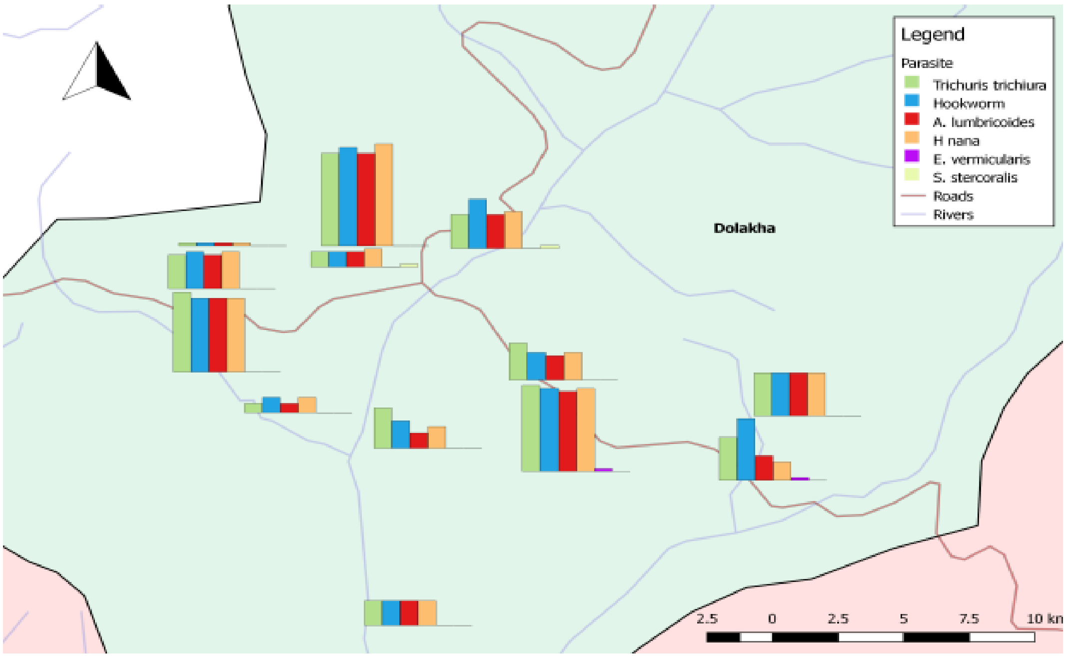

Figure 7.1: Intestinal parasites in 13 schools of Dolakha District.

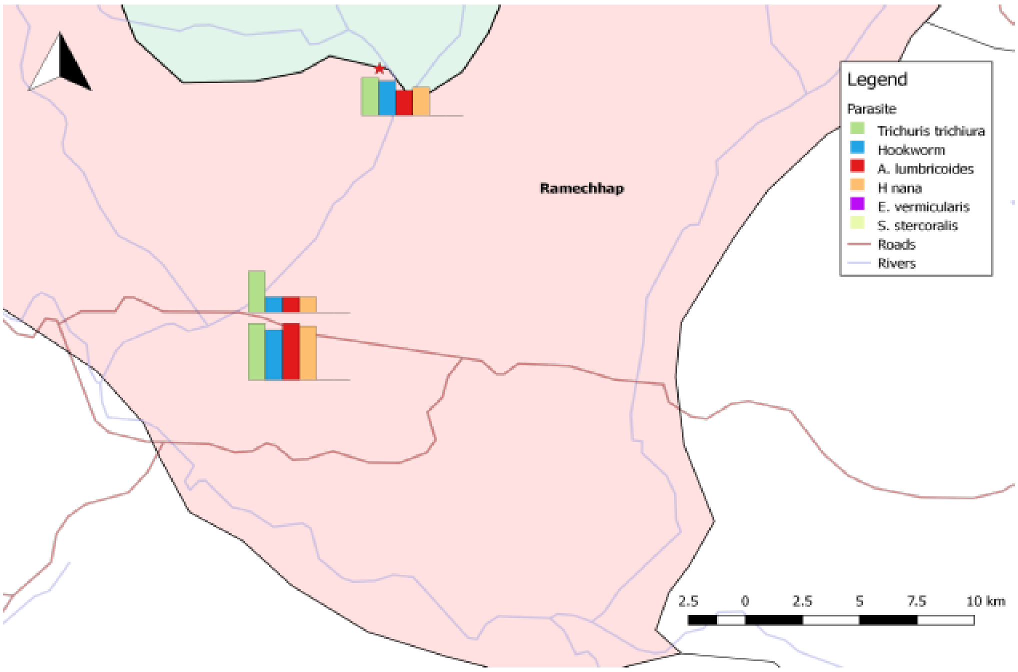

Figure 7.2: Intestinal parsites in three schools of Ramechhap District

The difference between the overall prevalence of intestinal parasite infection by district was not significant (43.5% in Dolakha *versus* 36.4% in Ramechhap; χ^2 = 1.89, P = 0.17). The overall prevalence of intestinal parasite infection was similar in males compared to females (40.4% *versus* 39.0%; χ^2 = 0.14, P = 0.71).

Table 7.3: Intestinal parasitic infections among schoolchildren in Dolakha and Ramechhap districts of Nepal between March and May 2015

Parasite	Prevalence [n (%)] [N=708]	Sex				Age group				District			
		Male [n= 339]	Female [n=369]	χ^2	*P*-value	8-12 years [n=108]	13-16 years [n=600]	χ^2	*P*-value	Dolakha [n=555]	Ramechhap [n=153]	χ^2	*P*-value
Nematodes													
Ascaris lumbricoides	185 (26.1)	87 (25.7)	98 (26.6)	0.07	0.79	21 (19.4)	164 (27.3)	2.95	0.09	154 (27.8)	31 (20.3)	3.48	0.06
Trichuris trichiura	219 (30.9)	109 (32.2)	110 (29.8)	0.45	0.50	27 (25.0)	192 (32.0)	2.10	0.15	176(31.7)	43 (28.1)	0.73	0.39
Hookworm	214 (30.2)	99 (29.2)	115 (31.2)	0.32	0.57	27 (25.0)	187 (31.2)	1.65	0.12	182 (32.8)	32 (20.9)	8.02	0.01
Strongyloides stercoralis	2 (0.3)	1 (0.3)	1 (0.3)	0.01	0.95	0 (0.0)	2 (0.3)	0.36	0.55	2 (0.4)	0 (0.0)	0.55	0.46
Enterobius vermicularis	2 (0.4)	1 (0.4)	1 (0.4)	0.01	0.96	0 (0.0)	2 (0.5)	0.39	0.53	2 (0.5)	0 (0.0)	0.62	0.43
Cestodes													
Hymenolepis nana	195 (27.5)	90 (26.6)	105 (28.5)	0.32	0.57	24 (22.2)	171 (28.5)	1.81	0.18	164 (29.6)	31 (20.3)	5.18	0.02
Total faecal-oral transmitted helminths	250 (35.3)	120 (35.4)	130 (35.2)	0.01	0.96	31 (28.7)	219 (36.5)	2.44	0.12	203 (36.6)	47 (30.7)	1.80	0.18
Intestinal protozoa													
Giardia intestinalis	216 (30.5)	101 (29.8)	115 (31.2)	0.16	0.69	22 (20.4)	194 (32.3)	6.18	0.01	176 (31.7)	40 (26.1)	1.75	0.19
Total intestinal protozoa	216 (30.5)	101 (29.8)	115 (31.2)	0.16	0.69	22 (20.4)	194 (32.3)	6.18	0.01	176 (31.7)	40 (26.1)	1.75	0.19

P- values were obtained by X^2 test

Risk factors for intestinal parasite infections

Results from the logistic regression analyses are given in Table 7.4. Age was significantly associated with the overall intestinal parasite infection (children aged 8–12 years had a lower odds of infection compared with their older counterparts (OR 0.61; 95% CI: 0.38–0.99, *P* = 0.04) (Table 4). Schoolchildren from households who do not have soap for handwashing were at a higher odds of intestinal parasite infection (aOR 1.81; 95% CI: 1.13–2.89, *P* = 0.01). No statistically significant association was found between intestinal parasite infection and sources of drinking water, containers used for fetching water or treatment of water. Children from households without sanitation facilities were negatively associated with *T. trichiura* (aOR 0.52; 95% CI: 0.29–0.92, *P* = 0.02). Domestic animals held outside the household were negatively associated with *G. intestinalis* and the association was statistically significant (aOR 0.52; 95% CI: 0.33–0.83; *P*=0.01).

Population-attributable risk analysis suggested that an estimated 11.3% of intestinal parasite infections might have been averted through handwashing with soap. An estimated 15.6% of *G. intestinalis* infections might have been averted if animals were not allowed to roam freely inside the household.

Table 7.4: Results from univariate and multivariate logistic regression analysis for parasitic infection

Risk factor		Any parasitic infection [n=236)]						*Trichuris trichuira* [n=185]						*Giardia lamblia* [n=181]					
	[n (%)]	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	[N=562]	OR	95% CI	*P*	aOR	95% CI	*P*	OR	95% CI	*P*	aOR	95% CI	*P*	OR	95% CI	*P*	aOR	95% CI	*P*
Sex																			
Female	282 (50.2)	1.00			1.00			1.00			*			1.00			*		
Male	280 (49.8)	1.10	0.77-1.58	0.60	1.09	0.75-1.59	0.64	1.15	0.79-1.67	0.47	1.10	0.75-1.62	0.63	0.99	0.68-1.44	0.96	0.96	0.65-1.41	0.82
Age																			
13-16 years	440 (78.3)	1.00																	
8-12 years	122 (21.7)	0.65	0.42-1.03	0.07	0.61	0.38-0.99	0.04	0.76	0.47-1.22	0.26	0.74	0.45-1.21	0.23	0.66	0.41-1.08	0.10	0.62	0.37-1.03	0.07
District																			
Dolakha	444 (79.0)	1.00			*			1.00			*			1.00			*		
Ramechhap	118 (21.0)	0.77	0.25-2.39	0.65	0.93	0.30- 2.90	0.90	0.90	0.29-2.80	0.86	0.87	0.28-2.64	0.80	0.78	0.28-2.22	0.64	0.86	0.38-1.93	0.71
Hygiene behavior																			
Lower category	245 (31.1)	1.00						1.00						1.00					
Middle category	142 (25.3)	0.87	0.55-1.38	0.56				0.96	0.61-1.52	0.87				1.22	0.78-1.91	0.39			
Higher category	175 (31.1)	0.89	0.57-1.37	0.59				1.07	0.66-1.71	0.79				1.08	0.67-1.74	0.75			
Drinking water consumption																			
From school	491 (87.4)	1.00						1.00						1.00					
From home	71 (12.6)	0.85	0.48-1.49	0.56				0.80	0.44-1.44	0.45				0.95	0.53-1.70	0.87			
Water risk behavior																			
Playing (yes vs no)	173 (30.8)	1.12	0.76-1.66	0.57				1.12	0.74-1.68	0.59				0.97	0.64-1.46	0.88			
Fishing (yes vs no)	68 (12.1)	1.04	0.59-1.82	0.89				1.11	0.62-1.97	0.73				1.27	0.72-2.25	0.41			
Laundry (yes vs no)	199 (35.4)	1.23	0.82-1.85	0.32				1.06	0.69-1.61	0.80				1.10	0.72-1.68	0.66			
Domestic chores (yes vs no)	142 (25.3)	1.18	0.75-1.87	0.48				1.02	0.63-1.64	0.94				1.24	0.77- 2.00	0.37			
Sanitary practices																			
Using latrine at school (yes vs no)	550 (97.9)	0.69	0.26-1.85	0.47				0.95	0.34-2.64	0.92				0.77	0.28-2.09	0.60			
Ethnicity of children																			
Tamang	213 (37.9)	1.00						1.00						1.00					
Brahmin	101 (18.0)	1.08	0.62-1.90	0.78	1.20	0.67-2.17	0.53	1.07	0.60-1.88	0.83	1.09	0.60-1.97	0.78	1.18	0.67-2.08	0.57	1.18	0.65-2.13	0.58
Chhetri	210 (37.4)	0.97	0.61-1.53	0.89	1.00	0.62-1.62	0.99	1.09	0.68-1.74	0.73	1.09	0.67-1.79	0.72	1.17	0.73-1.88	0.51	1.17	0.72-1.89	0.52
Newar	33 (5.9)	1.32	0.55-3.16	0.54	1.29	0.52-3.17	0.58	1.36	0.54-3.40	0.52	1.30	0.51-3.32	0.58	0.92	0.35-2.36	0.86	0.99	0.38-2.59	0.99
Janajati	5 (0.9)	3.60	0.50-25.97	0.20	3.98	0.52-30.54	0.18	1.92	0.26-13.97	0.52	2.17	0.28-16.8	0.46	6.47	0.87-48.26	0.07	7.94	0.96-65.44	0.05
Caregiver`s education																			
Never went to school	210 (37.4)	1.00						1.00						1.00			*		
Primary education	144 (25.6)	0.91	0.55-1.51	0.71	1.00	0.60-1.69	1.00	0.79	0.46-1.35	0.39				0.77	0.45-1.30	0.33	0.82	0.47-1.41	0.47
Secondary education	143 (25.4)	0.98	0.54-1.79	0.96	1.27	0.67-2.41	0.47	1.18	0.64-2.18	0.60				0.80	0.44-1.47	0.47	1.05	0.55-1.99	0.88
Higher education	65 (11.6)	0.70	0.31-1.62	0.41	0.90	0.38-2.16	0.82	0.77	0.33-1.82	0.56				0.71	0.31-1.63	0.42	0.75	0.32-1.77	0.51
Caregiver`s occupation																			
Farmer	458 (81.5)	1.00			*			1.00			*			1.00					
Public services	39 (6.9)	0.62	0.29-1.37	0.24	0.55	0.24-1.25	0.15	0.56	0.23-1.37	0.21	0.43	0.17-1.11	0.08	0.71	0.31-1.63	0.42	0.67	0.28-1.61	0.37
Business	40 (7.1)	0.87	0.39-1.93	0.73	0.87	0.37-2.04	0.75	1.15	0.51-2.59	0.74	1.10	0.46-2.64	0.83	0.51	0.21-1.26	0.14	0.47	0.18-1.21	0.12
Other	25 (4.5)	0.36	0.13-0.96	0.04	0.35	0.13-0.99	0.05	0.44	0.16-1.24	0.12	0.37	0.13-1.08	0.07	0.32	0.11-0.95	0.04	0.29	0.10-0.88	0.03
Socioeconomic status																			

Poor	298 (53.0)	1.00						1.00						1.00					
Average	215 (38.3)	1.34	0.91-1.98	0.14	1.29	0.86-1.92	0.22	1.12	0.75- 1.68	0.59	1.08	0.71-1.64	0.72	1.16	0.77-1.73	0.48	1.10	0.73-1.67	0.65
High	49 (8.7)	1.02	0.53-1.99	0.95	0.88	0.45-1.76	0.73	0.98	0.49-1.96	0.96	0.95	0.46-1.97	0.90	1.05	0.53-2.09	0.89	1.04	0.51-2.11	0.92
Drinking water in dry season																			
Private tap	287 (51.1)	1.00						1.00						1.00					
Protected spring	13 (2.3)	1.84	0.50-6.81	0.36				1.79	0.48-6.62	0.39				2.37	0.58- 9.68	0.23			
Public tap	36 (6.4)	1.31	0.51-3.26	0.53				1.06	0.43-2.60	0.90				1.17	0.50-2.74	0.72			
Other	226 (40.2)	1.16	0.75-1.79	0.50				1.29	0.82-2.02	0.27				1.02	0.65-1.60	0.94			
Drinking water in rainy season																			
Private tap	285 (50.7)	1.00						1.00			*			1.00					
Protected spring	1 (0.2)	na						na			na			na					
Public tap	44 (7.8)	1.12	0.49-2.54	0.79				1.05	0.45-2.47	0.91				1.02	0.44-2.34	0.96			
Other	232 (41.3)	1.31	0.86-2.01	0.21				1.38	0.89-2.15	0.15	1.23	0.51-2.98	0.64	1.14	0.73-1.76	0.57			
Water sufficiency for drinking and household chores	439 (78.1)	0.85	0.50-1.42	0.53				0.99	0.59-1.66	0.97				1.05	0.63-1.74	0.85			
Frequency of washing drinking water container with soap																			
Daily	347 (61.7)	1.00						1.00						1.00					
Never	40 (7.1)	1.41	0.65-3.06	0.39				0.80	0.34-1.91	0.62				1.72	0.77-3.83	0.18	1.62	0.72-3.66	0.25
Weekly	175 (31.1)	1.36	0.85-2.17	0.20				0.95	0.59-1.55	0.85				1.39	0.86-2.23	0.18	1.36	0.84-2.20	0.21
Container for fetching water																			
Metal	264 (31.1)	1.00						1.00						1.00					
Plastic	258 (61.7)	1.22	0.79-1.89	0.37				1.13	0.73-1.76	0.58				1.14	0.73-1.77	0.57			
Clay pot	40 (7.1)	0.56	0.23-1.37	0.21				0.57	0.22-1.48	0.25				0.54	0.21-1.39	0.20			
Status of drinking water container																			
Covered	417 (74.2)	1.00						1.00						1.00					
Uncovered	145 (25.8)	1.01	0.64-1.61	0.96				0.74	0.45-1.22	0.24				0.90	0.54-1.48	0.68			
Drinking water container used for other activity (yes *vs* no)	111 (19.8)	1.35	0.78-2.33	0.29				1.35	0.76-2.42	0.31				1.67	0.92-3.04	0.09	1.43	0.77-2.65	0.26
Water treatment prior to consumption (yes *vs* no)	76 (13.5)	0.74	0.41-1.35	0.33				0.89	0.48-1.67	0.72				0.77	0.41-1.47	0.44			
Water contamination with thermotolerant coliform (yes *vs* no)	154 (27.4)	1.06	0.70-1.60	0.79				1.09	0.71-1.68	0.68				0.93	0.61-1.43	0.75			
Sanitation in the household																			
Water seal latrine	283 (50.4)	1.00						1.00						1.00					
No latrine	168 (29.9)	0.92	0.55-1.54	0.76				0.53	0.30-0.91	0.02	0.52	0.29-0.92	0.02	0.65	0.38-1.12	0.12	0.66	0.38-1.13	0.13
Open pit latrine with slab	97 (17.3)	0.75	0.41-1.35	0.33				0.60	0.32-1.13	0.12	0.58	0.30-1.12	0.11	0.76	0.41-1.42	0.39			
Open pit latrine without slab	14 (2.5)	1.07	0.33-3.46	0.90				0.64	0.18-2.31	0.50				0.32	0.07-1.57	0.16	0.32	0.06-1.58	0.16
Soap for handwashing available (no *vs* yes)	417 (74.2)	1.85	1.18-2.92	0.01	1.81	1.13-2.89	0.01	1.48	0.92-2.38	0.10	1.53	0.93-2.52	0.09	1.33	0.83-2.13	0.24			
Waste disposal (yes *vs* no)	273 (48.6)	1.03	0.70-1.52	0.88				0.99	0.66-1.48	0.96				1.05	0.70-1.57	0.83			
Domestic animals																			
Possession of domestic animals (yes *vs* no)	507 (90.2)	1.07	0.57-2.02	0.83				1.22	0.64-2.35	0.55				1.30	0.69-2.46	0.42			

Animals held outside the house (yes *vs* no)	307 (54.6)	0.89	0.57-1.39	0.62	0.84	0.54-1.32	0.46	0.59	0.38-0.92	0.02	0.52	0.33-0.83	0.01

The multivariate global model includes a random intercept at the level of school adjusting sex, age, district where all the variables were assessed one by one and retained for the global model if their P-value is <0.2. The final model was obtained by using backward selection with the same level of <0.2

7.5. Discussion

Our data confirm that intestinal parasite infections are prevalent among schoolchildren in Dolakha and Ramechhap districts of eastern Nepal, which contrasts with a decline of intestinal parasite infections reported in other districts of Nepal [37]. Indeed, stool samples subjected to duplicate Kato-Katz thick smears and single wet mount and formalin-ether concentrated methods showed that one out of three schoolchildren were infected with at least one helminth and/or pathogenic intestinal protozoa infection. Soil-transmitted helminth infections were slightly more prevalent than intestinal protozoa infections. The predominant helminth species were *T. trichiura* and hookworm, while *G. intestinalis* was the predominant intestinal protozoa infection. Our observations contrast with previous epidemiological surveys that revealed intestinal protozoa being more prevalent than helminths [18, 38]. Unsafe WASH exacerbates parasite infections in general and helminth infections in particular [12, 39]. This might have governed the high prevalence of intestinal parasite infections in a setting where almost one-third of households reported not having any sanitation facilities at home and almost one-third of the children reported not washing their hands with soap after defecation. Our study also showed a considerable number of schoolchildren infected with *A. lumbricoides*, which is in line with studies carried out in another part of Nepal [40]. This could be due to the fact that transmission of *A. lumbricoides* is through the faecal-oral route with re-infections occurring quickly after treatment [41]. Since open defecation is widely practised, efforts must be made to improve sanitation practices, which are likely to have ramifications on soil-transmitted helminthiasis, intestinal protozoa infections and other neglected tropical diseases [38–42].

Our study revealed that multiparasitism was common, as reported elsewhere in Asia [14]. Indeed, we found that 39.7% of the children harboured multiple species of intestinal parasites concurrently, which is a major public health concern as multiple species parasite infections may increase susceptibility to other infections [48]. Reasons that might explain the high level of multiparasitism are the low socioeconomic status and lack of awareness of the surveyed schoolchildren and their caregivers about the transmission of intestinal parasites and how such infections can be prevented. Interestingly, most of the children diagnosed with a single helminth species were characterised by low infection intensity profiles, which is in contrast to findings from the baseline cross-sectional survey of the VgtS study in Burkina Faso. Indeed, in Burkina Faso, many of the infected children showed moderate helminth intensities and there was a higher percentage of children with intestinal protozoa infections compared to our study in Nepal [36]. Yet, it should be noted that low-intensity helminth infections might negatively impact on children's health and wellbeing [45–47]. Despite this, we are not aware of any large-scale, regular deworming activities carried out in our study area, although our findings suggest that such interventions are indicated.

Our study also determined risk factors for intestinal parasite infections, including the influence of age, sex and study setting. Of particular note is the negative association between any intestinal parasite infection and age with an adjusted OR below one. With regard to sex, both males and females showed similar infection rates, which corresponds with a study conducted in the Lalitpur District [52]. Behavioural and socioeconomic factors might explain the observed similarity. We found a significantly higher prevalence of the overall intestinal parasite infection and *G. intestinalis* among children whose caregivers were involved in farming activities. These observations are in line with a previous study conducted in the District of Lalitpur, where children belonging to farmer parents were most commonly infected [52]. Interestingly, our study revealed a significant negative association between *T. trichiura* infection and having no latrine, compared to having a water seal latrine in the household. There are no immediate explanations for this negative relationship, but other confounding factors such as hygiene behaviour may account for this observation. Even without improved sanitary facilities, adequate hygiene practices could make a difference in children's infection status with intestinal parasites. Further in-depth studies in the two study districts are warranted to deepen the understanding of health benefits of improved WASH. For instance, we found a significant association between those children living in households that did not have soap for handwashing after defecation and intestinal parasite infections, which corroborates prior studies conducted in Nepal [16]. Furthermore, in our study, higher odds were found for intestinal parasite infection for children from households without soap for handwashing, as compared to those households that had soap for handwashing. Additionally, we found a significant association between domestic animals kept outside the households, as there were lower odds of infection with *G. intestinalis* among those children. A similar association between children's proximity to livestock and *G. intestinalis* infection was found in a study conducted in rural India [53].

