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Schistosoma haematobium infection and asymptomatic bacteriuria in young South African females

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ABSTRACT

Schistosoma haematobium eggs can induce lesions in the urinary and genital tract epithelia, as eggs pass through or get trapped in the tissue. Local inflammatory reactions induced by *S. haematobium* eggs might affect the ability of bacteria to establish mucosal super-infection foci.

S. haematobium infection and asymptomatic bacteriuria can both portray haematuria, proteinuria and leukocyturia. This shared set of proxy diagnostic markers could fuel routine misdiagnosis in *S. haematobium* endemic areas. Furthermore, *S. haematobium* infected individuals might be at a higher risk of contracting bacterial urinary tract infections, which could manifest either as symptomatic or asymptomatic bacteriuria.

The aim of the current study was to explore whether schistosomal lesions are susceptible to super-infection by bacteria measured as asymptomatic bacteriuria. *S. haematobium* infection was determined by microscopy of urine samples. Furthermore, urine samples were tested with dipslides for asymptomatic bacteriuria and with dipsticks for haematuria, proteinuria and leukocytes. We found no association between asymptomatic bacteriuria and *S. haematobium* infection in a sample of 1040 female primary and high school students from a schistosomiasis endemic area in KwaZulu-Natal, South Africa. Furthermore, it was demonstrated that asymptomatic bacteriuria is not a bias for use of micro-haematuria as a proxy diagnostic measure for *S. haematobium* infection in this population.

1. Introduction

Urogenital schistosomiasis caused by the blood-dwelling digenean trematode *Schistosoma haematobium* remains a poverty related disease burden for an estimated 112 million people living in Sub-Saharan Africa (Hotez and Kamath, 2009; WHO, 2013). The associated morbidity poses diverse challenges and a heavy impact on local public health services. *S. haematobium* associated morbidity range from subtle to severe (Vennervald and Dunne, 2004; Khalaf et al., 2012). It can typically present with anaemia, stunted growth, and cognitive impairment in children, as well as with organ specific manifestations such as genital and urinary tract lesions. In severe cases it may lead to kidney damage and bladder cancer (Gryseels et al., 2006). Furthermore, female genital schistosomiasis has been hypothesised to be associated with HIV-transmission through genital mucosal lesions (Kjetland et al., 2006).

Tissue reactions induced by *S. haematobium* eggs migrating through or getting trapped in urinary tract epithelial tissues frequently invoke symptoms and paraclinical signs such as dysuria, pollakisuria, proteinuria, and haematuria (Gryseels, 2012). Haematuria is generally accepted as a good diagnostic measure for urogenital schistosomiasis in children, where micro-haematuria correlates well with the number eggs shed in urine (King and Bertsch, 2013). However, haematuria, proteinuria, dysuria, and pollakisuria are also common denominators for symptomatic urinary tract infections (UTI) (Wang et al., 2013), making misdiagnosis possible. Asymptomatic bacteriuria and *S. haematobium* infection also share a set of overlapping paraclinical signs such as haematuria, proteinuria and leukocyturia commonly observed by urine dipstick measures as proxy diagnostic parameters (King and Bertsch, 2013; Deville et al., 2004).

As uro-genital schistosomiasis can result in granuloma formation and hence epithelial inflammation in the urinary and genital

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tracts, local conditions of importance to bacterial colonisation may have changed (Foxman, 2010). Especially the early inflammatory phase of *S. haematobium* egg induced immune reactions is poorly described in humans. In a mouse model Fu et al. (2012) showed a distinct Th2 cytokine driven cell profile rich in activated macrophages, mixed eosinophil and neutrophil infiltrates accompanied by IgE isotype switch associated with *S. haematobium* eggs (Fu et al., 2012). Furthermore, other aspects like increased urine voiding frequency, granuloma formation, urinary tract fibrosis and dysfunction also resembled human disease. Thus, schistosome eggs seem to be accompanied by an antibacterial cell arsenal as they reach the mucosal surfaces in the genitourinary tracts; potent phagocytes as macrophages, neutrophils and also eosinophils, which pack granule proteins with antibacterial properties like major basic protein and eosinophil cationic protein (Malik and Batra, 2012; Persson et al., 2001; Ramarokoto et al., 2014). In contrast a recent mouse model demonstrates *S. haematobium* egg mediated IL-4 induction associated with an UTI susceptible phenotype (Hsieh et al., 2014). Eggs penetrating the epithelial surface could also be creating entry points for bacteria before the local inflammatory response is sufficiently expanded and able to control the infection focus. Furthermore, calcification of trapped eggs in chronic infections can build up with repeated exposure to infection and result in alterations and obstructions in the urinary tract, which are known risk factors for UTI (Lee and Neild, 2007). These scenarios underline that comprehensive understanding of the local immune dynamics elicited by *S. haematobium* eggs and potential bacterial superinfection is yet far from achieved. Successful establishment of bacterial infection possibly depends on a range of factors including *S. haematobium* egg infection intensity, re-infection rate and host immune status (Mduluza et al., 2003).

