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Lack of soil-transmitted helminths in pilgrims at the Grand Magal de Touba, in Senegal

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Abstract: Introduction: The Grand Magal de Touba (GMT) is the largest Muslim mass gathering in Senegal with a potential for infectious disease transmission. The objective of this study was to investigate pilgrims participating in this event for the presence of intestinal parasites, including soil-transmitted helminths (STHs). **Method:** The carriage of gastrointestinal STHs was assessed on rectal swabs (n = 513) taken with their consent, from cohorts of GMT pilgrims from two villages located in southern Senegal (2017–2022), and from patients consulting at the Mbacké health center in 2018 to 2021. RT-qPCR identification was performed to detect the presence of *Ascaris lumbricoides, Trichuris trichiura, Necator americanus, Ancylostoma duodenale and* Stongyloides stercoralis. **Result:** Among cohort participants, only one pilgrim was positive for *Entomoeba histolytica* in a pre-Magal sample, and an acquisition rate of 2.8% for *Giardia lamblia* was noted. Among Mbacké patients suffering from diarrhea, 9.2% were positive for *G. lamblia*, 5.1% for *Cryptosporidum* sp. and 2.0% for *E. histolytica*. No samples tested positive for any helminths, either in the cohorts or in patients at the Mbacké health center. **Conclusion:** Our results confirm that STHs appear to have been eliminated in Diourbel. Empirical treatment of patients suffering from diarrhea at the GMT should not be based on drugs that are only active on helminths in the absence of obvious signs of helminthic infestation. However, infections with protozoa and notably with *G. lamblia* are still prevalent, both in asymptomatic pilgrims and in those suffering from diarrhea.

Keywords: eradication; helminths; protozoa; Grand Magal; Touba; Senegal

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Introduction

Soil-transmitted helminths (STHs) are a group of human parasitic nematodes mainly confined to the world's tropical and subtropical climates. Helminth infections constitute a major public health problem worldwide and are among the most important neglected tropical diseases in terms of morbidity. Recent estimates suggest that 1.5 billion people are infected with at least one helminth species, most of whom reside in low- and middle-income countries in endemic regions of Asia, Latin America, the Caribbean and sub-Saharan Africa [1]. *Ascaris lumbricoides* is the largest of the roundworms (up to 400 mm in length) and the most widespread STHs [2]. *Trichuris trichiura* is the main whipworm to infect humans [3]. *Necator americanus* and *Ancylostoma duodenale* belong to the hookworms, with a predominance of *N. americanus*, which is particularly common in Southern China, Southeast Asia, the Americas and most of Africa [4]. *Strongyloides stercoralis* development engenders other free-living and parasitic pathways following environmental stimuli [5]. A cross-sectional study carried out in Senegal from November to December 2014 in the polarized households of the Keur Soce and Lamarame health posts showed that among the 392 children under 10 years selected for the study, 137 (34.9%) were carriers of at least one intestinal parasite, and that protozoan infections were more frequent than helminthic infections (93.4% vs. 2.2%, respectively) [6]. In another Senegalese study conducted in Saraya and Diourbel communities among children aged 1–14 years, in 2021, an overall prevalence of 51.1% intestinal protozoa was observed with most cases due to *Giardia lamblia, Blastocystis hominis and Entamoeba coli*, while a low STH prevalence of 1.2% was found mostly due to *T. trichiura* (1.0%) and *A. duodenale* (0.1%), with no cases of *A. lumbricoides, Hymenolepis nana* and *Enterobius vermicularis* [7].

The Grand Magal de Touba (GMT) is the largest Muslim mass gathering in Senegal. It brings together between four and five million people from all over the world every year. The acquisition rate of bacterial pathogens diagnosed by PCR in cohorts of pilgrims following their participation in the event in 2017–2021 was 32.3%, with enteroaggregative *Escherichia coli* (18.9%) and enteropathogenic *E. coli* (10.5%) being the most frequent. The acquisition of viral pathogens was low (1.7%) and also that of protozoa (2.9%), with all cases being due to *G. lamblia* [8]. The PCR results for patients consulting in Mbacké for diarrhea, in 2018–2021, showed the presence of bacterial pathogens in 63.1% patients with most cases having been due to enteroaggregative *E. coli* (45.3%), enteropathogenic *E. coli* (21.1%) and *Shigella*/enteroinvasive *E. coli* (18.9%). Only 8.5% of patients tested positive for viruses, with adenovirus being the most frequent (6.3%), and 14.7% for protozoa with *G. lamblia* as the most frequent (9.5%), as well as rare cases of *Cryptosporidium* sp. (4.2%) and *Entamoeba histolytica* (2.1%) [9].