We did not find any significant associations between intestinal parasite infections and clinical signs, but this observation requires further in-depth studies on whether and to what extent intestinal parasite infections can impact on children's health and wellbeing [6]. Without a deeper mechanistic understanding of how intestinal parasite infections might influence children's health and development, the effectiveness of parasitic disease control programmes are compromised [54].

We observed a high level of water contamination with TTC, which is an indicator of pollution of drinking water sources or drinking water vessels by organic means or domestic effluents. This might illustrate the inadequacy of the cleanliness of the storage containers and drinking vessels. Additionally, this may be due to constructional defects of water infrastructures, poor sanitation and the existence of animal or human waste in close proximity to open freshwater sources. Prior to the April 2015 earthquake, drinking water was mainly supplied by private pipes in Dolakha District and by gravity water supply schemes in Ramechhap District. After the earthquake, the proportion of supply by private pipe has dropped in Dolakha District from 56 to 47%, while only 44% of the gravity water supply remained functional after the earthquake in Ramechhap District [55, 56]. The communities in our study areas had access to water from springs, rivers and private pipes with shared taps. However, we did not find a significant association between TTC in drinking water and intestinal parasite infections, which is in line with observation by the VgtS project in Burkina Faso [36].

Our study has several limitations that are offered for consideration. First, our data were obtained from a relatively small number of schools in two districts of Nepal, and hence wider generalisation is not possible. Secondly, the number of schools selected in Dolakha was considerably higher than in Ramechhap District (13 *versus* 3), which is a problem for elucidating regional differences. Thirdly, children's age was determined by verbal reporting of children and their caregivers without definitive proof (such as a birth certificate). Fourthly, the diagnosis of intestinal parasites was based on single stool samples that were subjected to a suite of methods. Clearly, examination of multiple consecutive stool samples and triplicate or quadruplicate (rather than duplicate) Kato-Katz thick smears would have resulted in higher diagnostic sensitivity [6, 26, 57]. Employing techLab enzyme-linked immunosorbent assays (ELISAs), and polymerase chain reaction (PCR) might have revealed additional infections not detected by our methods. However, such tests were not available. Fifthly, we used an Oxfam Delagua kit for water quality assessment. An important limitation of this kit is that it does not detect the presence of parasitic elements. Sixthly, anaemia can be caused by multiple and complex factors, and hence it must be noted that by using a B-haemoglobin photometer device for Hb measurement, the identification of the exact type of anaemia was not possible [6, 58, 59]. Seventhly, due to a major earthquake that occurred in the midst of our cross-sectional survey in April 2015, we failed to obtain the targeted number of 800 children. Indeed, we were unable to collect data in three schools and the final number of children in the 16 surveyed schools was 708. This decreased the statistical power and precision of our data.

Despite these shortcomings, a major strength of our study is the appraisal of morbidity including self-reported signs and symptoms (e.g. fever, watery diarrhoea, bloody diarrhoea and mucus in stool), clinical morbidities (e.g. hepatomegaly and pale conjunctiva), and assessment of anaemia, as indirectly determined by quantification of Hb levels. An additional strength is the analytical approach taken (i.e. multivariate analysis) that allowed adjustments of potential confounders such as demographic, socioeconomic, regional differences and personal behavioural information. Moreover, although the diagnostic approach consisted of the collection of only a single stool sample per child, it was complemented by multiple diagnostic methods (i.e. wet mount, formal-ether concentration and Kato-Katz methods), which enhanced diagnostic sensitivity [6].

7.6. Conclusions

We conclude that intestinal parasite infections are a public health problem in Nepal. We found a high prevalence of soil-transmitted helminths and intestinal protozoa infections among children of 8–16 years. Our observations and results call for specific preventive measures and control interventions targeting schoolchildren. We believe that the morbidities caused by intestinal parasite infections can be overcome or prevented if adequate integrated control measures were to be promoted and implemented, such as the provision of soap for handwashing and regular deworming. These measures could minimise the burden of intestinal parasite infections in the two study districts. Additionally, emphasis should be placed on health promotion programmes at a regional level. The findings from our study provide setting-specific information for designing and implementing preventive programmes for overcoming the burden of intestinal parasite infections in Nepal and other similar countries in Southeast Asia.

Abbreviations

aOR: adjusted odds ratio; CI: confidence interval; DHS: Demographic Health Service; EKNZ: Ethikkommission Nordwest- and Zentralschweiz; epg: eggs per gram of stool; Hb: haemoglobin; KAP: knowledge, attitudes and practices; LMICs: low- and middle-income countries; MoE: Ministry of Education; MoHP: Ministry of Health and Population; ODK: open data kit; OR: odds ratio; SD: standard deviation; SDG: sustainable development goal; SOP: standard operating procedure; Swiss TPH: Swiss Tropical and Public Health Institute TTC: thermotolerant coliforms; WASH: water, sanitation and hygiene; WHO: World Health Organization; VDC: village development committees; VgtS: Vegetables go to School

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Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the "Ethikkommission Nordwest- und Zentralschweiz" (EKNZ) in Switzerland (reference no. UBE-15/02; approval date 12 January 2015), the institutional review board of Kathmandu University, School of Medical Sciences, Dhulikhel Hospital, Nepal (reference no. 86/14; approval date 24 August 2014) and the institutional review board, Nepal Health Research Council (reference no. 565; approval date 11 November 2014). The study is registered at International Standard Randomised Controlled Trial Number Register (identifier: ISRCTN30840; date assigned: 17 July 2015). Written informed consent was obtained from the school principals of each of the selected schools and the District Education Office. The teachers, schoolchildren and their caregivers were informed about the purpose and procedures of the study. Verbal consent was received from teachers and written informed consent (fingerprints of illiterates) was obtained from children's caregivers. Children were informed that participation was voluntary, and hence they could withdraw at any time without further obligation. At the end of the study, each infected child was provided with appropriate medicines free of charge. Deworming of the children with a single oral dose of albendazole (400 mg) was provided against soil-transmitted helminth infections. Children infected with *G. intestinalis* were treated with metronidazole (250 mg/kg) for five consecutive days. Children identified with anaemia were referred to the health centre for further diagnosis and treatment.

Consent for publication

Not applicable.

Availability of data and materials

The dataset analysed for this study are not publicly available due to use in the PhD study of the first author, but they are available from the corresponding author upon reasonable request and signature of mutual agreement. The questionnaires in English are available upon request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed to the study design. AS coordinated the field and laboratory work, collected data, supervised research assistants, performed the statistical analysis under the supervision of CS, and drafted the manuscript. CS, JG, PO, JG, SE, SS, RK, JU and GC contributed to the interpretation of the data, manuscript writing and revisions. All authors read and approved the final manuscript.

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8. Dietary pattern measured by principal component analyisis and its association with stunting among Nepalese schoolchildren in Nepal

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8.1. Abstract

Background: There is a large gap of knowledge on the link between major dietary patterns and stunting among schoolchildren in Nepal. The present study aimed to identify dietary patterns in rural Nepalese households and assess their association with stunting among schoolchildren in the frame of “Vegetable go to school” (VgtS) project.

Methods: This study used data from 708 schoolchildren aged 8-16 years participating in the baseline survey of the VgtS project in the districts of Dolakha and Ramechhap, of Nepal. We derived major dietary patterns from a principal component analysis of reported intake from a food frequency questionnaire completed through interviews with the caregivers. Statistical analysis was conducted using mixed logistic regression with random intercepts at the level of schools and adjusting for socio-demographic and behavioural indicators.

Results: The diet of surveyed schoolchildren mainly comprised of starchy staples and legumes. Five dietary patterns score were derived: mixed food, vegetables and lentils, milk and beverages, salty snacks, and processed food. The vegetables and lentils pattern scores were negatively associated with stunting (aOR 0.84; 95% CI: 0.66-1.08, *p*=0.17) after adjusting for regional differences, demographic and behavioural risk factors.

Conclusion: Our results suggest that adherence to dietary patterns high in vegetables and animal protein might be associated with reduced odds of being stunted among schoolchildren. Therefore, the promotion of dietary diversification strategies to improve schoolchildren’s food consumption is required in the study area.

Key words: Dietary diversity, Nepal, Stunting, Schoolchildren
Word count: 230

8.2. Background

Stunting or short height-for-age contribute to the mortality and the burden of disease among children [1, 2]. It is a resolvable problem, common especially in low-and middle-income countries (LMICs), and is more prevalent than underweight (low weight-for-age; 20%) or wasting (low weight-for-height, 10%) which is possibly because height gain is even more sensitive to dietary quality than weight [3, 4]. Stunting affected one third of children under-five in LMICs: a total of 178 million children, where the majority of stunted children was in Asia (112 million) [1]. Stunting often goes unrecognised by families where short stature is so common that it seems normal but people are unaware that the stunted children experience little or no catch-up in growth later in life [5]. Causes of stunting may be genetic, hormonal, pharmaceutical, psychological and/or nutritional [4]. The major consequences of childhood stunting were found to be short adult stature, reduced lean body mass, diminished intellectual functioning, lower birth weight and higher mortality of infants (i.e., of infants born to women who were stunted as a children) [6,7]. Additionally, the babies born to stunted women are themselves more likely to be stunted and could pass from one generation to other [8]. The growth retardation, which is the manifestation of chronic malnutrition, can be developed from lack of food, inappropriate diet quality, or a combination of both [9, 10]. Hence, in-order to prevent stunting among schoolchildren in Nepal, one of the low income countries in South Asia, were not available so far. Available information was mostly focused on children aged <5 years old [11]. In 2016, a study conducted among children aged 0-89 months in a remote and food-insecure region in the mountains of Nepal reported that increasing dietary diversity for children reduced the risk of stunting [12]. Similarly, another study reported 43% of children aged 6-59 months being stunted, but no association with dietary patterns score was found [11]. Meanwhile, another study examined the risk factors of stunting among children aged 6-59 months, reporting low dietary diversity to be one of the risk factors for stunting [13]. A study conducted in Kathmandu valley reported the prevalence of stunting among schoolchildren aged 6-10 years to be 45.6% in boys and 43.4% in girls [14]. Unfortunately, none of these studies have examined stunting and its risk factors, especially pertaining to diet at the unit of household.

Nutritional epidemiologists have suggested the use of dietary pattern analysis as a comprehensive method to understanding diet-disease relationship. It identifies sets of correlated foods and provides a score for each set according to the frequency of intake of foods. These scores are then used as surrogate for the food variables [15]. Dietary patterns defined with the use of cluster or factor analysis were previously reported to be associated with stunting in a study conducted in Tehran. The study reported that the dietary pattern score high in “carbohydrate-protein” was associated with a reduced risk of stunting among the first grade students in Tehran [9]. However, evidence is still limited, and most studies have been performed either in high or middle income countries targeting adults in relation to numerous outcomes such as cardiovascular diseases or obesity / overweight [16-18]. Limited information is available about major dietary patterns of schoolchildren and their association to the prevalence of stunting particularly in Nepal.

In order to overcome the problems of low nutrition and diet diversity among schoolchildren, the “Vegetables go to school” (VgtS) project was implemented with an objective of improving nutritional status of schoolchildren through agricultural diversification. The overall goal of the VgtS project is to improve nutritional security of the schoolchildren through school vegetable gardens linked to other school-based health, nutrition and environmental initiatives [19]. This study was therefore conducted to identify the major dietary patterns among schoolchildren in a rural community of Nepal and to investigate their associations with the prevalence of stunting among schoolchildren participating in VgtS project.

8.3. Methods

Ethical considerations

Ethical approval was granted by the *“*Ethikkommission Nordwest- und Zentralschweiz*”* (EKNZ) in Switzerland (reference number UBE-15/02; date of approval January 12, 2015); the “institutional review committee of Kathmandu University School of Medical Sciences, Dhulikhel Hospital, Nepal” (reference no. 86/14, date of approval August 24, 2014); and the “institutional review committee, Nepal Health Research Council” (reference no 565; date of approval November 11, 2014). The study is also registered at the international standard randomised controlled trial number register ISRCTN30840 (date assigned: July 17, 2015). Verbal consent was received from teachers and written informed consent (fingerprints of illiterates) was obtained from the caretakers of the study participants. All the participants of the study were informed about volunteer participation and freedom to withdraw at any time without further obligations. For the severe malnutrition, school-aged children were referred to the health centre for further diagnosis and treatment in the health centre.

Study design and subjects

The cross-sectional study used data from the first wave of the baseline survey of the VgtS project. The VgtS aims to improve nutritional security of the schoolchildren through school vegetable gardens linked to other school-based health, nutrition and environmental initiatives. The study population consisted of the children in 6th and 7th grade, between the ages of 8-16 years from Dolakha and Ramechhap district. The 16 out of 30 schools were randomly selected in consultation with the National Agricultural Research Council (NARC), the Ministry of Education (MOE) and the Ministry of Health and Population (MOHP).

Dietary assessment

We used a food frequency questionnaire (FFQ) for assessing the usual dietary intake. The FFQ contains questions on the average consumption frequency during the past year for 99 commonly consumed food items. The respondents indicated their answers in times per day, week, month and year or as never. The questionnaire was supplemented with coloured photographs of four differently sized portions of foods developed by a professional photographer under controlled conditions of light, distance, angles and presentations. Different sizes of glasses or bowls were presented to estimate the amount of liquids. The respondent indicates one of the amounts presented in the photographs. Other items were asked as a number of specified units (number, spoons) [20].

Food component derivation

Twenty one food groups were created according to the macronutrient composition [21]. The frequency of food group consumption was converted into the number of servings per week. Factor analysis with varimax rotation was then performed to derive optimal non-correlated components (food patterns).The factors retained were determined based on eigenvalue and interpretability [22].

Assessment of stunting

The measurements were taken by trained health workers following standard guideline [23]. Participant's height was measured without shoes, using a standard scale with a length of 2 m and a precision of 0.1 cm. Weight was measured using digital portable calibrated SECA weighing scale (SECA; Hamburg, Germany) having sensitivity of 0.1 kg with a capacity of 130 kg. The weighing machine was calibrated to zero before taking each measurement. The children were weighed wearing a light uniform, without shoes and with empty pockets. All measurements were taken by the same person. The growth and development status of children were evaluated by height-for-age ratio *Z* score (HAZ; stunting) according to WHO`s child growth standards 2006 and the HAZ < -2 are defined as stunting [24].

Assessment of other variables

The additional variables, including age (in years), sex, ethnicity (Brahmin/ Chettri/Newar/Tamang/Janajati), education (primary/secondary/higher/no-education), occupation (farmer/business/service/no-occupation), religion (Hindu/non-Hindu) and past breastfeeding (yes/no) were assessed through interviews conducted with the caregivers, using standardized questionnaires.

Statistical methods

Continuous variables were summarized using means and standard deviations (SD) if they were close to normally distributed and using medians and interquartile ranges (IQR) otherwise. Categorical variables were described using absolute frequencies and percentages. Dietary factor scores were analysed as potential predictors of stunting as main outcome. Mixed linear and logistic regression models with random school intercepts were used for this purpose. For each food pattern, three incremental models were computed: (i) without any adjustment; (ii) with adjustment for demographic variables (age, sex, education, occupation and district) and (iii) with further adjustment for lifestyle variables (breastfeed the school-aged children, food security). Associations are expressed as odds ratios with 95% confidence intervals. All analyses were carried out using STATA, version 14 (Stata Corporation; College Station, TX, USA).

8.4. Results

A total of 750 schoolchildren were eligible for the survey out of which 708 were included in the baseline study. However, due to the 2015 earthquake in the midst of the survey, only 562 (79.4%) households could be contacted by the data collectors (Table 8.1) [25].

Table 8.1: Characteristics of study population in the two districts of Nepal in March-May 2015

Characteristics [N=708]	[n, (%)]	Dolakha [n, (%)]	Ramechhap [n (%)]
Sex			
Male	339 (47.9)	261 (47.0)	78(51.0)
Female	369 (52.1)	294 (53.0)	75 (49.0)
Age of children			
Age group 1 (8-12 years)	108 (15.2)	86 (15.5)	22 (14.4)
Age group 2 (13-16 years)	600 (84.8)	469 (84.5)	131 (85.6)
Mean (SD) values in Dolakha and Ramechhap	12.8 (1.2)		
Grade			
Class 6	333 (47.0)	258 (46.5)	75 (49.0)
Class 7	375 (53.0)	297 (53.5)	78 (51.0)
Caregivers demographic characteristics [N=562]			
Age of caregivers			
18-24 years	2 (0.4)	1 (0.2)	1 (0.9)
24-40 years	239 (42.5)	184 (41.4)	55 (46.6)
>40 years	321 (57.1)	259 (58.3)	62 (52.5)
Median age (IQR)	39.5 (11)		
Education level of caregivers			
No formal schooling	210 (37.4)	174 (39.2)	36 (30.5)

Primary education		144 (25.6)	130 (29.3)	14 (11.9)
Secondary education		143 (25.4)	82 (18.5)	61 (51.7)
Higher education		65 (11.6)	58 (13.0)	7 (5.9)
Ethnicity of caregivers				
Brahmin		101 (17.9)	97 (21.9)	4 (3.4)
Chhetri		210 (37.4)	154 (34.7)	56 (47.5)
Newar		33 (5.9)	22 (4.9)	11 (9.3)
Tamang		213 (37.9)	166 (37.4)	47 (39.8)
Janajati		5 (0.9)	5 (1.1)	0 (0.0)
Main occupation of caregivers				
No occupation		25 (4.5)	10 (2.2)	15 (12.7)
Farmer		458 (81.5)	391 (88.1)	67 (56.8)
Public service		39 (6.9)	29 (6.5)	10 (8.5)
Business owner		40 (7.1)	14 (3.2)	26 (22.0)
Religion				
Hindu		448 (79.7)	341 (76.8)	107 (90.7)
Non Hindu		114 (20.3)	103 (23.2)	11 (9.3)
Socio-economic characteristics of caregivers [n=562]				
Roof material	Corrugated iron roof	415 (73.8)	325 (73.2)	90 (76.3)
	Wood and tiles	147 (26.2)	119 (26.8)	28 (23.7)
Wall material	Wood	66 (11.7)	61 (13.7)	5 (4.2)
	Corrugated iron	407 (72.4)	331 (74.6)	76 (64.4)
	Bricks	89 (15.9)	52 (11.7)	37 (31.4)
Floor material	Mud	524 (93.2)	430 (96.9)	94 (79.7)
	Cement	38 (6.8)	14 (3.1)	24 (20.3)
Energy for cooking	Charcoal/wood	473 (84.2)	390 (87.8)	83 (70.3)
	Electricity	89 (15.8)	54 (12.2)	35 (29.7)
Socioeconomic status	High	49 (8.7)	39 (8.8)	10 (8.5)
	Middle	215 (38.3)	163 (36.7)	52 (44.1)
	Poor	298 (53.0)	242 (54.5)	56 (47.4)
Owing agricultural land		511 (90.9)	412 (92.8)	99 (83.9)
Total production	≤ 10%	44 (7.8)	30 (6.8)	14 (11.9)
	10-30%	20 (3.6)	6 (1.4)	14 (11,9)
	>30%	498 (88.6)	408 (91.9)	90 (76.3)
Possession of domestic animals		507 (90.2)	401 (90.3)	106 (89.8)

**Socioeconomic status was derived from a factor analysis of variables indicating the possession of household assets such as radio, television, mobile phone, table, stove, petrol lamp, motorbike, car or truck, watch, iron, bike, cupboards etc. The score of the first factor was then divided into three categories using the k-means procedure.*

Among the 708 schoolchildren, the prevalence of stunting was 27.0%, higher in males than in females (31.6% versus 22.8%). The socio-demographic characteristics of the study populations are shown in Table 8.1. The mean age of the study participants was 12.8 years with a range between 8 and 16 years. Tamang was the largest ethnic group (37.9%). The median age of the caregivers was 39.5 years with an interquartile range of 11 years. More than one third of the caregivers had no formal education (37.4%) and only 11.6% had a level of education above secondary school. The mean (SD) of height-for-age was -1.38 (1.0). Mean consumption of animal products such as red meat, poultry, fishes and milk was low among schoolchildren i.e., 1.18, 1.96, 0.81 and 0.90 respectively. The mean consumption of vegetable is 3.79 and of fruit were 4.09 per week. The mean consumption of white cereals was high i.e., 9.12 servings per week (Table 8.2).

Table 8.2 : Consumption/ servings of food groups per week among schoolchildren in two districts in March-May 2015

Food group	Mean	Standard deviation	Median	Interquartile range	Consumption per week (minimum)	Consumption per week (maximum)
Whole cereals	3.18	5.57	0.00	7.00	0	31.5
White cereals	9.12	3.72	7.00	3.50	0	24.5
Lentils	3.32	5.40	0.00	7.00	0	35.0
Oils	6.41	5.37	7.00	3.50	0	28.0
Fatty food	3.28	5.99	0.00	3.50	0	38.5
Vegetable	3.79	23.90	0.00	0.00	0	206.1
Fruits	4.09	6.72	1.73	5.47	0	58.1
Roots and tubers	4.72	4.07	5.63	7.00	0	16.6
Nuts	1.82	5.34	0.00	0.00	0	42.0
Poultry	1.96	3.94	0.00	3.50	0	24.5
Redmeat	1.18	3.44	0.00	0.00	0	29.8
Fishes	0.81	2.69	0.00	0.00	0	17.5
Milk drinks	0.91	1.54	0.00	3.50	0	3.5
Milk products	1.18	2.66	0.00	0.00	0	17.5
Western	1.42	3.66	0.00	0.00	0	28.0
Processed cereals	2.75	3.93	0.00	7.00	0	21.0
Noodles	2.51	4.02	0.00	0.00	0	21.0
Salty snacks	2.8	4.20	0.00	3.50	0	24.5
Beverages	0.78	1.70	0.00	0.00	0	7.0
Medium beverages	5.02	3.61	3.50	3.50	0	21.0
Sugary food	4.83	5.78	3.50	7.00	0	28.0

The factor analysis revealed five factors explaining 40% of the variation in the total food intake and reflecting different dietary patterns. The loadings of the different dietary variables on each of the factor are presented in table 8.3. The five patterns were labelled based on the food items that loaded highest on them:

- mixed food pattern score (red meat, while cereals, fruits, poultry, western and fishes);
- vegetable and lentils pattern score (lentils, vegetables, nuts);
- milk and beverages pattern score (milk products, beverages such as juice);
- salty snacks pattern score (pickles); and
- processed food pattern scores (noodles, biscuits).

