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Prevalence and Distribution of *Cryptosporidium* spp. and *Giardia lamblia* in Rural and Urban Communities of South Africa

Güney Afrika'nın Kırsal ve Kentsel Topluluklarında Cryptosporidium spp. ve Giardia lamblia'nın Yaygınlığı ve Dağılımı

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ABSTRACT

Objective: Enteric diseases remain a serious health problem globally. High prevalence is evident in regions with poor socioeconomic conditions, poor sanitation, and inadequate clean water supply, such as South Africa. Designing an effective strategy, however, requires local knowledge, which can be particularly challenging to acquire in low- and middle-income countries. As the first step in this process, we investigated the prevalence and distribution of protozoan parasites *Cryptosporidium* and *Giardia* in the rural and urban gastrointestinal clinics of South Africa.
Methods: A cross-sectional study was conducted to assess the prevalence of enteric parasites *Cryptosporidium* and *G. lamblia* in rural and urban communities of South Africa. Stool samples were collected from November 2013 to June 2015 from patients with diarrhea (n=227) and without diarrhea (n=257). DNA was extracted and a diagnostic Taqman qPCR assay was performed to detect these protozoan parasites, which was further confirmed by the Sanger sequencing of a few samples.
Results: Of the 484 stool specimens collected, 34% (166/484) were positive for either *Cryptosporidium* spp. or *Giardia lamblia* parasites, with only 5% containing both parasites (22/484). In both study populations, *Cryptosporidium* was the most prevalent parasite (overall 25%) followed by *Giardia* (19%).
Conclusion: This study discovered that both *Giardia* and *Cryptosporidium* parasites might contribute to diarrheal disease in South Africa and are more prevalent in rural communities. Future studies are needed to identify the source of the infection and design appropriate interventions to reduce the burden of the disease.
Keywords: Intestinal parasites, protozoa, *Cryptosporidium* spp., *Giardia* spp.

ÖZ

Amaç: Enterik hastalıklar küresel olarak ciddi bir sağlık sorunu olmaya devam etmektedir. Güney Afrika gibi düşük sosyo-ekonomik koşulların, kötü sanitasyonun ve yetersiz temiz su kaynaklarının olduğu bölgelerde yüksek prevalans görülmektedir. Ancak etkili bir strateji tasarlamak için, düşük ve orta gelirli ülkelerde edinilmesi özellikle zor olabilecek yerel bir bilgi gerektirmektedir. Bu süreçte biz ilk adım olarak, Güney Afrika'nın kırsal ve kentsel gastrointestinal kliniklerinde protozoan parazitler *Cryptosporidium* ve *Giardia'*nın prevalansını ve dağılımını araştırdık.
Yöntemler: Güney Afrika'nın kırsal ve kentsel topluluklarında *Cryptosporidium* ve *G. lamblia* enterik parazitlerinin sıklığını araştırmak için kesitsel bir çalışma yapıldı. İshali olan (n=227) ve olmayan (n=257) hastaların Kasım 2013-Haziran 2015 tarihleri arasında dışkı örnekleri toplandı. DNA ekstrakte edildi ve bu protozoan parazitleri saptamak için tanısal bir Taqman qPCR tahlili kullanılarak, birkaç örnek Sanger dizilimi ile daha da doğrulandı.
Bulgular: Toplanan 484 dışkı örneğinin %34'ü (166/484) *Cryptosporidium* spp. veya *Giardia lamblia* parazitleri için pozitifti ve örneklerin sadece %5'i her iki paraziti de içeriyordu (22/484). Her iki çalışma popülasyonunda da *Cryptosporidium* en yaygın parazitti (toplam %25) ve bunu *Giardia* (%19) izledi.
Sonuç: Bu çalışma, hem *Giardia* hem de *Cryptosporidium* parazitlerinin Güney Afrika'daki ishal hastalığına katkıda bulunabileceğini ve kırsal topluluklarda daha yaygın olduğunu göstermiştir. Hem enfeksiyonun kaynağını belirlemek hem de hastalığın yükünü azaltmak için uygun müdahaleleri tasarlamak için gelecekteki çalışmalara ihtiyaç vardır.
Anahtar Kelimeler: Bağırsak parazitleri, protozoa, *Cryptosporidium* spp., *Giardia* spp.