The objective of this study was to investigate pilgrims participating in the event for the presence of intestinal parasites including STHs.

Methods

On inclusion, the procedure was to collect data using a standardized questionnaire. The survey covered demographic data (age and sex), chronic diseases and gastrointestinal symptoms. Rectal auto-sampling was carried out, with their consent, by pilgrims from two villages (Dielmo and Ndiop) located in southern Senegal from 2017 to 2022 before and after participating in the GMT, and from diarrheal patients consulting at the Mbacké health center near Touba (Diourbel discrict) from 2018 to 2022, most of whom were locals. Samples were taken using commercial rigid cotton swab applicators (Medical Wire & Equipment, Wiltshire, UK). Samples were kept at +4 °C before being transported to the Dakar laboratory for storage in a −80 °C freezer and then transferred to Marseille on dry ice for processing. We tested available samples for the presence of *G. lamblia, E. histolytica*, *Cryptosporidium* sp., *A. lumbricoides*, *T. trichiura*, *N. americanus*, *A. duodenale* and *S. stercoralis*, targeting the 18S, hsp70, Alum, Trich, Neca, Anduo and Angui genes, respectively (Table 1). Nucleic

acid extraction (DNA and RNA) was performed by Kingfisher, followed by RT-qPCR identification of the parasites. Genes were amplified using the Light Cycler 480 Probes Master Kit (Roche Diagnostics, France) for bacteria. A C1000 Touch thermal cycle (Bio-rad, Hercules, CA, USA) was used to perform each real-time quantitative PCR. Negative controls (mix mix) and positive controls (DNA from a bacterial strain) were included in all runs. Positive pathogen amplification results were evaluated against a cycle threshold (CT) value of $\leq$35. Acquisition rate was calculated as the proportion of patients who tested positive after participating in the GMT, and who were negative before the event.

Table 1: Primers and probes used for PCR amplification of nucleic acids from gastrointestinal pathogens tested in cohorts of pilgrims and in patients at the Mbacké health center during the Grand Magal of Touba.

Pathogens	Target Sequences	Primers/Probes	Sequences (5′ 3′)
Entamoeba histolytica	18S	Eh_R	AACAGTAATAGTTTCTTTGGTTAGTAA AA
		Probe	CTTAGAATGTCATTTCTCAATTCAT
Giardia lamblia	18S	Giardia-80F	6FAM – GACGGCTCAGGACAACGGTT
		Giardia-127R	TTGCCAGCGGTGTCCG
		Probe	6FAM – CCCGCGGCGGTCCCTGCTAGTAMRA
Cryptosporidium sp	hsp70 gene	1PS_F	AACTTTAGCTCCAGTTGAGAAAGTACT C
		1PS_R	CATGGCTCTTTACCGTTAAAGAATTCC
		Probe	6FAM – AATACGTGTAGAACCACCAACCAATAC
Ascaris lumbricoides	Alum	Alum_F	GAT ATA GCA GTC GGC GGT TTC TT
		Alum_R	GCC CAA CAT GCC ACC TAT TC
		Probe	6FAM -TTG GCG GAC AAT TGC ATG CGA T-TAMRA
Trichuris trichiura	Trich	Trich_F	TTG AAA CGA CTT GCT CAT CAA CTT
		Trich_R	CTG ATT CTC CGT TAA CCG TTG TC
		Probe	6FAM -CGA TGG TAC GCT ACG TGC TTA CCA TGG-TAMRA
Necator americanus	Neca	Neca_F	CTG TTT GTC GAA CGG TAC TTG C
		Neca_R	ATA ACA GCG TGC ACA TGT TGC
		Probe	6FAM -CTG TAC TAC GCA TTG TAT AC-MGB
Ancylostoma duodenale	Anduo	Anduo_F	GAA TGA CAG CAA ACT CGT TGT TG
		Anduo_R	ATA CTA GCC ACT GCC GAA ACG T
		Probe	6FAM -ATC GTT TAC CGA CTT TAG- MGB
Stongyloide stercoralis	Angui	Angui_F	GAA TTC CAA GTA AAC GTA AGT CAT TAG C
		Angui_R	TGC CTC TGG ATA TTG CTC AGT TC
		Probe	6FAM -ACA CAC CGG CCG TCG CTG C-TAMRA

