Hindawi

## *Research Article*

# Epidemiologic Investigation of Intestinal Parasite Infection and Associated Risk Factors among Primary Schoolchildren in the Manzini and Lubombo Provinces, the Kingdom of Eswatini

**Ai-Wen Yin,**[1,2] **Yueh-Lun Lee** [ID],[3] **Sindisiwe Dlamini,**[4] **Gugu Maphalala,**[5] **Chien-Wei Liao,**[2,6] **and Chia-Kwung Fan** [ID][1,2,6]

[1]*Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, 110 Taipei, Taiwan*
[2]*Department of Molecular Parasitology and Tropical Diseases, School of Medicine, College of Medicine, Taipei Medical University, 110 Taipei, Taiwan*
[3]*Department of Microbiology and Immunology, School of Medicine, College of Medicine, Taipei Medical University, 110 Taipei, Taiwan*
[4]*National Health Laboratory Service, Ministry of Health, Mbabane, Eswatini*
[5]*National Blood Transfusion Services, Ministry of Health, Mbabane, Eswatini*
[6]*Research Center of International Tropical Medicine, College of Medicine, Taipei Medical University, 110 Taipei, Taiwan*

Correspondence should be addressed to Chia-Kwung Fan; tedfan@tmu.edu.tw

Although the deworming program has been executed since 2000, the intestinal parasitic infection (IPI) rates among primary schoolchildren (PSC) in the two provinces of the Kingdom of Eswatini investigated in 2010 remained high, reaching 32.2%. In this study, we monitored the IPI status along with the associated risk factors for PSC in two provinces—Manzini and Lubombo. After consent from their parents/guardians, a total of 316 samples collected from PSC with grades 1 to 3 from four primary schools in Manzini and Lubombo were examined by the Merthiolate-Iodine-Formaldehyde (MIF) method. In addition, demographic characteristics and risk factors acquired by questionnaire surveys were included to be statistically analyzed. The overall prevalence was 40.5% (128/316), of which the infection rate in Manzini and Lubombo was 28.8% (19/66) and 58.3% (74/140), respectively. Pathogenic protozoa had the highest infection rate of 20.6% (65/316), including *Entamoeba histolytica/dispar* (8.5%, 27/316), *Giardia duodenalis* (14.6%, 46/316), and *Blastocystis hominis* (9.8%, 31/316). In terms of helminth infection, the infection rate was quite low, 1.6% only, and these five infected cases included four cases of *Hymenolepis nana* and one case of *Enterobius vermicularis* infection. Present study showed that 27.8% (88/316) of PSC were infected by more than one pathogenic parasite. Personal hygiene like washing hands before a meal has a significant protection effect (OR = 0.32, 95% CI = 0.14–0.75, $p = 0.009$). Rain or well water and the type of water supply from which they drank also showed a considerable risk factor (OR = 2.44, 95% CI = 1.25–4.79, $p = 0.04$). The IPI rate in PSC seems unlikely changed compared to that of the previous survey conducted in 2010, especially when the pathogenic protozoan infection rate remains high. Treatment of infected PSC with appropriate medication to reduce intestinal pathogenic protozoan infection should be seriously considered by Eswatini Health Authority.

## 1. Introduction

Intestinal parasitic infections (IPIs) influence one-third of the world population [1], and millions of people suffer from both helminths and protozoa due to poor personal hygiene and lack of clean water or proper sewage disposal [2]. Several studies showed that a more severe situation and worm burden occur in school-age children [3, 4]. Persistent infections may lead to chronic malnutrition and iron deficiency anemia that can cause lower study performance [5]. IPIs consisted of both helminths and protozoan infections, including mainly *Ascaris lumbricoides,* over 819 million

Figure 1: Primary schools' localities in Highveld and Lowveld regions of Manzini and Lubombo provinces, the Kingdom of Eswatini.

people were infected and followed by *Trichuris trichiura* (464.6 million) and then hookworms (*Ancylostoma duodenale* and *Necator americanus*) (438.9 million) [6], also referred to as soil-transmitted helminths (STHs), while pathogenic protozoan infections include *Entamoeba histolytica* (48 million), *Giardia intestinalis* (2.8 million), and *Blastocystis hominis* [7].