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Intestinal parasitic infections constitute a serious public health problem in low and middle-income countries globally. The risk of diarrheal disease is high in areas of poor sanitation, low socio-economic conditions, inadequate water supply and poor hygiene practices (1). Diarrheal infections are documented as the second leading cause of death worldwide, particularly in children under the age of five (2). Of those who survive the diarrhoea, the morbidity burden can affect their development (growth, cognitive performance, and physical fitness) (3). Several people are thought to be affected by neglected tropical diseases, of which many of the infections are due to protozoan parasites, such as *Cryptosporidium* spp. and *Giardia* (4,5).

Identifying people who are at high risk of infection is an important aspect of parasitic disease control programmes. Immunocompromised individuals are most vulnerable with incidence of diarrheal infections. Protozoa infections in humans are commonly caused by consumption of contaminated water and food containing the infecting oocysts (*Cryptosporidium*) and cysts (*Giardia*) in the environment (4). Geographical location therefore may play a role in pathogen exposure, and it has been shown previously that disease causality as well as prevalence of diarrhoea can vary greatly depending on region/community.

South Africa has been greatly impacted by diarrheal diseases caused by viruses, bacteria as well as parasitic organisms. In the work of (6), on the prevalence of intestinal parasitic and bacterial pathogens in Limpopo Province showed that *Cryptosporidium* spp. and *G. lamblia* were the most common cause of diarrhoea. A recent study of the enteric pathogens which included South African rural children <2 years in age detected *Cryptosporidium* spp. in diarrhoeal stool with *G. lamblia* being mainly present in asymptomatic samples (7) and several recent reviews have been published on this topic. However, in general, these have focused on the epidemiology and transmission dynamics of the parasites in humans ignoring the fact that S. African communities in which these parasites are endemic can be highly diverse and the exposure to domestic animals can vary greatly (8,9).

Much remains to be learned about the epidemiology of these pathogens in African rural communities where it is feasible that domestic animals could be an important reservoir of zoonotic pathogens. In the present study, we investigated the prevalence of two zoonotic pathogens (*Cryptosporidium* spp. and *Giardia lamblia*) in rural Giyani (Mopani district of Limpopo) and in urban Pretoria, (Soshanguve district of Gauteng) where we had previously discovered that contamination of the water supply with the human specific enteric parasite *E. histolytica* seemed to be comparable.

METHODS

Ethical Approval and Consent to Participate

The research and Ethics Committee of the University of Venda Granted Institutional approval. The study received ethical clearance from the Department of Health and Welfare in Polokwane, Limpopo province, South Africa (17/08/2009). Then we obtained permission to collect samples from the ethic committee of the hospitals and the clinics. The objectives and concepts of the study were clearly explained to the potential participants and a signed consent form was obtained before a participant enrolls in the study. Confidentiality of the participants was kept by giving each sample a code and their information was kept confidential. Parental consent was also obtained in case of minors.

Study Area, Population, and Sample Collection

Stool samples were collected from urban and rural populations of moderate to low socio-economic status. They were collected at a rural Nkomo Clinic, Giyani, and an Urban Clinic within the Dr. George Mukhari Hospital, Soshanguve district of Gauteng, Pretoria. Giyani is rural with people of different religious, educational, and socio-economic backgrounds, living in neighborhoods with distinctly different level of sanitation and Pretoria is a city in the northern part of Gauteng Province, South Africa. It is one of the country's three capitals. It covers an area of 1.644 km^2 of the total surface area of Gauteng province.