Results

A total of 731 individuals were included, including 634 from the cohorts comprising 312 males and 322 females, with the remaining 97 from Mbacké with 53 males and 44 females, over the study period. The mean age of the pilgrims in the cohorts was 25 years, while it was 16 years for the Mbacké patients. Among the cohort of pilgrims, eleven suffered from chronic respiratory disease, four from chronic heart disease and seven from arterial hypertension. Additionally, there were 26 cases of diarrhea (4.1%), 9 cases of nausea (1.4%), 36 cases of abdominal pain (5.6%), 20 cases of vomiting (3.1%), 15 cases of constipation (2.3%) and 8 cases of fever (1.2%). All patients from Mbacké were suffering from diarrhea with 47.4% experiencing vomiting, 42.2% abdominal pain, 4.1% nausea and 22.6% fever. The PCR results are presented in Table 2. Among the cohort participants, 5.0% tested positive for

Table 2: Prevalence of gastrointestinal pathogens.

	Cohorts															Mbaké Health Care Center					
Giardia lamblia	2017 N = 110		2018 N = 101		2019 N = 93		2020 N = 106		2021 N = 125		2022 N = 99		Total Before N = 634	Total After N = 634	Total Acquisition N = 634	2018 N = 23	2019 N = 54	2020 N = 15	2021 N = 3	2022 N = 2	Total N = 97
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	-	-	-	-	-	-	-	-	-
Cryptoporidium spp.	8 (7.2%)	2 (1.8%)	5 (4.9%)	6 (5.9%)	1 (1.0%)	1 (1.0%)	9 (8.4%)	7 (6.6%)	6 (4.8%)	5 (4.0%)	3 (3.0%)	8 (8.0%)	32 (5.0%)	29 (4.5%)	18 (2.8%)	2 (8.6%)	5 (9.2%)	2 (13.3%)	0	0	9 (9.2%)
Entomoeba hystolitica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (4.3%)	3 (5.5%)	0	0	1 (50%)	5 (5.1%)
Ascaris lumbricoides	0	0	1 (0.9%)	0	0	0	0	0	0	0	0	0	1 (0.1%)	0	0	1 (4.3%)	1 (1.8%)	0	0	0	2 (2.0%)
Trichirus trichiura	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Necator americanus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ancylostoma duodenale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strongyloides stercoralis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

G. lamblia before participating in the GMT and 4.5% tested positive afterwards with an acquisition rate of 2.8%. Only one pilgrim was positive for *E. histolytica* in a pre-Magal sample. None tested positive for *Cryptosporidium* sp. Among Mbacké patients suffering from diarrhea, while 9.2% were positive for *G. lamblia*, 5.1% for *Cryptosporidum* sp. and 2.0% for *E. histolytica*.

No samples tested positive for any helminths, either in the cohorts or in patients at the Mbacké health center.

Discussion

While STHs have almost been eliminated in most districts in Senegal, Diourbel was the area where parts of the community had a persistent, high burden of helminths [10]. The control program for STHs has been implemented annually in Africa since 2014 [11]. An antihelminthic mass preventive treatment with albendazole was carried out throughout Senegal in 2015, 2017, 2019 and 2021. Our results confirm that STHs appear to have reached the status of STH elimination as a public health problem (EPHP) in Diourbel, Ndiop and Dielmo, as defined by the 2030 World Health Organization (WHO) Neglected Tropical Disease (NTD) road map of attaining a prevalence of <2% for moderate-to-heavy-intensity infections [12]. Low rates of patients are, however, still infected with protozoa. Most NTD control programs deploy the WHO recommended albendazole or mebendazole as the preventive chemotherapy of choice for STH control, which, however, is not effective against intestinal protozoa. Given the need to control intestinal protozoan infections, an addition of a single dose of tinidazole to the current NTD preventive chemotherapy may be justified due to the challenges of poor compliance with multiple doses of metronidazole [7]. This is of particular concern in rural areas such as in Dielmo and Ndiop, and also in urban areas such as the one in Diourbel, whereby both lack optimal sanitation, including the absence of a sewage system for wastewater. In addition, during the GMT, the population in Touba is temporarily increased by a factor of 5, with participants from all Senegalese regions leading to a risk of introduction of intestinal protozoa with a potential for dissemination in the community. The concentration of people in Touba during the GMT has already proven to favor epidemics of intestinal infections like that of cholera in the past [13,14]. In addition, empirical treatment of patients suffering from diarrhea during the GMT should be based on drugs active on protozoa, while the use of drugs only active on helminths should be discontinued in the absence of obvious signs of helminthic infestation.

