Enteric pathogens of food sellers in rural Gambia with incidental finding of Myxobolus species (Protozoa: Myxozoa)

Richard S. Bradburya,*, Barbara Barbéb, Jan Jacobsb,c, Amadou T. Jallowa, Karamo C. Camaraa, Musa Colleya, Rita Wegmüllera, Babucarr Jasseyd, Yorro Chamd, Ignatius Baldehd and Andrew Prenticea,e

aMRC Keneba, MRC The Gambia, Keneba, The Gambia; bDepartment of Clinical Sciences, Institserovarute of Tropical Medicine, Antwerp, Belgium; cKU Leuven, Department of Microbiology and Immunology, University of Leuven, Belgium; dMinistry of Health & Social Welfare, Banjul, The Gambia; eMRC International Nutrition Group, London School of Hygiene & Tropical Medicine, London, UK

*Corresponding author: Present address: School of Medical and Applied Sciences, Central Queensland University, Rockhampton, QLD, Australia. Tel: +61 7 4930 9000; E-mail: r.bradbury@cqu.edu.au

Received 1 December 2014; revised 15 February 2015; accepted 16 February 2015

Background: Ongoing surveillance of enteric pathogens of public health significance among casual food sellers is undertaken in many resource-limited countries. We report the results of a survey in Kiang West province, The Gambia, and provide an exemplar methodology for such surveys in resource-limited laboratories.

Methods: Unpreserved, unrefrigerated stool samples were subjected to Salmonella, Shigella and agar plate culture for rhabditoid nematodes. Direct microscopy, formalin-ethyl acetate concentration and iron-hematoxylin staining was performed later, following preservation.

Results: Of 128 specimens received, no Shigella spp. was recovered, while four serovars of non-typhoidal Salmonella enterica, including Chandans, were isolated. Pathogenic parasitic infections were Necator americanus 10/128 (7.8%), Strongyloides stercoralis 3/128 (2.8%), Blastocystis species 45/128 (35.1%), Entamoeba histolytica complex 19/128 (14.8%) and Giardia intestinalis 4/128 (3.1%). A single case each of Hymenolepis diminuta and S. mansoni infection were detected. In one participant, myxozoan spores identical to those of Myxobolus species were found.

Conclusions: Rare parasitoses and serovars of Salmonella enterica may occur relatively commonly in rural Africa. This paper describes intestinal pathogens found in a cohort of food sellers in such a setting. Furthermore, it describes two parasites rarely recovered from humans and demonstrates the need for methods other than microscopy to detect S. stercoralis infections.

Keywords: Africa, Food sellers, Gambia, Myxozoa, Parasitology, Salmonella

Introduction

Diarrhea is the most common cause of death for children under 5 years of age in developing countries and remains a significant problem in The Gambia. Furthermore, typhoidal and non-typhoidal strains of Salmonella enterica are the primary cause of bacteremia in sub-Saharan Africa. Therefore, in developing countries, interventions to control the spread of pathogenic bacteria and parasites, particularly those that are orally transmitted, remain a paramount public health activity. Laboratory surveys of potential sources of such infections, such as food sellers, form an important part of public health control measures for these pathogens.

Most ready-made food purchased for human consumption in Kiang West province, Lower River Region of The Gambia, West Africa is prepared by casual food sellers. Food sellers typically congregate outside schools, and workplaces such as the Karantaba government clinic and MRC Keneba research station. The group also includes shop keepers in villages who prepare food for sale. Such casual food sellers represent a potential source of infection with enteric pathogens for many people within their province, and for this reason the Gambian Ministry of Health and Social Welfare mandates ongoing surveillance of pathogens of public health significance amongst casual food sellers. Food sellers must submit a single stool for analysis and be cleared of any food-transmissible enteric pathogens in order to be issued permits to sell food.

In the context of public health, and in the absence of availability of molecular or antigen detection methods, protozoa such as Entamoeba histolytica and Giardia intestinalis (syn. G. duodenalis, G. lamblia) are detected by microscopic techniques. The methodology employed is an important consideration when undertaking...
such surveys. Often fecal parasite surveys rely on the WHO approved Kato-Katz method. This method is highly effective for the detection of most helminths, but does not allow for the detection and identification of protozoa. In this context, a combination of the formol ethyl acetate concentration method combined with a permanent stain is superior to Kato-Katz to detect all parasites of public health relevance.