The study population consisted of both adults and children of all ages, in rural areas and urban areas. A consent form was used to collect data such as the age, gender, and origin. The stool consistency was recorded by checking the stool type, whether diarrheal or non-diarrheal, this was done based on the physical presentation of the sample as defined by the Bristol stool form scale (diarrhea: Types 6 and 7; non-diarrheal: Type 1 to 5) (10). Samples were labelled with unique participant identifier codes, aliquoted in 2 mL tubes and then shipped to the University of Virginia Infectious Diseases Research Laboratory for analysis.

It is also important to note that most people living in urban settings do not have access to proper urban health care facilities, and thus cannot receive an adequate treatment of diarrhea. Although it is believed that urban areas have better access to an improved water source, sanitation facility and better knowledge about the prevention and control of diarrheal disease in comparison to rural populations an uncontrolled growth of informal settings with adverse housing conditions makes these advantage less visible.

Genomic DNA Purification

The genomic DNA extraction protocols used were as previously described by (11). QIAamp DNA Stool Mini Kit (Qiagen) was used to extract the DNA; this was done following the manufacturer's recommended procedures with the modifications previously described (11,12). To monitor possible contamination during the extraction process, a stool sample that was tested and found negative for the target parasites was included in each batch of extraction. The resulting DNA was used for identification of *Cryptosporidium* spp. and *Giardia lamblia* spp.

Species Identification

A diagnostic Taqman qPCR assay (with Taqman probes and species or genus specific primers) to detect the presence of *Cryptosporidium* spp. and *Giardia lamblia* in the extracted faecal genomic DNA was used for molecular detection of protozoan parasites. The following probes/primers and cycling conditions were used for the amplification (Table 1) (13).

Statistical Analysis

Descriptive analysis was used for data description and Graphpad prism was used to perform statistical analysis, using contingency table with chi-square test. A p-value less than 0.05 was considered significant.

Table 1. Primers and probes used for species identification

Primers	Probe/primer sequence	Fluorophore	Cycling conditions
Cryptosporidium F primer	GGGTTGTATTTATTAGATAAAGAACCA	TEXAS RED	1 cycle, 95 °C for 3 minutes, 40 cycles, 95 °C for 10 seconds 40 cycles 60 °C for 1 minute
Cryptosporidium R primer	AGGCCAATACCCTACCGTCT		
Cryptosporidium probe	GTGACATATCATTCAAGTTTCTGAC		
Giardia F primer	GACGGCTCAGGACAACGGTT	VIC	
Giardia R primer	TTGCCAGCGGTGTCCG		
Giardia probe	CCCGCGGCGGTCCCTGCTAG-MGB		

RESULTS

A total of 484 patients were recruited in this study, of which 227 (47%) were from Giyani (rural) and 257 (53%) were from Pretoria (urban). In rural areas, the patients were aged 2-73 (19.2±17.71) year(s). Most of the study participants were males (37%) while 46% were females, 39 (17%) gender of the samples was unidentified and most of the patients were aged 6-64 (60%). About 125 (49%) were diarrheal and 132 (51.3%) were non-diarrheal. Most of these patients were aged 6-64 (36.1%) and the age of 47 (18.3%) was unidentified. Of the 227 stool samples collected, 61 (27%) were diarrheal and 166 (73%) were non-diarrheal (based on the physical presentation of the sample). In urban areas, the patients were aged 1-90 (41.7±22.13). Many of the patients were aged 6-64 (41%). One hundred and four (40.5) of these patients were males while 103 (40%) were females; the gender of 50 (19.5%) of the patients was unidentified. Table 2 shows the demographic data of the study population.

The Overall Prevalence of Protozoan Parasites in the Study Population

Of the 484 stool specimens collected, 34% (166/484) were positive for either *Cryptosporidium* spp. or *Giardia lamblia* parasites while 66% (318/484) were negative. Of the 166 positive samples, 49% (112/227) were from the rural and 21% (54/257) from the urban cohort. *Cryptosporidium* (25%) was the most prevalent parasite in the study population, followed by *Giardia* (19%) with 5% of samples containing both parasites.