Our study has some limitations. First, the number of participants was small and pilgrims form Ndiop and Dielmo may not be representative of all pilgrims participating in the GMT. Also, the sampling methods used in our study (rectal swab) may have possibly accounted for the low prevalence of parasites. Stool and rectal swab samples have shown excellent correlation for the investigation of the gut bacterial microbiota by PCR in healthy individuals [15] and for pathogen detection in patients suffering from gastroenteritis, including for protozoa [16,17]. We, however, found no publication addressing the comparison of stool and rectal sample usefulness for helminth detection by PCR in humans. We cannot therefore formally exclude that rectal sampling may have accounted for the negativity of helminth detection; however, our results are in line with other works using stool samples that were conducted in the Diourbel area in 2021, showing a low prevalence of STHs by PCR (1.2%) after control programs for STHs were implemented in 2019 [7]. Unfortunately, no data are available for the Ndiop and Dielmo areas.

Conclusions

Our results confirm that STHs have been likely eliminated from rural villages in Ndiop and Dielmo and in Diourbel. However, infection with protozoa and notably with *G. lamblia* are still prevalent, both in asymptomatic pilgrims and in those suffering from diarrhea. The use of drugs active against protozoa

should therefore be considered for both preventive chemotherapy and for the treatment of patients with diarrhea who are resistant to antibiotics.

Author Contributions: C.D.: Writing of the original draft, contributed to the experimental design, wrote the manuscript, conducted the qPCR method, administered questionnaires, followed patients and collected samples. I.O. contributed to the experimental design. N.G. conducted the qPCR technique, administered questionnaires, followed patients and collected samples. M.S., H.B., and A.A. contributed to the experimental design. P.G. Writing of the original draft, contributed to the experimental design, wrote the manuscript, and coordinated the work. C.S. contributed to the experimental design and coordinated the work. All authors contributed to and approved the current version of the manuscript.

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Conflicts of Interest: No conflicts of interest relevant to this article were reported.

Ethics: The protocol was approved by the National Ethics Committee for Health Research in Senegal (SEN17/62). It was performed in accordance with the good clinical practices recommended by the Declaration of Helsinki and its amendments.

