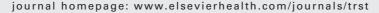


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# Geophagia is not associated with *Trichuris* or hookworm transmission in Zanzibar, Tanzania

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## **KEYWORDS**

Pica; Geophagia; Helminthiasis; Pregnancy; Parasites; Zanzibar **Summary** Geophagia may be harmful as a method for the transmission of geohelminths. In this study, we pose two questions in a representative sample of 970 pregnant women from Pemba Island, Zanzibar, Tanzania. Can consumed earth be a vector for geohelminth infection? And do geophagists have differential parasitic infection? The parasitological content of 59 non-food substance samples was analysed. Cross-sectional data regarding pica behaviour were collected through interviews conducted by local researchers. Ascaris, Trichuris and hookworm status was ascertained through Kato-Katz smears. The prevalence of geophagia at baseline was 5.6% and the overall prevalence of Ascaris, Trichuris and hookworm infection was 5.6%, 33.2% and 32.9%, respectively. No consumed soil samples contained infectious parasitic stages, and only one of the consumed pica substances (charcoal) contained parasites of potential risk to human health. In bivariate analyses, neither the prevalence nor the intensity of infection with Ascaris, Trichuris or hookworm differed significantly by geophagia status. Furthermore, in multivariate models, geophagia was not a significant predictor of helminth infection status. We conclude that geophagia is not a source of Trichuris or hookworm infection among pregnant women in Pemba (insufficient power to evaluate the effect of Ascaris), which is in contrast to existing findings of helminth infection and geophagia.

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# 1. Introduction

Pica is the purposive consumption of non-food substances. These substances include, but are not limited to, ash, charcoal, chalk, earth (geophagia), ice (pagophagia) and

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laundry or cooking starch (amylophagia). The healthfulness of pica continues to be debated. Proponents of pica suggest potential benefits such as mineral supplementation (especially iron and calcium), relief of gastrointestinal distress and detoxification, particularly of plant secondary compounds (Johns, 1999; Profet, 1992; Wiley and Katz, 1998). Others posit a range of harmful effects, including geohelminth infection, caloric displacement, constipation, dental damage, eclampsia, fatigue, hypertension, intestinal blockage, iron deficiency, lead poisoning, peritonitis and, most frequently, anaemia (Halsted, 1968; Key et al., 1982; Rothenberg et al., 1999; Sayetta, 1986).

It has been postulated that pica, namely geophagia, increases the likelihood of transmission of orally transmitted parasitic nematodes (Anell and Lagercrantz, 1958; Glickman et al., 1999; Halsted, 1968; Hooper and Mann, 1906). Whilst there is strong epidemiological and biological evidence that geophagia is not a significant mechanism for hookworm infection (Geissler et al., 1998a; Gelfand, 1945; Saathoff et al., 2002; Vermeer and Frate, 1979), there is insufficient evidence to determine whether there is a relationship between geophagia or the consumption of other non-food substances and non-hookworm nematode infections, namely Trichuris and Ascaris. For causality to be demonstrated, a vector that links pica to nematode infection must first be demonstrated (i.e. the substance must contain viable nematode eggs or larvae). An association between earth consumption and increased Trichuris and Ascaris burden in earth consumers has been shown in several studies (Geissler et al., 1998a; Luoba et al., 2005; Saathoff et al., 2002; Wong et al., 1991) but not all (Vermeer and Frate, 1979). Yet, to date, measurement of the parasite content of the soil has been investigated in only two sites (Geissler et al., 1998a; Wong and Bundy, 1990; Wong et al., 1991): no studies have determined the parasite content of earth consumed by adults and no studies have looked at the parasitic content of non-earth pica substances.