Microscopy is ineffective for the detection of Strongyloides stercoralis infection, a helminth with significant long-term individual and public health implications. In settings where serology is unavailable, such as this, addition of a culture method for rhabditoid nematodes also allows speciation of hookworms, the identification of other helminths with hookworm-like eggs, and differentiation of S. stercoralis from other Strongyloides species infecting humans in cases where eggs of the latter have hatched and only rhabditiform larvae are present in stool by the morphology of the free-living adult female worm. However, these further identification activities require unpreserved, unrefrigerated stool, and any delay in preservation may have a significant negative impact on detection of trophozoites of protozoa by microscopy. In settings where molecular methods for parasite confirmation are not available, the microscopic skills and expertise of the parasitologist become paramount for accurate species identification and detection of rare parasites (those rarely described in the literature as causing human infections). Such competences are however fading in resource-rich nations due to the advent of advanced molecular diagnostic tools and the relatively low prevalence of parasitic infections. Those wishing to undertake parasitology in a resource-limited setting are encouraged to refer to textbooks and journal articles where morphological techniques are described and discussed. By employing these methods in a parasite survey and combined with education of laboratory staff, a more comprehensive picture of enteric disease and the rates of so-called rare parasitoses (some of which are quite common in specific areas of the developing world) will be achieved.

In 2012–2013, the MRC Keneba laboratory assisted in the enteric survey of casual food sellers for potential food-borne pathogens. The results of this survey are presented here. We also report our methodology and processes for identification of enteric pathogens by microscopic methods and culture, and report several cases of infection with rare parasites.

**Materials and methods**

**Sample collection**

Field workers from the MRC and the Department of Health and Social Welfare actively recruited each adult casual food seller in the district to submit a stool sample. Between the months of January and March 2012 (dry season), unpreserved stool samples were collected in the morning by field workers and transported at ambient temperature to the MRC Keneba laboratory within 5 hours, for analysis. Analysis at MRC Keneba was performed free of charge as a donation to the Gambian community.

**Detection of bacterial pathogens**

Upon receipt, stools were cultured directly on desoxycholate and MacConkey agar (Oxoid, Basingstoke, UK) for detection of Salmonella and Shigella. One gram of stool was cultured in 10 ml of Selenite broth (Oxoid, Basingstoke, UK) overnight, then sub-cultured onto MacConkey agar for further recovery of Salmonella. All incubations were done at 37°C for 18–24 hours. Urease negative, non-lactose fermenting isolates were identified using the API 20E system (bioMérieux, Marcy-l’Etoile, France). Salmonella serotyping was performed by the Institute of Tropical Medicine, Antwerp, Belgium.

**Detection of enteric parasites**

Direct microscopy in saline wet preparation was performed on all samples at receipt. Samples were then preserved in sodium-acetate formalin for further parasite detection and identification. Parasite concentration using the formalin-ethyl acetate concentration method was carried out by washing the SAF preserved specimens in normal saline, preparing a slide for permanent stain from the deposit and then correcting the volume of deposit to 1 ml prior to resuspension in 9 ml of 10% formalin (Sigma-Aldrich, St. Louis, MO, USA). The formalin was allowed to infuse for 30 minutes, followed by the addition of three drops of triton X-100 (Sigma-Aldrich, St. Louis, MO, USA). This solution was shaken vigorously, followed by the addition of 3 ml of ethyl acetate (Sigma-Aldrich, St. Louis, MO, USA), and then centrifuged at 500 g for 10 minutes. One saline and one lugol’s iodine preparation of the concentrated deposit was then reviewed for the presence of cysts, eggs and larvae. Smears for permanent staining were prepared by mixing one drop of the faeces/saline deposit sediment with a half drop of Mayer’s albumin (Meridian Bioscience, Cincinnati, OH, USA) on a glass microscope slide. These slides were then allowed to air dry. Prepared smears were stained using the iron haematoxylin method, as previously described, but without the addition of a carbol fuchsin step. Faecal concentration and permanent stain analysis was undertaken on all specimens in this study.

**Culture for Rhabditoid nematodes**

Koga Strongyloides agar was prepared on site using the traditional formula, as previously described. Culture of approximately 1 g of unpreserved, unrefrigerated faeces at 30°C was performed upon receipt of stool samples. Agar plates were checked for larval track daily, up to 7 days. Where larval tracks were seen, direct microscopy for larvae was performed using a dissection microscope, then the plate was flooded with sterile water for 5 minutes. A 7 ml aliquot of this water was decanted into a 15 ml conical tube and 7 ml of 100% ethanol added to kill the filariform larvae and free-living adult S. stercoralis. This solution was then centrifuged at 500 × g for 10 minutes, followed by identification of larval species by standard light microscopy.

