AFRICAN JOURNAL OF MEDICINE
and medical sciences

VOLUME 15, NUMBERS 3/4, SEPTEMBER/DECEMBER 1986

EDITORS: T.A. JUNAID
O. BADEMOSI and D.D.O. OYEBOLA

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Palo Alto Melbourne

ISSN 0309-3913

Afr. J. Med. med. Sci. (1986) **15**, 85–92.

Ascaris lumbricoides as a vehicle of bacterial infections

S. O. ADEDEJI* AND E. O. OGUNBA
Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria

Summary

Investigations were conducted on microflora on the body surface and internal organs of adult *Ascaris lumbricoides*. The investigations involved examination of wet faecal preparations of over 100 pupils using light microscope. The body surface of the adult *Ascaris* worms was cultured on selective media. Adult worms were dissected and different parts of the worms' gut were cultured for isolation of micro-organisms. The results of the bacteriological examination of the body surface of *A. lumbricoides* yielded many genera of bacterial organisms. The results from internal organs of dissected adult *Ascaris* worm yielded varying percentages of organisms similar to those found on the surface of the worm. The possibility of linking *Ascaris* infection with this aetiology of pyrexia of unknown origins as commonly seen in tropical regions was discussed.

Résumé

Des investigations ont été conduites sur les microbes résidents sur la surface du corps et les organes internes de l'adulte *Ascaris lumbricoides*. Des investigations incluent un examen des échantillons fécaux à l'état frais non coloré au microscope. Plus de cent élèves ont été ainsi examinés. Des cultures de la surface de l'*Ascaris* ont été disséqué et plusieurs sections de l'intestine ensemencé pour l'isolement des microorganismes. La culture des surfaces a révelé plusieurs genres de bactérie tandis que les organes internes ont révelé de variables pourcentages des organismes similaires aux bactéries de la surface. La possibilité de faire un 'cause-effet' de cette étiologie dans la fièvre d'origine indéterminée comme le voyant dans les régions tropicales a été discutée.

*To whom correspondence should be addressed.

Introduction

Organisms including many bacteria normally resident in the human bowel show their appearance over the course of a few days after delivery of a baby. Most of the bacteria which are normal flora of the bowel are non-pathogenic in their normal habitat and are thus regarded as commensals.

Micro-organisms alone do not have the exclusive ability to colonize the human body, but larger organisms, including members of the group Helminthes and Arthropoda as well, commonly invade and colonize various tissues of man. The nematodes are an especially important group because many of their members have successfully invaded and established themselves in almost all the tissues of man.

Although both the micro-organisms and the nematodes are individual entities and they are capable of independent existence, they all inhabit a common environment of the gut and are subjected at times to similar circumstances. Some of them may have entered into relationships that are mutually beneficial, others may be antagonistic and still others may just have an accidental association and act as transport agents. For instance, Markell and Kuritsubo (1967) recorded that the trophozoites of *Entamoeba coli* do ingest *Giardia intestinalis* and thereby assist in keeping down their population in the intestinal lumen. Hutchinson (1965) established a relationship between Neoascaris egg and Toxoplasma transmission in which Toxoplasma organisms were incorporated into the egg of Neoascaris worm inhabiting the intestinal lumen of infected individuals. The excited *Ascaris lumbricoides* has often been incriminated as being a passive transport

agent of contamination of different tissues of the body with pathogenic bacteria from the intestinal tract.

In this study, efforts have been made to find out the extent of invasion of the different tissues of intestinal nematodes by micro-organisms inhabiting the human gut with a view to determining the relationship between these micro-organisms and the nematodes.

Materials and methods

Six hundred and twenty school children of ages between 8 years and 14 years from Ibadan Municipal Council School, Oje, Ibadan, Nigeria were examined. The sanitary condition of the school was very poor and provided an excellent environment for the development of nematode eggs and larvae. There was no pipe-borne water in the school but there were three pit latrines.