Although the deworming program has been executed since 2000, the outcome of this campaign remained largely unclear due to no monitoring of its effectiveness. In 2010, we analyzed a survey focusing on IPIs among primary schoolchildren (PSC) and we found that the overall IPI rate was 32.2% (86/267), of which the infection rate of helminths was quite low (0.4%, 1/267) [8]. Only one primary schoolchild was infected by one helminthic parasite—*A. lumbricoides*. Nevertheless, the pathogenic protozoan infection rate, in contrast, was pretty high, reaching 20.6% (55/267). Among the pathogenic protozoan infections, the main species were *Ent. histolytica/dispar*, *B. hominis*, and *G. intestinalis*, whereas the main species of non-pathogenic protozoa were *Ent. coli*, *Endolimax nana*, *Iodoentamoeba butschlii*, and *Chilomastix mesnili* [8].

The main purpose of this study was to monitor the IPI prevalence and treatment efficiency in PSC after an annual regular deworming campaign for 10 years. In addition, IPI status in spatial distribution in two different altitudes was also investigated.

## 2. Methods

### 2.1. The Geography Description of the Kingdom of Eswatini.
Eswatini is surrounded by South Africa in the north, west, and south and Mozambique in the east. Determined by altitude, Eswatini is divided into four regions with diverse landscapes. From west to east, the average altitude descended and separated Eswatini into three different areas. The capital, Mbabane, is located on Highveld with an average altitude of 1,200 meters. An average 700 meters high area is called Middleveld and the largest city is Manzini and Lowveld, mainly about 250 meters is less populated than the other areas (Figure 1).

### 2.2. Study Population and Subject Selection.
The sample size was estimated using the general formula, $n = z^2 p\ (1 - p)/d^2$, under 95% confidence level, 5% margin of error, and 21% response distribution, and getting at least 285 samples is recommended [9]. According to the geographic locality, four elementary schools located in two different regions, Lowveld and Highveld, were selected by Bilharzia and Deworming Program, Eswatini (Figure 1). Seventy-five PSC from grades 1 to 3 from each school would be recruited, and ideally a total of 300 students should participate in the present study.

### 2.3. Diagnostics and Assessment of Risk Factors Associated with IPIs and Treatment.
Present study was undertaken from July 19 to August 10, 2019. To compare (IPI) outcomes and associated risk factors, we first designed a questionnaire and items included personal basic data (student's gender, family members, and parents' occupation) and hygiene status including washing hands after using toilet facilities, washing hands before eating, fingernail trimming, finger sucking, the way and frequency of bathing, the type of floor, and the frequency of cleaning the bedding. Microscopic examination of eggs or cysts or trophozoites in feces was performed by using the Merthiolate-Iodine-Formaldehyde (MIF) method (Shin-Yung Co., Ltd., Taiwan) which is routinely conducted in Taiwan CDC and Hospitals. The excellence of MIF method is capable of reducing the interference by stool particles, dismissing the disgusting smells during fecal specimen processing and particularly important, to improve the identification efficiency of intestinal helminthic eggs and protozoan cysts or trophozoites in clinical laboratory practice [10]. Each primary schoolchild was given a wide-opening container for stool collection and the stool specimen was immediately transported to Parasitology Laboratory of Mbabane Government Hospital for further examination. Stool samples containing eggs, cysts, or trophozoites as detected by the MIF method were considered positive, and infected PSC were treated appropriately by physicians in the respective province hospital.

### 2.4. Statistical Analysis.
Differences in the prevalence of infection based on independent variables including gender, age group, residence, and parents' employment status were determined by a chi-squared ($X^2$) test. The univariate crude odds ratio (OR) and 95% confidence interval (CI) were used to determine associations between independent variables, risk factors, and infection, and $p$ value of <0.05 was considered statistically significant. All statistical analysis of data from the questionnaire and parasitological examination

TABLE 1: Demographic characteristics and intestinal parasitic infections among primary schoolchildren from Manzini and Lubombo provinces, the Kingdom of Eswatini.

