BRIEF COMMUNICATION

ASSESMENT ON THE STATUS OF *SALMONELLA* AND *SCHISTOSOMA* COINFECTIONS IN THE MIDDLE AWASH VALLEY AND ZIWAY, ETHIOPIA"

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ABSTRACT

BACKGROUND: *Salmonellosis and schistosomiasis are prevalent diseases in Ethiopia. Although both are prevalent, the presence and magnitude of Salmonella and schistosome co-infections has not been assessed. The aim of this cross-sectional study was, therefore, to assess co-infection with Salmonella and Schistosoma in the Middle Awash Valley and Ziway, Ethiopia.*
METHODS: *A total of 423 stool samples from Bochesa village around Ziway and 211 urine samples from Asoba village in the Middle Awash Valley, were diagnosed for Schistosoma mansoni (using Kato-Katz method) and Schistosoma haematobium (using dipstick) in 2004, respectively. Out of these, 158 stool and 127 urine specimens from both schistosomiasis positive and negative subjects were cultured for Salmonella infection.*
RESULTS: *The prevalence of Schistosoma mansoni among students and residents in Bochesa was 71.5% and 54.1%, respectively, while the prevalence of Schistosoma haematobium among residents in Asoba Village was 48.8%. Non-lactose fermenting bacteria were found in 19 S. mansoni positive and 6 S. mansoni negative stool specimens. In the urine specimens positive for S. haematobium infection, 45 lactose fermentors and 52 non-lactose fermentors were found. On the other hand, out of S. haematobium negative urine specimens, 3 lactose fermentor and 27 non-lactose fermentor bacteria were encountered. However, Salmonella was not identified among all non-lactose fermentor colonies.*
CONCLUSION: *The results indicated the absence of association between salmonellosis and schistosomiasis in this particular study. Nevertheless, further study in other schistosome endemic areas of Ethiopia is recommended prior to concluding the absence of association between salmonellosis and schistosomiasis in Ethiopia.*

Key words: *Ethiopia; Coinfection; Salmonellosis; Schistosomiasis*

INTRODUCTION

In 2000, typhoid fever caused an estimated 21.7 million illnesses 217,000 deaths, and paratyphoid fever caused an estimated 5.4 million illnesses worldwide (1). Salmonella infection in humans can be categorised into two broad types, that caused by low virulence serotypes of *Salmonella enterica* which cause food poisoning, and that caused by the high virulence serotypes *Salmonella enterica typh*i (*S. typhi*), that causes typhoid, and a group of serovars, known as *S. paratyphi* A, B and C, which cause Paratyphoid (2). Several studies have shown that *Salmonella* infection is prevalent in Ethiopia (3-6). Schistosomiasis, due to *Schistosoma, mansoni* and *S. haematobium are* also known to be prevalent in Ethiopia. Chitsulo et al. (7) estimated that about 30 million people are exposed to both forms of schistosomiasis.

Schistosomes are parasitic helminths that infect humans through dermo-invasion while in contaminated water. Several species of *Schistosoma* infect humans: *S. haematobium*, which is responsible for urinary schistosomiasis; *S. mansoni*, *S. japonicum*, *S. mekongi*, and *S. malayi*, which are responsible for gastrointestinal (GI) and hepatosplenic schistosomiasis; and *S. intercalatum*, which also affects the GI tract but which is associated with lower morbidity. All species have been associated with *Salmonella* bacteremia (8).

Concurrent *Schistosoma-Salmonella* infections are common and are complicated by the bacteria adhering to adult schistosomes present in the mesenteric vasculature.

Salmonella can evade certain antibiotics by binding to *Schistosoma*. As a result, effective bactericidal concentrations of antibiotics are unfortunately above the achievable therapeutic levels of the drugs in co-

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infected individuals. Salmonella-Schistosoma binding is analogous to the adherence of *Salmonella* to cells lining the mammalian intestine. Perturbing this binding is the key to eliminating *Salmonella* that complicate schistosomiasis (8)

Salmonellosis and schistosomiasis co-infections are assumed to have harmful effect on human patients. In studies in animal model, it has been shown that schistosoma parasites alter phagocytosis and decrease intracellular destruction of *Salmonella* (10). Moreover, co-cultivation of *Salmonella in vitro* with schistosome enhances the bacterial growth, indicating that nutritional factors are also involved in the relationship (11). These findings support the observation that patients with schistosomiasis co-infected with *Salmonella* are more likely to become carriers of *Salmonella* even after treatment with antibiotic (8).