Table 8.3: Factor loading values of pattern of dietary intake derived from principal component analysis

Food groups	Factor				
	1	2	3	4	5
Whole cereals	0.427	0.524	-0.119	0.137	-0.066
White cereals	0.681	0.089	-0.339	0.159	-0.037
Lentils	0.195	0.697	0.068	0.127	0.031
Oils	-0.011	-0.004	0.077	0.701	0.084
Fatty food	0.391	0.418	0.364	0.214	0.158
Vegetable	0.011	0.688	0.109	-0.142	0.293
Fruits	0.644	0.267	0.211	0.079	0.248
Roots and tubers	-0.006	-0.056	-0.147	0.622	0.296
Nuts	0.460	0.450	0.262	0.104	0.061
Poultry	0.568	0.094	0.208	0.165	0.261
Redmeat	0.795	0.153	0.159	-0.045	0.105
Fishes	0.656	-0.024	0.299	-0.027	0.032
Milk drinks	0.179	-0.216	0.409	0.101	0.295
Milk products	0.224	0.244	0.575	0.072	-0.039
Western	0.693	0.062	0.201	-0.019	0.155
Processed cereals	0.173	0.110	-0.039	0.022	0.736
Noodles	0.091	0.101	0.127	0.147	0.653
Salty snacks	0.107	0.167	0.248	0.628	-0.058
Beverages	0.316	0.119	0.587	0.042	0.121
Medium beverages	0.174	0.104	0.479	0.318	0.047
Sugary food	0.255	0.202	0.167	0.340	0.490

Extraction method: principal component analysis; rotation method: varimax with Kaiser normalisation.
Score coefficients with highest values in each component are shown in bold
Whole cereals: wheat, millet, maize, wheat bread
White cereals: sooji, beaten rice, rice, maida
Lentils: soyabean, beans, sprout, whole pulse
Oils: Mustard oil, sunflower oil, soyabean oil
Fatty food: Deep fried local food swaari, donought, pakauda, malpa, fries
Vegetable: both dark green and other vegetables
Fruits: all vitamin A rich and non-rich fruits
Vegetable roots: potato, sweet potato, yam
Nuts: peanut, cashew, walnut, pista, dryfruit, almonds
Poultry: eggs, chicken
Red meat: buff, pork, mutton
Fish: fried and non-fried fish
Milk drinks: milk
Milk products: cheese, paneer, yogurt
Western: sausages
Processed cereals: biscuits, bread
Noodles: noodles, choumin
Salty snacks: pickles, bhujiya
Beverages: fruit juice
Medium beverages: coffee and tea (with and without milk)
Sugary food: ice-cream, sweet, chocolate and jam

The associations of each dietary factor score with socio-demographic characteristics such as age, sex, religion, education and occupation are shown in table 8.4. Female schoolchildren had a higher average score for the mixed food pattern (coef. =0.16, 95% CI:-0.01-0.32, p=0.05). The coefficients for children with well-educated parents were mostly positive, the only exception being the score for the salty snacks pattern score and processed food pattern scores (coef.=-0.15, 95% CI=-0.24-0.06, *p*=0.01; coef.=0.07, 95% CI=-0.17-0.21, *p*=0.13 respectively).

Table 8.4: Association of socio-demographic characteristics (age, sex, religion, education and occupation) with the derived food pattern among schoolchildren in Dolakha and Ramechha districs, Nepal (March-May, 2015)

	Mixed food pattern score			Vegetable and lentils pattern scores			Milk and beverages pattern score			Salty snacks pattern score			Processed food pattern score		
	Coefficient	95% CI	p-value	Coefficient	95% CI	p-value	Coefficient	95% CI	p-value	Coefficient	95% CI	p- value	Coefficient	95% CI	p-value
Age	-0.02	-0.22- 0.17	0.80	0.05	-0.15-0.26	0.60	0.08	-0.12-0.27	0.46	-0.08	-0.29- 0.11	0.39	-0.01	-0.21- 0.19	0.94
Sex															
Male	Ref														
Female	0.16	-0.01-0.32	0.05	-0.01	-0.16-0.16	0.98	0.13	-0.04-0.29	0.12	-0.01	-0.17-0.16	0.97	-0.06	-0.22-0.10	0.46
Religion															
Non Hindu	Ref														
Hindu	-0.14	-0.36- 0.07	0.20	0.04	-0.17-0.26	0.70	0.03	-0.18- 0.25	0.75	0.23	0.01-0.44	0.04	0.02	-0.19-0.24	0.82
Education															
Non educated	Ref														
Educated	0.01	-0.08- 0.10	0.86	0.12	0.03-0.20	0.01	0.06	-0.04-0.15	0.24	-0.15	-0.24- -0.06	0.01	-0.07	-0.17-0.21	0.13
Occupation															
No occupation	Ref														
Has occupation	-0.01	-0.15- 0.13	0.90	0.02	-0.12- 0.16	0.75	0.02	-0.12-0.16	0.77	0.01	-0.13- 0.15	0.92	.04	-0.10-0.18	0.56

The association of food patterns scores with the prevalence of stunting is shown in Table 8.5. The vegetables and lentils pattern score showed a negative association with stunting (aOR: 0.84, 95% CI: 0.66-1.07; *p*=0.17) after adjusting for district, and demographic and behavioural related variables (such as food security at the household, breastfeeding and consumption of breakfast prior to the survey). A negative association was also observed with the mixed food pattern score; however, these associations were not statistically significant (aOR 0.90, 95% CI: 0.72-1.13, *p*=0.36).

Table 8.5: Association of stunting with the derived food patterns among schoolchildren

	Model 1			Model 2			Model 3		
	Univariate analysis			Adjusted for demographic variables*			Aditionally adjusted for other variables**		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Stunting									
Mixed food pattern score	0.89	0.71-1.12	0.33	0.91	0.72-1.14	0.39	0.90	0.72-1.13	0.36
Vegetables and lentils pattern score	0.84	0.66-1.08	0.18	0.84	0.66-1.08	0.17	0.84	0.66-1.07	0.17
Milk and beverages pattern score	0.98	0.79- 1.20	0.83	0.99	0.80-1.22	0.93	1.00	0.81-1.23	0.99
Salty snacks pattern score	1.05	0.86-1.30	0.62	1.07	0.87-1.32	0.53	1.09	0.89-1.35	0.39
Processed food pattern score	1.07	0.87-1.31	0.52	1.07	0.87-1.31	0.53	1.07	0.87-1.31	0.52

** Adjusted for age, sex, education and occupation. **Adjusted for age, sex, education, occupation, food security, breastfeed and breakfast day before the survey*

8.5. Discussion

In the present cross-sectional study, we examined the dietary patterns associated with stunting among schoolchildren in Dolakha and Ramechhap district of Nepal. Our findings revealed five major food patterns scores that characterised the dietary habits of rural Nepalese community: mixed food, vegetables and lentils, milk and beverages, salty snacks and processed food pattern scores. There was a protective association between adherence to “vegetables and lentils” and stunting among the schoolchildren in Nepal, even after taking potential confounders such as age, sex, districts, and behavioural factors into account. To the best of our knowledge, this study is the first study examining the association between major dietary patterns scores and stunting among schoolchildren in Nepal.

Overall, the association between vegetables lentils pattern scores and stunting was protective; however not significant. The beneficial effect of vegetables may have been counterbalanced by detrimental effects of other food items, resulting in the insignificant association between vegetables and lentils patterns and stunting in our study. Overall, no significant association was observed between also “mixed food pattern score” and “milk and beverages pattern scores” and stunting in this population. The vegetables and lentils pattern is negatively associated with female children and is positively associated with children having educated parents with occupation.

Our study did not derive the same dietary patterns as of other studies because of variation of Nepalese diet from the other population and some characteristics were different according to the dietary pattern [20]. The schoolchildren of higher age (13-16 years) had a higher score for the “vegetable and lentils” and “milk and beverages” whereas lower score for the “salty snacks and processed food”. This may be because of the more learning regarding importance of vegetables by the higher aged study population and the caregivers. Similarly, “processed food item consumption” is higher in those children whose caregivers were involved into any occupation. This may be due to affordability and adequate income or may be the caregivers were likely to be busier so the schoolchildren buy processed food by themselves. In terms of food and food groups’ intake, we found that the stunted children had lower intake of animal products, dairy, poultry or fishes. Based on these findings, it seems that the diet quality is not diversified as the predominant food for Nepalese children are mostly big portion of cereals with high heat exposure while cooking [26].

The dietary pattern score approach that includes food behaviours of individuals may provide greater information on nutritional aetiology. In this study, we sought to identify dietary patterns scores associated with stunting in schoolchildren of Nepal. Although, stunting might be prevalent among schoolchildren, few studies have assessed the role of diet in its aetiology. Additionally, the nutritional status of children aged <5 years is widely researched and interventions are continuously being sought to prevent and treat malnutrition as a nationwide project. However, no information is available in terms of the association between dietary pattern scores and stunting among the schoolchildren in Nepal. Yet, studies were available on the association between dietary pattern and obesity elsewhere [27]. Additionally, the biological plausibility of eating vegetables and lentils pattern score and its association with stunting is not available. Furthermore, the epidemiological studies suggest that consumption of vegetables; whole grain cereals and whole grain cereals products have many beneficial health effects [28]. However, we did not found the significant association between theses healthy food pattern scores which might be due to the different other confounding factors being associated.

The strength of our study is the use of advanced multivariate analysis to control for different socio-economic (e.g. age, sex, education, religion, occupation of caregivers, food security at household) and behavioural factors (e.g. breastfeeding and consumption of breakfast before the survey). Other strength of our study is the use of data-driven food pattern score analysis.

This approach has emerged as a complementary approach for examining diet-outcome relationships and is more predictive of disease risk than individual food or nutrients [29].

The current study provides useful information elucidating the association between dietary patterns and stunting in schoolchildren. However, several limitations should be considered while interpreting these results. First, our study was of a cross-sectional design, and it is not possible to determine the temporality and causality of variables in this setting; therefore, the exact association between major dietary patterns and stunting could be rather confirmed by prospective studies. Second is the reliance on FFQ as a measure of dietary intake. The method is susceptible to recall bias, both for frequency of foods eaten and for quantification of portion sizes although various amount estimation tools were used in collecting more accurate data. Third, stunting is a heterogeneous multi-factorial disorder and beside dietary factors, other variables, such as hereditary factors and metabolic conditions could be considered. Fourth, the analysis of micronutrient deficiencies, including iron, copper and vitamins that could indicate the nutrients intake was not explored. Fifth, the PCA method itself has limitations that arise from subjective choices made in deciding the variable scale, the number of variables and factors with their interpretation contributing to the inconsistency [29]. Sixth, we cannot generalise our findings to the entire Nepalese schoolchildren as Nepal is a multi-ethnic country with vast diversity in food habits and culture [30].

8.6. Conclusion

Our results suggest that higher intake of vegetable and lentils pattern score is protective for decreased odds of stunting in Nepalese schoolchildren. Although, the association is not significant, and a further exploration is required, the vegetables and lentils pattern should be promoted for the schoolchildren. This study adds to the existing literature by identifying relationship between dietary patterns scores and stunting among the understudied population residing in low-resource setting of Nepal. Identifying food-based dietary patterns scores may be useful in improving dietary habits in the soon to be adult population and public health efforts at decreasing stunting. Further studies are required to confirm our findings. Additionally, efforts are needed to promote healthy and balanced dietary habits among schoolchildren.

8.7. References

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9. Nutritional and health status of children 15 months after integrated school garden, nutrition, and water, sanitation and hygiene interventions: a cluster-randomised controlled trial in Nepal

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9.1. Abstract

Background: It has been suggested that specific integrated ninterventions delivered through the education sector might contribute in improving schoolchildren's health and wellbeing. This cluster-randomised controlled trial aimed to evaluate the effects of a school garden programme and complementary nutrition, and water, sanitation and hygiene (WASH) interventions on children's health and nutritional status in two districts of Nepal.

Methods: The trial included 682 children aged 8-17 years from 12 schools. The schools were randomly allocated to one of three interventions: (i) school garden programme (SG; 4 schools, n=172 children); (ii) school garden programme with complementary WASH, health and nutrition interventions (SG+; 4 schools, n=197); and (iii) no specific intervention (control; 4 schools, n=313). The same field and laboratory procedures were employed at the baseline (March 2015) and end-line (June 2016) surveys. Questionnaires were administered to evaluate WASH conditions at schools and households. Water quality was assessed using a Delagua kit. Dietary intake was determined using food frequency and 24-hour recall questionnaire. Haemoglobin levels were measured using HemoCue digital device and used as a proxy for anaemia. Stool samples were subjected to a suite of copro-microscopic diagnosis for intestinal protozoa and helminths. The changes in key indicators between the baseline and end-line surveys were analysed by mixed logistic and linear regression models.

Results: Anaemia slightly decreased in SG+ (33.0% to 32.0%; $p<0.01$, compared to control) and markedly increased in the control (22.7% to 41.3%) and SG arm (20.7% to 43.9%; $p=0.56$, compared to control). Handwashing with soap before eating strongly increased in SG+ arm (from 74.1% to 96.9%; $p=0.01$, compared to control where only a slight increase was observed from 78.0% to 84.0%). A similar observation was made for handwashing after defecation (SG+ increase from 77.2% to 99.0% *versus* control with increase from 78.0% to 91.9%; $p=0.36$). While prevalence of parasite infections strongly declined in SG+ (37.1% to 9.4%; $p<0.01$, compared to control), a minor decline was found in SG (33.5% to 27.4%; $p=0.42$, compared to control) and in the control arm (43.9% to 42.4%). Stunting was slightly lowered in SG+ (19.9% to 18.3%; $p=0.92$) and in the control arm (19.7% to 18.9%) and slightly increased in SG arm (17.7% to 19.5%; $p=0.54$).

Conclusion: An integrated intervention consisting of WASH, nutrition and health with school garden (SG+) can improve some of the indicators of health and nutritional status among schoolchildren, such as anaemia, intestinal parasitic infections and hygiene components.

Trial registration: ISRCTN17968589 (date assigned: 17 July 2015)
(*http://www.isrctn.com/ISRCTN17968589*)

Key words: Anaemia, Intestinal parasitic infections, Malnutrition, Nepal, Schoolchildren, School garden, Water, sanitation and hygiene (WASH)

9.2. Background

Childhood is a critical period for the development of eating patterns that persist into adulthood, particularly with regards to fruit and vegetable consumption [1]. Hence, it is vital that schoolchildren learn early about the importance of a balanced diet, including fruits and vegetables [2]. Considering the importance of adequate nutrition in childhood to achieve healthy growth and development, giving children opportunities to learn about fruits and vegetables, including their benefits, may help to facilitate the increase in their intake that could prevent malnutrition [1]. School gardens are considered as an ideal setting to facilitate dietary behaviour change among children. They offer a potential to increase children's exposure to, and consumption of, fruits and vegetables [3]. Studies indicate positive effects on children's food preferences and dietary habits, including fruits and vegetables consumption, and about knowledge, benefit towards good health and prevention of malnutrition [4, 5]. School garden education also provides a context for understanding seasonality, what needs to be eaten and where food comes from [6, 7]. Furthermore, it provides an opportunity to teach life skills to schoolchildren, including gardening and working cooperatively on planting and harvesting [6].

Malnutrition, inadequate water, sanitation and hygiene (WASH) conditions and intestinal parasitic infections are intricately linked. Severe malnutrition in schoolchildren has been documented in association with inadequate sanitation, poor hygiene and improper child feeding practices [8]. Inadequate WASH conditions are also important risk factors for intestinal parasitic infections that are transmitted through the faecal-oral route [9, 10]. Parasitic infections contribute to stunting by loss of appetite, diarrhoea, mal-absorption and/or an increase in nutrient wastage [11, 12]. Furthermore, infections with intestinal parasites may cause internal bleeding, leading to a loss of iron and anaemia [13], exacerbate the effects of malnutrition, and hence, compromise the development of cognitive abilities [11]. An inadequate dietary intake could lead to weakened immunity, weight loss, impaired growth and increased susceptibility to intestinal parasitic infections [11]. Hence, it is crucial to consider the inter-linkages of malnutrition, intestinal parasitic infections, and WASH for preventive action.

In Nepal, studies related to the inter-linkage of WASH, health and nutrition interventions focusing on increased knowledge and consumption of adequate diet, especially fruits and vegetables, are limited. Efforts to control malnutrition were predominantly targeted to children under the age of 5 years [14]. Deworming campaigns are mainly focussing on school-aged children; however, drug therapy alone might be only a short-term measure for reducing worm infection among the target population [15]. It has been shown that the prevalence of intestinal parasitic infection returns to the pre-treatment levels within 6 to 18 months after treatment cessation [16–18]. A school garden programme with integrated nutrition education, health and WASH interventions, and increasing knowledge about diet diversity, could address the underlying determinants of nutritional and health problems among schoolchildren [19]. A multi-country, multi-sectorial project entitled "Vegetables go to School: improving nutrition through agricultural diversification" (VgtS) was developed and implemented in five countries of Asia and Africa (Bhutan, Burkina Faso, Indonesia, Nepal and the Philippines) to address schoolchildren's nutrition and health problems in an interdisciplinary approach [20]. The objective of the current study was to evaluate whether a school garden and education programme and a school garden with complementary WASH, health and nutrition interventions would improve nutritional and health indices among schoolchildren in two districts of Nepal.

9.3. Methods

Study design

We undertook a randomised controlled trial in 12 schools. Four schools received a school garden and an education about fruits and vegetables only (SG). Four schools received school garden, coupled with nutrition, health and WASH interventions (SG+). The remaining four schools did not receive any specific interventions (control schools). The two main impact pathways assessed were whether: (i) children's knowledge about, and intake of, fruits and vegetables will increase by growing fruits and vegetables in both SG and SG+ which, in turn, will improve their nutritional status; and (ii) the prevalence of malnutrition, anaemia and intestinal parasite infections among children in SG+ will be reduced, compared to SG and control schools.

Interventions

School gardens with education component (SG)

The first intervention component consisted of a school garden for the cultivation of nutrient-dense vegetables. Teachers were trained in theoretical and practical skills on how to establish and manage school gardens (e.g. levelling and raising land beds, construction of drainage, plantation and caring by children). The trainings were offered twice for one week and conducted by project teams, including representatives from the National Agricultural Research Council (NARC), the Ministry of Health and the Ministry of Education. Teachers received different varieties of vegetable seeds and gardening tools and equipment [21]. The school gardens were set up in April 2015. The second intervention consisted of the development and implementation of a curriculum to teach children about gardening (duration:

23 weeks; mainly theory). Teachers received specific training about the use of curriculum by a local project team. The teaching took place once a week during a 90 min class with an emphasis on learning by doing in the school gardens.

Children's caregivers were invited to visit the school at least twice a year to receive a briefing about the school garden project. Children received small packets of seeds to grow vegetables at home and teachers visited some of the children's homes for observation of the garden [21]. Two technical staff were recruited with a background in agriculture. They monitored the school gardens weekly and provided technical assistance as requested by the students and teachers. Single school gardens produced, on average, about 150 kg of vegetables per school year, which were distributed among the children and teachers [22].

School garden and complementary interventions (SG+):

In addition to the school garden programme, complementary WASH, health and nutrition interventions were implemented in four schools. The intervention package included the following components:

- Health promotion activities, such as the development of an educational comic book that incorporated information about school gardens, nutrition and WASH targeted to schoolchildren. Formative research was conducted with children and their caregivers to develop this booklet.
- Provision of a nutrition booklet and hand-outs, incorporating information for children related to fruits and vegetables. The booklet was developed in collaboration with health personnel.
- Development of a poster to display information related to nutrition, handwashing and waste management for children.
- Demonstration of adequate handwashing with soap. The demonstration was done by health personnel, delivered to children and their caregivers.
- Developing songs related to sanitation and hygiene. Teachers, in collaboration with local organisation, drafted the songs in the schools.
- Audio-visual aids related to nutrition and WASH for children and their caregivers.
- Construction of at least three latrines per school and six to 12 handwashing facilities with the weekly provision of soap (50 bars per week).
- Weekly health education programmes related to nutrition and WASH for caregivers and community stakeholders with the distribution of soap once a week over a 5-month period.

- Organisation of informative sessions for caregivers to explain the school garden programme, highlighting the importance of school gardening and replicating the learnt gardening skills at home to set up home gardens.

These interventions were implemented in combination with health education. They were intended to be implemented over a 12-month period. However, due to a major earthquake and a series of aftershocks that hit Nepal in April and May 2015, the duration was shorter than planned.

Study sites, study population and sample size

This study was conducted in the Dolakha and Ramechhap districts. Dolakha is located approximately 180 km and Ramechhap approximately 150 km from Kathmandu, the capital of Nepal. The study population consisted of schoolchildren aged 8-17 years at the baseline survey. A Monte Carlo simulation showed that 800 children, with 50 children per school and four schools per intervention arm would provide at least 75% power for finding simultaneous significant effects of the two implemented type of interventions under the following assumptions:

- the prevalence of intestinal protozoan and helminth infections is about 30% and remains constant in the absence of any intervention;
- the probability of new intestinal protozoa and helminth infections at follow-up is 15%;
- the same effect odds ratios (ORs) apply to incidence and persistence of intestinal protozoa and helminth infection; and
- each of the two interventions reduces the odds of infection by 50%, and their effects are additive on the logit-scale.

The study was registered as a cluster randomised controlled trial with study ID ISRCTN17968589 (date assigned: 17 July 2015). The study intended to measure and compare the impact of SG and SG+ interventions on schoolchildren's nutritional and health indices in comparison to control schools. At baseline, a total of 12 schools (10 in Dolakha and 2 in Ramechhap) were selected randomly among 30 schools that met the following inclusion criteria: (i) schools located within one-hour walking distance from a main highway; and (ii) water available at school for vegetable cultivation. Only two schools were included in the Ramechhap district, as the two criteria were difficult to meet. The schools were then randomly allocated to one of the three study arms (Figure 1). In the first arm, schools received the school garden and education component about gardening only (SG); in the second arm they additionally received WASH, health and nutrition interventions (SG+), while no specific interventions were implemented in the third arm; hence, serving as control.

Outcome indicators

The outcome indicators and expected results are presented in Table 1. The presented outcomes were based on the project's impact pathway that assumes stepwise changes in the children's knowledge of fruits and vegetables and intake via school garden that might lead to a change in children's nutritional and health status.

Data collection procedures

The same instruments were employed in the baseline and end-line surveys. The school directors, district and village authorities, parents and children were informed about the purpose and procedures of the study. Enumerators with a background including higher secondary education and health sciences were recruited for the questionnaire survey. The enumerators were not involved in the implementation of the project and were blinded to the intervention status of the school. Written informed consent was obtained from the children, parents or legal guardians of the children. The voluntary nature of participation in the research activities was emphasised. Children aged 8-17 years were enrolled at baseline. At the follow-up survey in June 2016, the same children were re-assessed. Each child was given a unique identification code for the different assessments at the onset of the study.