If pica is indeed a route by which geohelminths establish infection, this would help to explain the frequent association between anaemia and pica (Geissler et al., 1998a, 1998b; Glickman et al., 1999; Kettaneh et al., 2005; Nchito et al., 2004; Rainville, 1998; Thomson, 1997; Young and Ali, 2005). There are other explanations for a pica—anaemia association, which include replacement of nutritious food in the diet with non-nutritious substances, chelation of dietary iron by the pica substance and reduction of iron absorption by the gut (Arcasoy et al., 1990; Berkel et al., 1970; Danford, 1982; Hooda et al., 2004). Determining the association of pica with parasitic nematode infections will help to direct research efforts seeking to understand the association of pica and anaemia as well as those to reduce the transmission of geohelminths.

The purpose of this paper was to examine the relationship of geohelminth infection with geophagia in Pemba Island, Zanzibar, Tanzania. Parasitological analyses of 59 pica substances and epidemiological and biological data on 970 pregnant women are presented to address two questions. Do pica substances contain geohelminths? And is there differential parasitic infection between geophagists and non-geophagists?

#### 2. Methods

The study was conducted in the northern area of Pemba Island from 2004 to 2006. Pemba is the second largest island in the Zanzibar archipelago located 50 km off the coast of Tanzania. The main economic activities are clove farming and fishing. Swahili is the principal spoken language and Islam is the predominant religion. In general, the culture and ecology of Zanzibar are similar to the rest of coastal East Africa (Middleton, 2004). Because pica is prevalent on Pemba (Young and Ali, 2005) and the parasitic nematode burden is high (Montresor et al., 2003), Pemba is ideal for a study of the relationship between pica and parasitic nematode burden.

The four types of earth consumed in Pemba are udongo, ufue, vitango pepeta and mchanga. Udongo is a fine reddish-brown clayey earth that is found close to the surface and is used in making structures such as house walls. Ufue is much whiter than udongo, and is found by digging several inches or feet into the earth's surface, e.g. from the construction of wells or latrines. Vitango pepeta (also called vitango mlima) are large soft white chunks of earth and are obtained from closer to the surface of the earth than ufue. Mchanga is the sandiest of the earths eaten and is on the top layer of the earth.

A purposive sample of 60 Pemban men and women who engage in pica were interviewed about their pica habits in 2004 or 2006 (approximately two-thirds of whom were participants in the trial). After the interview, a researcher accompanied participants to the source of the pica substance if they were still engaging in pica. The consumer then collected two types of samples: (1) the exact type of material that they consumed in the amount that they would eat in a day, termed 'acceptable'; and (2) the same type of substance, but material that they did not consider acceptable to consume, termed 'rejected'. These semistructured interviews and the observation of sample collection revealed useful information about how the samples are collected, stored and prepared.

To determine the role of pica substances in the transmission of parasitic nematodes, the concentration of parasitic nematode stages present in pica substances from Pemba was measured. Approximately 15g of the materials was preserved in 10% formalin within 24h of collection. Samples were then transported to Johns Hopkins University in the USA where sample examination was performed as follows. The entirety of a sample was passed through a double layer of 1 mm steel mesh into a Petri dish. Water was then added to the dish. When there was too much sediment to read a dish accurately, the sample was split into halves or quarters and examination was repeated until the entire sample was examined. The entire dish was scanned using an inverted microscope with a mechanical stage and a  $10\times$  objective. Eggs and larvae were confirmed and speciated using a  $40\times$ objective.

This method is based on a protocol developed on Pemba for identifying diagnostic parasitic nematode stages in stool, when egg concentrations are expected to be low (Goodman et al., 2007). This protocol, when applied to stool samples where low concentrations of eggs and larvae are present, has proven to have greater or equal sensitivity to the sugar and flotation techniques commonly used to examine parasitic

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stages in soil. Whilst this is the first time this protocol has been applied to measuring parasitic stages in soil, it was a reasonable choice given the limitations imposed by formalinfixed samples and the expectation that methods would not behave differently between soil and stool sample types.