The Distribution and Prevalence of Parasites by Origin and Stool Type

The distribution of parasites was also investigated by host origin; the parasites occurred at different frequencies in the urban and rural populations (Table 3). The high prevalence of both the parasites was seen in rural areas than urban and the difference was significant. In the case of occurrence by stool type, *Cryptosporidium* was the most common organism isolated and the frequency of its occurrence was not statistically different in diarrheal and non-diarrheal patients. Interestingly, *Giardia lamblia* was found to be more common in non-diarrheal than diarrheal patients and the difference was significant (p=0.046). There was no significant difference in the number of co-infections with both parasites in the diarrheal patients (p=0.0805) (Table 3). Of the diarrheal samples, 13 were associated with *Cryptosporidium* spp. and 7 with *Giardia lamblia* from rural areas and from urban areas, 15 were associated with *Cryptosporidium* spp. and 4 with *Giardia lamblia*.

Parasite Burden

In this study we hypothesized that patients with high parasite burdens would show symptoms upon parasite infection. Comparing the parasite burden of each parasite with diarrheal and non-diarrheal stool specimens challenged this hypothesis. Statistically significant increase in parasite load occurred in symptomatic cases due to *Cryptosporidium* spp. but not *Giardia lamblia* (Figure 1).

Co-infections

Among the infected patients, 73% (122/166) were infected by a single intestinal parasite and 13% (22/166) had two infections.

DISCUSSION

Protozoan parasites such as *Cryptosporidium* spp. and *Giardia lamblia* are the causes of diarrheal infections worldwide and are important enteric pathogens in developing countries. A few studies have been conducted on the prevalence and distribution of these protozoan parasites in South Africa (13-15). Communities particularly at risk are those with poor sanitation services

Table 2. Demographic characteristics of the study population

Characteristics		Sample set 1 (rural) n=227	Sample set 2 (urban) n=257
Gender	Male Female Unidentified gender	83 (37%) 105 (46%) 39 (17%)	104 (40.5%) 103 (40%) 50 (19.5%)
Age groups	Age range £5 years 6-64 years ³65 years Unidentified age	2-73 (19.2±17.71) 37 (16.3%) 93 (41%) 6 (3%) 90 (39.7%)	1-90 (41.7±22.13) 19 (7.4%) 154 (60%) 37 (14.4%) 47 (18.2)
Stool type	Diarrheal stools (type 6 and 7) Non-diarrheal stools (type 1-5)	61 (27%) 166 (73%)	125 (49%) 132 (51%)

Table 3. The distribution and prevalence of parasites by origin and stool type

Pathogen	Rural (n=227)	Urban (n=257)	p	Diarrheal stool (n=186)	Non-diarrheal stool (n=298)	p
Cryptosporidium spp.	56 (25%)	41(16%)	0.0129	32 (17%)	65 (22%)	0.243
Giardia lamblia	55 (24%)	14 (5%)	<0.0001	19 (10%)	50 (17%)	0.046
Co-infections	18 (8%)	4 (2%)	0.0008	4 (2%)	18 (6%)	0.0702

and inadequate water supplies (16,17). These highly infective parasites can be transmitted between species and in the present study we aimed to determine if zoonotic transmission could be a significant contributor to the amount of these parasites present in S. African communities.

To investigate this, we compared the frequency of *Cryptosporidium* spp. and *Giardia lamblia* in a rural (Giyani, Limpopo) and urban (Pretoria, Gauteng) clinics. The gastrointestinal clinics involved in our study had comparable rates of the human specific protozoan pathogen *E. histolytica,* suggesting that exposure to infected humans or human contaminated water supplies were similar. *Cryptosporidium* infection rates can also however be altered by host immune status as evidenced by the work of Bartelt et al. (18) in 2013 who reported a higher prevalence of *Cryptosporidium* (75.3%) in HIV-infected patients in Limpopo, South Africa. A limitation of this study however was that it was observational in nature and only focused on the frequency of the disease occurring in human hosts.