The sampled children provided fresh mid-morning, post-exercise stool sample, which were processed and analysed the same day by using the Kato-Katz technique, a formalin-ether concentration and a saline wet mount concentration method. Furthermore, the intensity of infection was calculated as the number of eggs per gram of stool (EPG). The selected schoolchildren were subjected to anthropometric measurements according to standard operating procedures, as described by the World Health Organization (WHO), using a digital scale and a height measuring board with a precision of 0.1 kg and 0.1 cm, respectively. The haemoglobin (Hb) level was measured and used as a proxy for anaemia, using a Hemocue portable device (HemoCue Hb 201+ System; HemoCue AB, Angelholm, Sweden). Drinking water samples were collected at the unit of schools, households and community water source [23]. The water samples were analysed *in situ* at the schools and households for turbidity, pH, chlorine residuals and microbial quality using the DelAgua Kit (Oxfam-DelAgua; Guildford, UK) using readily available standard operating procedures. Details of the data collection procedure are described in a previously published study protocol [20].

Statistical analysis

Data were described using percentages, frequencies and means. To characterise household socioeconomic status, we conducted a factor analysis to group households into three socioeconomic strata from a list of 18 household assets and construction material of the house wall, roof and floor [24]. Three factors reflecting household socioeconomic status were retained and each of them divided into three strata (high, middle and poor) using the k-means procedure. As the children who are symptomatic at baseline often systematically differ from children who were asymptomatic at baseline, we decided to not just study change in prevalence but to distinguish change in children who were asymptomatic (i.e. by studying incidence) and change in children who were symptomatic (i.e. by studying "remission" or "persistence" which equals "1-remission"). Mixed logistic regression models with random intercepts of schools were used to estimate intervention effects on incidence and persistence of binary outcomes, such as intestinal parasite infections (primary outcome), anaemia (primary outcome), stunting (primary outcome) and thinness, between baseline and end-line. These models also included the factors district, sex and age group. To address change in prevalence, repeated measures analyses with additional random intercepts at the level of children were used. Models of change in prevalence additionally involved a survey indicator variable to address potential period effects and interactions of this variable with the intervention indicator variables to estimate and compare changes in prevalence across the different study arms. The change in prevalence is determined by the persistence (e.g. children who were stunted at baseline and were still stunted at end-line and whether there was a difference between groups) and incidence along with the baseline prevalence according to the formula:

*Prevalence at follow-up = (prevalence at baseline) * persistence + (1-prevalence at baseline) * incidence.*

All effect estimates regarding dichotomous outcomes are reported as OR with 95% confidence intervals (CIs).

Mixed linear regression models with random intercepts for schools were applied to assess intervention effects on longitudinal changes of continuous variables such as dietary diversity scores (DDS), height and weight, and Hb level. These models included the baseline value of the respective outcome as one of the predictor variables along with age, sex, district and socioeconomic status. Differences were considered statistically significant if *p*-values were <0.05. All analyses were carried out using STATA, version 14 (STATA Corporation; College Station, TX, USA).

9.4. Results

Study compliance and characteristics of study population

Of the 708 children who were enrolled at the March 2015 baseline survey, 682 children completed the questionnaire survey and 624 children completed all aspects of health and nutritional examination (anthropometry, stool examination, Hb measurements) at the June 216 end-line survey. Of four schools allocated to receive the SG intervention, a total of 172 children completed the follow-up. For the four schools allocated to receive the SG+, a total of 197 children completed the end-line and for the four schools allocated to the control group without any intervention, 313 children completed the end-line survey in both districts. Due to the proximity of the earthquake epicentre to the study area, which destroyed around 75% of schools and households in May 2015, 26 children were lost from baseline and 89 of 562 households were no longer accessible at the end-line survey in both districts. Hence, complete data were available from 433 households. Therefore, the final analysis included 433 households, 682 schoolchildren for socio-demography and knowledge, attitude and practice (KAP) and 624 for clinical examination (anthropometry, stool and Hb) (Figure 9.1). We compared the baseline socioeconomic status of the households having participated in the follow-up with those households which were lost at follow-up. From the 31.2% households classified with a high socioeconomic status at baseline, only 8.7% remained in this class at end-line. The percentage of households with an average socioeconomic status increased from 30.9% to 38.3%, while households with poor socioeconomic status increased from 37.9% to 53.0% over the 15-month study period.

Table 9.1: Outcome indicators and expected results among schoolchildren in three intervention arms (SG, SG+ and control) in a randomised controlled trial conducted in two districts of Nepal between March 2015 and June 2016

Outcome	Description of outcome	Expected results
Outcome 1 (Primary outcome)	Change in knowledge about fruits and vegetables, malnutrition, anaemia and intestinal parasitic infection	Schoolchildren know about: • the average daily requirement of intake of fruits and vegetables • malnutrition and its causes • importance of consuming fruits and vegetables for improved health • WASH and related diseases including intestinal parasitic infections
Outcome 2	Change in dietary diversity and fruits and vegetables intake	• the dietary diversity score (DDS) and the average fruits and vegetables consumption will increase among school children among SG+ • the dietary behaviour translates into behaviour change towards increased fruits and vegetables consumption

Outcome 3	Change in nutritional status and haemoglobin level	• the improvement in children's weight and height among schoolchildren in the SG+ arm • the increase of blood haemoglobin levels among schoolchildren in the SG+ arm
Outcome 4 (Primary outcome)	Change in intestinal parasitic infection	• the incidence of intestinal parasitic infections among schoolchildren from intervention schools will be decreased
Outcome 5	Change in water quality, sanitation and hygiene conditions	• WASH conditions will be improved with well-tailored package of interventions implemented at the unit of schools and households

The characteristics (e.g. sex, age) of children and caregivers who completed the follow-up study are described in Table 9.2. More than half of the surveyed children were male (52.7%). There was substantial heterogeneity in the educational status of caregivers across study arms, with 51.4% of caregivers being without formal education in SG+ compared to 26.6% in the control arm, which has also been taken into account in the statistical analysis. The primary occupation of caregivers was farming across all study arms (90.7% in SG+; 79.3% in SG; 78.1% in control; $p<0.01$). More than three quarter of the schoolchildren from all groups had domestic animals in their households (85.0% SG+; 86.8% SG and 94.0% control; $p<0.01$). Most of schoolchildren's households had agricultural land (82.9% SG+; 92.7% SG and 94.0% control; $p<0.01$), and the self-food production was slightly lower in the SG+ arm (82.1%; compared to 90.1% in SG and 91.0% in control; $p<0.01$).

Table 9.2: Characteristics of schoolchildren and caregivers in Dolakha and Ramechhap districts, Nepal at baseline, 2015

Characteristics	Categories	Control [n, (%)]	SG-intervention* [n, (%)]	Complementary intervention (SG+)** [n, (%)]	Total	*p-value*
Children's demographic characteristics						
Sex						
Female		156 (47.3)	77 (43.0)	106 (53.3)	339 (47.9)	0.13
Male		174 (52.7)	102 (57.0)	93 (46.7)	369 (52.1)	
Age groups						
Age group 1 (8-12 years)		47 (14.2)	29 (16.2)	32 (16.1)	108 (15.3)	0.78
Age group 2 (13-16 years)		283 (85.8)	150 (83.8)	167 (83.9)	600 (84.7)	
Mean age of the respondents		14.0 (1.1)				
Caregivers demographic characteristics						
Caregivers education						
No formal schooling		80 (26.6)	58 (48.0)	72 (51.4)	210 (37.4)	<0.01
Primary education		72 (24.0)	36 (29.8)	36 (25.7)	144 (25.6)	
Secondary education		94 (31.2)	22 (18.2)	27 (19.3)	143 (25.4)	
Higher education		55 (18.3)	5 (4.1)	5 (3.4)	65 (11.6)	
Caregivers ethnicity						
Brahmin		28 (9.3)	52 (37.1)	21 (17.4)	101 (18.0)	<0.01
Chhetri		102 (33.9)	56 (46.3)	52 (37.1)	210 (37.4)	
Newar		15 (5.0)	14 (11.6)	4 (2.9)	33 (5.9)	
Tamang		152 (50.5)	30 (24.8)	31 (22.1)	213 (37.9)	
Janajati		4 (1.3)	0 (0.0)	1 (0.7)	5 (0.9)	
Caregivers occupation						
No occupation		21 (7.0)	0 (0.0)	4 (2.9)	25 (4.5)	<0.01
Farmer		235 (78.1)	96 (79.3)	127 (90.7)	458 (81.5)	
Public service		17 (5.7)	17 (14.1)	5 (3.6)	39 (6.9)	
Business owner		28 (9.3)	8 (6.6)	4 (2.9)	40 (7.1)	
Socioeconomic characteristics						
Roof materials	Corrugated iron roof	272 (90.4)	59 (48.8)	84 (60.0)	415 (73.8)	<0.01
	Wood and tiles	29 (9.6)	62 (51.2)	56 (40.0)	147 (26.2)	
Wall materials	Wood	41 (13.6)	15 (12.4)	10 (7.1)	66 (11.7)	0.05
	Corrugated iron	47 (15.6)	12 (9.9)	30 (21.4)	89 (15.8)	
	Bricks	213 (70.8)	94 (77.7)	100 (71.4)	407 (72.4)	

Floor materials	Mud	270 (89.7)	115 (95.0)	139 (99.3)	524 (93.2)	<0.01
	Cement	31 (10.3)	6 (5.0)	1 (0.7)	38 (6.8)	
Energy for cooking	Charcoal/wood	254 (84.4)	96 (79.3)	123 (87.9)	473 (84.2)	0.17
	Electricity	47 (15.6)	25 (20.7)	17 (12.1)	89 (15.8)	
Socioeconomic status	High	28 (9.3)	15 (12.4)	6 (4.3)	49 (8.7)	<0.01
	Middle	96 (31.9)	62 (51.2)	57 (40.7)	215 (38.3)	
	Poor	177 (58.8)	44 (36.4)	77 (55.0)	298 (53.0)	
Own agricultural land		283 (94.0)	112 (92.7)	116 (82.9)	511 (90.9)	<0.01
Total production	≤10%	13 (4.3)	9 (7.4)	22 (15.7)	44 (7.8)	<0.01
	10-13%	14 (4.7)	3 (2.5)	3 (2.1)	20 (3.6)	
	≥30%	274 (91.0)	109 (90.1)	115 (82.1)	498 (88.6)	
Possession of domestic animals		283 (94.0)	105 (86.8)	119 (85.0)	507 (90.2)	<0.01

**SG: School garden intervention*

***SG+: School garden, nutrition and WASH interventions*

p-values were obtained by χ^2 test

Socioeconomic status was derived from a factor analysis of variables indicating the possession of household assets such as a radio, a television, a mobile phone, a table, a stove, a petrol lamp, a motorbike, a car or truck, a watch, an iron, a bike, a cupboard etc. The score of the first factor was then divided into three categories using the k-means procedure.

Change in dietary behaviour and consumption of vegetables and fruits

The changes in dietary behaviour in our cohort of schoolchildren are shown in Table 9.3. There was an improvement in the dietary diversity scores, i.e. from 7 at baseline to 9 at follow-up in all three arms. An increase of knowledge regarding the importance of consuming ≥5 portion of vegetables and fruits per day was found mostly among SG+ schoolchildren (7.1% to 24.9% in SG+; 12.2% to 28.5% in SG; and 10.9% to 26.5% in control). The improvement in knowledge about requirement of vegetables in diet also translated into behavioural change by increasing in the intake of vegetables i.e. SG+ (33.5% to 74.6%), SG (37.2% to 74.4%) and control arm (33.9% to 77.0%). Similarly, the percentage of schoolchildren who heard about malnutrition increased in all schools, but most strongly in SG+ (44.2% to 88.3%), followed by SG (25.6% to 70.9%) and the control arm (26.5% to 68.0%). The changes in dietary consumption at the unit of households are shown in Table 10.4. The consumption of poultry showed a stronger increase in the complementary intervention (SG+) group than in the SG group (28.7% to 77.8 *vs.* 27.3% to 55.5%, $p<0.001$). Moreover, unlike in SG, there was no decrease in the consumption of fruits and lentils in SG+, with the former consumption increasing slightly and the latter one significantly.

Table 9.3: Dietary behaviour of schoolchildren at baseline and follow-up across the different study arms in Dolakha and Ramechhap districts, Nepal (March-May 2015 and June 2016)

Nutrition variables	Categories	Control		SG-intervention		Complementary intervention (SG+)		Effect of SG-intervention (95% CI)	*p*-value	Effect of complementary interventions (SG+) (95% CI)	*p*-value
		Baseline (n=313)	Follow-up (n=313)	Baseline (n=172)	Follow-up (n=172)	Baseline (n=197)	Follow-up (n=197)				
Dietary diversity score[(a)]	1	2 (0.6)	28 (9.0)	2 (1.2)	24 (14.0)	0 (0.0)	14 (7.1)	-0.9 (-2.2-0.3)[(c)]	0.13	0.4 (-1.0-1.9)[(c)]	0.53
	2	30 (9.6)	33 (10.5)	26 (15.1)	27 (15.7)	11 (5.6)	25 (12.7)				
	3	106 (33.9)	32 (10.2)	53 (30.8)	21 (12.2)	50 (25.4)	21 (10.7)				
	4	109 (34.8)	29 (9.3)	53 (30.8)	21 (12.2)	72 (36.6)	22 (11.2)				
	5	50 (16.0)	30 (9.6)	32 (18.6)	22 (12.8)	49 (24.9)	29 (14.7)				
	6	15 (4.8)	27 (8.6)	5 (2.9)	20 (11.6)	14 (7.1)	29 (14.7)				
	7	1 (0.3)	29 (9.3)	0 (0.0)	13 (7.6)	1 (0.5)	34 (17.3)				
	8	0 (0.0)	44 (14.1)	1 (0.6)	13 (7.6)	0 (0.0)	19 (9.6)				
	9	0 (0.0)	61 (19.5)	0 (0.0)	11 (6.4)	0 (0.0)	4 (2.0)				
Self-reported daily consumption of fruit and vegetable consumption[(a)]	0	34 (10.9)	0 (0.0)	29 (16.9)	0 (0.0)	17 (8.6)	0 (0.0)	0.1 (-0.3-0.6) [(c)]	0.56	-0.2 (-0.7-0.3) [(c)]	0.51
	1	7 (2.2)	64 (20.5)	7 (4.1)	25 (14.5)	11 (5.6)	28 (14.2)				
	2	20 (6.4)	166 (53.0)	18 (10.5)	98 (57.0)	19 (9.6)	120 (60.9)				
	3	117 (37.4)	0 (0.0)	68 (39.5)	0 (0.0)	98 (49.8)	0 (0.0)				
	4	101 (32.3)	0 (0.0)	29 (16.9)	0 (0.0)	38 (19.3)	0(0.0)				
	≥5	34 (10.9)	83 (26.5)	21 (12.2)	49 (28.5)	14 (7.1)	49 (24.9)				
Opinion about fruits and vegetables consumption[(b)]	It is not good	24 (7.7)	27 (8.6)	15 (8.7)	11 (6.4)	20 (10.1)	3 (1.5)	2.0 (0.6-6.4)[(d)]	0.41	5.0 (0.9-27.3) [(d)]	0.23
	It am not sure	58 (18.5)	12 (3.8)	61 (35.5)	0 (0.0)	38 (19.3)	0 (0.0)				
	It is good	231 (73.8)	274 (87.5)	96 (55.8)	161 (93.6)	139 (70.6)	194 (98.5)				
Heard about malnutrition		83 (26.5)	213 (68.0)	44 (25.6)	122 (70.9)	87 (44.2)	174 (88.3)	1.2 (0.7-1.1) [(d)]	0.50	2.8 (1.4-5.3) [(d)]	<0.01
Caregivers perceiving malnutrition as a problem		67 (21.4)	189 (88.7)	34 (19.8)	115 (94.3)	73 (37.1)	165 (94.3)	3.3 (0.3-33.8) [(d)]	0.31	0.7 (0.1-10.4) [(d)]	0.83
Responses related to the causes of malnutrition:	Disease	0 (0.0)	44 (14.1)	0 (0.0)	3 (1.7)	2 (1.02)	5 (2.5)	0.1 (0.0-1.2) [(d)]	0.07	2.7 (0.1-58.3) [(d)]	0.53
	Lack of food	19 (6.1)	95 (30.3)	11 (6.4)	36 (20.9)	11 (5.6)	63 (32.0)	0.6 (0.3-1.4) [(d)]	0.27	1.8 (0.8-4.1) [(d)]	0.18
	Unbalanced food intake	19 (6.1)	108 (34.5)	14 (8.1)	46 (26.7)	30 (15.2)	83 (42.1)	0.7 (0.3-1.6) [(d)]	0.45	1.9 (0.8-4.5) [(d)]	0.16
	Poorly prepared food	2 (0.6)	47 (15.0)	1 (0.6)	14 (8.1)	3 (1.5)	19 (9.6)	0.5 (0.2-1.8) [(d)]	0.34	1.2 (0.3-4.8) [(d)]	0.81

	Lack of means to afford good food	3 (1.0)	36 (11.5)	0 (0.0)	10 (5.8)	5 (2.5)	24 (12.2)	0.5 (0.2-1.8) [(d)]	0.30	2.4 (0.6-9.1) [(d)]	0.21
Consumption of green vegetables prior to day of survey (24 hours)		123 (39.3)	177 (56.5)	50 (29.1)	98 (57.0)	87 (44.2)	102 (51.8)	1.0 (0.7-1.69 [(d)]	0.87	0.8 (0.5-1.3) [(d)]	0.32
Consumption of other vegetables prior to day of survey (24 hours)		106 (33.9)	241 (77.0)	64 (37.2)	128 (74.4)	66 (33.5)	147 (74.6)	0.9 (0.5-1.5) [(d)]	0.63	1.0 (0.6-2.0) [(d)]	0.90
Consumption of fruit prior to the day of survey (24 hours)		138 (44.1)	134 (42.8)	64 (37.2)	66 (38.4)	105 (53.3)	79 (40.1)	1.15 (0.3-4.4) [(d)]	0.83	1.00 (0.2-4.6) [(d)]	1.00

SG=School garden

SG+= School garden, nutrition , health and WASH

[(a)] *These variables were treated as numerical* [(b)]*The odds ratio was calculated by comparing "good" versus "other" in a mixed logistic regression model adjusted for clustering within schools*

[(c)] *Intervention effects were estimated by mixed linear models for the changes in the respective outcome variables, with the respective intervention indicator variable as main predictor and adjustment for clustering within schools. They can be interpreted as adjusted differences in the mean changes of the respective variables between the given intervention group and the control group*

[(d)] *Odds ratio of desired follow-up outcome between the respective intervention group and the control group from a mixed logistic regression model adjustment for clustering within schools.*

Table 9.4: Changes in key indicators on WASH, health and nutrition at households levels in Dolakha and Ramechhap districts, of Nepal, March/May 2015 and June 2016

Predictors	Group	Baseline (%)	Follow-up (%)	Change in prevalence (%)	Intervention effect *	*p*-value
Water, sanitation and hygiene (WASH)						
Water sufficiency	Control	73.4	78.0	4.6	1.00	
	SG-Intervention	83.8	98.2	14.4	8.3 (1.74-39.28)	0.01
	Complementary interventions (SG+)	82.6	90.9	8.3	1.6 (0.65-4.02)	0.30
Water treatment*	Control	21.2	71.8	50.6		
	SG-Intervention	9.9	65.8	55.9		
	Complementary interventions (SG+)	0.1	41.3	41.2		
Soap available for hand-washing in household	Control	71.8	78.8	7.0	1.00	
	SG-Intervention	78.4	91.0	12.6	2.0 (0.80-5.13)	0.14
	Complementary interventions (SG+)	80.2	87.6	7.4	1.2 (0.52-2.77)	0.67
Health and nutrition						
Heard about night-blindness	Control	52.3	65.3	13.0	1.00	
	SG-Intervention	55.8	64.0	8.2	0.9 (0.39-1.99)	0.77
	Complementary interventions (SG+)	31.4	50.4	19.0	1.2 (0.60-2.34)	0.63
Heard about anaemia	Control	53.1	51.0	-2.1	1.00	
	SG-intervention	44.1	32.4	-11.7	0.6 (0.32-1.28)	0.23
	Complementary interventions (SG+)	20.7	37.2	16.5	2.6 (1.30-5.35)	0.01
Heard about intestinal parasitic infection	Control	20.7	82.6	61.9	1.00	
	SG-Intervention	37.8	59.4	21.6	0.1 (0.06-0.27)	0.01
	Complementary interventions (SG+)	33.9	67.8	33.9	0.2 (0.10-0.49)	0.01
Food supplements to surveyed child	Control	40.2	49.8	9.6	1.00	
	SG-Intervention	18.0	77.5	59.5	15.6 (6.33-38.7)	0.01
	Complementary interventions (SG+)	5.8	64.5	58.7	30.0 (10.61-84.81)	0.01
Prepare vegetable for child	Control	91.3	93.7	2.4	1.00	
	SG-intervention	81.1	86.5	5.4	1.4 (0.42-4.66)	0.58
	Complementary interventions (SG+)	70.2	95.0	24.8	6.8 (2.02-22.94)	0.01
Give fruits to child	Control	85.1	93.7	8.6	1.00	
	SG-intervention	82.3	92.8	10.5	1.3 (0.40-4.56)	0.62
	Complementary interventions (SG+)	66.1	98.3	32.2	21.2 (3.99-112.83)	0.01

SG: School garden

SG+: School garden, nutrition and water, sanitation and hygiene
**Odds ratios are referring to the interaction between period (represented by an indicator variable for end line observations) and the respective intervention (with control group serving as the reference category) and were estimated by mixed logistic regression models adjusted for clustering within schools and children.*
**Chlorination water treatment was introduced in all three arms during the study period from different humanitarian organisations as well in the households, thus no statistical comparisons were made.*

Changes in KAP on WASH among schoolchildren

The change in KAP on WASH among schoolchildren is shown in Table 9.5. Handwashing with soap (a) before eating and (b) after defection showed stronger increases from baseline to end-line in SG+ compared to the control arm, with (a) 74.1% to 96.9% *vs*. 78.3% to 84.0% (p <0.01), and (b) 77.2% to 99.0% *vs.* 78.0% to 91.0% (p = 0.06). The proportion of children bringing drinking water from home decreased in the SG+ (21.8% to 11.7%), while it increased in SG (11.0% to 27.3%) and control (11.2% to 43.1%). The intervention had no effect on knowledge related to the diseases such as diarrhoea and cholera.