Epidemiological data regarding helminths and pica behaviour were collected as part of a trial investigating the prevention of severe maternal anaemia during pregnancy in approximately 2367 women. The mean gestational age at baseline was 25 weeks (range 20–30 weeks). For a subset of study participants (n = 970), stool samples were examined by Kato-Katz at baseline to determine infection status of Ascaris, Trichuris and hookworm (WHO, 1994). Study participants were asked about their current and recent past pica behaviour at four time points in pregnancy as well as about their pica behaviour in past pregnancies and when not pregnant. The fear of chastisement for engaging in geophagia has likely led to some underreporting, but ethnographic interviews revealed that geophagia is tolerated as 'normal' during pregnancy, leading us to believe that the geophagy behaviour reported in pregnancy is reasonably accurate. A woman was classified as a geophagist if during the baseline interview she reported purposely consuming one or more types of earth at any point in that pregnancy. All study participants gave their informed consent.

Student's t-test was used to assess differences in means. The  $\chi^2$  test and Fisher's exact test were used to test differences in proportions. Differences between the logged values of egg intensity as well as the untransformed observed values were calculated. Differences in median were calculated by Mann—Whitney U-test. Logistic regression was used to predict infection status. A P-value  $\leq$ 0.05 was considered statistically significant. All analyses were performed using Intercooled Stata 9.1 for Macintosh (Stata Corp., College Station, TX, USA).

### 3. Results

# 3.1. Description of pica samples

Ash and charcoal came from participants' kitchen hearths or store of charcoal. All but two *udongo* soil samples were obtained from the walls of houses. One *udongo* sample was dug up in a field, the other came from the inside of a well by scraping the well bucket along the inside of the well to loosen soil from the interior wall. The rest of the soil samples were collected from fields on the island. The whitewash was scraped from house walls.

Those who engaged in pica were particular about the substances they ate. When participants were asked about what made the samples that they did eat acceptable for consumption, they remarked on their attractive smell and texture. They also emphasised its location, typically noting it came from a 'good and clean' area. For example, soil from the walls of houses was taken from higher up on the wall, where animals and children 'could not reach to make it dirty'. Even the whitewash that one participant consumed had to be from a section near the top of the wall for it to be acceptable. No one approved of eating, just any, earth or any kind of even one type of earth. The samples that were rejected were chosen because they were from 'dirty'

or 'contaminated' areas, where humans and animals could tread and/or defecate.

The ash, cement powder and whitewash were eaten without preparation. A few of the earth samples were eaten without preparation, one-third were sun-dried and most were brushed (*kupangusa*) or scraped off (*kuparapara*). Two of the charcoal samples had been recently cooked in the hearth, the other three had not been exposed to flame since the charcoal was made.

All but three consumers were female; they stated that they ate these substances only when pregnant. *Vitango pepeta*, or soft white stones, were consumed by one male child and one adult male, and one adult male also consumed *ufue*.

# 3.2. Prevalence of pica

The non-food substances most frequently consumed by the 970 participants at any point during their current pregnancy were unripe mango (84.1%), uncooked rice (55.1%), large quantities of ice (20.5%) and earth (7.0%). No non-food substances were consumed by 13.3% of women. Other non-food substances consumed by fewer than 2% of the study participants included ash, chalk, cement powder, charcoal and powdered limestone (whitewash).

## 3.3. Parasitology of pica samples

Nematodes in the 'acceptable' and 'rejected' samples of ash, powdered cement, charcoal, whitewash and the four types of earth were examined. Unripe mango and uncooked rice were excluded because they are unlikely vectors of parasite transmission. Although ice is a potential source of infection through contaminated water, it was excluded from the analysis because it is not thought to be an important source of infective parasitic nematode stages. Furthermore, because we were interested in the ways that pica could be related to helmintic infection, and non-pica-eating and pica-eating participants alike are exposed to similar water (some frozen, some not), the nematode content of water would not explicitly test this link.