Moreover, *Salmonella-Schistosoma* co-infection has shown its influence on treatment. Treatment for both infections had shown no relapse while antibiotics alone resulted in *Salmonella* relapse after 1 month. No relapse occurred after a second course of antibiotics together with treatment for the *S. intercalatum* infection (8).

Although both salmonellosis and schistosomiasis are prevalent in Ethiopia, the presence and magnitude of *Salmonella* and schistosome co-infections has not been assessed. Hence, the aim of this study was to assess the carrier state of *Salmonella* infections in patients with intestinal and urinary schistosomiasis and to determine the level of co-infection.

MATERIALS AND METHODS

***Study area*:** Stool specimens were collected from school children in Bochesa Elementary School and residents of Bochesa village on the shore of Lake Ziway in Ethiopia. It is located about 160 km to the south of Addis Ababa. The population is about 2500. For *S. haematobium* infection, urine specimens were collected from residents of Asoba village in Middle Awash, which is found at a distance of some 250 km east of Addis Ababa. It has a population of 1650. Women in their menstrual period were excluded from *S. haematobium* study, while children under 5 years of age were excluded from both schistosome studies.

The design was cross sectional. The sample size was estimated for each study site using Daniel's formula $[n=Z^2 P (1-P)/d^2]$, where n was the sample size, Z statistic for level of confidence, P expected prevalence or proportion, and D precision. However, the number of study cases for Bochessa was higher than what was calculated and that of Assoba was lesser than the calculated figure due to the number of persons who showed up for screening. For the recovery of *Salmonella*, stool and urine specimens were collected from those whose intensity of infection was relatively higher.

The study was conducted after getting verbal consent and all *S. mansoni* and *S. haematobium* positive cases in this investigation were treated with Praziquantel at a dose of 40mg/Kg body weight by a health professional.

***Stool and urine specimen collection and examination*:** Small plastic sheets were distributed to all subjects in Bochesa Primary School and Bochesa village. A total of 423 stool samples were collected and the Kato-Katz method was used to diagnose *S. mansoni* eggs in the stool specimens (12). Out of the specimens diagnosed for *Schistosoma mansoni*, 125 positive, for experimental, and 33 negative, for control, were transported in a Cary Blair Transport Medium to the laboratory for detection of *Salmonella*.

For the collection of urine specimens for *Schistosoma haematobium*, plastic cups were distributed to each subject. Females in their menstrual period were excluded to avoid false positive results. The Combur $\frac{10}{}$ Test (Roche) dipstick was used to detect haematuria. The dipstick was used following the manufacturer's instructions. After collecting the urine, the dipstick was dipped in the urine for one second and taken out by removing the extra urine by rubbing to the rim of the cup. It was then placed horizontally for about a minute and the result was read. A total of 211 subjects were screened and 97 urine specimens of $\geq$ 2+ haematuria (13) value and 30 negative specimens were transported to the laboratory in a test tube containing the Cary Blair transport medium as experimental and negative control, respectively.

Salmonella culturing: Nutrient Broth (Oxoid) was inoculated with cotton swab containing stool specimen and incubated for 24 hours at 37^{o}C. The cotton swab containing urine specimen was also processed similarly.

After 24 hours of incubation, from each nutrient broth culture of stool and urine specimens, a sample of 1 ml was transferred to approximately 10 ml of Selenite-F broth (Oxoid) and 0.1ml to 10ml Rappa-

port-Vassilliadis (Merck) and incubated at 37°C for 24 hours and at 42°C water bath for 24-48 hours, respectively.