Table 9.5: Change in knowledge , attitude and practices regarding water, sanitation and hygiene among schoolchildren in Dolakha and Ramechhap districts, Nepal, (March-May 2015 and June 2016)

Outcomes	Categories	Baseline (2015) Control (n=313)	SG-Intervention (n=172)	Complementary intervention (SG+) (n=197)	Follow-up (2016) Control (n=313)	SG-Intervention (n=172)	Complementary intervention (SG+) (n=197)	Effect of SG-intervention* (95% CI)	*p-value*	Effect of complementary intervention (SG+)* (95% CI)	*p-value*
Hand washing:											
Before eating		244 (78.0)	115 (66.9)	146 (74.1)	263 (84.0)	149 (86.6)	191 (96.9)	1.36 (0.71-2.18)	0.44	4.41 (1.34-14.52)	0.01
After playing		186 (59.4)	90 (52.3)	127 (64.5)	203 (64.9)	125 (72.7)	141 (71.6)	1.48 (0.68-3.23)	0.32	0.95 (0.39-2.30)	0.90
After defecation		244 (78.0)	117 (68.0)	152 (77.2)	285 (91.0)	168 (97.7)	195 (99.0)	4.37(1.50-12.76)	0.01	2.21 (0.40-12.24)	0.36
Children bringing drinking water from home		35 (11.2)	19 (11.0)	43 (21.8)	135 (43.1)	47 (27.3)	23 (11.7)	0.60 (0.18-2.03)	0.42	0.25 (0.06-1.06)	0.06
Children using hygienic latrine at school		308 (98.4)	156 (90.7)	190 (96.4)	306 (97.8)	171 (99.4)	196 (99.5)	5.04 (0.43-59.51)	0.12	0.96 (0.04-20.99)	0.98
Dirty water causing:	Diarrhoea	196 (62.6)	104 (60.5)	140 (71.1)	281 (89.8)	163 (94.8)	196 (99.5)	3.77 (0.22-65.9)	0.36	5.48 (0.12-251.73)	0.38
	Cholera	36 (11.5)	45 (26.2)	54 (27.4)	313 (100.0)	172 (100.0)	197 (100.0)	n/a		n/a	
	Skin Irritation	26 (8.3)	7 (4.1)	14 (7.1)	56 (17.2)	15 (8.7)	6 (3.0)	0.45 (0.12-1.61)	0.22	0.29 (0.05-1.53)	0.14
	Typhus	22 (7.0)	10 (5.8)	9 (4.6)	42 (13.4)	8 (4.6)	12 (6.1)	0.45 (0.79-2.59)	0.37	1.41 (0.19-10.22)	0.73
	Eye irritation	2 (0.6)	0 (0.0)	7 (3.5)	22 (7.03)	1 (0.6)	1 (0.51)	n/a		n/a	
	Worms/parasites	28 (8.9)	16 (9.3)	26 (13.2)	52 (16.6)	29 (16.9)	21 (10.7)	1.02 (0.62-1.67)	0.95	0.58 (0.32-1.07)	0.08

SG: School garden

SG+: School garden, nutrition and water, sanitation and hygiene

**Odds ratio from a mixed logistic regression model of outcome at follow-up as a function of outcome at baseline and group, with a random effect for school.*

Changes in anthropometric indicators and anaemia among schoolchildren

The changes in anthropometric indicators and anaemia among schoolchildren are shown in Table 9.6. Stunting was slightly lowered in SG+ (19.9% to 18.3%; p=0.92) and in the control arm (19.7% to 18.9%) and slightly increased in SG (17.7% to 19.5%; p=0.54), however, without any significant difference. Thinness increased both in SG+ (5.7% to 9.9%; p<0.01 compared to control) and SG (9.7% to 10.4%, p=0.12 compared to control) and decreased in the control arm (12.3% to 7.1%). There was a slight reduction in anaemia in SG+ (33.0% to 32.0%) but a major increase was observed in SG (20.7% to 43.9%) and the control arm (22.7% to 41.3%), the difference in change of prevalence between SG+ and control arm being statistically significant (p<0.01).

Table 9.6: Change in prevalence of targeted health outcomes in cohort of schoolchildren in Dolakha and Ramechhap districts Nepal (March/May 2015 and June 2016)

Outcomes	Group	Baseline Prevalence (%/mean /sd)	Follow-up Prevalence (%/mean/sd)	Change in Prevalence (%/mean/sd)	Interaction OR* (95% CI)	*p-value*
Intestinal parasitic infections	Control	43.9	42.4	-1.5	1.00	
	SG-Intervention	33.5	27.4	-6.1	0.78 (0.43-1.42)	0.42
	Complementary intervention (SG+)	37.1	9.4	-27.7	0.17 (0.34-0.89)	<0.01
Anaemia	Control	22.7	41.3	18.6	1.00	
	SG-Intervention	20.7	43.9	23.2	1.23 (0.62-2.45)	0.56
	Complementary intervention (SG+)	33.0	32.0	-1	0.31 (0.16-0.59)	<0.01
Hb level	Control	12.6 (1.2)	11.9 (1.8)	-0.7	1.00	
	SG-Intervention	12.7 (1.2)	12.0 (1.5)	0.7	0.04 (-0.53-0.62)	0.89
	Complementary intervention (SG+)	12.3 (1.4)	12.5 (1.3)	0.2	0.61 (0.04-1.17)	0.04
Stunting	Control	19.7	18.9	-0.8	1.00	
	SG-Intervention	17.7	19.5	1.8	1.28 (0.58-2.84)	0.54
	Complementary intervention (SG+)	19.9	18.3	-1.6	0.96 (0.45-2.06)	0.92
HAZ score	Control	-1.4 (1.1)	-1-4 (1.4)	0	1.00	
	SG-Intervention	-1.3 (0.9)	-1.4 (1.4)	0.1	-0.12 (-0.53-0.29)	0.57
	Complementary intervention (SG+)	-1.4 (1.0)	-1.5 (1.1)	0.1	0.23 (-0.18-0.63)	0.27
Wasting	Control	12.3	7.1	-5.2	1.00	
	SG-Intervention	9.7	10.4	0.7	2.31 (0.79-6.77)	0.12
	Complementary intervention (SG+)	5.7	9.9	4.2	4.51 (1.46-13.9)	<0.01
WHZ	Control	-1.02 (0.9)	-0.56 (1.0)	0.46	1.00	
	SG-Intervention	-0.95 (0.9)	-0.83 (1.1)	-0.12	-0.27 (-0.68-1.36)	0.19
	Complementary intervention (SG+)	-0.44 (0.9)	-0.74 (0.96)	0.30	-0.31 (-0.71-0.99)	0.13

Data are in percentage

Control: n=313; SG-intervention: n=172; Complementary intervention: n=197

SG: School garden

SG+: School garden, nutrition and water, sanitation and hygiene

**Odds ratios are referring to the interaction between period (represented by an indicator variable for end line observations) and the respective intervention (with control group serving as the reference category) and were estimated by mixed logistic regression models adjusted for clustering within schools and children.*

a: Cut off point for Anaemia: haemoglobin lower than 80 grams per litre

b: Stunting: height for age <-2 SD of the WHO Child Growth Standards Median

c: Wasting: weight for height <-2 SD of the Child Growth Standards Median

The persistence and incidence of anthropometric indicators and anaemia at end-line are shown in Table 9.7. The persistence of stunting was slightly lower in SG+ (36.8%) than in the control arm (37.7%). The incidence of stunting was slightly higher in SG (16.3%) than SG+ (13.7%) and control arm (14.3%). The mean increase in height and weight were highest in SG+ (6.8 cm and 5.8 kg, respectively), intermediate in the control (5.2 cm and 6.2 kg, respectively) and lowest in SG (3.2 cm and 3.5 kg, respectively). The height and weight gains in the SG arm were significantly lower than the ones in the control arm. Persistence of anaemia was higher in SG (67.6%) than in SG+ (47.6%) and the control arm (52.5%). The mean change in Hb level was significantly higher in SG+ than in the control arm (Δ=0.61, 95% CI: 0.04-1.17; *p*=0.04).

Table 9.7: Differences in nutritional indicators for the study cohort at follow-up by group (control, SG-intervention and combined intervention (SG+) in Dolakha and Ramechhap districts, Nepal (June 2016)

Outcomes	Follow-up			Effect of SG-intervention* (95% CI)	*p-value*	Effect of complementary interventions (SG+)* (95%CI)	*p-value*
	Control	SG-intervention	Complementary interventions (SG+)				
*Logistic models (binary outcomes)**							
Persistence** of stunting	20 (37.7)	10 (34.5)	14 (36.8)	0.88 (0.32-2.39)	0.80	0.97 (0.39-2.42)	0.95
Persistence of thinness	8 (24.2)	4 (25.0)	4 (36.4)	1.00 (0.25-4.01)	1.00	1.71 (0.40-7.43)	0.47
Persistence of overweight	4 (66.7)	0 (0.0)	1 (14.3)	n/a		n/a	
Persistence of anaemia	32 (52.5)	23 (67.6)	30 (47.6)	2.01 (0.74-5.49)	0.17	0.87 (0.38-2.02)	0.75
Incidence of stunting	31 (14.3)	22 (16.3)	21 (13.7)	1.24 (0.62-2.46)	0.55	0.97 (0.49-1.92)	0.94
Incidence of thinness	11 (4.70)	13 (8.8)	15 (8.3)	1.86 (0.72-4.75)	0.20	1.81 (0.74-4.14)	0.19
Incidence of overweight	14 (5.3)	6 (3.7)	6 (3.3)	0.77 (0.21-2.74)	0.69	0.62 (0.18-2.18)	0.46
Incidence*** of anaemia	79 (38.0)	49 (37.7)	31 (24.2)	0.86 (0.36-2.01)	0.72	0.46 (0.19-1.10)	0.08
Linear model (continuous outcomes)$^{\Delta}$							
Change in height-for-age (for stunting)	-0.02 (-0.24, 0.19)	-0.16 (.0.44, 0.13)	0.19 (-0.09, 0.46)	-0.14 (-0.49-0.22)	0.46	0.21 (-0.14-0.56)	0.24
Change in BMI-for age (for wasting)	1.58 (1.14, 2.02)	0.94 (0.35, 1.54)	1.02 (0.44, 1.59)	-0.64 (-1.38-0.10)	0.09	-0.57 (-1.29-0.15)	0.12
Height gain (cm)	5.20 (3.98, 6.43)	3.20 (1.59, 4.81)	6.84 (5.33, 8.35)	-2.01 (-4.03-0.02)	0.05	1.64 (-0.30-3.58)	0.10
Weight gain (kg)	6.16 (5.11, 7.21)	3.50 (2.09, 4.91)	5.75 (4.42, 7.09)	-2.65 (-4.41- (-0.89))	<0.01	-0.40 (-2.10-1.29)	0.64
Change in haemoglobin level (g/dl)	-0.64 (-0.98, -0.30)	-0.60 (-1.06, -0.13)	-0.03 (-0.48, 0.42)	0.42 (-0.54-0.62)	0.89	0.61 (0.04-1.17)	0.04

SG: School garden
SG+: School garden, nutrition and water, sanitation and hygiene
**Logistic regression models: odds ratio from a mixed logistic regression model of outcome at follow-up as a function of outcome at baseline with a random effect for school.*
Δ- First three columns contain estimates and 95% confidence intervals of average changes from baseline to follow-up in the respective study arms, obtained using mixed linear regression model with random intercepts at the level of schools. Column 4 to 7 contain intervention effects (SG vs control and SG+ vs control) estimated by mixed linear models for the changes in the respective outcome variables, with the respective intervention indicator variable as main predictor and adjusted for clustering within schools. They can be interpreted as adjusted differences in the mean changes of the respective variables between the given intervention group and the control group
*** Persistence: Children who were still symptomatic at follow-up (i.e. did not have a remission)*
**** Incidence: Occurrence of new cases*

Change in intestinal parasitic infections in schoolchildren

At baseline, the prevalence of intestinal parasitic infections, among schoolchildren in the three arms, were all high (37.1% in SG+, 33.5% in SG, and 43.9% in the control arm). At the end-line, there was a strong decline to 9.4% in SG+, while the prevalence showed only minor changes in SG and the control arm (Table 9.6).

The persistence and incidence of intestinal parasitic infections at the end-line are presented for all study arms in Table 9.8. The persistence of overall intestinal parasitic infection was significantly lower in SG+ than in the control arm (8.4% *vs*. 45.8%, $p<0.01$). The incidence of overall intestinal parasitic infections was highest in the control arm (39.7%), intermediate in SG (25.7%, $p = 0.05$ compared to the control arm) and lowest in SG+ (10.0%, $p<0.01$ compared to the control arm). The persistence of overall intestinal protozoa was lowest in SG+ (0.0%), comparable in SG (9.1%) and the control arm (10.3%). Similarly, the incidence of overall intestinal protozoa was lowest in SG+ (1.5%, $p<0.01$ compared to control), intermediate in SG (5.8%, $p=0.17$ compared to control) and highest in the control arm (10.4%). Similar patterns were observed for the persistence (a) and incidence (b) of overall soil-transmitted helminthic infections, with values for (a) of 10.3% (SG+), 28.3% (SG) and 47.5% (control arm), and for (b) of 7.3% (SG+), 18.0% (SG) and 28.5% (control arm).

Table 9.8: Intestinal parasitic infections change during follow-up across the different study arms in Dolakha and Ramechhap districts, Nepal (March-May 2015/ June 2016)

Outcomes	Follow-up (2016) Control (n=151/118)#	SG-intervention (n=109/55)#	Complementary interventions (SG+) (n=120/71)#	Effect of SG-intervention (95% CI)	*p-value*	Effect of complementary interventions (SG+) (95% CI)	*p-value*
Persistence of overall intestinal parasitic infections	54 (45.8)	17 (30.9)	6 (8.4)	0.51 (0.20-1.27)	0.15	0.10 (0.32-0.30)	<0.01
Persistence of overall intestinal protozoa	9 (10.3)	4 (9.1)	0 (0.0)	0.83 (0.24-2.88)	0.78	n/a	n/a
Persistence of *Giardia intestinalis*	9 (10.3)	4 (9.1)	0 (0.0)	0.83 (0.24-2.88)	0.78	n/a	n/a
Persistence of overall soil transmitted helminth	56 (47.5)	15 (28.3)	7(10.3)	0.42 (0.18-0.98)	0.05	0.12 (0.05-0.33)	<0.01
Persistence of overall cestodes	3 (4.0)	4 (10.0)	0 (0.0)	n/a	n/a	n/a	n/a
Persistence of *Hymenolepis nana*	3 (4.0)	4 (10.0)	0 (0.0)	n/a	n/a	n/a	n/a
Persistence of overall nematodes	53 (46.1)	11 (22.0)	7 (11.1)	0.32 (0.13-0.78)	0.01	0.14 (0.05-0.38)	<0.01
Persistence of *Ascaris lumbricoides*	19 (21.8)	5 (12.2)	2 (3.5)	0.49 (0.15-1.58)	0.23	0.13 (0.03-0.62)	0.01
Persistence of *Trichuris trichiura*	21 (22.8)	4 (9.5)	2 (3.6)	0.34 (0.09-1.28)	0.11	0.12 (0.22-0.60)	0.01
Persistence of hookworm	18 (20.9)	2 (4.8)	3 (4.8)	0.19 (0.04-0.88)	0.03	0.20 (0.06-0.71)	0.01
Incidence of overall intestinal parasitic infections	60 (39.7)	28 (25.7)	12 (10.0)	0.49 (0.24-1.00)	0.05	0.16 (0.07-0.36)	<0.01
Incidence of overall intestinal protozoa	19 (10.4)	7 (5.8)	2 (1.5)	0.53 (0.23-1.31)	0.17	0.14 (0.03-0.59)	<0.01
Incidence of *Giardia intestinalis*	19 (10.4)	7 (5.8)	2 (1.5)	0.53 (0.23-1.31)	0.17	0.14 (0.03-0.59)	<0.01
Incidence of overall soil transmitted helminths	43 (28.5)	20 (18.0)	9 (7.3)	0.48 (0.19-1.20)	0.12	0.16 (0.06-0.48)	<0.01
Incidence of overall cestodes	10 (5.1)	5 (4.0)	0 (0.0)	n/a		n/a	
Incidence of *Hymenolepis nana*	10 (5.1)	5 (4.0)	0 (0.0)	n/a		n/a	
Incidence of overall nematodes	39 (25.3)	15 (13.2)	9 (7.0)	0.35 (0.12-1.02)	0.05	0.17 (0.05-0.56)	<0.01
Incidence of *Ascaris lumbricoides*	1 (0.5)	0 (0.0)	1 (0.7)	n/a		n/a	
Incidence of *Trichuris trichiura*	18 (10.2)	5 (4.1)	5 (3.7)	0.33 (0.09-1.22)	0.10	0.33 (0.10-1.10)	0.07
Incidence of hookworm	35 (19.1)	10 (8.2)	3 (2.3)	0.35 (0.13-0.94)	0.04	0.10 (0.02-0.36)	<0.01

SG: School garden
SG+: School garden, nutrition and water, sanitation and hygiene
#: The first number (n) is for the children having been without the respective parasite at baseline and the second one (n) for children having been infected by the respective parasite at baseline.
**Odds ratio from a mixed logistic regression model of outcome at follow-up as a function of outcome at baseline and type of intervention, with a random effect for school.*
Persistence was analysed in the sample of children who had the outcome at baseline and incidence among children who were free of the outcome at baseline.

Changes in key indicators on WASH, health and nutrition in households

The changes in key indicators from the questionnaire related to WASH, health and nutrition in the surveyed schoolchildren's households are presented in Table 9.9. The proportion of water sufficiency increased significantly in SG compared to control (83.8% to 98.2%; *p*=0.01). The proportion of caregivers having "heard about intestinal parasitic infections" increased in all three arms (33.9% to 67.8%, *p*=0.01 in SG+; 37.8% to 59.4%, *p*=0.01 in SG; and 20.7% to 82.6% in the control arm). The proportion of households providing food supplements increased significantly in SG (18.0% to 77.5%, *p*=0.01) and SG+ (5.8% to 64.5%; *p*=0.01), compared to control (40.2% to 49.8%). The proportion of households preparing vegetables increased in all three arms (from 70.2% to 95.0%, *p*=0.01 in SG+, from 81.1% to 86.5%, *p*=0.58 in SG and from 91.3% to 93.7% in the control arm). The same was true for the proportion of households giving fruits to children (from 49.0% to 51.0%, *p* <0.001 in SG+, from 50.4% to 14.2% in SG and from 54.6% to 76.6% in the control arm).

Table 9.9: Changes in key indicators on WASH, health and nutrition at households levels in Dolakha and Ramechhap districts, of Nepal, March/May 2015 and June 2016

Predictors	Group	Baseline (%)	Follow-up (%)	Change in prevalence (%)	Intervention effect *	*p*-value
Water, sanitation and hygiene (WASH)						
Water sufficiency	Control	73.4	78.0	4.6	1.00	
	SG-Intervention	83.8	98.2	14.4	8.3 (1.74-39.28)	0.01
	Complementary interventions (SG+)	82.6	90.9	8.3	1.6 (0.65-4.02)	0.30
Water treatment*	Control	21.2	71.8	50.6		
	SG-Intervention	9.9	65.8	55.9		
	Complementary interventions (SG+)	0.1	41.3	41.2		
Soap available for hand-washing in household	Control	71.8	78.8	7.0	1.00	
	SG-Intervention	78.4	91.0	12.6	2.0 (0.80-5.13)	0.14
	Complementary interventions (SG+)	80.2	87.6	7.4	1.2 (0.52-2.77)	0.67
Health and nutrition						
Heard about night-blindness	Control	52.3	65.3	13.0	1.00	
	SG-Intervention	55.8	64.0	8.2	0.9 (0.39-1.99)	0.77
	Complementary interventions (SG+)	31.4	50.4	19.0	1.2 (0.60-2.34)	0.63
Heard about anaemia	Control	53.1	51.0	-2.1	1.00	
	SG-intervention	44.1	32.4	-11.7	0.6 (0.32-1.28)	0.23
	Complementary interventions (SG+)	20.7	37.2	16.5	2.6 (1.30-5.35)	0.01
Heard about intestinal parasitic infection	Control	20.7	82.6	61.9	1.00	
	SG-Intervention	37.8	59.4	21.6	0.1 (0.06-0.27)	0.01
	Complementary interventions (SG+)	33.9	67.8	33.9	0.2 (0.10-0.49)	0.01
Food supplements to surveyed child	Control	40.2	49.8	9.6	1.00	
	SG-Intervention	18.0	77.5	59.5	15.6 (6.33-38.7)	0.01

	Complementary interventions (SG+)	5.8	64.5	58.7	30.0 (10.61-84.81)	0.01
Prepare vegetable for child	Control	91.3	93.7	2.4	1.00	
	SG-intervention	81.1	86.5	5.4	1.4 (0.42-4.66)	0.58
	Complementary interventions (SG+)	70.2	95.0	24.8	6.8 (2.02-22.94)	0.01
Give fruits to child	Control	85.1	93.7	8.6	1.00	
	SG-intervention	82.3	92.8	10.5	1.3 (0.40-4.56)	0.62
	Complementary interventions (SG+)	66.1	98.3	32.2	21.2 (3.99-112.83)	0.01

SG: School garden
SG+: School garden, nutrition and water, sanitation and hygiene
**Odds ratios are referring to the interaction between period (represented by an indicator variable for end line observations) and the respective intervention (with control group serving as the reference category) and were estimated by mixed logistic regression models adjusted for clustering within schools and children.*
**Chlorination water treatment was introduced in all three arms during the study period from different humanitarian organisations as well in the households, thus no statistical comparisons were made.*

Changes in drinking water quality in households

The thermo-tolerant coliforms (TTC) in the drinking water showed considerably higher percentages in all study groups at the end-line compared to baseline (increase from 0.0% to 13.7% in SG+; increase from 2.4% to 9.5% in SG and increase from 3.9% to 14.8% in the control arm) (Table 9.10).