None of the acceptable earth samples contained any detectable helminths of risk to human health (Table 1). One of the five acceptable charcoal samples contained a non-parasitic free-living nematode and another contained two *Strongyloides* spp. rhabditiform larvae. Three of the rejected samples contained helminths. One of the rejected *ufue* (earth) samples contained a *Taenia* spp. egg and a rejected *udongo* (earth) sample had one *Toxascaris* egg. Of these, only the *Taenia* spp. egg is of potential significant health risk to humans.

# 3.4. Sociodemographic characteristics of geophagists

Geophagists did not differ significantly in age, number of past pregnancies, urban residence, marital status, formal education of self or husband, cash-earning employment of self or husband, or number of household durable goods owned (bicycle, radio, home lit by electric and/or glass

Table 1 Parasitology of pica samples	gy of pica samples				
Substance	No. of 'acceptable' samples analysed	Mean grams consumed daily	No. of 'acceptable' samples with helminths of risk to humans	No. of 'rejected' samples analysed	No. of 'rejected' samples with helminths of risk to humans
Ash	_	14.9	0	-	0
Cement	_	39.1	0	_	0
Charcoal	5	7.5	_	5	0
Earth					
Mchanga	_	12.5	0	_	0
Udongo	12	25.1	0	12	0
Ufue	m	26.5	0	m	_
Vitango pepeta	2	28.1	0	5	0
Whitewash	_	5.0	0	2	0
Total	29		1	30	1

lanterns and a metal roof) (Table 2). The only significant sociodemographic difference detected between geophagists and non-geophagists was duration of pregnancy: geophagists were approximately 2 weeks later in pregnancy at the time of the interview (Table 2). A difference in anthelmintic intervention is that significantly fewer geophagists than non-geophagists received the enhanced care.

#### 3.5. Geohelminth burden at baseline

There were no statistically significant differences in helminth infection prevalence or intensity by geophagia status at baseline (Table 3).

To assess possible confounding factors, logistic regression was then performed with *Ascaris*, *Trichuris* and hookworm as outcomes (Table 4). The independent variables were geophagia during current pregnancy, age, urban or rural residence, ownership by household of four durable goods (bicycle, radio, home lit by electric and/or glass lanterns and a metal roof), presence of pit toilet in the home and whether the woman had received any formal education.

Increasing age, urban residence, ownership of a pit toilet and formal education were all significantly negatively associated with *Trichuris* infection. Only ownership of a pit toilet was significantly negatively associated with *Ascaris* infection. Geophagia was not a significant predictor of either *Ascaris* or *Trichuris* infection.

Urban residence, ownership of a pit toilet and formal education were all significantly negatively associated with hookworm infection. The likelihood of hookworm infection was 74% higher in the geophagous group, but the strength of evidence for this association was only modest.

### 4. Discussion

Our results from the soil analysis and the behavioural and epidemiological data do not support geophagia as a vector for *Trichuris* or hookworm infection among adults in Pemba. The study is not sufficiently powered to conclude about the role of geophagia in the transmission of *Ascaris*.

Parasitological analyses of the acceptable earth samples revealed no parasitic nematode stages. Two of the rejected earth samples contained geohelminth eggs. It appears that in Zanzibar, consumers of pica substances tend to consume earth that is parasite-free, which is in contrast to previous studies where the earth consumed by Jamaican and Kenyan children was contaminated (Geissler et al., 1998b; Wong et al., 1991). It is worth noting that two acceptable charcoal samples each contained a single parasitic nematode stage. Of these two samples, only one contained a helminth considered potentially detrimental to human health (Strongyloides spp. larvae). This is the first time that non-soil pica substances have been shown to contain geohelminths.

