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# Lower prevalence of *Entamoeba* species in children with vertically transmitted HIV infection in western Kenya

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A cross-sectional molecular epidemiological study of *Entamoeba* species was conducted among asymptomatic Kenyan children with (n=123) and without (n=111) HIV infection. The prevalence of *E. histolytica* was low (0.4%). *Entamoeba* species infection was inversely related with HIV infection [HIV(+): 29.3% vs. HIV(-): 55.0%, $P<0.001$]: multiple-species infection was related to higher $CD4^+$ T-cell counts. Thus, HIV infection is not a risk factor for amebic infection, and multiple-species infection can be an indicator of better immune status.

Of the six *Entamoeba* species (spp.) reported in humans, only *Entamoeba histolytica* is pathogenic, and the remaining five (*E. dispar*, *E. moshkovskii*, *E. coli*, *E. hartmanni*, and *E. polecki*) are considered commensal [1]. *E. histolytica* infections are highly prevalent among HIV-infected men who have sex with men (MSM) [2, 3], however, it remains unclear whether HIV infection is a risk factor for *E. histolytica* infection. Also, little attention has been paid to commensal *Entamoeba* spp. infections even in HIV-infected population. In the present study, we recruited children with vertically-transmitted HIV infection and children without HIV infection in Kenya to minimize the MSM confounding effects. We, then, determined the prevalence of *E. histolytica* and other representative commensal *Entamoeba* spp. among them using systematic molecular screening methods and evaluated the reliability of *Entamoeba* spp. infection as an indicator of HIV infection.

A cross-sectional survey was conducted at the Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) in Kisumu, Kenya in December 2013. We recruited 234 children {median age, 10 years [interquartile range (IQR), 7.0–12.0]} comprising 123 HIV-vertically infected [72 females; median age, 10 years (IQR, 7.0-12.0)] and 111 HIV-uninfected children [60 females; median age, 10 years (IQR, 7.0–12.0)]. All of the children had neither history of anti-intestinal parasite drug administration in the last three months nor any symptoms of disease. Of the 123 HIV-infected children, 118 were on reverse transcriptase inhibitor-based first-line antiretroviral therapy. $CD4^+$ T-cell counts were available for 110 HIV-infected children [median: 926 cell/μl (IQR, 603–1344)]. The study protocol was approved by the Ethics Committees of the Kenya Medical Research Institute, JOOTRH, and Kanazawa University in Japan. After assent and informed consent were obtained from each child and

his/her guardian, stool samples were collected at the hospital (JOOTRH) and processed by the DNAzol® reagent (Thermo Fisher Scientific, MA) according to the manufacture's instruction. The extracted DNA was screened for *Entamoeba* spp. (*E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. hartmanni*, and *E. coli*) using the universal PCR, and subsequent species-specific PCRs. For the universal screening, a part of the 18S ribosomal RNA subunit gene of *Entamoeba* spp. was amplified using nested PCR with the following primers, annealing temperatures, and cycles: TN21′ (5′-AAGATTAAGCCATGCATGTSKA-3′)/TN14′ (5′-GATACCTTGTTACGACTTCTY-3′), 58°C, and 30 cycles in the first round, and MA115 (5′-GACATCGGAGAGGGAGCT-3′)/ SA12 (5′-GCGTGCRGCCCAAGATG-3′), 57°C, and 35 cycles in the second round. For *Entamoeba* species-specific screening, the multiplex PCR was used for *E. histolytica* and *E. dispar with* specific primers (Eh-L, Eh-R, Ed-L, and Ed-R) [4]; and the remaining species-specific PCRs were performed using the first PCR product of the universal PCR as a template with the following primers, annealing temperature, and cycles: MW27 (5′-CTCTTCACGGGGAGTGCG-3′)/TA28 (5′-CACTATTGGAGCTGGAATTAC-3′) for *E. moshkovskii*, MA67 (5′-TTGGATGTAGAGATACATTC-3′)/TA28 for *E. hartmanni*, MA113 (5′-GCCAAGAGAATTGTAGAAATCG-3′)/TA28 for *E. coli;* 54°C, and 35 cycles. All PCRs were conducted according to the standard protocol of LA-Taq® polymerase (TaKaRa Bio Inc., Shiga, Japan), except adding 0.1% dimethyl sulfoxide in the reaction mixture. PCR positive results of *E. hartmanni* and *E. coli* were confirmed by sequencing as previously reported [5]. Statistical analyses were conducted using R 3.1.3 software [6] .