| | Total examined ($N = 316$) | Number and prevalence (%) | | |
|---|---|---|---|---|
| | | Helminth ($N = 5$) | Protozoa ($N = 126$) | All ($N = 128$) |
| Age (years) | | | | |
| ≦7 | 134 | 4 (2.99) | 53 (39.55) | 55 (41.04) |
| 8–9 | 127 | 1 (0.64) | 56 (44.09) | 56 (44.09) |
| ≧10 | 51 | 0 (0) | 17 (33.33) | 17 (33.33) |
| Unknown | 4 | 0 | 0 | 0 |
| Gender | | | | |
| Boys | 201 | 2 (1.0) | 79 (39.3) | 79 (39.3) |
| Girls | 115 | 3 (2.61) | 47 (40.87) | 49 (42.61) |
| Residence | | | | |
| Lowveld | 169 | 3 (1.78) | 63 (37.28) | 63 (37.28) |
| Highveld | 147 | 2 (1.36) | 63 (42.86) | 65 (42.22) |
| Employment of parents | | | | |
| Both | 120 | 2 (1.67) | 52 (43.33) | 52 (43.33) |
| Either | 161 | 3 (1.86) | 58 (36.02) | 60 (37.27) |
| None | 35 | 0 (0) | 16 (45.71) | 16 (45.71) |
| Living environment | | | | |
| Brick house | 230 | 3 (1.32) | 97 (42.17) | 98 (42.61) |
| Mud house | 71 | 2 (2.82) | 25 (35.21) | 26 (36.62) |
| Hut | 4 | 0 | 2 (50.00) | 2 (50.00) |
| Wooden house | 7 | 0 | 0 | 0 |
| Homeless | 1 | 0 | 1 (100) | 1 (100) |
| Unknown | 3 | 0 | 1 (33.33) | 1 (33.33) |

were performed using SAS version 9.3 software (SAS Institute, Cary, NC, USA) [11].

*2.5. Ethical Consideration.* Informed consent was obtained from every parent/guardian who agreed with their kids to participate in the present study, and approval was granted by the Eswatini Health and Human Research Review Board (SHR172/2019).

## 3. Results

After consent from parents/guardians, although 380 PSC were recruited, only 316 samples from 201 boys and 115 girls, whose ages ranged from 5 to 15 years (mean age of $8.00 \pm 1.62$ years), were valid to be used for further analysis because they gave their fecal specimens along with complete questionnaire data. Overall, 128 IPI cases were detected among 316 subjects, thus resulting in the prevalence rate of 40.5%. Age group of 8–9 y old had the highest prevalence of 44.09% (56/127), followed by 41.04% (55/134) in the age group of ≦7 y old and then 33.33% (17/51) in the age group of ≧10 y old. The prevalence by gender showed that boys had 39.30% (79/201) and girls had 42.61% (49/115). The prevalence of PSC who lived in Lowveld (Lubombo) area was 37.28% (63/169) and those who lived in Highveld (Manzini) area had prevalence of 42.22% (65/147) (Table 1). The prevalence of PSC whose parents were both unemployed was 45.71% (16/35), followed by 43.33% (52/120) in both had jobs and 37.27% (60/161) in either parent hired.

The prevalence of PSC who lived in brick houses was 42.98% (98/228) and that of PSC who lived in mud houses was 36.62% (26/71) (Table 1).

Single infection was the most commonly found among PSC (28.48%, 90/316), followed by dual infection of 8.86% (28/316) and multiple infection was the least found (2.85% (9/316)). The prevalence of boys in Lowveld and Highveld was 33.60% (36/109) and 45.70% (43/94), respectively, while the figure in girls was 43.55% (26/62) and 41.50% (22/53), respectively (Table 2). Totally, nine different intestinal parasites including two helminths, e.g., *E. vermicularis* and *H. nana*, while three pathogenic protozoa including *Ent. histolytica/dispar, G. intestinalis, and B. hominis* and four non-pathogenic protozoa including *Ent. coli, E. nana, I. buetschii,* and *E. hartmanni* had been detected in the present study. *E. coli, G. duodenalis,* and *B. hominis* were the top three with high infection rate of 18.7% (59/316), 14.6% (46/316), and 9.8% (31/316), respectively (Table 2).