**Follow-up of positive results**

All food sellers found to harbor enteric bacteria or parasites pathogenic to themselves or others were treated at the MRC Keneba clinic and re-screened within 2 weeks to confirm clearance of the pathogen prior to a food seller permit being issued. Due to ethical constraints, it was not possible to interview participants harboring rare parasitoses to gain a more comprehensive history and clinical picture.
Results

A total of 128 samples (118 female, 10 male) were received from all villages in Kiang West, 88 samples were from Keneba village, with the remaining samples from the province being relatively equally distributed in proportion to village population. Twenty-four out of 128 (18.7%) study participants harbored no enteric parasites, two participants did not harbor parasites but were carriers of *Salmonella enterica*; four serovars was observed. These serovars were Offa (group S), Chester (group B), Chandans (group F) and Worthington (group G). No *Shigella* spp. was recovered from any participants.

In only one case were hookworm eggs seen in the direct wet preparation, the remainder (10/128; 7.8%) were detected only in the formalin-ethyl acetate concentrate. Rhabditoid nematodes were positive in 13/128 (10.1%) samples, with 3/128 (2.3%) identified as *Necator americanus*, based upon the morphology of the filariform larvae and adult free-living females in the case of *Strongyloides*. All culture-positive cases of hookworm showed eggs in the fecal concentrate, but none of the *S. stercoralis* infections were detected by microscopy-based methods. A single case of *Hymenolepis diminuta* was detected. In addition, a single case of the cestode *Hymenolepis diminuta* (Figure 1) was detected.

A total of 102/128 (79.6%) of food sellers tested had one or more intestinal parasites detected in their stool sample. A single parasite was identified in 36/128 (28.1%) participants; 38/128 (29.6%) harbored two parasites; 20/128 (15.6%) had three parasites; 4/128 (3.1%) showed four parasites, while two each had five or six parasites detected in a single stool.

Sixty out of 128 (46.8%) samples contained pathogenic protozoa, with 45/128 (35.1%) having *Blastocystis* species, 19/128 (14.8%) harboring *Entamoeba histolytica* complex and 4/128 (3.1%) with *Giardia intestinalis*. *Dientamoeba fragilis* was detected in 2 out of 128 samples (1.5%). Spores of a myxozoan were detected in one participant, a woman co-infected with *Giardia intestinalis* and *Blastocystis* species. These spores were morphologically identical to a *Myxobolus* species (protozoa: Myxozoa; Figure 2). Five cases of *Pentatrichomonas hominis* were detected in saline wet preparation, but only one of these remained visible in permanent stain. Five cysts belonging to the *Entamoeba* genus could not be identified to species level due to shrinkage on permanent stain and difficult morphology in wet preparation.

Follow-up stool samples, taken at 1 week following treatment at MRC Keneba clinic of participants found to be colonized with bacteria or parasites pathogenic to themselves or that might represent a public health risk to their customers, were universally negative.

Discussion

This survey did not intend to provide a representative sample of the population in Kiang West, as it was carried out on adult food sellers. The female predominance in the study reflects the traditional gender roles of casual food sellers. A bias towards Keneba village existed as the MRC field station in this village has led to it becoming the economic center of the district. Colonization with *Salmonella* is uncommon in West Kiang food sellers, though a risk does exist. Pathogenic protozoa (*Giardia intestinalis*, *Entamoeba histolytica* complex and *Blastocystis* species) pose the highest risk for customers of unscreened casual food sellers. Helminths were present, but due to their life cycle these pose a limited risk to customers.

Detection of bacterial pathogens

Four serovars of *Salmonella* were identified in Kiang West food sellers: Offa (group S), Chester (group B), Chandans (group F) and Worthington (group G). It is known that non-typhoidal *Salmonella* (NTS) are an important cause of invasive bacterial disease in African adults and children, and are associated with a case fatality rate of 20–25%. In rural Gambia, NTS was found to be the second most
important blood isolate (27.9%) after *Streptococcus pneumoniae* in children with invasive bacterial disease,\(^1\) while in an urban setting in The Gambia, NTS accounted for 8.6% of blood isolates from patients with suspected bacteremia.\(^1\) Amongst Gambian children with severe acute malnutrition, NTS was the major cause of bacteremia (13%).\(^1\) Besides *Salmonella* Enteritidis and *Salmonella* Typhimurium that are the most commonly isolated NTS from humans in Africa,\(^1\) other NTS serotypes have also been isolated, from human as well as from animal sources. *Salmonella* Offa has been previously isolated in The Gambia from domestic poultry,\(^1\) and from a child with invasive bacterial infection.\(^1\) In addition, this serovar has been isolated from captive wild animals in Nigeria\(^6\) and from sesame seed products.\(^7\) *Salmonella* Chester has been associated with frozen meals after a multistate outbreak in the US (CDC, 2010) and in Canada in 2010.\(^8\) This serovar had also been isolated from animal feedings in Namibia\(^9\) and from animals in South Africa.\(^10\) *Salmonella* Worthington was the most commonly isolated NTS in a prevalence study among human and animal species in India between 2001 and 2005,\(^11\) and has been associated with neonatal (meningitis) outbreaks and childhood diarrhea in India,\(^12\) and infection in elderly people due to contaminated milk powder in France in 2005.\(^13\) In addition, this serovar has also been isolated from turkey production facilities and poultry products.\(^14\)