From each enrichment medium of the stool specimens, a loop full of sample was streaked on Xylose-lysine-deoxycholate (XLD) media (Oxoid) and incubated for 24 hours at 37°C. A total of 106 non-lactose fermenting colonies that were red and centrally black were transferred to the nutrient broth and incubated. Several *Salmonella* suspect colonies were picked from every plate, and the purity of isolates was checked on XLD media. Suspected *Salmonella* colonies were screened based on their minimum biochemical profile.

The urine specimen washes were also processed following a similar procedure. A total of 132 non-lactose fermenting colonies were isolated and purified from the urine specimens.

On the other hand, as a positive control, 35 Swiss mice were exposed to the *S. mansoni* cercariae after completing the patent period for the development of adult worms, were orally infected with 0.1ml of 10^2CFU, non-lethal dose, laboratory isolate group D *Salmonella* bacteria. The *Salmonella* were incubated in the Brain Heart Infusion. The mice were then kept for 15 days post exposure. During their stay, starting from day 9 to15, nine mice died. Of the remaining, twenty mice were found appropriate for the experiment. Then, the mice were killed with ether and dissected to harvest adult schistosoma worms.

Out of the twenty, 16 were found to be positive for adult *Schistosoma* worm. The adult schistosoma worms from each mouse were washed with normal saline independently, and the first and second washes were collected separately. A third wash was made after crushing the worms. A total of 48 samples were then separately transferred to the nutrient broth and incubated for 24 hours at 37 °C. Similarly, a sample of 1ml and 0.1ml was transferred to approximately 10ml of Selenite-F broth (Oxoid) and Rappaport-Vassilliadis and incubated at 37 °C for 24 hours and at 42 °C water bath for 24 hours, respectively. From each broth, a loop of sample was streaked on XLD media.

RESULTS

***Parasitological findings*:** Out of the 253 students and 170 residents examined using the Kato-Katz method, 181 (71.5%) and 92 (54.1%), respectively, were found to be positive for *S. mansoni* egg (Table 1). The egg per gram (epg) count ranged between 24 and 3816. The mode of epg was 24 with a mean value of 284.32. There was an association between age and *S. mansoni* infection as well as sex and *S. mansoni* infection at a=0.01 (99% CL).

Of the 211 urine samples tested using the urine dipstick, haematuria at 1+, 2+, 3+ and 4+ values was in a proportion of 2.8%, 10.9%, 10% and 25.1%, respectively. The overall prevalence of haematuria was 48.8%. The proportion of *S. haematobium* positive females to males was 57.4%. It was also observed that *S. haematobium* infection was associated to age and sex at (a=0.012; 98.8%) and (a=0.022; 97.8%), respectively.

Table 1: Prevalence of schistosomiasis mansoni by age in Bochesa Elementary School and Bochesa Village in Ziway, 2004

Study subject	Age group (years)	% (Number positive/ Number examined)
Bochesa Elementary School children	≤10	56.3(27/48)
	10 – 19	76.1(153/201)
	20 – 29	25.0(1/4)
	Total	**71.5(181/253)**
Residents (Bochesa Village)	≤10	0.0(0/2)
	10 – 19	63.6(28/44)
	20 – 29	55.0(33/60)
	30 – 39	45.0(9/20)
	40 – 49	46.7(7/15)
	≥ 50	48.3(14/29)
	Total	**54.1(92/170)**

Table 2: Prevalence of haematuria/schistosomiasis haematobium among residents in Asoba Village in the Middle Awash, 2004

Study subjects	Age group (years)	% (Number positive/ Number examined)
Residents (Asoba Village)	≤ 10	56.8(21//37)
	10 – 19	54.5(30/55)
	20 – 29	44.4(32/72)
	30 – 39	38.5(10/26)
	40 – 49	46.2(6/13)
	≥ 50	50.0(4/8)
	Total	**48.8(103/211)**

***Bacteriological findings*:** Non-lactose fermenting bacteria were found in 19 *S. mansoni* positive and 6 *S. mansoni* negative stool specimens. None of the 25 non-lactose fermenting colonies belonged to *Salmonella*.