The frequency of *Cryptosporidium* spp. and *Giardia* infections was significantly lower in the urban setting versus the rural one. Since these are different areas, it is believed that variance could be due to the population living in urban areas having better access to an improved water source, sanitation facilities, health care facilities and better knowledge about the prevention and control of diarrheal disease in comparison to rural populations. Another possible reason could be that people living in rural areas tend to be poorer than their urban counterparts are, a factor known to have an impact on the level of hygienic practice.

Giyani is mostly rural, and it is possible that transmission of zoonotic parasites could occur through ingestion of water contaminated with animal feces. Our hypothesis is therefore that a substantial reservoir of these parasites in animals contributes to the prevalence of this parasite in Giyani and zoonotic carriage can contribute to the geographical heterogeneity in the frequency of the protozoan parasitic infections of *Cryptosporidium* and *Giardia* (19). Therefore, we recommend that studies on zoonotic transmission be conducted in order to design preventive measures to reduce morbidity and other consequences of infections by these protozoan parasites in the Limpopo Province.

Studies in other parts of the African continent have reported infection rates of *Cryptosporidium* spp. and *Giardia lamblia* in patients with and without gastrointestinal symptoms to be as low as 6.3% and 2.7% respectively (20). To examine the generalizability of our finding of high *Cryptosporidium* infection

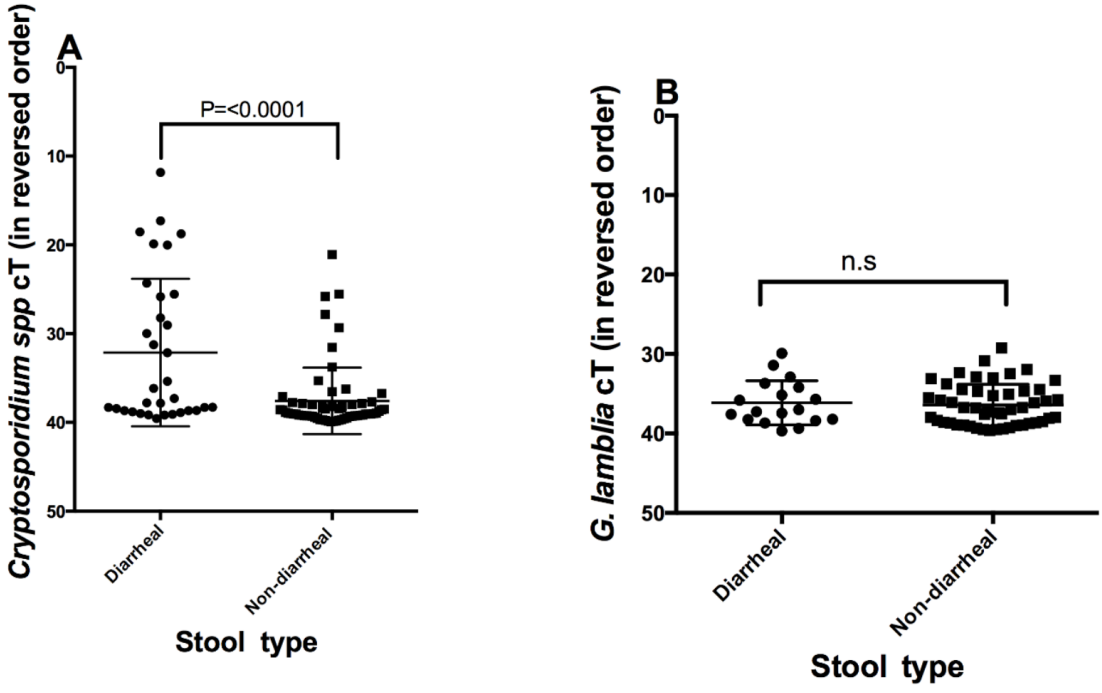

Figure 1. The impact of parasite burden in the outcome of the infection. In the scatter plots the *y*-axis indicates the threshold value of the qPCR assay results in samples positive for each parasite. Horizontal lines indicate the data means and vertical lines the standard deviation X axis indicates if the stool was diarrheal or non-diarrheal A) Cq values of *Cryptosporidium* positive samples B) Cq values of *Giardia* positive samples
PCR: Polymerase chain reaction