Table 9.10: Water quality parameters of households at baseline and its change during follow-up across the different study arms in Dolakha and Ramechhap districts, Nepal (March-May 2015 and June 2016)

Category	Parameters	Unit	Range	Baseline (2015) Control	SG-intervention	Complementary interventions (SG+)	Follow-up (2016) Control	SG-intervention	Complementary interventions (SG+)	Effect of SG-intervention (95% CI)	*p-value*	Effect of complementary interventions (SG+)(95% CI)*	*p-value*
										□ change*			
Physical characteristics	Turbidity	NTU	>5	7 (3.0)	3 (3.6)	0 (0.0)	46 (20.0)	26 (30.9)	23 (31.5)	0.53 (-0.67-1.72)	0.39	0.59 (-0.76-1.94)	0.39
			2-5	223 (97.0)	81 (96.4)	73 (100.0)	184 (80.0)	58 (69.1)	50 (68.5)				
	pH		6.5-8.5	230 (100.0)	84 (100.0)	73 (100.0)	230 (100.0)	84 (100.0)	73 (100.0)				
Chemical characteristics	Free residual chlorine	mg/L	0.3-0.5	0 (0.0)	0(0.0)	0(0.0)	42 (18.3)	26 (30.9)	18 (24.7)	0.02(-0.15-0.58)	0.26	0.01 (-0.03-0.05)	0.73
			0.1-0.2	230 (100.0)	84 (100.0)	73 (100.0)	188 (81.7)	58 (69.1)	55 (75.3)				
	Total residual chlorine	mg/L	0.2-0.499	230 (100.0)	84 (100.0)	73 (100.0)	2 (0.9)	0 (0.0)	0 (0.0)	-0.01 (-0.36-0.02)	0.74	0.02 (-0.02-0.05)	0.32
			0-0.199	0 (0.0)	0(0.0)	0(0.0)	3 (1.3)	4 (4.8)	0 (0.0)				
			≥0.5	0 (0.0)	0(0.0)	0(0.0)	225 (97.8)	80 (95.2)	73 (100.0)				
										OR**			
Microbiological characteristics	Thermo-tolerant coliforms	CFU/100 mL	<1	170 (73.9)	68 (80.9)	68 (93.2)	171 (74.3)	59 (70.2)	52 (71.2)	1.34 (0.51-3.52)	0.55	0.95 (0.27-3.27)	0.93
			1-10	37 (16.1)	10 (11.9)	5 (6.8)	9 (3.9)	13 (15.5)	4 (5.5)				
			11-100	14 (6.1)	4 (4.8)	0 (0.0)	16 (7.0)	4 (4.8)	7 (9.6)				
			>100	9 (3.9)	2 (2.4)	0 (0.0)	34 (14.8)	8 (9.5)	10 (13.7)				

SG: School garden

SG+: School garden, nutrition and water, sanitation and hygiene

** Estimated by a mixed linear regression model for the change in the respective outcome variable, with the given intervention indicator variable as main predictor and adjusted for clustering within schools.*

*** Odds ratios are referring to the interaction between period (represented by an indicator variable for end-line observations) and the respective intervention (with control group serving as the reference category) and were estimated by mixed logistic regression models adjusted for clustering within schools and children*

9.5. Discussion

Our study assessed the effects of school gardens and complementary nutrition and WASH interventions on children's KAP about fruits and vegetables, their dietary diversity, intestinal parasite infections and nutritional status in the districts of Dolakha and Ramechhap, Nepal within the frame of the VgtS project. Only few studies have investigated an effect of SG and SG+ interventions on children's nutritional practices, anthropometric indices and intestinal parasitic infections. The novelty of our approach was to assess a number of behavioural, health and nutritional outcome indicators in the frame of a school garden programme.

Effects on intestinal parasitic infections, anaemia, anthropometry and KAP on WASH

Our results indicate that the SG+ intervention significantly reduced intestinal parasite infections and anaemia in comparison to control, which might be partly due to the impact of applied interventions such as increase in knowledge in handwashing before eating and deworming within 6 months intervals. Consistently, the strongest increase in the schoolchildren's handwashing before eating was observed in the SG+ arm. Furthermore, significant improvements in caregivers' knowledge on nutrition indicators, such as preparation of vegetables and giving fruits to children, increased in the SG+ arm. Stunting has slightly decreased in the SG+ and SG arms, but these changes were not significantly different from the slight increase observed in the control arm. No measurable improvements were observed for thinness.

The significant decrease in anaemia and intestinal parasite infections could be partially explained by deworming in 6 months interval that increased Hb level among children in SG+. The decrease could also be explained by the number of complementary interventions to the schoolchildren and their caregivers leading to increased knowledge on handwashing before eating and after defecation. Similar programmes that combined WASH and nutrition interventions in Bangladesh and Peru have shown impressive results with respect to health (increased access to safe water, improved sanitation and enhanced handwashing), reduced anaemia and improved nutritional indicators (increased dietary diversity scores, reduced stunting) [25]. Our study showed no effect of the intervention on stunting and thinness and this might be explained by the duration of intervention before the end-line assessment. Of note, height and weight may not be ideal indicators for schoolchildren because of unequal growth during adolescence [26]. A study conducted in Bangladesh reported that the odds of being stunted in adolescence could be explained by the combined effect of being stunted in childhood and having mothers whose height was <145 cm [26]. Furthermore, the same study reported that girls were more likely to be stunted in childhood than boys, whereas boys were more likely than girls to be stunted in adolescence and this might be due to the difference in pace of maturation [26]. As a limitation, we did not explore the history of stunting among children in their childhood, which could be considered in future studies.

Effects on fruits and vegetables consumption

The intervention studies conducted among children and youths have suggested that gardening can lead to improvements in fruit and vegetables consumption [27–29]. Published studies have measured the relationship between schoolchildren's fruits and vegetables intake and participation in a school garden programme. The results were, however, inconsistent for comparison with our study that only revealed a minor effect [1, 2, 27, 28, 30, 31]. Studies conducted among schoolchildren reported significant beneficial effect on fruits and vegetables intake [28, 31]; one study reported a significant beneficial effect of school garden on vegetable consumption only [30]; another study reported only minor effects of school garden on fruits and vegetables intake [2]; one study found a significant beneficial effect on fruits and vegetables consumption in boys only [27]; while, yet another one study reported no differences between boys and girls in fruits and vegetables intake [1]. Christian *et al*. (2014) found little evidence to support that school gardens alone could improve students' fruits and vegetables intake. The authors though reported that when the school garden programme was integrated within an educational component (curriculum), students' daily fruits and vegetables consumption significantly increased, which is in line with the findings of our study, showing a small effect on the consumption of fruits and vegetables and growth indicators.

Effects on the school curriculum and involvement of children and teachers for school gardening

The main aim of SG in the VgtS project was to introduce children to basic gardening skills such as land levelling, raising beds for drainage and easy planting, watering, weeding and harvesting. Two weeks on every Friday, 90 minutes were allocated to school garden education. Previous successful gardening interventions used to involve additional elements to the gardening activities, such as health promotion programmes [1, 2, 31, 32]. In our study, we found positive impacts on children's fruits and vegetables intake, anaemia status and intestinal parasite infections when schools integrated gardening activities throughout their curriculum and implemented additional complementary interventions (SG+). However, experiences and lessons learned are that for sustainability of the programme, schools need continued support for the provision of regular refreshment trainings on knowledge related to the gardening, health, nutrition and WASH. Of note, the successful interventions in prior trials were implemented by teachers [1, 2, 30, 31], which was partly the case in our study.

Effects on water quality

In our survey, some water samples from both SG and SG+ households exceeded the national tolerance limit for TTC contamination (<1 colony forming unit (CFU)/100 ml). The microbiological analyses of water samples revealed the presence of TTC in 25 water samples of SG with eight of these samples having TTC >100 CFU/100 ml; and 17 water samples of SG+ with 10 of these samples having TTC >100 CFU/100 ml that call for specific treatment. Of note, despite households reporting of obtaining water from improved sources and treating water, faecal contamination was still observed in most of the water samples. The increased water contamination with TTC might have been caused by garbage discarded in open spaces in close proximity to drinking water points, open defecation practices or cross-contamination between water supply and sewage system, leaky pipes contaminating the water via runoff or behavioural practices during transportation. Similar findings of cross-contamination and leakage points, old pipelining and drainage system and back siphoning have been reported in a study conducted in Myagdi district and a mountainous region of Nepal [33, 34].

Taken together, our study showed that combining school garden, WASH, regular deworming and nutrition interventions resulted in decreased intestinal parasitic infection and anaemia and increased knowledge of children about requirement of consumption of more than five portions of fruits and vegetables per day. This approach addressed the immediate causes of undernutrition (e.g. providing awareness about requirement of consumption of nutrient-dense fruits and vegetables via school garden) as well as the underlying contributing factors that included lack of access to clean water and sanitation, recurrent infectious diseases and lack of awareness on health and hygiene.

Study limitations

The main issues encountered were related to difficulties in implementing SG and SG+ interventions in our study, explained by the relatively short implementation period (5 months only). It is conceivable that school gardens require longer term commitment, and a supportive team for protecting and maintaining garden over the regular days as well as during school holidays. More specifically, there are several limitations to our study.

First, although, the number of clusters in the intervention and control arms was the same, the numbers of children within the clusters and between the two districts were different. This is mainly explained by the challenge posed by the April 2015 earthquake, which affected particularly the Ramechhap district. Indeed, 26 children and 89 households were lost during follow-up. Approximately one out of six households (15.8%) were not found in the post-earthquake emergency crises and a number of villages were severely destroyed during the earthquake. In addition, around 3.7% of the school-aged children were lost to follow-up, due to the aftermath of the earthquake, mostly in the intervention schools, which resulted into a loss of statistical power. Second, the numbers of schools selected in Dolakha and Ramechhap districts were not equal, which might be a limiting factor in generalizing the regional differences. Third, only two of the schools had a school meal programme which, due to limited resources, targeted only schoolchildren up to fourth grade. Fourth, the integrated agriculture, nutrition and WASH interventions were implemented only for a relatively short period (5 months) due to delayed project implementation, a major earthquake, an economic blockage between India and Nepal and the end of the project in 2016; that might have limited the larger benefits for children's health and nutritional status. Fifth, we did not explore the history of stunting among children in their childhood, which should be investigated in future studies. Sixth, we did not collect data in different seasons. Instead, the data were collected over a bit more of a single calendar year with different fruits and vegetables being abundant in different periods of the year. This suggests that the true relationships between school gardens and nutrition outcomes, including fruits and vegetable consumption, may have been underestimated for some schools, if data were collected during the low production month. In the meantime, it is possible that schools, opting to maintain a vegetable garden, may be generally more interested in creating a healthier school environment [35]. Seventh, nutritional and WASH practices of children were self-reported and changes in behaviour were not closely observed, which may have resulted in over- or under-reporting. Similarly, it is conceivable that households tend to under- or over-report their dietary consumption patterns and either over- or underestimate their consumption of healthy foods, such as fruits and vegetables, thus resulting in biases of food intake assessment [36]. Eighth, the results from selected schools, households and communities in the Dolakha and Ramechhap districts may not be considered as representative for other parts of Nepal. Ninth, our diagnostic approach consisted of the collection of a single stool sample per child, which was subjected to duplicate Kato-Katz thick smear examination. Clearly, the collection of multiple consecutive stool samples (instead of single specimens) and examination of triplicate or quadruplicate Kato-Katz thick smears would have resulted in higher sensitivity of the diagnostic methods [37]. Although our diagnostic approach for helminth consisted of the collection of a single stool sample per child, stool samples were subjected to duplicate Kato-Katz thick smear examination using multiple diagnostic methods (e.g. Kato-Katz plus formalin ether-concentration and wet mount methods), which enhanced diagnostic accuracy. Tenth and finally, a limitation is that anaemia can be caused by multiple and complex factors. Thus, by using a HemoCue device for Hb measurement, the identification of the exact type of anaemia was not possible and we did not collect data on other important risk factors for anaemia, such as vitamin A, riboflavin and folate deficiencies [38].

Despite these limitations, the current research provides some evidence that SG+ interventions improve direct and indirect determinants of children's nutritional and health indices, by reducing intestinal parasitic infections, improving Hb levels and improving certain hygiene practices. Our model of interventions implemented in these pilot schools could be readily replicated and scaled-up. The study thus holds promise to impact on public health. The methodology used for the study presents a suitable approach for evaluating impacts of school-based programme in settings where there is paucity of information related to schoolchildren's health and nutrition [39]. School gardens and complementary nutrition and WASH interventions could sustainably impact children's dietary and hygiene behaviour in the longer term, if they are linked with a greater involvement of their parents/caregivers.

9.6. Conclusion

Our study suggests that a holistic approach of school gardens, coupled with complementary education, nutrition, WASH and health interventions holds promise to increase children's fruit and vegetable consumption and decrease intestinal parasite infections, stunting and anaemia. We recommend that engaging children into high quality gardening interventions that can also incorporate additional intervention components, such as regular deworming and educational activities (e.g. health promotion programmes and teaching children and their caregivers about healthy foods and hygiene practices) are essential for improving children's dietary intake, health and the nutritional status.

List of abbreviations

CFU, colony forming unit

CI, confidence intervals

EPG, eggs per gram of stool

Hb, haemoglobin

KAP, knowledge, attitude and practices;

NARC, Nepal Agricultural Research Council

OR, odds ratio

SG, school garden

SG+, School garden with complementary intervention

TTC, thermo-tolerant coliforms

VgtS, Vegetables go to School (project)

WASH, water, sanitation and hygiene

WHO, World Health Organization

Declarations

Ethical approval

Ethical approval was obtained from the "Ethikkommission Nordwest- und Zentralschweiz" (EKNZ) in Switzerland (reference number UBE-15/02; approval date: January 12, 2015), the institutional review board of Kathmandu University, School of Medical Sciences, Dhulikhel Hospital, Nepal (reference no. 86/14; approval date: August 24, 2014) and the institutional review board, Nepal Health Research Council (reference no 565; approval date: November 11, 2014). The study is registered at International Standard Randomised Controlled Trial Number register (identifier: ISRCTN 30840; date assigned: July 17, 2015). Participants (children and their parents/caregivers) provided written informed consent, with the opportunity to "opt-out" of the study at any time without further obligation.

Availability of data and material

The data analysed for this study are not publicly available, as they are part of the PhD study of the first author. However, the data are available from the corresponding author upon reasonable request and signature of a mutual agreement. The questionnaires in English are available upon request from the corresponding author.

Competing interest

The authors declare that they have no competing interests.

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Authors contributions

All listed authors contributed to the study design. AS coordinated the field and laboratory work, collected data, supervised research assistants, performed the statistical analysis under the supervision of CS and drafted the manuscript. CS, PO, JG, SE, SS, RK, JU and GC contributed to the interpretation of the data and manuscript writing. All authors read and approved the final version of the manuscript prior to submission.

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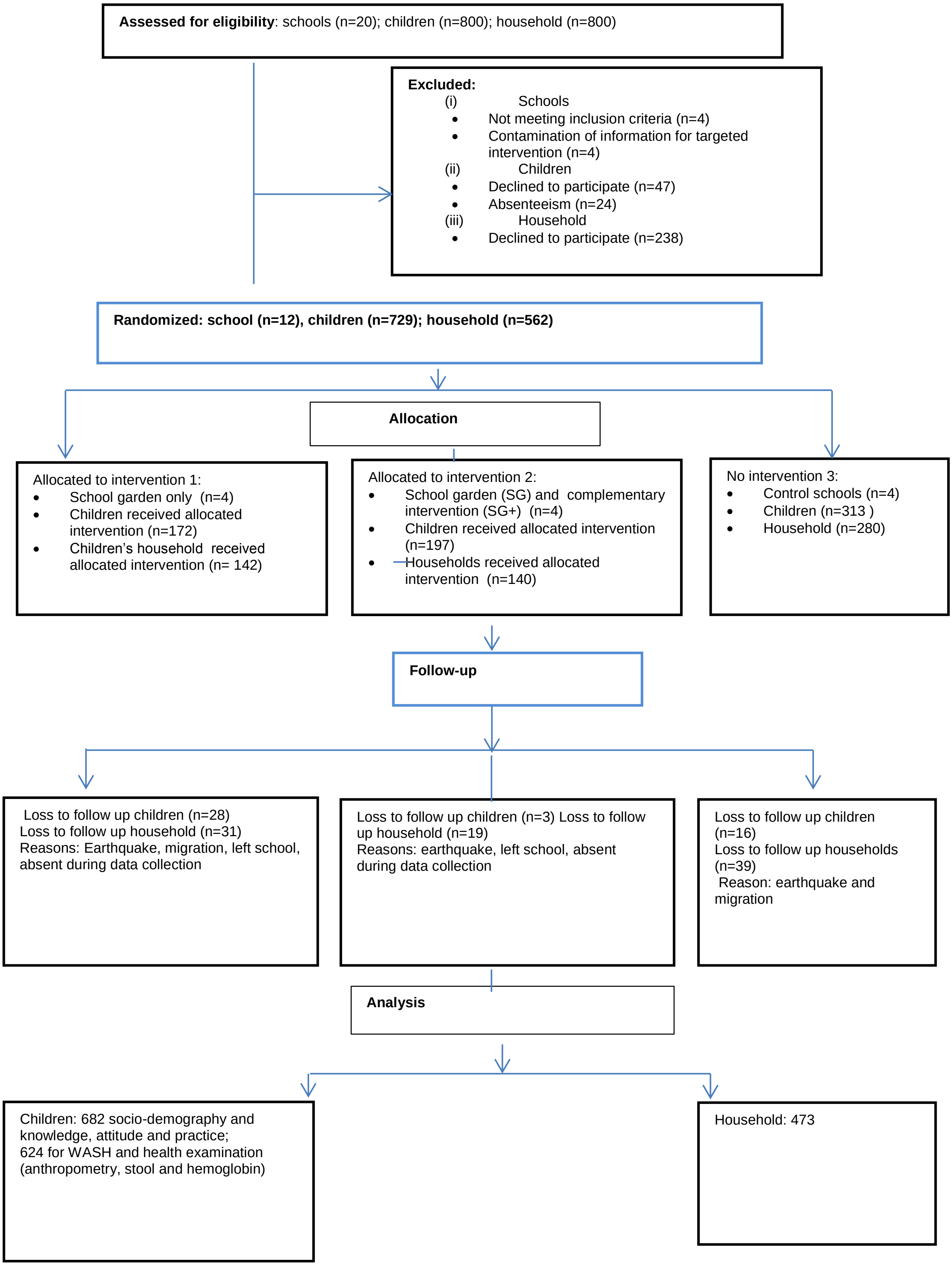
SORT 2010 Flow Diagram

Figure 9.1: Study compliance of the study population



10. Discussion and conclusion

10.1. Outline of the discussion

The overall goal of this PhD thesis was to investigate malnutrition, anaemia and intestinal parasitic infections among schoolchildren in two districts of Nepal and to evaluate the effects of complementary interventions (i.e. school garden, nutrition and water, sanitation and hygiene (WASH) on improving children's nutritional status and mitigating the health issues. The PhD study used interdisciplinary approaches (i.e. applied descriptive and analytical epidemiology linking field and laboratory work) in order to better understand the multiple underlying risk factors for malnutrition, anaemia and intestinal parasitic infections among schoolchildren. The study pursued in the frame of a multi-country research development project entitled "Vegetables go to School: improving nutrition through agricultural diversification" (VgtS). A cluster-randomised controlled trial (RCT), including a baseline and follow-up surveys, was employed. A pack of integrated WASH, nutrition and health interventions, complementary to a school garden program, were implemented and the study then assessed their effects on selected nutrition and health outcomes. The study entailed four specific objectives. The first objective was "to investigate the WASH conditions at the unit of selected schools, households and community" (Chapter 5). The second objective was "to determine the local epidemiology of malnutrition, anaemia and intestinal parasitic infection among schoolchildren" (Chapters 6, 7 and 8). The third objective was "to assess the knowledge, attitude and practices (KAP) of schoolchildren and caregivers regarding nutrition, health and WASH conditions (Chapters 6, 7 and 8). The fourth objective was "to generate evidence on the effects of complementary interventions on selected key indicators for schoolchildren's nutritional and health status fifteen months after a baseline cross-sectional survey" (Chapter 9). The study gave an opportunity, of piloting the implementation of various tailored operational activities, from which a number of helpful lessons can be drawn for similar projects in other parts of Nepal and elsewhere in the future.

This discussion chapter will now highlight, for each objective the key findings, lessons learned and major recommendations. The explanation on PhD work contributing to the three main pillars of the Swiss TPH, in the field of public health; namely innovation, validation and application, are outlined. Finally, a set of conclusions, research needs and recommendations, for further improving schoolchildren's nutrition and health status in Nepal and other low-and middle- income countries, are put forward.

10.2. Overall summary of the research findings

The assessment of health risks and effects has been undertaken by combining (i) environmental assessment to measure microbial and chemical contamination of the water quality and sanitation status (Chapter 5); (ii) epidemiological cross-sectional surveys to identify existing health risks related to malnutrition, anaemia and intestinal parasitic infections (Chapters, 6, 7 and 8); and (iii) development of interventions and applications. The results stemming from our baseline survey confirmed that WASH is not adequate at the unit of the school, households and communities (Chapter 5). We found high prevalence of malnutrition, anaemia and intestinal parasites at baseline (Chapters 6 and 7). Moreover, our study revealed an important association of lack of meals prepared in the household, not having supper and risk of anaemia; households lacking soap for handwashing and intestinal parasitic infections; freely roaming domestic animals; cereal pattern being associated with prevention of stunting. The findings from our cross-sectional survey revealed that there is a discrepancy of knowledge among the schoolchildren and caregivers regarding malnutrition, anaemia and intestinal parasitic infections. The discrepancy was observed between scientifically recognised causes of malnutrition, anaemia and intestinal parasitic infections and local culturally reported causes about the targeted outcomes. This issue is important and is of public health relevance, since a misunderstanding of health messages from the population might blunt any attempted health intervention or education programmes. After the intervention, the prevalences of anaemia, and intestinal parasitic infections were significantly lower at the complementary intervention arm (June 2016), compared to school garden and control arms (Chapter 9). Similarly, preparation of vegetables at the household level also increased in the complementary intervention arm compared to the control. In summary, the study findings showed that changes observed before and after the different types of interventions commencement were likely to contribute to the significant impact on child health and nutrition behaviour (Chapter 9).

10.3. Overall significance of research

Our findings are of considerable importance from a public health and control point of view. The set of generated results can inform caregivers, teachers, local communities, stakeholders and decision makers on potential health benefits of an integrated school gaden program for schoolchildren in Nepal. Moreover, the study released a book "*Binu dreams of school vegetable garden*" and "*Food for fitness,*" a WASH manual "*Water, sanitation and hygiene (WASH) training manual for school health awareness program*" and varieties of information, education and communication (IEC) materials that could be replicated nationwide. A more detailed discussion of each PhD objective is presented below.

Objective 1: To investigate the water, sanitation, and hygiene conditions at the unit of selected schools, households and community

The baseline cross-sectional study, highlighted several WASH challenges at the unit of school, households, and community. Although Nepal was on track of achieving the MDG of ensuring safe water quality and better sanitation facilities; the government still need to ensure proper monitoring mechanisms for systems which are already in place. The water sources of the households were mostly tap and the improved spring water, however, an improved spring water source with a spring box might not be a sufficient protection measure against faecal contamination. Edberg (1997), acknowledges the importance of a spring box, emphasizes the indispensability of further protection measures such as avoiding agriculture, livestock and wastewater runoff close to a spring and on its catchment zone (Edberg et al., 1997). A contamination of the water source is possible in the surveyed districts, being located on a hill and immediately surrounded by agrarian land. Furthermore, as none of the water sources are surrounded by fences, animals can freely enter the catchment zone, thereby causing contamination. Additionally, contamination may come from the garbage discarded in open spaces in close proximity to drinking water points, open defecation practices, or cross-contamination between water points and sewage system. The described conditions are thus likely to favour contamination of water sources and necessitate further protective measures.

For the water quality, we examined turbidity, percentage of hydrogen (pH), residual chlorine and TTC. The water quality analysis could be carried out under good conditions including running and distilled water, stable electricity supply and laboratory ware in the Kirnetar Health Center of DH-KUH. The laboratory was opened 24 hours a day, allowing an uninterrupted sample analysis within 24 hours of sampling.