Crude logistic regression analysis showed that rain water and other types of water supply had a significant relevance in the infection rate (OR = 2.37, 95% CI = 1.19–4.66, $p = 0.04$), compared to tap water. Personal hygiene like washing hands before meal showed a considerable protection effect (OR = 0.39, 95% CI = 0.16–0.95, $p = 0.04$). In different residences, Highveld area showed more risk than Lowveld for PSC in the acquisition of pathogenic parasites (OR = 1.90, 95% CI = 1.05–3.41, $p = 0.03$) (Table 3).

## 4. Discussion

Neglected tropical diseases (NTDs) have been formally recognized as targets for global action towards the Sustainable Development Goals (SDGs) aiming to end the epidemics of HIV, tuberculosis, malaria, and NTDs by 2030 [12]. The present study intended to help Eswatini Health

Table 2: Polyparasitism status among primary schoolchildren from Manzini and Lubombo provinces, the Kingdom of Eswatini.

| Area | Status of intestinal parasite infection | | | Subtotal (%) |
| --- | --- | --- | --- | --- |
| | Single (%) | Dual (%) | Multiple (%) | |
| Lowveld | | | | |
| Boys ($N = 107$) | 24 (22.4) | 12 (11.2) | 0 (0) | 36 (33.6) |
| Girls ($N = 62$) | 22 (35.5) | 4 (6.5) | 1 (1.6) | 27 (43.5) |
| Highveld | | | | |
| Boys ($N = 94$) | 31 (33.0) | 8 (8.5) | 4 (4.3) | 43 (45.7) |
| Girls ($N = 53$) | 13 (24.5) | 5 (9.4) | 4 (7.5) | 22 (41.5) |
| Residence | | | | |
| Lowveld ($N = 169$) | 46 (27.2) | 16 (9.5) | 1 (0.6) | 63 (37.3) |
| Highveld ($N = 147$) | 44 (29.9) | 13 (8.8) | 8 (5.4) | 65 (44.2) |
| Helminth | | | | |
| E. vermicularis | 0 (0) | 1 (0.3) | 0 (0) | 1 (0.3) |
| H. nana | 2 (0.6) | 2 (0.6) | 0 (0) | 4 (1.3) |
| Pathogenic protozoa | | | | |
| E. histolytica/dispar | 10 (3.2) | 12 (3.8) | 5 (1.6) | 27 (8.5) |
| G. duodenalis | 29 (9.2) | 11 (3.5) | 6 (1.9) | 46 (14.6) |
| B. hominis | 15 (4.7) | 10 (3.2) | 6 (1.9) | 31 (9.8) |
| Non-pathogenic protozoa | | | | |
| E. coli | 32 (10.1) | 18 (5.7) | 9 (2.8) | 59 (18.7) |
| E. nana | 0 (0) | 1 (0.3) | 1 (0.3) | 2 (0.6) |
| I. buetschlii | 2 (0.6) | 1 (0.3) | 2 (0.6) | 5 (1.6) |
| E. hartmanni | 0 (0) | 0 (0) | 3 (0.9) | 3 (0.9) |
| Total | 90 (28.5) | 56 (17.7) | 32 (10.1) | 178 (56.3) |

Ministry to monitor the outcome of the annual deworming campaign regarding pathogenic IPIs among PSC through mutual collaboration with Public Health Department to fulfill the missions of SDG 3 and SDG 17. Among NTDs, IPIs including soil-transmitted helminthiasis (Ascariasis, Hookworm Diseases, Trichuriasis, and Strongyloidiasis) and protozoan infections (Amebiasis and Giardiasis) have influenced 3.5 billion persons and caused clinical morbidity in nearly 450 million people [7]. Furthermore, over 600 million school-aged children were vulnerable due to IPIs [7].