rates in rural clinics (25%) we compared our study results to those of other studies in Limpopo. The *Cryptosporidium* infection rate in rural Giyani is similar to the frequency (26.5%) reported in Bela-Bela rural clinic (21) and 21% reported in Turkey by polymerase chain reaction (22). In contrast to our findings, a study by Polat et al. (23) and Yilmazer et al. (24) in Turkey reported low prevalence rate (1.94%) and (8.93%) respectively. This low rate of infection may be due to environmental factors and sample size which was less compared to our study population.

The prevalence of these parasites is not however necessarily similar throughout Africa and other countries. As shown by a study in a rural area of Zanzibar, Tanzania that the prevalence of *Giardia* was as high as 53.4% (25). The occurrence of *Giardia* infection in our study (19%), also agrees with the findings reported in Turkey (26). Another study in Turkey reported a lower infection rate as compared to our study findings. Other studies in Africa reported the prevalence of *Giardia* to be 13.9% in the rural Agboville area of Côte d'Ivoire (27) and 14.2% in rural Equatorial Guinea (28). This variability reflects the need to continue to monitor the frequency of infectious diseases in the S. African population and identify the risk factors in both rural and urban African communities. Both asymptomatic and symptomatic infections with the *Giardia* and *Cryptosporidium* parasites have been shown to negatively affect child growth and development and are linked to other negative effects on the long-term health of the human host. The high prevalence of protozoan parasite reported in this study, particularly in rural settings, revealed poor sanitation and environmental contamination as a public health problem among individuals in this area.

While *Cryptosporidium* spp. and *Giardia lamblia* are associated with moderate to severe diarrhoea and increased mortality in Africa, in our study neither the *Cryptosporidium* nor the *Giardia* parasites were significantly more common in diarrheal cases. Although this result is at odds with most previous studies, it is not completely unheard of. In the present study, we examined the parasite load in diarrheal and non-diarrheal stools, and the results showed that a higher parasite burden was significantly associated with diarrhoea due to *Cryptosporidium*. The reason for the high rate of asymptomatic *Giardia* infections however remained unclear. While it was expected that parasite co-infections would increase the risk of diarrheal disease no significant association between cases where co-infections with both *Giardia* and *Cryptosporidium* parasites occurred and diarrheal symptoms was observed. Our future work involves examining the synergistic effect of concomitant co-infections of *Giardia* with other enteric pathogens that may be endemic within this population.

CONCLUSION

In conclusion, infections with both *Cryptosporidium* and *Giardia* parasites were observed in the study, with a significantly higher prevalence occurring in rural areas compared to urban areas. As these parasites spread by eating and drinking of contaminated water, research on the source of this contamination must be conducted to improve prevention measures.

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*Ethics

Ethics Committee Approval: The research and Ethics Committee of the University of Venda Granted Institutional approval. The study received ethical clearance from the Department of Health and Welfare in Polokwane, Limpopo province, South Africa (17/08/2009).

Informed Consent: The objectives and concepts of the study were clearly explained to the potential participants and a signed consent form was obtained before a participant enrolls in the study.

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*Authorship Contributions

Concept: R.N., C.G., A.S., Design: R.N., A.S., Data Collection or Processing: R.N., Analysis or Interpretation: R.N., C.G., A.S., Literature Search: R.N., A.S., Writing: R.N., C.G., A.S.

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