The aim of the study was to explore, whether urinary tract schistosomiasis alters susceptibility to superinfection with bacteria in schistosome infected individuals. Furthermore, the study investigates whether asymptomatic bacteriuria constitute a bias, due to overlapping paraclinical signs, for use of urine dipsticks to detect micro-haematuria as a proxy diagnostic measure for *S. haematobium* infection in primary and high school students from an endemic area with a highly focal infection distribution in rural South Africa (Pillay et al., 2014).

2. Methods and materials

2.1. Study design, area and population

A cross-sectional study nested in a larger mass treatment dependent study in randomly selected schools was carried out from May to August 2012 in Ugu District, KwaZulu-Natal, South Africa. Schools, which had not yet undergone mass treatment for schistosomiasis, were included. Exclusion criteria were male gender, clinical illness assessed by local authority district nurses, and delivery of less than 20 ml urine. Fig. 1 illustrates the participant recruitment process. Furthermore, samples from menstruating young women were excluded. However, occasionally it was not possible to enquire about period status due to spectators.

The majority of the Ugu population is of Zulu ethnicity and more than 80% live in rural settings, many without potable water, basic sanitation and electricity (Khumalo et al., 2008). Many gorges and river systems traverse the poverty challenged rural inlands facilitating focal schistosomiasis epidemiology (Pillay et al., 2014). South African registered praziquantel is expensive and there is limited availability at local clinics, which render it unlikely, that but a few study participants could have received recent treatment prior to study enrolment.

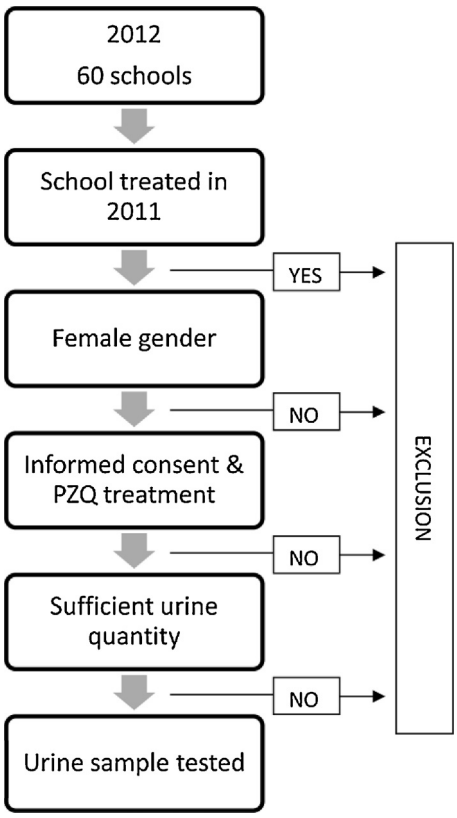

Fig. 1. Flow-chart showing sample selection.

2.2. Urine collection

Participants were invited to give a urine sample in 400 ml wide-mouthed screw capped plastic containers. Collection took place 10 am–2.30 pm. The urines were immediately put into cooler boxes with icepacks to prevent egg hatching and sun exposure.

2.3. Parasitology

Egg preservation for standard microscopy of *S. haematobium* infection egg intensity was done in the field. 10 ml swirled urine was poured directly into 15 ml tubes containing 1 ml 5% formalin solution (formaldehyde solution 38% w/v, Medicolab) and kept out of sunlight. At a later stage in the laboratory, the preserved urines were centrifuged (Beckman Coultier, Allegra X-22), the supernatant decantered and the entire residual was mounted onto glass slides for standard counting by light microscopy. One in ten urine samples was tested twice for quality control. Egg counts were done blinded to bacteriuria and dipstick results.

2.4. Dipstick parameters and bacteriuria

Urines were tested on site for haematuria ($\geq$10 rbc/μl), leukocytes ($\geq$20–25 wbc/μl) and proteinuria ($\geq$10–15 mg/dl) with dipsticks (Neotest 4, Occidem Biotech) as recommended by the manufacturer. Each urine sample was swirled before testing. Immediately after dipstick testing, Uricult dipslides (Orion Diagnostica) were used to sample for presence of significant, per definition, asymptomatic bacteriuria ($\geq 10^5$ CFU/ml) according to the manufacturer's recommendations. If doubt arose as to whether a slide was negative, dipslides were incubated for an additional 24 h and re-read. Polymicrobial growth was considered as contamination. All dipstick and dipslide readings were carried out by the same investigator.