Present prevalence (40.5%) was lower than that of PSC in Ethiopia (52%) [13], Burkina Faso (60.8%) [14], Cote d'Ivoire (55.2%) [15], Nigeria (86.2%) [16], São Tomé and Príncipe (64.7%) [17], and Pakistan (52.8%) [18], while it was higher than that in Western Saudi Arabia (12%) [19], Iran (21.5%) [20], India (27.5%) [20], Nepal (31.5%) [21], Marshall Islands (22.8%) [22], and Senegal (35%) [23]. The difference in prevalence can be attributed to the following reasons: the timing of the survey due to deworming schedule, environmental conditions of the target areas, or contamination of water supplies [24].

Prevalence of IPIs in the present study was 40.5%, indicating no significant difference between the present and previous studies (41.9%) of 10 years ago. Helminth infection rate was still low, and this achievement may be due to the regular biannual deworming program whereby each primary schoolchild was given one tablet of albendazole or praziquantel for STHs or Bilharzia control [25]. Nevertheless, pathogenic protozoan infections seem likely long ignored, thus resulting in a still high infection rate.

This situation did not change and improve as such the infection status was similar to that found in a survey which was conducted by our team 10 years ago.

Substantial studies in different countries have also found similar situation that helminth infections have been greatly reduced, but pathogenic protozoan infections like Ent. Histolytica/dispar, B. hominis, or G. intestinalis are predominant IPIs [26]. It has been well known that amoebas, B. hominis, and G. intestinalis are able to cause gastrointestinal problems of varying severity and outcomes, including rendering children unable to attend school due to diarrhea or severe abdominal pain, consequently leading to low academic performance [27, 28]. It is time to address the importance as how to prevent PSC from pathogenic protozoan infections as well as treatment regimen [29].

Noteworthy, the predominant helminth infection in the present study was H. nana (4/5). Some studies have found that H. nana infection has a high prevalence in PSC and 50–75 million cases were estimated globally [30]. Although the symptom of H. nana infection is usually benign or asymptomatic [31–33], anemia and eosinophilia have been often reported [32]. Strikingly, a report indicated that H. nana in a HIV-infected man causes malignancy because transformed cells derived from H. nana may develop with morphologic features and invasive behavior compatible with cancer [34]. Although extra-intestinal H. nana infections are rare, cysticercoids have been reported in whole-blood preparations from glucocorticoid-treated children [35] and a case of fatal invasive H. nana infection with atypical morphologic features was described in a HIV-positive man [36, 37]. Moreover, H. nana is known to develop abnormal, enlarged, and ballooned cysticercoids in immunosuppressed mice [38]. Since HIV infection in Eswatini is very common [39], whether H. nana infection may cause malignancy in immunocompromised patients is a serious concern.

Table 3: Logistic regression analysis of intestinal pathogenic parasitic infections among primary schoolchildren from Manzini and Lubombo provinces, the Kingdom of Eswatini.

