



# Food-Borne Protozoan Infection in HIV<sup>+</sup>/AIDS Patients and Healthy Individuals: A Case-Control Study in Iran

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## HIGHLIGHTS

- Prevalence of protozoa in HIV<sup>+</sup>/AIDS patients and healthy individuals were 34.2% and 4.1%, respectively.
- A meaningful relationship was seen between age and incidence rate of protozoan infection ( $p < 0.05$ ).
- HIV<sup>+</sup>/AIDS individuals should be educated regarding importance of food and water safety.

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## ABSTRACT

**Background:** Intestinal protozoa transmitted by food are the most common opportunistic parasites among the HIV<sup>+</sup>/AIDS patients. As, there is no report about prevalence of food-borne intestinal parasites in such patients in Yazd, so this study was performed to determine the prevalence of intestinal protozoa in the HIV<sup>+</sup>/AIDS patients in comparison with HIV<sup>-</sup> individuals.

**Methods:** A case-control study was conducted from July 2015 to March 2016. Totally, 73 patients (group I) were involved from the Prevention of Behavioral Disorders Center, Yazd, central of Iran. A control group (group II) comprised 147 HIV<sup>-</sup> individuals. After collecting the stool samples; wet mount, concentration method, and modified Ziehl-Neelsen staining was performed. Statistical analysis was done using SPSS 16.0 by Chi-Square and Fisher tests.

**Results:** Detected food-borne protozoa in HIV<sup>+</sup>/AIDS patients were included *Giardia lamblia*, *Entamoeba histolytica*, *Blastocystis hominis*, *Chilomastix mesnili*, *Endolimax nana*, and *Entamoeba coli*. Overall, out of 73 cases in group I, 34.2% were infected with intestinal food-borne protozoa compared with 4.1% (out of 147 cases) infection rate in group II, which showed significant difference ( $p < 0.05$ ). There were no significant differences between sex and incidence rate of parasitic protozoan infection between groups I and II. However, a meaningful relationship was found between age and incidence rate of parasitic infection ( $p < 0.05$ ).

**Conclusion:** It is emphasized the necessity of increasing awareness among clinicians regarding the occurrence of the food-borne parasites in this population. Considering their susceptibility, HIV<sup>+</sup>/AIDS individuals should be educated regarding high importance of food and water safety and disinfecting protocols of suspected and hazardous foods (especially unwashed vegetables and fruits) to prevent intestinal parasitic diseases.

## Introduction

Intestinal parasitic infections transmitted by food have worldwide spread (Savioli et al., 1992). Food poverty,

poor sanitation, lack of access to safe drinking water, and hot and humid weather of tropical area are some major factors involved in the spread of food-borne parasitic

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infections (Nihar Dash et al., 2010). It is known that the incidence of these infections is much higher in developing countries in comparison with the developed ones (Al-Megrin, 2010; Genta et al., 1989).

Parasitic infections, especially opportunistic intestinal parasites are the main problem of immunocompromised individual, particularly patients with HIV<sup>+</sup>/AIDS (Genta et al., 1989; Xiao et al., 2001). People with HIV<sup>+</sup>/AIDS compared to normal individuals are more vulnerable to various infections and suffer more complications (Togeh et al., 2000). High incidence rate of various gastrointestinal infections in the form of diarrhea has been reported in HIV<sup>+</sup>/AIDS individuals (Al-Megrin, 2010). The incidence of these infections in the patients in developed countries are about 50%, while in developing countries such as Haiti and some African countries has been reported up to 95% (Smith et al., 1988). Until August 2016, a total of 36900000 people with HIV has been identified in the world and 30727 people with HIV<sup>+</sup>/AIDS has been identified in Iran which 6016 patients have died (NCAP, 2016). Opportunistic intestinal parasites in patients with immune deficiency cause diarrhea, severe malabsorption in the small intestine, and even significant mortality (Berenji et al., 2010; Meamar et al., 2007). Several studies conducted in different parts of the world have suggested a relatively high prevalence of these infections with immunocompromised patients (Lindo et al., 1998). Despite the increasing number of HIV infected patients in Iran in recent years, little attention has been paid to food-borne protozoa in these persons (Meamar et al., 2007). Since, there is no report on the prevalence of food-borne protozoa in HIV<sup>+</sup>/AIDS patients from Yazd, central of Iran, therefore we decided to research about incidence of food-borne protozoa in HIV<sup>+</sup>/AIDS patients in comparison with healthy individuals in this region.

## Materials and methods

### Ethics

This research was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (No. IR.SSU.MEDICINE.REC.1394.150).

### Sampling

This case-control study was conducted from July 2015 to March 2016 in Yazd, central of Iran. Two groups of individuals were included; HIV<sup>+</sup>/AIDS patients refer to Prevention of Behavioral Disorders Center (group I=73) and HIV seronegative and clinically healthy individuals (group II=147). Before sample collection, the informed consent was completed by individuals of either group I or group II. Also, a simple structured questionnaire was

signed by each person to ensure neither antiparasitic nor antibiotics drugs were administrated in the last month. The fresh stool sample was collected from each individual early morning discharge and transferred to laboratory for next preparation and examination.

### Direct wet-mount

Specimens were examined for intestinal parasites by direct wet-mount with placing a small amount of feces on two different points of a microscopic slide and adding a drop of either saline or Lugol's solution. After mixing thoroughly, each sample was examined for any protozoa.