From the *S. haematobium* positive urine specimens, 45 lactose and 52 non-lactose fermentors were found. On the other hand, 3 lactose fermentor and 27 non-lactose fermentor colonies were obtained among *S. haematobium* negative urine specimens. However, no *Salmonella* was encountered among the 79 non-lactose fermentor colonies of the urine specimens.

Similarly, from the positive control study, all the 48 samples of the adult *Schistosoma* worm washes, obtained from the Swiss mice cultured on XLD media, show growth of lactose fermenting colony. Thus, no *Salmonella* was recovered.

DISCUSSION

In the present study, the prevalence of *S. mansoni* among school children and residents of Bochesa village was 71.5% and 54.1%, respectively. Previously, a prevalence of 41.3% was reported for Bochesa village (14). We presently found the overall prevalence of urinary schistosomiasis to be 48.8% in Asoba Village in Middle Awash using a urinalysis dipstick. In a previous study on *S. haematobium* infection among school children in Middle Awash Valley, the prevalence of the disease was reported to be 9.9% (13). A comparison of the prevalence of schistosomiasis from the two areas with the previous prevalence of infections showed that the disease was increasing in magnitude.

Various studies have indicated the occurrence of association between *Salmonella* and schistosome worms (7, 15). It has also been shown that schistosome infected individuals serve as carriers of *Salmonella* and shed the bacteria in their faeces or urine (16).

Although the infection rate of *S. mansoni* and *S. haematobium* was found to be high in this study, no *Salmonella* was recovered from both the experimental and negative controls. Our findings agree with a previous study from Nigeria, where Pugh and Gilles (17) found no association between bacteriuria and *Schistosoma haematobium* infection in the Malumfashi area, northern Nigeria. This lack of association between urinary bacterial infection and schistosomiasis in Malumfashi area of northern Nigeria was assumed to be due to the low intensity of *S. haematobium* infection. In a study conducted in two rural Nigerian communities, one with a higher and the other with a low endemicity of urinary schistosomiasis, Soyannwo et al. (18) showed proteinuria, haematuria, pyuria and bacteriuria to be significantly more frequent in the area of high than low endemicity. However, in the present study the infection rate of *S. haematobium* was not low. Hence, prevalence level alone may not be a factor for the absence of association.

The ability of *Salmonella* to cause infection and survive in a susceptible host and find a way to attach with a schistosome adult worm is dependent on its phase. Isaacson et al. (19) showed the existence of two phenotypes in *Salmonella.* The phenotype that is called adhesive was shown to produce pili, is adhesive to porcine enterocytes, is readily phagocytized, and survives intracellularly in phagocytes. The other phenotype termed non-adhesive does not produce pili, is not adhesive to enterocytes, is phagocytized less efficiently, and does not survive within the phagocyte. Cells in each phenotype can freely switch to the other phenotype at a fairly high frequency, thus the shift between each phenotype is phase dependent (19).

It is also well understood that schistosome maturity, its metabolic activity, and the action of antibodies such as anti-*Salmonella* can influence the bacterial fixation on the worm (20). Evidence has been accumulating for variability among and within schistosome populations. Such variability has been seen in both larval and adult stages (21). Thus, variation among schistosomes should also be considered as a factor for the maintenance of association.

Therefore, the above mentioned bacterial characteristics, schistosomal variations, genetic and adaptive factors of the human host with environmental factors may have contributed to the absence of *Salmonella* from the stool and urine samples of both *Schistosoma mansoni* and *Schistosoma haematobium* positive specimens analyzed in this study. Moreover, the feeding behaviour and occupation of the study subjects as well as the season the study was conducted might have a confounding effect on the absence of association between salmonella and schistosoma co-infection.

Although the association between *Salmonella* and *Schistosoma* was not observed in the present study, the possible occurrence of such association should be considered by health facilities during case manage-

ment in order to avoid disease complication and minimize the *Salmonella* reservoir in *Schistosoma* positive individuals as it was indicated by other studies. As there are variations in *Salmonella* strain as well as *S. mansoni* and *S. haematobium*, there is still a need to carry out similar studies in different schistosome endemic areas to determine the status of *Salmonella* and *Schistosoma* co-infection in Ethiopia.

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