| Variables | Prevalence | | | | | |
| | All | | | Pathogenic parasites | | |
| | OR | 95% CI | $p$ value | OR | 95% CI | $p$ value |
|---|---|---|---|---|---|---|
| Age (y) | | | | | | |
| ≦7 | 1.00 | | | 1.00 | | |
| 8-9 | 1.61 | 0.90–2.88 | 0.21 | 1.31 | 0.70–2.46 | 0.69 |
| ≧10 | 1.27 | 0.57–2.83 | 0.99 | 1.35 | 0.57–3.21 | 0.68 |
| Gender | | | | | | |
| Boys | 1.00 | | | | | |
| Girls | 1.14 | 0.66–1.97 | 0.65 | 1.45 | 0.79–2.62 | 0.22 |
| Residence | | | | | | |
| Lowveld | 1.00 | | | 1.00 | | |
| Highveld | 1.49 | 0.66–1.97 | 0.16 | **1.90** | **1.05–3.41** | **0.03** |
| Employment of parents | | | | | | |
| None | 1.00 | | | 1.00 | | |
| Either | 0.58 | 0.23–1.45 | 0.08 | 0.62 | 0.23–1.66 | 0.19 |
| Both | 1.01 | 0.74–2.49 | 0.38 | 0.92 | 0.34–2.48 | 0.63 |
| Living environment | | | | | | |
| Others | 1.00 | | | 1.00 | | |
| Brick house | 1.36 | 0.74–1.61 | 0.31 | 1.17 | 0.61–2.26 | 0.63 |
| Raw meat | | | | | | |
| No | 1.00 | | | 1.00 | | |
| Yes | 0.78 | 0.38–1.61 | 0.51 | 1.14 | 0.53–2.43 | 0.74 |
| Raw vegetable | | | | | | |
| No | 1.00 | | | 1.00 | | |
| Yes | 0.39 | 0.12–1.28 | 0.12 | 0.66 | 0.18–2.42 | 0.53 |
| Water supply | | | | | | |
| Tap water | 1.00 | | | 1.00 | | |
| Well | 1.68 | 0.88–3.18 | 0.76 | 1.62 | 0.78–3.27 | 0.67 |
| Rain and others | **2.37** | **1.19–4.66** | **0.04** | 2.01 | 0.97–4.17 | 0.16 |
| Eating ground | | | | | | |
| No | 1.00 | | | 1.00 | | |
| Yes | 1.21 | 0.54–2.69 | 0.65 | 1.65 | 0.68–4.01 | 0.27 |
| Washing hands before meal | | | | | | |
| No | 1.00 | | | 1.00 | | |
| Yes | 0.52 | 0.22–1.22 | 0.13 | 0.39 | **0.16–0.95** | **0.04** |
| Washing hands after toilet | | | | | | |
| No | 1.00 | | | 1.00 | | |
| Yes | 1.44 | 0.61–3.41 | 0.41 | 1.62 | 0.63–4.18 | 0.31 |
| Wearing shoes | | | | | | |
| No | 1.00 | | | 1.00 | | |
| Yes | 1.61 | 0.91–2.82 | 0.10 | 1.58 | 0.86–2.94 | 0.14 |
| Touching soil | | | | | | |
| No | 1.00 | | | 1.00 | | |
| Yes | 0.99 | 0.55–1.81 | 0.99 | 0.86 | 0.45–1.64 | 0.58 |
| Pet | | | | | | |
| No | 1.00 | | | 1.00 | | |
| Yes | 1.47 | 0.69–3.15 | 0.32 | 1.26 | 0.55–2.91 | 0.58 |

The association of age with infection was not significant, indicating that all age groups had almost equal exposure opportunity. This could be as a result of the general contamination of the environment, even though younger children are generally reported to have higher prevalence due to their poor personal hygiene like geophagia/pica [40]. Prevalence seemed likely to exist in different genders in different ecological environments in the present study, e.g., boys in Lowveld showed lower prevalence than girls, while the situation in Highveld was opposite. It could be explained that the environmental contamination by intestinal parasites is serious, thus making PSC susceptible to acquire the IPIs through frequent exposure to the contaminated environments, regardless of the different ecological environments. Moreover, on the whole, boys and girls had similar prevalence, so the infection was not gender-dependent in this study, which is consistent with other studies [16, 22], indicating boys or girls had equal opportunity in acquisition of IPIs. The occupation of parents/guardians also did not show a significant association with infection in the logistic analysis

and such a similar finding was also recorded in Nigeria [16]. However, living in Highveld area than Lowveld area had significantly higher risk of acquisition of pathogenic parasites by logistic regression analysis. In Eswatini, annual rainfall was recorded highest in the Highveld, between 1,000 and 2,000 mm, while in the Lowveld, 500 to 900 mm per annum was recorded. The Highveld temperature is temperate and seldom uncomfortably hot, while the Lowveld may record temperatures around 40°C in summer [41]. Alternatively, it can be explained that more abundant rain water with temperate temperature in Highveld than Lowveld may provide more suitable environments for parasites to survive plus a lack of good sanitation. Also, contact with contaminated soil and infected water when taking care of body hygiene and domestic activities may lead to higher opportunities for PSC to acquire these parasites via fecal-oral route.

Therefore, regardless of living in different ecological environments, key measures would be to educate PSC the better personal hygienic habits as well as improve environmental sanitation to prevent intestinal pathogenic protozoan and helminthic infections. Also, proper treatment of infected PSC by considering adding praziquantel and/or metronidazole to mebendazole- or albendazole along with regular deworming regimens to Eswatini PSC is urgently needed.

## Data Availability

The raw data can be acquired upon request.

## Disclosure

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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