*Salmonella* Chandans is a rare serovar, it was first isolated from a lizard\(^1\) and shortly afterwards described as a cause of septicaemia.\(^1\) It then disappeared from the peer-reviewed literature for almost 50 years before being isolated in beef cattle in Northern Ireland in 2007.\(^15\) Four years later, the serovar was isolated from a healthy control child out of 495 sampled in The Gambia.\(^1\) This second isolation from a human in The Gambia raises the question of whether this serovar may be relatively more common in that country or region than other areas around the globe.

Detection and identification of rare parasitoses

A single case of *Hymenolepis diminuta* infection was detected in this survey. *H. diminuta* is a cestode of rats that can occasionally present as a zoonosis in humans following ingestion of the insect intermediate host (beetles, fleas and moths).\(^1\) Eggs resemble those of *H. nana*, but are larger (average size 58 to 86 μm, compared to 37–42 μm), lack the polar filaments seen in *H. nana* eggs and have a thicker outer membrane compared to the latter.\(^16\)

The finding of passage of the spores of a myxozoan, a parasite of fresh fish, in a human was unexpected. As PCR confirmation of species was not possible, the spores observed were identified based upon morphology alone. Each spore was pyriform in shape and smooth, showing bilateral symmetry. Two pyriform polar capsules each side of the central axis of the thinner end of the spore were observed. Sporoplasm occupied the remaining part of spores, and they did not take up iodine stain, a slight separation occurred at the apex of the spore. Based upon this morphology and size (8–9 μm×11–12 μm), they were identified as probably being of the genus *Myxobolus* (*Myxozomatidae: Myxozoa*). The morphological appearance of this isolate compared exactly to that described by Boreham and colleagues in 1998 of a *Myxobolus* species being passed in human stool in Queensland, Australia.\(^17\) Other anecdotal observations of transient passage or true human infection with myxozoan parasites have been described throughout the literature and this genus has been implicated as a true parasite of immune-compromised humans.\(^18\)

Incidentally, toxins from another myxozoan, *Kudoa septempunctata* have recently been identified as the cause of food poisoning outbreaks in Japan, but in the absence of intestinal colonization.\(^19\) Follow-up testing did not show any further spores, and it is likely that this case represented transient passage of spores rather than true intestinal colonization.

Study limitations

As to the sampling, a major limitation was that only single fecal sample was analyzed for each participant. It has been established that up to six negative faecal cultures may be required to rule out asymptomatic carriage of *Salmonella* in food sellers.\(^20\) Similarly, multiple samples greatly improve the detection rate of enteric parasites.\(^21\) The Gambian health authorities only require a single stool examination to issue a food-seller’s permit. This, combined

Detection of enteric parasites

The relative absence of *S. mansoni* infection is a recognized phenomenon in the Lower River Region, attributable to the salinity of The Gambia river and its tributaries east to Farafenni during the dry season (November–June).\(^1\) This salinity precludes growth of the intermediate host water snail (*Biomphalaria*) species in these waterways. It is therefore likely that the present case represented an infection acquired outside of the Kiang West region.

The absence of the soil-transmitted helminths (STH) *Trichuris trichiura*, *Ancylostoma* species and *Ascaris lumbricoides* in this group was notable. Several factors may have contributed to this finding. This was as survey of adult food sellers and adults have a lower incidence of non-hookworm STH compared to children.\(^22\) Necator americanus is the most prevalent of the human hookworms in Africa, and the relative resistance of *N. americanus* to dry and dusty environments such as Kiang West would provide a selective advantage for this species of hookworm over the *Ancylostoma* species. However, *T. trichiura* and *A. lumbricoides* are neither commonly seen in the routine clinical samples referred to the MRC Kenema clinic laboratory for parasite investigations. The only previously published systematic parasitic survey in this region, undertaken 50 years ago, found in 69.5% of the adult population (n=4,42) hookworm infections, as well as 4.3% *Ascaris lumbricoides* infection (n=27).\(^23\) The cause of this noticeable drop in hookworm infection rates and the absence of *Ascaris* infection are likely multifactorial; greatly improved education and improved sanitation, plus far superior access to healthcare are supposed to be the major factors. Another may be the widespread use of single dose mebendazole only as mass drug administration for helminth infections. This drug is effective against *A. lumbricoides* and *T. trichiura*, but has limited efficacy against *N. americanus*.\(^24\)