References

1. Gazzinelli-Guimaraes, P.H.; Nutman, T.B. Helminth parasites and immune regulation. *F1000Research* **2018**, *7*, F1000 Faculty Rev-1685. [CrossRef] [PubMed]
2. Elkins, D.B.; Haswell-Elkins, M.; Anderson, R.M. The epidemiology and control of intestinal helminths in the Pulicat Lake region of Southern India. I. Study design and pre- and post-treatment observations on Ascaris lumbricoides infection. *Trans. R. Soc. Trop. Med. Hyg.* **1986**, *80*, 774–792. [CrossRef] [PubMed]
3. Else, K.J.; Keiser, J.; Holland, C.V.; Grencis, R.K.; Sattelle, D.B.; Fujiwara, R.T.; Bueno, L.L.; Asaolu, S.O.; Sowemimo, O.A.; Cooper, P.J. Whipworm and roundworm infections. *Nat. Rev. Dis. Primer.* **2020**, *6*, 1–23. [CrossRef] [PubMed]
4. Loukas, A.; Maizels, R.M.; Hotez, P.J. The yin and yang of human soil-transmitted helminth infections. *Int. J. Parasitol.* **2021**, *51*, 1243–1253. [CrossRef] [PubMed]
5. Krolewiecki, A.; Nutman, T.B. Strongyloidiasis: A Neglected Tropical Disease. *Infect. Dis. Clin.* **2019**, *33*, 135–151.
6. Sylla, K.; Tine, R.; Sow, D.; Lelo, S.; Ndiaye, L.; Faye, B.T.; Ndiaye, M.; Dieng, T.; Faye, B.; Gaye, O. Epidemiological Profile of Intestinal Parasitic Infection among Preschool and School Children Living in a Rural Community in Senegal: A Cross-Sectional Survey. *J. Bacteriol. Parasitol.* **2018**, *9*, 1–7. [CrossRef]
7. Afolabi, M.O.; Sow, D.; Mbaye, I.; Diouf, M.P.; Loum, M.A.; Fall, E.B.; Seck, A.; Manga, I.A.; Cissé, C.; Camara, B.; et al. Prevalence of malaria-helminth coinfections among children living in a setting of high coverage of standard interventions for malaria and helminths: Two population-based studies in Senegal. *Front. Public Health* **2023**, *11*, 1087044. [CrossRef] [PubMed]
8. Goumballa, N.; Hoang, V.T.; Diouf, F.S.; Mbaye, B.; Parola, P.; Sokhna, C.; Gautret, P. Risk factors for symptoms of infection and the acquisition of pathogens among pilgrims at the Grand Magal of Touba, 2017–2021. *Travel. Med. Infect. Dis.* **2022**, *49*, 102418. [CrossRef] [PubMed]
9. Goumballa, N.; Sambou, M.; Samba, D.F.; Bassene, H.; Bedotto, M.; Aidara, A.; Dieng, M.; Hoang, V.T.; Parola, P.; Sokhna, C.; et al. PCR investigation of infections in patients consulting at a healthcare centre over a four-year period during the Grand Magal of Touba. *Travel. Med. Infect. Dis.* **2023**, *52*, 102515. [CrossRef] [PubMed]

10. Senegal ESPEN—World Health Organization. 2022. Available online: https://espen.afro.who.int/countries/senegal (accessed on 31 January 2024).
11. Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN). 2020. Available online: https://espen.afro.who.int/ (accessed on 31 January 2024).
12. WHO. *Ending the Neglect to Attain the Sustainable Development Goals: A Road Map for Neglected Tropical Diseases 2021–2030*; World Health Organization: Geneva, Switzerland, 2020.
13. Manga, N.M.; Ndour, C.T.; Diop, S.A.; Dia, N.M.; Ka-Sall, R.; Diop, B.M.; Sow, A.I.; Sow, P.S. Cholera in Senegal from 2004 to 2006: Lessons learned from successive outbreaks. *Med. Trop.* **2008**, *68*, 589–592.
14. Finger, F.; Genolet, T.; Mari, L.; de Magny, G.C.; Manga, N.M.; Rinaldo, A.; Bertuzzo, E. Mobile phone data highlights the role of mass gatherings in the spreading of cholera outbreaks. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 6421–6426. [CrossRef] [PubMed]
15. Radhakrishnan, S.T.; Gallagher, K.I.; Mullish, B.H.; Serrano-Contreras, J.I.; Alexander, J.L.; Miguens Blanco, J.; Danckert, N.P.; Valdivia-Garcia, M.; Hopkins, B.J.; Ghai, A.; et al. Rectal swabs as a viable alternative to faecal sampling for the analysis of gut microbiota functionality and composition. *Sci. Rep.* **2023**, *13*, 493. [CrossRef] [PubMed]
16. Kabayiza, J.C.; Andersson, M.E.; Welinder-Olsson, C.; Bergström, T.; Muhirwa, G.; Lindh, M. Comparison of rectal swabs and faeces for real-time PCR detection of enteric agents in Rwandan children with gastroenteritis. *BMC Infect. Dis.* **2013**, *13*, 447. [CrossRef] [PubMed]
17. Kotar, T.; Pirš, M.; Steyer, A.; Cerar, T.; Šoba, B.; Skvarc, M.; Poljšak Prijatelj, M.; Lejko Zupanc, T. Evaluation of rectal swab use for the determination of enteric pathogens: A prospective study of diarrhoea in adults. *Clin. Microbiol. Infect.* **2019**, *25*, 733–738. [CrossRef] [PubMed]