The overall prevalence of *Entamoeba* spp. was significantly lower in the HIV-infected

children than in the HIV-uninfected children (29.3% vs. 55.0%, $P<0.001$, Fig. 1a). In HIV-infected and HIV-uninfected children, the following *Entamoeba* species were detected: *E. histolytica*, in 0% and 0.9%; *E. dispar*, 3.3% and 5.4%; *E. coli*, 26.8% and 51.4% ($P<0.001$); *E. hartmanni*, 14.6% and 27.9% ($P=0.016$); and *E. moshkovskii*, in 0% and 0%, respectively. Multiple logistic regression analysis showed that HIV-infected children were nearly three times less likely to be infected with any species of *Entamoeba* than HIV-uninfected children (adjusted odds ratio=0.33, 95%CI=0.19–0.60, P<0.001). Thus, HIV infection was found to be inversely related with *Entamoeba* spp. infections. These findings clearly indicate that HIV infection is not a risk factor for *Entamoeba* spp. infections in Kenyan children. However, in this study, the prevalence of *E. histolytica* was too low (0.4%, 1/234) to conclude the relationship between *E. histolytica* infection and HIV infection.

The observed inverse association between *Entamoeba* spp. and HIV infections among Kenyan children may be as a result of the medical interventions in HIV-infected children. In Kenya, HIV-infected children are managed by the local health sector with regular monitoring of their $CD4^+$ T-cell counts [7], which may strengthen their consciousness of hygiene and restrict their daily activities. We also found a higher prevalence of *Entamoeba* spp. infection in HIV-uninfected female children than in HIV-infected females (63.3% vs. 27.8%, $P<0.001$; Fig. 1a), but not in males ($P=0.132$). The predominant distribution of *Entamoeba* spp. infection in female may be explained by the probable *Entamoeba*-infection risks, such as housekeeping work and nursing care [8-11]. A sociobehavioral analysis would be needed for better understanding of these findings.

Multiple *Entamoeba* spp. infection was found more in HIV-uninfected children than in HIV-infected children (27.9% vs. 13.0%, $P$=0.005). Among the HIV-infected children, $CD4^+$ T-cell counts were significantly higher in those with multiple *Entamoeba* spp. infection (median, 1261 cells/μl) than in those without the infection (918 cells/μl, $P$=0.03) or those with the single *Entamoeba* spp. infection (787 cells/μl, $P$=0.03) (Fig. 1b). Considering that $CD4^+$ T-cell counts of HIV-uninfected children are generally higher than those of HIV-infected children, these results suggest that better immunological status may play a role in the establishment of multiple-*Entamoeba* spp. infections. Further studies targeting symptomatic and immune-compromised population would be needed to clarify the role of host immune responses in amebic infections, particularly in multi-species infections.

This is the first molecular epidemiological study of *Entamoeba* spp. infection in Kenya. Our findings clearly indicate that the HIV infection is not a risk factor for *Entamoeba* spp. infections, and suggest that the multiple-species infection can be an indicator of better immune status in children.

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Contribution: E.M., M.T., and H.I. participated in the design and performance of the entire study; T.N., T.M., M.C.S., and E.M. conducted the molecular analyses; J.O., P.O., B.L., and Y.K., and M.T. collected the samples and data in the field and E.M., B.X., and M.T. analyzed the data; E.M.S., W.S., and H.I. provided critical review for the research design and for this paper; E.M., M.T., and H.I. wrote this paper; and all authors checked the final version of the manuscript.

**Conflicts of Interest**

The authors have declared that no competing interests exist.