### Formalin-ethyl acetate concentration

At first, about 5 ml of each diluted stool sample was strained through wet gauze into a centrifuge tube. This content was adjusted to achieve about 0.5 to 0.75 ml sediment after primary centrifugation stage. The supernatant was poured away, and then distilled water was added to the tube to reach about 10 ml. Ethyl acetate (3 ml) was added to each tube, shaken for 30 s, and after that the obtained mixture was centrifuged at 300 x g for 2 min. Next, the three upper layers were poured away, the pellet was put in residual water and then homogenized with slow stirring. Finally, the slides were provided for the examination step (Truant et al., 1981).

### Modified Ziehl-Neelsen technique

Fecal smear for each specimen was made. Each sample was fixed with methanol for 3 min, and stained with carbol fuchsin for about 15-20 min. Decolourise was done with acid alcohol (1% HCl in methanol) for 15-20 s and counterstained with 0.4% malachite green for 30-60 s. The prepared slide was examined using x40 and x100 objectives.

### Statistical analysis

Data were analyzed using SPSS (v. 16.0) by Chi-Square and Fisher tests.

## Results

Detected food-borne protozoa in HIV<sup>+</sup>/AIDS patients were included *Giardia lamblia*, *Entamoeba histolytica*, *Blastocystis hominis*, *Chilomastix mesnili*, *Endolimax nana*, and *Entamoeba coli*. Overall, out of 73 cases in group I, 34.2% were infected with intestinal food-borne protozoa compared with 4.1% infection rate (147 cases) in group II, which showed significant difference ( $p<0.05$ ). The prevalence rate of various food-borne protozoan parasites in each group was summarized in Table 1.

The relationship between infection rate and sex in both groups I as well as II showed no significant difference ( $p>0.05$ ). In groups I and II, 38.1% and 2.9% women were infected with protozoa, respectively; while the rate of infection among men in groups I and II was 32.7% and

5.1%, respectively. The highest range of food-borne protozoa in HIV<sup>+</sup>/AIDS patients was shown in age group over 40 years (53.6%) and the lowest in the age group of less than 30 years (5.1%) indicating significant difference ( $p<0.05$ ).

**Table 1:** Prevalence of intestinal protozoa in HIV<sup>+</sup>/AIDS patients and non-HIV infected individuals in Yazd, central of Iran

Protozoa	Infection rate in HIV <sup>+</sup> /AIDS patients	Infection rate in HIV <sup>-</sup> individuals
	No. (%)	No. (%)
<i>G. lamblia</i>	8 (10.8)	1 (7.0)
<i>E. histolytica</i>	1 (1.4)	3 (2)
<i>B. hominis</i>	2 (2.7)	0 (0)
<i>C. mesnili</i>	3 (4.1)	0 (0)
<i>E. nana</i>	3 (4.1)	2 (1.4)
<i>E. coli</i>	4 (5.5)	0 (0)
Co- infection	4 (5.5)	0 (0)
Total	25 (34.2)	6 (4.1)

## Discussion

Intestinal parasitic infections especially food-borne protozoa among HIV infected persons are one of the major causes of morbidity and mortality in developing countries (WHO, 1987). In the present study, we showed that HIV positive persons had high risk to be infected with food-borne intestinal parasitic infection. Prevalence rate of protozoan parasites in groups I and II were 34.2% and 4.1%, respectively. The incidence rates of infection achieved in this study were consistent with findings of some previous researches (Buyukbaba et al., 2003; Hailemariam et al., 2004; Mohandas et al., 2002; Ramakrishnan et al., 2007; Zali et al., 2004).

In our research, the highest and lowest prevalence rates of protozoan infection in HIV<sup>+</sup>/AIDS people were related to *G. lamblia* (10.8%) and *B. hominis* (2.7%), respectively that confirmed other studies by Gupta et al. (2008) and Meamar et al. (2007) who reported *G. lamblia* as the most prevalent intestinal parasite in HIV infected patients. In contrast, Kurmiawan et al. (2009) reported lower prevalence rate of *B. hominis* in Indonesian patients. These differences in prevalence rates may be due to the various condition of weather (hot and humid in Indonesia and hot and dry in central of Iran) which may influence the epidemiology and incidence rate of various parasitic diseases in the environment as well as human population. However, in the present work, the prevalence of co-infection of *G. lamblia* and *B. hominis* was 5.5% with similarity to findings by Karnyavan et al. (2009).

In the current investigation, we found no significant difference between sex and incidence rate of parasitic protozoan infection in both groups I and II. So, it seems that sex is not a risk factor for acquiring infection in this regard. In contrast, a meaningful relationship was found between age and infection rate. This finding could be attributed to the fact that older people are mainly more

susceptible to the infectious diseases probably due to suppression of immune system.

## Conclusion

This study showed that the risk of infection in patients with weakened immune systems due to AIDS was more than the normal persons. Also, due to higher prevalence of pathogenic protozoa in people with AIDS comparing to the healthy ones, the diagnosis and treatment of these infections is particularly important. Therefore, it is emphasized the necessity of increasing awareness among clinicians regarding the occurrence of the food-borne parasites in this patient population. Considering their susceptibility, HIV<sup>+</sup>/AIDS individuals should be educated regarding high importance of food and water safety and disinfecting protocols of suspected and hazardous foods (especially unwashed vegetables as well as fruits) to prevent intestinal parasitic diseases.

## Conflicts of interest

All authors of this manuscript declare that there is no conflict of interest.

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