The school was visited ten different times between the months of September 1980 and January 1981. Stool cartons were distributed to the students with specific instructions as to their use in order to avoid contamination of their contents. All the stool specimens were collected at 08.30 h on each collection day from the school and were brought to the laboratory and examined fresh both macroscopically and microscopically and with no preservative added. Saline preparations of the fresh stool samples were examined with light microscope and later the specimens were examined by the modified zinc sulphate concentration method. Children that harboured *A. lumbricoides* worms were dewormed with a broad-based anthelmintic drug, Antepar (piperazine citrate). A single dose of 120 mg per kg body weight to a maximum total dose of 4 g Piperazine citrate was found effective for expelling the worms. In 5 h, the worms were easily dislodged from their positions by movement to the gut and were expelled in the faeces.

The adult worms found in the stool specimens were washed several times in sterile distilled water until clean. With the aid of a pair of sterile forceps, each worm was rubbed onto selective agar plates and later rinsed in nutrient broth medium and Robertson's cooked meat medium to ensure the recovery of all bacteria adhering into the surface of the worm. The adult worm was killed by dipping it in hot water, which also helps to sterilize the body surface of the worm as was done by Przjalkowski (1961). Both the male and female adult *A. lumbricoides* were dissected. Using a sterile Pasteur pipette with rubber teat, the peritoneal fluid from the worm was taken and cultured onto selective solid media and also into selective liquid media; the abdominal fluid was also taken and cultured accordingly. The intestine was removed and washed in sterile distilled water to remove adhering fluid and it was then cut into the fore-gut, the mid-gut and the hind-gut. Each part was then opened and the contents cultured on and in selective media accordingly. The ovary, the uterus and the oviduct, and the vagina were extracted separately and cultured separately on various selective media and then incubated for 24–72 h both aerobically and anaerobically under carbon dioxide.

The male worm was also opened and different parts of the body including the peritoneal fluid, abdominal fluid, the fore-gut, the mid-gut, the hind-gut, the testes the vas deferens, and seminal vesicle were cultured after extraction, using selective media.

The bacteria in the contents of intestine and other parts of the worms were identified. Studies of the anaerobic flora were conducted separately and both the worm's body surface and the contents of intestine and internal organs were examined. The Gaspak method of anaerobiosis using fresh blood agar plates was used. After incubation of cultures at 37°C overnight culture plates were examined both macroscopically to study the colonial appearance and microscopically to study the morphology of the bacteria. The spore staining method was done on all characteristic colonies on anaerobic culture plates. Biochemical and carbohydrate fermentations were carried out. The colonies on MacConkey agar medium that resembled enterococci were further tested using the aesculin bile test and the heat-resistant test. The staphylococcal pathogenicity test was determined by using fresh human citrated plasma for both the slide and the tube techniques.

Results

Stool examination

Out of a total of 140 stool specimens examined, seventy specimens were collected from boys

and the remaining seventy from girls. Table 1 gives the results of the microscopic examination. The infection rate for each nematode recorded was higher in boys than in girls. Some of the pupils had polyparasitism with nematode infections.

Table 2 shows that only adult worms of *A. lumbricoides* and hookworm were recorded. There were no adult *Trichuris trichiura* recorded.

Table 3 shows that adult *A. lumbricoides*, both male and female, carry on their body surface different genera of bacteria. But lactobacilli and aerobic spore-bearers were not isolated on the body surface. The summary of the dissection results is shown in Tables 4 and 5. There was no anaerobic organism isolated in all the specimens. The microscopic preparations from the materials stained for the presence of spores to demonstrate anaerobic spore-forming bacilli were also negative.

Discussion

Intestinal nematode infection was common amongst the school children examined, and the higher incidence occurred with *A. lumbricoides* followed by *T. trichiura* and then hookworm (Table 1). Polyparasitism was also commonly recorded and this confirms earlier reports from Ibadan about the frequency of occurrence of the three: *A. lumbricoides*, *T. trichiura* and hookworm (Cowper & Woodward, 1961; Ogunba, 1974). Infection through the oral route is also important with *A. lumbricoides* occurring in over 80% of both the male and female children (Table 1). Although the environment favours development of hookworm eggs to larval stages, relatively fewer children were infected with hookworm.

Table 2 shows that *T. trichiura* is not easily dislodged by piperazine citrate. The drug was chosen because of its safety in use. However, it proved fairly effective in dislodging adult worms of *A. lumbricoides* and hookworm from the intestinal lumen.