Observations of sample collection and ethnographic interviews indicate that Pembans who eat earth are aware that eating earth can be 'dirty' and take precautions (with sample selection and preparation) as they consume pica materials. These practices (e.g. pan heating, sun drying, brushing off loose material on exterior) reduce the likelihood of helminth transmission. The caustic substances, with the harshest environments for geohelminths, were not

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	No geophagia at baseline (n = 916)	Geophagia at baseline $(n = 54)$
Age (years)	28.1	29.3
Number of past pregnancies	4.2	4.9
Duration of pregnancy (weeks)	25.0	27.4 <sup>*</sup>
No. of valuable household goods (0-4)	2.0	1.8
Primiparous (%)	12.8	5.6
Urban (%)	31.2	33.3
Married (%)	98.3	98.1
Women with any formal education (%)	55.9	61.1
Husbands with formal education (%)	69.7	61.5
Women with cash-earning jobs (%)	7.6	11.1
Husbands with cash-earning jobs (%)	68.4	66.7

<sup>&</sup>lt;sup>a</sup> Comparison of means was performed using Student's t-test; comparison of proportions was done with  $\chi^2$  test.

prepared at all prior to consumption. Preparation of pica substances by consumers has not been discussed in any of the other epidemiological studies of geophagic soils; it is our hope that future studies will include such descriptions.

The epidemiological data supported these findings. There was no significant difference between the prevalence of Ascaris, Trichuris or hookworm infection at baseline by geophagia status (Table 3). In the multivariate models of helminth infection, geophagy had no predictive power, even after controlling for potential confounders (Table 4). It is possible that some Ascaris and Trichuris infections may not have been detected if women had very recently been infected, since the period of time between infection and egg-shedding is several weeks.

Strikingly, in the multivariate model for hookworm infection (Table 4), geophagia was nearly significantly positively correlated (odds ratio (OR) 1.74; P=0.066). Because the physiological basis for geophagia causing hookworm is not strong (Geissler et al., 1998a; Gelfand, 1945; Saathoff et al., 2002), we looked for other explanations of a relationship. One such explanation comes from the primate world, where parasitic infection stimulates animals to eat earth. Regular ingestion of soil counteracted endoparasitic infections in rhesus macaques and howler monkeys, although the physiological mechanism is not fully understood (Krishnamani and Mahaney, 2000). Humans have also reported consuming clay to treat hookworm infection (Bateson and Lebroy, 1978).

We explored statistically the relationship between hookworm and geophagia further and found that inclusion of the women's baseline haemoglobin mitigates the magnitude and

significance of geophagia in the hookworm infection model (OR 1.5; P=0.127, full model not shown). Thus, geophagia is associated both with hookworm and haemoglobin level. When haemoglobin and geophagia are included in the same model, the effect of hookworm but not haemoglobin falls away, leading us to believe that haemoglobin may be mediating the association between hookworm and geophagia, i.e. it is in the causal pathway. Haemoglobin status may be on the causal pathway from hookworm infection to geophagia if, as many argue, geophagia is undertaken to obtain needed micronutrients caused by hookworm-induced blood loss. Because of the likely low bioavailability of micronutrients in soils, this explanation is only modestly plausible (Young, 2007).

There are several differences between the present study and previous studies of the relationship between geophagia and geohelminth infection that could account for different findings. The prevalences of pica and helminth infection are both lower in Pemba than they were in other study sites. The prevalence of geophagia among pregnant women at baseline in Pemba was only 5.6% (increasing slightly to 7.0% if the definition expands to earth consumption at any point in the pregnancy), whilst in all the other studies of geophagia and helminth infection the prevalence of geophagia at baseline was >46%. The baseline prevalence of Ascaris, Trichuris and hookworm was also slightly lower in the present study than in other studies. (A table detailing the differences in all study methodologies and results is available upon request from the corresponding author.) This lower prevalence means that this study has less power than the others.

Table 3 Prevalence a	and intensity of helmin	th infection at baselin	e	
	Prevalence (%)		Mean intensity (IQR) eggs/g	ı
	No geophagia at baseline (n = 916)	Geophagia at baseline (n = 54)	No geophagia at baseline (n = 916)	Geophagia at baseline (n = 54)
Ascaris lumbricoides	5.5	7.4	3951 (504–2880) (n = 50)	5346 (504–10 188) (n=4)
Trichuris trichiura	33.2	33.3	348 $(72-384)$ $(n=304)$	411 (72–600) ( <i>n</i> = 18)
Hookworm	32.3	42.6	1292 (120—960) (n = 296)	874 (144—1080) (n=23)

IQR: interquartile range

<sup>\*</sup> P < 0.05.