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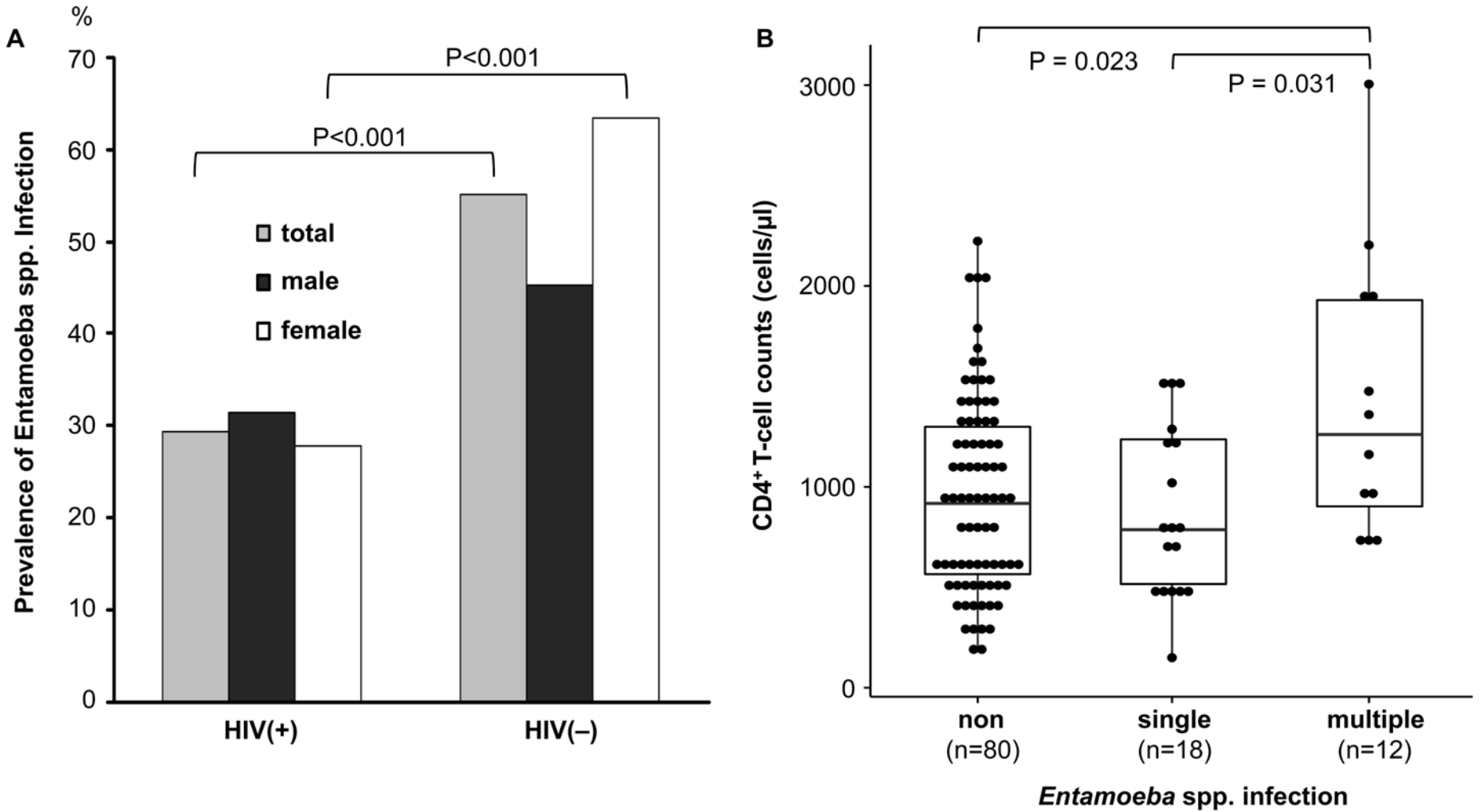
**Figure legends**

**Fig. 1 Distribution of *Entamoeba* species in HIV-infected and HIV-uninfected children.** (a) Prevalence of *Entamoeba* spp. infection in HIV-infected HIV(+) and HIV-uninfected HIV(–) children. Significance was assessed using Fisher's Exact Test. (b) Box plot of the $CD4^+$ T-cell counts among HIV-infected children with non-, single- and multiple-*Entamoeba* spp. infection. The distribution of $CD4^+$ T-cell counts in HIV-infected children with or without *Entamoeba* infection was compared using the Kruskal–Wallis test and subsequently assessed using the Mann–Whitney U Test.


A
%
70
60
50
40
30
20
10
0
Prevalence of Entamoeba spp. Infection
P<0.001
P<0.001
total
male
female
HIV(+)
HIV(–)
B
3000
2000
1000
0
CD4+ T-cell counts (cells/μl)
P = 0.023
P = 0.031
non
(n=80)
single
(n=18)
multiple
(n=12)
Entamoeba spp. infection