The recovery of many bacteria species with *E. coli* having the highest incidence from the cuticle of adult *Ascaris* is significant. Both the lactobacilli and the aerobic spore-bearers were however, not isolated from the body surface although these were isolated from the gut, and *Lactobacillus* spp. were recovered from other tissues of adult *Ascaris* worm (Tables 4 and 5). The non-recovery of lactobacilli and aerobic

Table 1. Microscopic examinations of stool specimens

	Sex of pupils	
	Male	Female
Number of pupils examined	70	70
Number of pupils with *Ascaris* eggs	65 (92.86%)	60 (85.71%)
Number of pupils with *Trichuris* eggs	31 (42.28%)	22 (31.42%)
Number of pupils with hookworm eggs	19 (27.14%)	15 (21.42%)

Table 2. Macroscopic examination of stool specimens after anthelminthic drug administration of pupils with nematode eggs

Number of children with *Ascaris* eggs dewormed	125
Number of children with adult *Ascaris*	84
Number of children with hookworm dewormed	34
Number of children with adult hookworm	21
Number of children with *Trichuris* eggs	53
Number of children with adult *Trichuris*	Nil

Table 3. Results of bacteriological examination of the body surface of adult *Ascaris lumbricoides*

	Number (and percentage) of positive results							
	Number of adult worm examined	Specimen positive for *E. coli*	Specimen positive for *Enterococci*	Specimen positive for *Staphylococci*	Specimen positive for *Proteus*	Specimen positive for *Pseudomonas*	Specimen positive for *Lactobacilli*	Specimen positive for aerobic spore-bearer
Male *Ascaris lumbricoides*	20	20 (100)	8 (40)	4 (20)	9 (45)	7 (35)	— (—)	— (—)
Female *Ascaris lumbricoides*	20	20 (100)	5 (25)	5 (25)	11 (55)	6 (30)	— (—)	— (—)

Table 4. Bacteriological examination of both the gut and internal organs of male ascarids

Type of specimen examined	Number of specimens examined	Number (and percentage) of positive results						
		Specimen positive for *E. coli*	Specimen positive for *Enterococci*	Specimen positive for *Staphylococci*	Specimen positive for *Proteus*	Specimen positive for *Pseudomonas*	Specimen positive for *Lactobacilli*	Specimen positive for aerobic spore-bearer
Peritoneal fluid	10	8 (80)	5 (50)	3 (30)	3 (30)	2 (20)	2 (20)	1 (10)
Abdominal fluid	10	6 (60)	4 (40)	3 (30)	5 (50)	2 (20)	1 (10)	3 (30)
Fore-gut	10	10 (100)	5 (50)	4 (40)	5 (50)	3 (30)	2 (20)	1 (10)
Mid-gut	10	10 (100)	5 (50)	4 (40)	5 (50)	3 (30)	2 (20)	1 (10)
Hind-gut	10	10 (100)	5 (50)	4 (40)	5 (50)	3 (30)	2 (20)	1 (10)
Testes	10	7 (70)	4 (40)	5 (50)	1 (10)	— (—)	1 (10)	— (—)
Vas deferens	10	7 (70)	4 (40)	5 (50)	1 (10)	— (—)	1 (10)	— (—)
Seminal Vesicle	10	6 (60)	2 (20)	3 (30)	2 (20)	1 (10)	1 (10)	— (—)

Table 5. Bacteriological examination of both the gut and internal organs of female ascarids