The significance of the detection of members of the *E. histolytica* complex in this study is difficult to interpret. The pathogenic *Entamoeba histolytica* is one species in a morphologically identical *E. histolytica* species complex. This complex of species also contains *E. dispar*, *E. moshkovskii* and *E. Bangladeshi*.\(^25\) *E. histolytica* is the primary pathogen in this group, though *E. moshkovskii* has been described as causing diarrhea in children.\(^26\) *E. dispar* is considered to be a non-pathogen, although a single case of confirmed *E. dispar* liver abscess has been reported from Brazil.\(^27\) Without an *E. histolytica* species-specific ELISA or PCR testing, the implications of 15% carriage of *E. histolytica* complex are difficult to determine.
with the logistical, resource and transport issues inherent with operating such a survey in an isolated region of West Africa, collection of multiple stool samples was not feasible. Furthermore, while samples were preserved within 2 hours of arrival in the laboratory, in some cases, several hours may have elapsed between collection and preservation. This average lag time was not recorded, but as samples were preserved on the morning of collection, it would not have exceeded 18 hours for any sample and for the majority of samples, would have been less than 12 hours. Such time delays prior to preservation may result in specific protozoa (D. fragilis, P. hominis and others) not being detected due to their rapid morphological deterioration outside of the host.6

As to the techniques used, urease as a screen for non-lactose fermenting Enterobacteriaceae suspected of being Salmonella or Shigella species is less selective than addition of a triple-sugar iron agar (TSIA) slope, but it allowed differentiation without this additional agar and simplified the procedure for laboratory staff. The use of TSIA is however recommended and was not performed in this case due to supply limitations. Direct microscopy was found to be useful in this case for the detection of P. hominis due to its characteristic motility and often poor staining in permanent stains,7 although this organism is considered a non-pathogen and thus for clinical purposes initial screening by a concentration method combined with permanent stain would suffice. Formalin-ethyl acetate concentration was chosen over the Kato-Katz as the initial screening method due to its improved recovery of protozoan cysts and trophozoites and resource constraints precluding the use of both methods. In a clinical context, presence or absence of parasitic infection is perhaps more relevant than egg count, as any load of infection warrants treatment, but this represents a weakness in the study from an epidemiological perspective. A further limitation of the study was the absence of a modified acid fast stain for coccidia, either alone or incorporated into the iron-hematoxylin stain. This precluded the accurate detection of Cryptosporidium spp. and Cyclospora cayetanensis.

Conclusions

The present survey provided the opportunity to gauge the level of colonization with enteric parasites and bacteria of adult food sellers from throughout the Kiang West area. Several rarely reported human parasites were detected in this group. This study also emphasizes the need for rhabditoid nematode culture to detect S. stercoralis infections and identify species of other rhabditoid nematodes infecting humans.

Author’s contributions: RSB conceived the study; RSB, AP, ATJ, KCC, MC, VC, BJ and IB designed the study protocol; RSB, KCC and MC carried out the wet preparation analysis; RSB and ATJ carried out Salmonella, Shigella and rhabditoid nematode culture and the formalin-ethyl acetate concentrate concentrate analysis; RSB undertook the iron haematoxylin stain analysis and the identification of rare parasites; BB and JJ undertook the Salmonella serotyping; RSB, JJ and BB undertook the analysis and interpretation of these data; RSB, BB and JJ drafted the manuscript; RSB, BB, JJ, IB, RW and AP critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. RB is the guarantor of the paper.

Acknowledgements: The author’s would like to acknowledge the Scientific Institute of Public Health, Brussels, Belgium and the Pasteur Institute, Paris, France for their assistance in confirmation of the Salmonella serotyping.

Funding: This work was supported by joint funding from the UK Medical Research Council [MRC, grant number MC-A760-5QX00] and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement.

Competing interests: None declared.

Ethical approval: This study was approved and undertaken in collaboration with the Ministry of Health and Social Welfare of The Gambia. Ethical approval was granted by the Gambia Government/MRC Joint Ethics Committee [L2013.51].

References