In our study, the low awareness level of schoolchildren and caregivers on harmful effects of poor WASH practices was very prominent. For example, schoolchildren washed their hands ineffectively, too quickly, not thoroughly enough and/or without soap. The findings related to the WASH in schools and households (presented in Chapter 5) were used for the planning and design of the WASH intervention at complementary intervention arm schools. This approach is similar to approaches outlined by UNICEF (3 Star approach) (GIZ and UNICEF, 2013).

A study, conducted in 2012 at the unit of household, found that most of the households did not treat water before drinking and hygiene behaviour, such as handwashing practices, were found to be more than 90% regardless of toilet availability (Aryal et al., 2012b). This finding is similar in the case of our study. Similarly, another recent study, conducted in the mid-western Nepal, regarding the hygiene behaviour reported of water scarcity and socio-economic disadvantage, being the most frequent constraints of adequate hygiene behaviour, which is also similar in our study (McMichael and Robinson, 2016b).

There are some methodological problems to take into consideration when interpreting the obtained results under objective 1. The information obtained in the thematic field of WASH was self-reported by schoolchildren, their caregivers and teachers. In addition, a major challenge during data collection was the aftermath of the earthquake of 2015 and the economic blockade, including challenges of unavailability of transportation and high expectation and demands of people in the surveyed area.

Objective 2: To determine the local epidemiology of malnutrition, anaemia and intestinal parasitic infection among schoolchildren

A major aspect of the thesis, involved the prevalence assessment of malnutrition, anaemia and intestinal parasitic infections, then the development, production and pilot testing of complementary intervention packages. Our findings, from the anthropometric measurements, showed that malnutrition is moderately prevalent at baseline among the 708 schoolchildren surveyed in the Dolakha and Ramechhap districts of Nepal. Overall 27.0% of the schoolchildren were classified as stunted, 11.3% wasted and 3.4% overweight. In rural areas, inadequate access and diversity of food is a major challenge, thus it is not surprising to find a moderate percentage of malnutrition in schoolchildren in surveyed rural areas. Yet, the comparison with the national previous studies is limited, as schoolchildren were not included in the Demographic and Health Survey as the national nutrition surveillance system in Nepal is focused mostly on under five children (Tiwari et al., 2014; Akhtar, 2016b; Gaire et al., 2016). Even though our study was conducted in a rural setting, our findings suggest that overweight could also be an important parameter to be considered and needs to be monitored in future studies as transition from under- to overweight can occur within the same individual (Drewnowski and Popkin, 1997; Lobstein et al., 2004).

In the same way, the overall prevalence of anaemia was also moderate in our study group with a prevalence of 23.6%. Of those cases, 15.8% had severe, 60.6% had moderate and 23.6% had mild anaemia. The dietary diversity scores was lower among anaemic, compared to non-anaemic children. Less than half of the children with anaemia, had a lower intake of both animal and plant source food. There is one recent study, conducted in Nepal in 2015 among schoolchildren in eastern part of Nepal. This study found 42.4% prevalence of stunting among schoolchildren, which is higher than our findings. The higher prevalence of anaemia found in the study, conducted in the eastern part of Nepal, might be partially explained by the availability and adequacy of food among schoolchildren.

Our findings from intestinal parasitic infection examination showed that the soil-transmitted helminth are widespread among schoolchildren in the studied districts which contrasts with the general decline of intestinal parasitic infections reported in the rest of the Nepal (Kunwar et al., 2016b). Indeed, unsafe WASH and inadequate management of the excreta in the environment may have exacerbated the helminth infection in our study. Additionally, one third of the households reported not having sanitation facilities in their households and almost one third of the children reported not washing their hands with soap after defecation. Furthermore, intestinal protozoa infections were also endemic throughout the survey areas with 30.9% of the schoolchildren harboured *Giardia intestinalis*. Our data showed both a higher and lower prevalence of intestinal protozoa compared to results from other studies in Nepal conducted between 2013 and 2014, which reported rates between 58.6% and 18.5% (Sah et al., 2013d; Pradhan et al., 2014a). We found that anticipation of better hygiene serves as a protective factor for preventing intestinal parasite infection; this might indicate that hygiene behaviour among our schoolchildren needs to be improved. Furthermore, we revealed an important finding of *G. intestinalis* being associated with "freely roaming" livestock in the household, which indicates a potential zoonotic transmission of *Giardia intestinalis*. Future research on the type of livestock and species-specific Giardia species is needed for a deeper understanding of morbidity patterns in children for designing prevention and treatment strategies of associated morbidities.

After baseline data collection and analysis of the targeted outcomes, as research approach, a community-based approach was chosen, involving interviews with schoolchildren, other key informants such as teachers, health workers, caregivers in order to identify potential health, nutrition and WASH risk factors in the study area and to formulate key messages for the educational package. This approach was chosen according to recommendations made by previous studies and behavioural theories (Klepp et al., 2005; Glanz et al., 2008; Taylor-Davydov and Markova, 2014; Robinson et al., 2015). These studies recommend presenting the information in an intellectually, socially and culturally appealing way towards the target group. Whereas common infectious and parasitic diseases pandemic remained major unresolved health problems within the country, emerging non-communicable diseases relating to diet and lifestyle have also been increasing over the last two decades, thus creating a double burden of disease (Amuna and Zotor, 2008).

The community-based mixed method that we applied was absolutely critical for the development of the culturally tailored educational package. The situation analysis, assessment of specific needs of the target population, local culture and customs, dietary pattern and health risk factors and integrating them in the educational package guided by behavioural theories proved to be very successful approach (Chapter 9). An earlier study entitled “The Magic Glasses,” a successful video designed for the prevention of soil-transmitted helminth inspired us to pilot test our package (Bieri, 2013).

Some of the caregivers have less contact with the health worker providing awareness due to the remoteness and their busy schedule after the earthquake. It was also difficult to convince the caregivers for attending the provided health awareness session due to the aftermath of the earthquake and frequent aftershocks as the project district was the epicentre of the earthquake.

Objective 3: To assess the knowledge, attitude and practices (KAP) of schoolchildren and caregivers, regarding nutrition, health and WASH

Inadequate knowledge of mothers leads to inadequate and improper feeding practices, such as giving more carbohydrates instead of fruits and vegetables. In addition, insufficient food portions and insufficient choices of food were observed in our study suggesting inadequate knowledge of the main principles of child feeding practices. However, unavailability of food, little diversity of food that parents could offer and the eating preferences of children were among the reasons for malnutrition and anaemia among children at baseline.

A recent study in Nepal, conducted in peri-urban community, reported about the behavioural foundation for healthy lifestyle beginning in early childhood, when caregivers play a key role in their children’s lives. However, in the meantime the study also reported that caregivers of children of peri-urban community of Nepal lack adequate and accurate understanding about the impact of healthy diet (Oli et al., 2015). The case is similar in our study as more than half of the caregivers are unaware regarding nutrition, intestinal parasites and WASH. Similarly, in our study, we found the low health seeking behaviour among the mothers at baseline is similar to the findings from the recent study conducted in Lele VDC of Nepal where 31% of mothers seek treatment from a traditional healer, when their children suffered from malnutrition and diarrhoea (Shrestha, 2015). Another research conducted in the terai region of Nepal, showed the remarkably lower knowledge about nutrition, and including anaemia among households and is similar to our study (Jones et al., 2005). Another study conducted in Jirel community of eastern Nepal reported a low awareness about the types, causes, and treatments of helminthic infection. People have a frequent inability to confirm the efficacy of drug therapy, this has led to a dissatisfaction with biomedical approaches (Williams-Blangero et al., 1998).

Objective 4: To evaluate the effects of integrated complementary school vegetable gardens, nutrition and WASH interventions on children's nutritional and health status

The rationale for assessing the effects of integrated complementary interventions on the children's nutritional and health status is based on the assumption that these intervention address the underlying factors of the children's nutritional status by:

i) improving dietary diversity and increasing consumption of varieties of fruits and vegetables;

ii) reducing and preventing incidence of intestinal parasitic infections;

iii) improving children's knowledge, attitudes in and practices of health and hygiene behaviours; and

iv) improving WASH conditions at schools.

As a result of these interventions, children would likely have reduced intestinal parasitic infections and a better nutritional status. Our study results show that the school garden and complementary interventions package we implemented and trialled resulted in a reduction of stunting among school children by 1%, anaemia by 1 %, intestinal parasitic infection by >20 %, consumption of fruits and vegetables by >10%. This was due to an increase in knowledge and improved hygienic practices. The study established proof of principle that the school garden and complementary interventions widen the student awareness and changes behaviour, resulting in less anaemia and intestinal parasitic infection cases, and increased consumption of vegetables. This positive impact on the outcomes was clearly attributable to the interventions implemented as no other efforts againsts malnutrition, anaemia and intestinal parasitic infections were implemented in the study area before. The dietary consumption of vegetables increased in the intervention schoolchildren household compared to the control arm.

Apart from the increase in consumption, there was also progress made in the percentage of water treatment, handwashing with soap at the unit of the household compared to baseline in intervention arm. In addition, the handwashing before eating among schoolchildren and after defecation increased in intervention schools. The children receiving complementary interventions showed significant impact on the awareness of malnutrition, intestinal parasitic infections, treatment of drinking water, and caregivers hearing about intestinal parasitic infections, food supplementation, preparing vegetables and giving fruits to the children.

However, herein also lies a challenging finding for water quality- the thermo-tolerant coliforms increased in all study arms drinking water samples although the reporting of water treatment increased in all arms after baseline. This might be very possible that the households tend to over report their water treatment information thus systematically biasing the water treatment result. In addition, further pollution of drinking water may have occurred between the water source and households during the transportation of water or storage.

Other non-studied factors, such as handling, might thus explain the observed deterioration of the water quality from water sources located outside households properties. Wright (2004) reviewed 60 studies and examined differences in drinking water quality between water collection points outside the houses of surveyed households and the final point of use. They found a significant reduction in water quality between the water collection point and the point of use (Wright et al., 2004). The study reported the water quality was observed to be higher in households that covered their storage vessels (Wright et al., 2004), whereas evidence for such an effect could not be found in the present study.

The two weeks prevalence of diarrhoea was found to be reduced among schoolchildren in complementary intervention schools compared to baseline. The implemented interventions might have worked; however on the other hand diarrhoea prevalence also may have been influenced by the climatic factors (i.e., temperature and rainfall)(Levy et al., 2009). The diarrhoea lowering effects of interventions, targeting community water sources as well as treatment of household drinking water, was summarized in a systematic review and meta-analysis conducted by Clasen (2007). The report of diarrhoea in children was significantly reduced by household water treatment and safe water storage (Clasen et al., 2007). Positive effects of household water treatment and improved storage were also reported in other subsequent reviews (Fewtrell et al., 2005; Zwane and Kremer, 2007). There is some evidence for a diarrhoea lowering effect of interventions targeting sanitary facilities (Clasen et al., 2010). Hence, the hygienic interventions, such as health promotion programs are often considered effective methods to reduce diarrhoeal morbidity (Clasen et al., 2010; Ejemot-Nwadiaro et al., 2015). The meta-analysis examining the potential of handwashing to reduce diarrhoea found a reduction in diarrhoea by 47% (Curtis and Cairncross, 2003). Similarly, Ejemot (2008) found the reduction of diarrhoeal prevalence by 30% among children with implemented hygienic intervention (Ejemot-Nwadiaro et al., 2008).

The results of our study also showed that children from the complementary intervention arm had not reduced thinness compared to children from the control arm. The lack of improvement in the children’s nutritional status might be explained by the inadequate dietary intake, since the national school feeding programme was not operational in our study sites and the dietary diversity score at the household was low. However, caregivers’ behaviour on nutrition related activities also depend on the socio-economic context. For example, due to poverty and lack of availability of fruits and vegetables, some of the children might be unable to consume it and also maintain adequate hygiene. Therefore, a significant increase in children’s weight or height gain was not expected.

The complementary intervention implemented arm was able to significantly reduce the anaemia and intestinal parasite infection prevalence. The interventions have proven to contribute to a decline in intestinal parasite infections among schoolchildren of the intervention group, which is in line with the previous findings conducted elsewhere (Freeman et al., 2016; Grimes and Templeton, 2016; Speich et al., 2016c). The study in Sri Lanka showed that anaemia is strongly associated with socio-economic factors and the mother's education level (Marsh et al., 2002; Menon et al., 2007).

The study findings revealed that the education component of the project was key to improving the children's nutritional and health outcomes. Generally, the caregiver's knowledge of the preparation of vegetables, provision of adequate WASH, and awareness of various illnesses such as anaemia, intestinal parasitic infections improved significantly in complementary intervention compared to the control arm. Thus, the study demonstrated that school, household and community based education components were the successful part of the implemented project. On the other hand, the training message was more practical and suitable in the local context.

The ability of this research to meet all its objectives was slightly challenged by the earthquake faced in the midst of the survey in the study site where Dolakha district was an epicentre. Furthermore, the economic blockade resulting fuel crisis was also another hindrance of full implementation of the designed interventions. As a result of the earthquake and economic blockade in addition with political unrest in Nepal, the interventions implemented got shortened. Additional hardship was the landslides and the flood in the roads of the project areas. Due to the time lost during this phase, the health awareness programs targeting schoolchildren and caregivers, including the communities and other stakeholders, could not be conducted smoothly. The attitudes of the people were also demanding as the demand for high incentives was requested per attendance into the health education session. Once, the fear of an aftershock was slowing down, the economic blockade made transportation again difficult, resulting a delay in construction of hardware of WASH (latrines and hand washing facilities) in the targeted schools.

Our results show that VgtS project-related interventions hold a promise for improving the health and nutritional status of schoolchildren. In order to achieve a more substantial change, an in-depth consideration and analysis of several factors must be carefully considered for designing future interventions and for choosing methods to assess the effects.

10.4. Public health significance of findings: translation into policy and practice

Research and policy dialogue

Initiated by the Swiss TPH VgtS project team, an inter-sectorial technical consultative workshop was held in Nepal in December 2016 to discuss the research findings and to consult stakeholders in relation to the public health priorities recommendations for future research (Technical report of Nepal, 2016). The workshop was attended by the representatives from the health services, universities and government administrations in Nepal. The details of our study interventions and the results of the trial were presented to different groups and stakeholders. For dissemination the materials related to the intervention package including a cartoon book was handed over to the MOHP, MOE, the NARC, DH-KUH and Kathmandu University-Aquatic Science Unit. The head and officials from each organisation; the VgtS Phase 2 project leader at Swiss TPH and head of unit, head and officials from each surveyed schools and officials from different organisations were present. All the participating organisations showed interest in integrating the designed interventions into their health education program. Conversely, the health, education and agricultural personnel valued the opportunity to establish a dialogue with the authorities regarding public health priorities and needs related to health, nutrition and health. The workshop received significant media attention.

Some of the baseline results of the study have already been showcased at one International congress and one national congress: a poster presentation at the 9th European Congress on Tropical Medicine and International Health (ECTMIH, Basel) in 2015 and at the Second National Summit of Health and Population Scientist in Nepal, in 2016. The discussion about the PhD project activities conducted to a media coverage by the National television. Furthermore, the overall integrated approach of complementary WASH, nutrition and health has been presented in a Policy brief, developed in support of the policy workshops organised in all VGtS project countries (Cissé et al. 2017).

Control strategies to date

Given the tremendous global burden of malnutrition, anaemia, and intestinal parasitic infections caused in the most deprived communities, the WHO announced a roadmap (WHO, 2012e) about these outcomes. For nutritional deficiencies such as malnutrition and anaemia, basic, clinical, epidemiologic, and operational research is required to optimize malnutrition and anaemia reduction strategies in the developing countries. In the same way, evidences to develop guidelines for iron supplementation programs in areas of high anaemia pressure are required through laboratory and field studies for an improved mechanistic and clinical understanding of this interaction. Rather than a population-wide coverage of iron interventions, directed interventions after point-of-care screening of iron status (e.g., of hepcidin) and infection might be a useful future strategy. Simpler strategies such as deferring iron supplementation to febrile children until convalescence should also be evaluated (Pasricha et al., 2013). Deficiencies of other micronutrients such as folate and vitamin B12 and A, infections such as chronic diseases, and disorders of haemoglobin may substantially contribute to the burden of anaemia in developing setting (Thurlow et al., 2005). Hence, there is an urgent need to clarify the relative contribution of these conditions to the overall burden of anaemia, in a different geographic setting. Meanwhile, the cut-offs for haemoglobin, that define anaemia remains uncertain, especially in children and where hemoglobinopathies are common. Optimal statistical and functional cut-offs for anaemia should be defined in diverse populations (Pasricha et al., 2013).

For neglected tropical diseases, in recent years, major pharmaceutical companies, the Bill and Melinda Gates Foundation, the government of the United States, United Kingdom and the World Bank announced substantial support for accelerating the control, elimination and possibly the eradication of those diseases. The strategies mainly included sustaining or expanding mass drug administration programs to meet the SDG by 2020 and by sharing expertise to promote research and development of new drugs (SDG, 2015). The “London Declaration on Neglected Tropical Diseases” was endorsed in which new levels of collaborative effort and tracking of progress in talking 10 out of 17 NTDs on WHO list were pledged. Even though, progress has been made in controlling NTDs over the past 10 years, the UN MDG of treating 75% of schoolchildren at risk by 2010 has not been reached. The WHO roadmap to overcome the global impact of NTDs, released in January 2012, has reset the goal to 75% treatment coverage for children in endemic areas by 2020.

The concerns regarding the sustainability of a mass drug administration remained as the rapid reinfection rates after treatment cessation, have repeatedly been reported (Shield et al., 1984; Hlaing et al., 1987; Knopp et al., 2009). Furthermore, the compliance issues may arise in areas where regular repeated treatment is required (Allen and Parker, 2012; Molyneux et al., 2016). Drug supply in remote areas is difficult and also distracts from other priorities in resource-poor local health centres, weakening local health systems (Cavalli et al., 2010). Furthermore, there is growing concern about the potential development of parasite resistance to anthelminthic as a result of continued treatment pressure (Keiser and Utzinger, 2010). The benefit of mass drug administration has been debated after a Cochrane review by Taylor (2015) that summarised the effects of deworming on nutritional indicators and haemoglobin in 42 RCTs. The review reported of insufficient information to determine whether deworming has an effect on nutritional and haemoglobin, hence, the justification for deworming programs is questionable (Taylor-Robinson et al., 2015).

Practical applications of the research findings

For all the reasons listed above, interventions preventing malnutrition, anaemia and intestinal parasitic infections reinfection, designed by VgtS project are urgently required to augment the effect of mass drug administration as a part of an integrated approach. Mass drug administration effectively reduces the prevalence of morbidity and infections, and preventive interventions such as a school garden and health promotion programs prevent reinfection, thereby reducing incidence. In the long term, this will lead to a reduction in treatment cycles required for effective management and control and reduce the treatment pressure, resulting in a more sustainable approach to control. Indeed, there are studies that have shown that interventions combining mass drug administration with health education and improved sanitation are more effective (Smits, 2009).

One major way to reach the SDGs is through integrated control efforts comprising a multi-component strategy including school garden, mass drug administration, improved sanitation, health education and a home garden. The need for health education and additional public health measures, including novel, effective and easy-to-use educational control tools, has repeatedly been advanced (Shang et al., 2010; Zhang et al., 2010; Ahmed et al., 2011; Prichard et al., 2012; Y. U. Ziegelbauer et al., 2012a). The literature indicates that strategies involving health education and behaviour change have been poorly implemented in practice and culturally sensitive and evidence-based control strategies that address the cultural, social and behavioural dimensions of disease are urgently required (Vandemark et al., 2010). The interventions package we developed is a novel, evidence-based and culturally sensitive educational tool that suitably complements the current approach to malnutrition, anaemia and intestinal parasitic infections control advocated by the WHO. It can readily be incorporated into on-going nutrition, WASH and intestinal parasitic infection control programs in Nepal (Bundy et al., 1990).

The developed interventions package could be scaled up within Nepal as a school-based intervention. The research methods and strategies developed to produce the intervention package and to test the efficacy of educational interventions on consumption of adequate fruits and vegetables, prevention and control of malnutrition and an intestinal parasitic infections could readily be applied to other endemic areas. Although the intervention package was specifically tailored for the district of Dolakha and Ramechhap study area, the interventions would likely be effective in other low fruits and vegetables consumption areas, malnutrition, anaemia and intestinal parasitic infections endemic areas in Nepal with similar prevalence. Furthermore, the intervention package can be tested and applied in other Asian countries where there is a high prevalence of malnutrition and intestinal parasitic infection.

For scaling up, the essential authorities and policy makers at the local, district and national levels should be involved from the beginning. In addition to scaling up on a school basis, the intervention can be expanded to the wider community through different communication channels such as mass media. Previous studies showed that mass media can improve knowledge in communities about the prevention of the diseases that afflict them, and promote behavioural changes that support interventions (Vaishnav and Patel, 2006). The effectiveness of designed interventions in higher prevalence settings and with different ethnic population needs further investigation. Hence, some major learned lessons of the research methods and results are emphasised below:

- the field data related to nutrition, health and agriculture will strengthen advocacy efforts between the ministries, health organisations, NGOs and INGOs. This is of particular relevance to the nutrition, health and agriculture targeted initiatives organisations;
- the findings relating to malnutrition, health and intestinal parasitic infections including consumption of fruits and vegetables will guide the design of future population-based, epidemiological studies on malnutrition and neglected tropical diseases. Additionally, this work will guide the design of future research related to nutrition and health in Nepal;
- the simple, pretested and interesting educational package targeting health, nutrition and requirement of consumption of fruits and vegetable among children developed and trialled in Nepal can be applied to other similar developing country setting; and
- by addressing concerns of the caregivers (e.g., causative agents of malnutrition and intestinal parasitic infections) as well as identified risk factors (e.g., self-medication practices, high consumption of junk food by the children, perception of intestinal parasites being caused by eating sugary products), sustainable strategies for improving the consumption pattern and health behaviour and health care access of the communities in Nepal can be developed.

10.5. Innovation, validation and application

This PhD work was conducted in the context of the public health continuum of “innovation, validation and application,” the three pillars of Swiss TPH research-cum-action. Innovation refers to “novel ideas, methods or approaches;” validation refers to “the testing of such an innovation;” and application refers to the “practical implementation of a validated innovation.” This thesis contributed to all of these pillars, as shown in Table 10.1.