Type of specimen examined	Number of specimens examined	Number (and percentage) of positive results						
		Specimen positive for *E. coli*	Specimen positive for *Enterococci*	Specimen positive for *Staphylococci*	Specimen positive for *Proteus*	Specimen positive for *Pseudomonas*	Specimen positive for *Lactobacilli*	Specimen positive for aerobic spore-bearer
Peritoneal fluid	10	9 (90)	3 (30)	3 (30)	3 (30)	1 (10)	1 (10)	— (—)
Abdominal fluid	10	9 (90)	3 (30)	3 (30)	3 (30)	2 (20)	— (—)	— (—)
Fore-gut	10	10 (100)	3 (30)	3 (30)	3 (30)	2 (20)	3 (30)	1 (10)
Mid-gut	10	10 (100)	3 (30)	3 (30)	3 (30)	2 (20)	3 (30)	1 (10)
Hind-gut	10	10 (100)	3 (30)	3 (30)	3 (30)	2 (20)	3 (30)	1 (10)
Ovary	10	5 (50)	1 (10)	1 (10)	4 (40)	1 (10)	1 (10)	— (—)
Oviduct	10	5 (50)	1 (10)	1 (10)	4 (40)	1 (10)	1 (10)	— (—)
Uterus	10	6 (60)	2 (20)	3 (30)	2 (20)	1 (10)	1 (10)	1 (10)
Vagina	10	5 (50)	2 (20)	3 (30)	2 (20)	1 (10)	1 (10)	— (—)

spore-bearers from the cuticle of *Ascaris* worms may be due to the initial treatment of the worms with hot water which probably made then non-viable.

Both Tables 4 and 5 show that almost all the internal organs of adult *Ascaris* worms harboured various types of bacterial organisms. However, significant amongst these are the reproductive organs namely; the testes, the ovaries, seminal vesicles, uterus and vagina. With so many organisms being isolated from the reproductive organs, one wonders how many of these bacteria would be incorporated in the *Ascaris* eggs during their formation and thus get transmitted trans-ovarially to the next host. If indeed this happens, then the *Ascaris* larva in its migratory phase within the human tissues would equally be an agent of bacterial dissemination into the tissues of its host.

The high association of *E. coli* and adult worms of *Ascaris* is highly significant especially because of the great activity of adult *Ascaris* worms within the intestinal lumen and tissues of man. Since the adult worm of *Ascaris* often invades the tissues from the intestinal lumen, especially when it gets excited as a result of the physiological changes in the intestinal lumen during wrong application of drugs, such excited *Ascaris* worms would most probably carry with them loads of other bacteria, including *E. coli*, from the gut lumen to the invaded tissues and start off bacterial infection in these unusual foci. In this manner, the unsuspecting *Ascaris* worm would act as an agent of transmission of deadly bacterial organisms into the various tissues of the body that normally would not be invaded by these bacterial organisms.

In Nigeria, established complications of Ascariasis in man include the disorder of small bowel pattern (Lagundoye, 1972), Ascaris pneumonitis, intestinal obstruction, intussuscpetion, intestinal perforation, appendicitis, inflammatory gall bladder disease, volvulus, pancreatitis and it is common practice to suspect and look for Ascariasis under these conditions (Oluwasanmi, 1968; Waller & Otherson, 1970; Romey, Lilly & McHardy, 1970; Piggot, Hansbarger & Neafie, 1970; Lagundoye, 1972; Ajao & Solanke, 1977; Ajao & Ajao 1979). However, clinical medical practice especially in the tropics has often recorded cases of pyrexia of unknown origin, many of which remained uninvestigated.

In some cases, these could be fatal, and the cause of death would remain unknown, especially since facilities for post-mortem examination are often not available and when available in Nigeria, the necessary permission to carry it out may not be granted. It is, therefore, desirable that clinicians in the tropics where ascariasis is prevalent should be mindful of the probability of having pyrexia of unknown origin caused by the transfer of normal bacteria inhabitants of the gut by migrant *Ascaris* worms into tissues where they could cause disastrous consequences. This is especially important because *A. lumbricoides* is normally a common parasite of many people in many rural tropical environments where its density of infection is often very heavy (Cowper & Woodward, 1961; Gilles, 1965; Ogunba, 1974; Obiamiwe, 1977; Adedeji, 1981).

It is, therefore, important that in cases of pyrexia of unknown origin with *A. lumbricoides* infection, the patient needs to be dewormed as well as given an antibiotic therapy to arrest the activity of bacteria that could have been transferred to the tissues by the active and migratory larval and adult *Ascaris* worm.

Acknowledgments

We would like to express out thanks to the Headmaster of Ibadan Municipal School, Oje, for allowing us to collect the specimens from the School children for these studies. We thank Mr 'Bayo Arewa and Mr Ikejiora for secretarial help.

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(*Received* 23 *January* 1986; *accepted* 5 *February* 1986)