<sup>&</sup>lt;sup>a</sup> Intensity is calculated as eggs per gram of stool for infected women only. The untransformed values are listed for ease of interpretation.

	Trichuris (n=	s (n = 970)		Ascaris	Ascaris $(n = 970)$		Hookwo	Hookworm $(n = 970)$	
	ORa	95% CI	P-value	ORa	95% CI	P-value	ORa	95% CI	P-value
Geophagia during current pregnancy (none as referent)	1.03	0.567-1.850	0.919	1.45	0.492-4.283	0.499	1.74	0.965-3.154	0.066
Age (years)	0.98	0.956-0.996	0.031	0.97	0.933-1.018	0.248	0.98	0.963 - 1.004	0.106
Urban (rural as referent)	1.24	0.987 - 1.458	0.036	0.96	0.597 - 1.543	0.865	09.0	0.482 - 0.742	<0.001
No. of durable goods (0-4)	0.98	0.817-1.088	0.771	0.90	0.662-1.215	0.482	0.92	0.785 - 1.068	0.263
Pit toilet in household (none as referent)	0.49	0.320 - 0.684	<0.001	0.33	0.130-0.823	0.018	0.65	0.449 - 0.969	0.028
Formal education (none as referent)	0.65	0.492 - 0.906	0.007	0.62	0.330-1.168	0.140	0.61	0.450 - 0.838	0.002

The participants in the present study were all adult consumers of pica. In contrast, the previous two studies testing the parasitological profile of earth involved earth that children consumed. It is likely that children are less discriminate in their selection of pica substances than adults and thus in child-preferred samples there may be a more significant presence of infectious parasitic nematode stages, although the Luo children in Geissler's (2000) study avoided 'contaminated' earth. A further difference is that our technique for identification of nematodes was different to those previously used in other studies. We do not expect it to be less sensitive than previous methods used and it was sensitive enough to be able to detect nematodes in the rejected samples. Finally, the quantity of sample that we analysed for each substance (approximately 15g) is much greater than the amounts analysed by Geissler et al. (1998a) or Wong and Bundy (1990), but the number of samples analysed is much smaller, limiting our interpretation.

# 5. Conclusions

Based on this preliminary work, it seems unlikely that geophagia is a source of parasitic nematode infection in adults on Pemba Island, and that other pica substances are of low risk as sources of infection. Thus, the role of pica as a vector for parasitic nematode infection may need to be re-evaluated in the pica—anaemia debate, at least in adult populations on Pemba Island. Because this study does not confirm prior findings, further research, preferably in other sites, is warranted.

We would like the findings of this study to encourage other researchers to perform parasitological analysis on pica materials and to do so on all pica substances consumed by their subjects, not just earths. Information regarding the parasite content of pica substances is vital to the debate about the healthfulness of engaging in pica as well as elucidation of the relationship between parasites, pica and anaemia.

**Authors' contributions:** SLY conceived the substudy design, oversaw pica sample collection, collected ethnographic data and drafted the paper; DG determined sample preservation methods, examined pica samples, interpreted parasitology results and wrote the parasitology methods; THF designed and supervised the parasitological sample collection and analysis, and assisted with data analysis and drafting the paper; SMA assisted with substudy design, researcher training and pica sample collection; MRK was a primary investigator in the epidemiological portion of the study and contributed to the study design; SSK assisted with data analysis and interpretation of the data; RJS and JMT were primary investigators in the epidemiological portion of the study, assisted with interpretation of the data and critically revised the article. All authors read and approved the final manuscript. RJS is guarantor of the paper.

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