Table 10.1: Manuscript summary and their contribution to the “innovation, validation and application” wings of Swiss TPH

<table>
<tr><th>Chapter</th><th>Title</th><th>Innovation</th><th>Validation</th><th>Application</th></tr>
<tr><td>5</td><td>"Complementary school garden, nutrition, water, sanitation and hygiene interventions to improve children’s nutrition and health status in Burkina Faso and Nepal: a study protocol"</td><td>The protocol presented the model of VgtS cluster-randomised trial with an incorporation of integrated school garden, nutrition and WASH interventions for multi-sectoral school-based projects in Nepal and Burkina Faso</td><td rowspan="4">Baseline and follow-up surveys conducted in the districts of Dolakha and Ramechhap in Nepal were a validation of the study protocol that had an objectives of (i) to investigate the water, sanitation, and hygiene conditions at the unit of selected schools, households and community;
(ii) to determine the local epidemiology of malnutrition and intestinal parasitic infection among schoolchildren;
(iii) to assess the knowledge, attitude, practices (KAP) of school children and caregivers regarding nutrition and WASH conditions; and
(iv) to evaluate the effectiveness of the impact of complementary and integrated school gardens, nutrition and WASH interventions on children nutritional and health status.</td><td></td></tr>
<tr><td>6</td><td>"Water quality, sanitation, and hygiene conditions in schools and households in Dolakha and Ramechhap districts, Nepal: results from a cross-sectional survey"</td><td>Some percentage of drinking water source samples and point-of-use samples at schools, water source samples in the community, and point-of-use samples at household were contaminated with thermo-tolerant coliforms. The presence of domestic animals roaming inside homes was significantly associated with drinking water contamination.</td><td rowspan="3">The baseline findings from the cross-sectional survey helped to analyse the situation in the ground and then to designing and application of locally applicable interventions packaged targeted to the schoolchildren for improvement of their nutritional, health and WASH conditions at the unit of school, households, and communities</td></tr>
<tr><td rowspan="2">7</td><td rowspan="2">"Prevalence of anaemia and risk factors in school children in Dolakha and Ramechhap districts, Nepal"</td><td>Anaemia is moderate public health problem in our study area.Lack of meals prepared in the household and not having supper was significantly associated with anaemia. The dietary diversity scores were lower among anemic compared to non-anemic children. Consumption of vitamin A-rich fruits and vegetables were negatively associated with anaemia. More than half of the children had at least one sign of nutritional deficiency.</td></tr>
<tr><td>Stunting is also moderate public health problem in our study area. We observed a significant difference of stunting between boys and girls and in the two districts. Dolakha had a higher stunting rate than Ramechhap district.</td></tr>
</table>

8	"Intestinal parasitic infections and risk factors among schoolchildren in Dolakha and Ramechhap districts, Nepal"	High intestinal parasite infections especially soil-transmitted helminth. Households lacking soap for hand washing were at higher risk. Households without freely roaming domestic animals showed protective agains intestinal parasitic infection. Considerable morbidity was found among the surveyed children including fever and watery diarrhoea.		
9	"Dietary pattern measured by principal component analysis and its association with stunting among Nepalese schoolchildren in Nepal"	Diet mainly comprised of starchy staples and legumes. Mean consumption of animal product was low. Five dietary patterns score were derived: mixed food; vegetables and lentils; milk and beverages; salty snacks; and processed food. The vegetables and lentils pattern scores were negatively associated with stunting.		
10	"Nutritional and health status of school-aged children 15 months after integrated school garden, nutrition and water, sanitation and hygiene interventions: a cluster-randomised controlled trial in Nepal"	Anaemia significantly decreased in complementary intervention (SG+WASH) compared to control arm. Hand washing with soap before eating strongly increased in SG+WASH compared to control arm. The prevalence of parasite infections was a strong decline in SG+WASH compared to control arm.		
11	Nutrition-sensitive interventions targeting schoolchildren was for the first time implemented among the schoolchildrens of targeted districts of Nepal for improving their nutritional and health status.			The designed and implemented interventions were effective for improving nutritional and health status of targeted schoolchildren especially anaemia and intestinal parasitic infection. Further multi-sectoral projects with the collaboration of MOHP, MOE and the NARC are required to replicate the project within

				whole country for improving the nutriton and health status in schoolchildren.

10.6. Limitations of study

Even though findings are plausible and comparable to results of other studies, there are a few methodological limitations.

WASH

Analysing drinking water quality from each household only once in two seasons (monsoon and summer) may not be sufficient to assess the drinking water quality usually found in a household. Taking several samples from each household and even in different seasons is expected to result in more reliable results. Furthermore, by sampling stored water, some differences in the observed drinking water quality between households might be explained by unequal storage durations (Roberts et al., 2001). Depending on the dominance of either sedimentation or regrowth of indicator organisms during storage, drinking water quality of water samples from households might thus have been over or underestimated. It would thus have been important to collect information related to the duration of water stored.

Microbial drinking water quality was assessed by measuring the number of TTC that indicates faecal contamination of water. Even though the use of TTC as an indicator organism is widely acknowledged as an appropriate method to study water quality, E. coli is considered as the most suitable indicator, because it is exclusively of faecal origin and many genera included in this group of TTC may be of environmental origin, such as vegetation or soil (Edberg et al., 2000; Leclerc et al., 2001). Additionally, water should be analysed more than once in order to obtain a more precise estimation of drinking water quality, where repeated measurements would allow for examining changes in water quality over time.

Diet

One of the limitations of this thesis is a lack of record of seasonal change of diet. Data for the both baseline and end-line surveys were collected during monsoon and summer.

Anthropometry

The précised estimation of the prevalence of stunting, thinness and underweight through anthropometry is challenging. The anthropometric indicators such as height-for-age (HAZ), weight-for-age (WAZ) and body mass index (BMI) z-scores are age dependent. At the baseline survey, we noted that many children had no accurate birthday dates. To overcome this limitation, we took a mid-year point as the date of birth which might have introduced a random bias, resulting in a lower or higher anthropometric prevalence estimates.

Intestinal parasites

For the parasitological survey, a single faecal sample was used and analysed with the Kato-Katz, formal ether and wet mount method. The literature indicates that a single Kato-Katz might underestimate infection prevalence (Knopp et al., 2008b). Again, should the infection prevalence be an underestimation of the true prevalence, this is the case for both interventions and control arms and does not influence the trial results. Therefore, we could not justify the extensive resources required for multiple Kato-Katz analysis. Following baseline assessment, all intervention and control schoolchildren were treated with albendazole (400 mg single dose, recommended by WHO) and monitored for treatment compliance. With this regimen, albendazole provides, on average, cure rates of 98% and 47% for *Ascaris* and *Trichuris*, respectively; however, efficacy can be 100% in those with low infection intensity (Horton, 2000; Vercruysse et al., 2011). Given the predominant species in the study area was *T. trichiura* and infection intensity was low, children were not re-checked for remaining infections after treatment. In the current study, diarrhoeal morbidity 2-week prevalence information was relied on reporting from the caregivers.

Intervention package and duration

The intervention package included school garden, classroom discussion, health promotion, educational book, songs, pamphlet with key messages, provision of health awareness sessions for mothers, provision of soap for hand washing, construction of latrines and hand washing facilities in schools. The effect of the intervention package was measured as a whole and we did not establish the impact of the educational book in isolation. In a future study, it would be valuable to measure the impact of an educational book alone, without additional input; however, this might be difficult to measure, since an individual teachers contribution in the intervention cannot be isolated and will vary in different schools. However, previous literature had recommended that combined interventions with other teaching methods are effective to achieve the maximum impact by the intervention (Hu et al., 2005; Yuan et al., 2005).

In the view of the multifactorial nature of targeted outcomes, the main limitation of our study related to measuring specific outcomes within the interlinked and lengthy impact pathways and a short intervention duration. In our case, the impact pathways are very long and interlinked to one another. For example, height and weight related indices, may not be sensitive enough for detecting the effects of changes in diseases or diet in short-term. The methodology used in this study is “best practice” for the implementation of RCT and the results indicated large and highly significant differences between interventions and control arms for all targeted outcomes measured. Hence, the limitations were minor and did not affect the main trial outcomes.

10.7. Conclusion

The overall goal of this PhD thesis was to generate evidence on the effects of integrated school gardens, nutrition and WASH interventions on the nutritional and health status of schoolchildren in rural schools of Nepal. To our best knowledge, this research is the first of its kind in the rural settings of Nepal.

We assumed that an integrated intervention model had the potential to change a few indicators (outcomes) related to malnutrition, anaemia, intestinal parasitic infections and WASH conditions and other underlying risk factors. The implementation of the VgtS project along with complementary interventions was found associated with improved child consumption of fruits and vegetables, reduction of anaemia status and intestinal parasitic infection and improvement of WASH behaviours at the unit of schools and households in the complementary interventions implemented schools. Therefore, long-term investment and strong multi-sectorial collaboration, including the close involvement of household and communities, will be essential in future nutrition-sensitive programs targeted to improve nutrition and health in school-aged children.

Recommendations arising from this research, as well as areas for further investigation, are presented below for each research outcome:

WASH and diarrhea

Only two measurements of drinking water quality per water source and the household at a different point of time have been made. It is thus recommended to analyse several water samples, in several times, for future studies.

Observing a fraction of the surveyed households during water fetching and water storing might allow identifying further factors that contribute to the deterioration of water quality from a source to a point-of-use. In the same way, with regard to hygiene, reported handwashing with soap is not ideal to assess practiced hand washing. Even though much more time intensive, it is recommended to include observation of children and their caregivers on their hand washing behaviour as this might result in more accurate results (Haas and Larson, 2007).

For future studies, reliance on period prevalence of diarrhoea (1 week or less) is recommended as the measurement is straightforward and yields higher study power (Schmidt et al., 2011).

Malnutrition, anaemia and intestinal parasitic infection

For nutrition, advocacy targeting governments, international organisations and funding bodies for greater investment in fruit and vegetables consumptions and malnutrition prevention and control is necessary. In future research, it would be useful to combine anthropometry with other indicators indicating nutritional deficiencies, such as a biochemical marker for micronutrient deficiencies and muscle strength to investigate morbidity related to malnutrition among schoolchildren. An integrated approach to nutrition involving collaborations of the MOHP and the MOE, are required.

For intestinal parasites, the development of field compatible diagnostics allowing a more accurate evaluation should be a top research priority. The current NTDs control programs are highly vulnerable due to the possibility of anthelmintic resistance development and spread, following years of mass drug administration. For future research, it may be necessary to construct a compulsory registration of children in rural parts of Nepal, much less likely to be registered in civil registration systems. The only use of the (mini-) FLOTAC techniques could be adopted for intestinal parasite infection, as helminths and intestinal protozoa species can be detected concurrently by this technique (Barda et al., 2015; Coulibaly et al., 2016). More emphasis needs to be placed on the transitional period between childhood and adulthood, which is marked by increased dietary requirement.

KAP regarding nutrition and WASH

Education of the schoolchildren, their caregivers and also the teachers regarding the ways in which to minimise the risk of exposure to malnutrition, anaemia and intestinal parasitic infections and to improve general hygiene, including handwashing before eating and after defecation is required. Verbal communication of health messages by the local health worker and visual tools would be appropriate, given the low level of literacy. Measures of cost-effectiveness, such as, for example, establishing how much the educational intervention costs per child and per outcomes averted should be studied. From the educational perspective, other knowledge gaps include the impact of the intervention on cognitive development of the children. The research needs scaling-up and for a potential scaling-up of the intervention package developed within this thesis and follow-up studies are required to establish to what extent the intervention package is culturally adaptable and effective in other malnutrition and intestinal parasitic infection endemic areas. Formal process for exchange between different ministries, health organisations and universities, ensuring a joint approach to situation analysis, disease surveillance, disease control interventions and broader control is required. This could be mediated through the MOE in collaboration with non-governmental organisations.

Future projects should explore the feasibility of converting the school garden educational book and the health promotion with posters into different multimedia formats, such as a video and television series making these accessible for the wider community. Even though RCTs provide the strongest evidence of a causal relationship between intervention and outcome, the combination of RCTs with observational approaches might be effective for future research to provide additional evidences on certain outcomes of interest. Furthermore, a modified cluster-RCT study design, including mid-term follow-up studies, could be employed in future studies. This will allow the researcher to assess long-term changes in targeted outcomes and re-infection rates.

The country efforts for SDGs (e.g., SDG-2 zero hunger; SDG-3 health and well-being; SDG-4 quality education and SDG-6 water and sanitation) provide an opportunity to increase schoolchildren's health through joint multi-sectorial approaches addressing nutrition and disease prevention (adequate WASH). The appropriate mechanisms or tools for operationalizing inter-sectorial collaboration in Nepal, such as frameworks or formal agreements need to be designed and supported for better health and well-being in all schools (SDG, 2015).

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EDUCATION

2017	Ph.D in Epidemiology University of Basel, Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland Dissertation title: "Complementary school garden, nutrition and water, sanitation and hygiene interventions to improve children's nutrition and health status in Nepal."
2013	Masters in Sociology and Anthropology Tri-chandra College, Tribhuwan University, Nepal
2012	Masters of Public Health Royal Tropical Institute, Vrije Universiteit, Amsterdam
2007	Bachelor in Public Health Nepal Institute of Health Science, Purbanchal University, Nepal

PROFESSIONAL EXPERIENCE

Research and Teaching

2019 till date	Research Project on "Preventing Infant Malnutrition with Early Supplementation (PRIMES) Pilot" in collaboration with University of California, San Francisco and Dhulikhel Hospital funded by Gates foundation, USA.
2019 till date	Research Project on "Climate change effects on infectious diseases and malnutrition among school children in Nepal" in collaboration with Swiss Tropical and Public Health Institute, Switzerland and Dhulikhel Hospital funded by ZHAW School of Management and Law, Switzerland.
2019 till date	Research Project on "Women's task of domestic water carrying and the implications for health: a cross-sectional study in Nepal" with Eawag- the aquatic research, Switzerland funded by SDC.
2019 till date	Research Project on "Assessing haemoglobin status in schoolchildren and their parents as biomedical project" add-on to the baseline survey of "Nudging children toward healthier food choices: An experiment combining school and home gardens" in collaboration with Leibniz Institute of Vegetables and Ornamental Crops GroBbeeen/Erfurt (IGZ) e.V, Germany.
2019 till date	Research project on "Effect of a Health Education Program on Salt Reduction in Cooked Foods at a University Cafeteria in Nepal" in collaboration with Harvard T. Chan University, USA.
2018 till date	Research project on "Nepal Pioneer Worksite Intervention Study focusing on Healthy Eating" in collaborations with Yale University and Dhulikhel Hospital funded by NIH grant, USA.
2017 till date	Postdoctoral Researcher in the study "Evaluation of the impact of water quality and hygiene interventions on the health status of children in the project area of Helvetas WARM-P Project in Nepal," Eawag- the aquatic research, Switzerland funded by SDC.

2014-2017 PhD Researcher, Epidemiology and Public Health Unit, Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland

Responsibilities:

- Responsible for the coordination and collaboration with different governmental organizations (National Agricultural Research Council, Minsitry of Health, Ministry of Education) including stakeholder meetings in the schools, households and communities;
- Overall planning, implementation and monitoring of the field work targeted to the nutrition, health and water, sanitation and hygiene to schoolchildren;
- Design and implementation of the interventions targeted to nutrition, health and water, sanitation and hygiene;
- Data collection, data management and data analysis;
- Writing manuscripts;
- Regular monitoring and evaluation of the implemented intervention programs in the school, household and communities focusing schoolchildren.

2012-2014 Public Health Manager, Dhulikhel Hospital, Kathmandu University Hospital, Dhulikhel, Nepal

Responsibilities:

- Responsible for the regular activities such as clinical and preventive programs of seventeen peripheral primary level health centers;
- Involvement into collaborating and creating new health centers where needed;
- Developing wider linkage between the departments of Dhulikhel Hospital and community stakeholders;
- Initiating partnerships with other community based health initiatives in which as a tertiary level hospital; Dhulikhel Hospital could give significant support;
- Overall management of the tasks conducted by the Department of Community Programs including public Health Programs, Community Development Programs and Health Service Programs;
- Human Resource Development and Management;
- Conduction and management of various national and district level workshops related to leadership, quality of healthcare;
- Conduction of the research related to different issues through the Department of Community Programs into the different rural areas of the Country;
- Facilitation and supervision for the students of different backgrounds coming for internship into the Dhulikhel Hospital into the areas such as research and global health programs targeting to microfinance program, school health programs, women's health programs, community development programs.

2008-2011 Instructor, Dhulikhel Medical Institute, Kathmandu University, Dhulikhell, Nepal

Responsibilities:

- Involved in academic program (General Medicine, Laboratory Technicians, and Physiotherapy) of Dhulikhel Medical Institute in subjects like Research, Health Education, Behavioral Science, Environmental Health and Epidemiology.

2008-2011 Community Supervisor, Department of Community Programmes, Dhulikhel Hospital

Responsibilities:

- Planning and implementing community based health programs in the rural areas of Nepal through the seventeen outreach centers in coordination with all the departments of the hospital, District Health Office, communities and other local stakeholders;
- Initiating and supervising the microfinance program amongst women's groups to strengthen the financially poor families and also mobilizing the same groups in health activities, particularly related to maternal and child and adolescent health;
- Making new partnerships and working structures for supporting other community based hospitals;
- Initiating new community based endeavors in health programs, including establishing outreach centers in rural Nepal, organizing health camps, organizing health promotion programs;
- Conduct various community based researches as principal investigator and also as co-investigator;
- Coordinating with the health sciences academic programs to facilitate the community based learning programs;
- Guiding visiting international students for community based health programs;
- Work as the hospital project manager for some joint projects, e.g. rural child health program (run with the support of Childreach International, UK, since July 2009);
- Rural adolescent and reproductive health program (run with the support of Scharf Stiftung, DSW, Germany, since Aug 2008).

PEER-REVIEWED PUBLICATIONS

Published

1. Shrestha A, Sharma S, Gerold J, Erismann S, Sagar S, Koju R, et al. Water quality, sanitation, and hygiene conditions in schools and households in Dolakha and Ramechhap districts, Nepal: results from a cross-sectional survey. Int. J. Environ. Res. Public. Health. 2017;14.
2. Shrestha A, Sharma S, Gerold J, Erismann S, Sagar S, Koju R, et al. Prevalence of intestinal parasitic infection and associated risk factors among schoolchildren in Dolakha and Ramechhap districts, Nepal. Parasites and vectors. 2018
3. Erismann S, Shrestha A, Diagbouga S, Knoblauch A, Gerold J, Herz R, et al. Complementary school garden, nutrition, water, sanitation and hygiene interventions to improve children's nutrition and health status in Burkina Faso and Nepal: a study protocol. BMC Public Health. 2016;16:244.
4. Erismann S, Knoblauch AM, Diagbouga S, Odermatt P, Gerold J, Shrestha A, et al. Prevalence and risk factors of undernutrition among schoolchildren in the Plateau Central and Centre-Ouest regions of Burkina Faso. Infect. Dis. Poverty. 2017;6:17.
5. Erismann S, Diagbouga S, Odermatt P, Knoblauch AM, Gerold J, Shrestha A, et al. Prevalence of intestinal parasitic infections and associated risk factors among schoolchildren in the Plateau Central and Centre-Ouest regions of Burkina Faso. Parasit. Vectors. 2016;9:554.

Manuscripts in progress

1. Shrestha A, Sharma S, Gerold J, Erismann S, Sagar S, Koju R, et al. Prevalence of anemia and associated risk factors among school-aged children in Dolakha and Ramechhap districts, Nepal. BMC Hematology (submitted to the BMC Hematology) (Submitted to BMC Paediatrics)
2. Shrestha A, Sharma S, Gerold J, Erismann S, Sagar S, Koju R, et al. Using a mixed-methods approach to develop and measure impact of a school- and household- based intervention on fruits and vegetable intake and intestinal parasitic infections of Nepalese schoolchildren. BMC Public Health
3. Shrestha A, Sharma S, Gerold J, Erismann S, Sagar S, Koju R, et al. Evaluation of health and nutritional status of schoolchildren one year after school garden, complementary nutrition and WASH intervention in Nepal: a cluster randomised controlled trial. International Journal of Behavioral Nutrition and Physical Activity (Submitted to BMC Public Health)
4. Shrestha A, Sharma S, Gerold J, Erismann S, Sagar S, Koju R, et al. Dietary patterns measured by principal component analysis and its association with stunting among schoolchildren in Dolakha and Ramechhap districts in Nepal. Public Health Nutrition

Book Publication

Shrestha A, Gerold J, Bhandari K. Binu dreams of vegetable gardens. Basel: Swiss Tropical and Public Health Institute, 2016

CONFERENCES

1. Shrestha A, Sharma S, Gerold J, Erismann S, Sagar S, Koju R, et al. Prevalence of intestinal parasitic infection and associated risk factors among schoolchildren in Dolakha and Ramechhap districts, Nepal. International congress and one national congress: a poster presentation at the 9th European Congress on Tropical Medicine and International Health (ECTMIH), Basel in 2015.
2. Shrestha A, Sharma S, Gerold J, Erismann S, Sagar S, Koju R, et al. Water quality, sanitation, and hygiene conditions in schools and households in Dolakha and Ramechhap districts, Nepal: results from a cross-sectional survey, Second National Summit of Health and Population Scientist, Nepal, 2016.
3. Shrestha A, Cissé Guéladio. Health and nutritional status of schoolchildren one year after complementary school garden, nutrition and WASH interventions: a cluster randomised controlled trail in Nepal.

SEMINARS AND TRAININGS

1. Participation in 'Health Information Management System Training' head at District Health Office, Dhulikhel on February 2008.
2. Participated in Training program on 'Sexual Harassment' organised by Heal Nepal (2007).
3. Training program on 'Early Warning and Reporting System' on five different diseases organised by Nepal Government.
4. As a coordinator of Program on Awareness of HIV/AIDS in World AIDS Day 2006.
5. Training program on 'Microfinance Praxis' (25th-29th Dec 2009) organised by International Network of Alternative Finance Institutions Nepal.

6. Numerous health camps and community awareness programs in various places of the country, organised by Dhulikhel Hospital Kathmandu University Hospital
7. As the manager in various trainings on 'First Aid Management' to the paramedical staffs, school teachers, bus drivers, youths, etc.
8. Participation in 'Management of Community Based Prevention and Care of HIV/AIDS' in October 10-22, AIHD, Thailand.
9. Participations into the different lectures related to Health Development and Maternal Health in World Health Organisation, Genva.
10. Participation into the lecture "Global Economy and Health" delivered by Global Fund, Geneva.

SCHOLARSHIPS

<u>Postdoc Research Fellowship, Eawag- the aquatic research, Zurich, Switzerland (2017-2018)</u>
<u>PhD Research Fellowship</u>, Swiss Agency for Development Cooperation, Switzerland (2014-2016)
<u>Master's in Public Health Fellowship</u>, Netherlands Fellowship Program, Nuffic, The Netherlands (2011-2012)
<u>Top-up Stipend</u>, PhD Program Health Science, University of Basel, Switzerland, (2015)
<u>Extended Stipend</u>, PhD Program Health Science, University of Basel, Switzerland, (2016)

EXTRA-CURRICULAR ACTIVITIES

- Organizing management of science exhibition during intermediate level.
- Chief coordinator of numerous free health camps in rural Nepal during studying Bachelor in Public Health.
- Students' coordinator in various cultural and social service activities in Bachelor in Public Health.
- Volunteered different programs related to street children and home maids organized by different NGOs.
- Chief coordinator of management for the mid-level health worker refresher training of Kavre district of Nepal.
- Involvement in data collection in adolescent, women and child related research conducted by CWISH, Kathmandu, Nepal.

TECHNICAL SKILLS

Computer application: Microsoft office
Statistical software: STATA, SPSS
Epidemiological software: EPI Info
Signature: