Acta Tropica xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Prevalence and risk factors of intestinal protozoan and helminth infections among pulmonary tuberculosis patients without HIV infection in a rural county in P. R. China

Q1 Xin-Xu Li[a,b,c,**], Jia-Xu Chen[a,b], Li-Xia Wang[c], Li-Guang Tian[a,b], Yu-Ping Zhang[d], Shuang-Pin Dong[d], Xue-Guang Hu[d], Jian Liu[d], Feng-Feng Wang[e], Yue Wang[e], Xiao-Mei Yin[e], Li-Jun He[f], Qiu-Ye Yan[f], Hong-Wei Zhang[f], Bian-Li Xu[f], Xiao-Nong Zhou[a,b,*]

[a] *National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai 200025, PR China*
[b] *Key Laboratory of Parasite and Vector Biology, Ministry of Health, WHO Collaborating Centre for Malaria, Schistosomiasis and Filariasis, Shanghai 200025, PR China*
[c] *National Center for Tuberculosis Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, PR China*
[d] *Gushi County Center for Disease Control and Prevention, Henan Province, Gushi 465200, PR China*
[e] *Anhui Province Institute of Parasitic Disease Control, Hefei 230061, PR China*
[f] *Henan Province Center for Disease Control and Prevention, Zhengzhou 450016, PR China*

ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form 22 March 2015
Accepted 4 May 2015
Available online xxx

Keywords:
Intestinal protozoan
Intestinal helminth
Tuberculosis
Prevalence
Risk factor
P. R. China

ABSTRACT

Although co-infection of tuberculosis (TB) and intestinal parasites, including protozoa and helminths, in humans has been widely studied globally, very little of this phenomenon is known in China. Therefore, a cross-sectional study was conducted in a rural county of China to investigate such co-infections. Patients with pulmonary TB (PTB) undergoing anti-*Mycobacterium tuberculosis* (anti-MTB) treatment were surveyed by questionnaires, and their feces and blood specimens were collected for detection of intestinal protozoa and helminths, routine blood examination and HIV detection. The χ^2 test and multivariate logistic regression model were used to identify risk factors. A total of 369 patients with PTB were included and all of them were HIV negative. Overall, only 7.3% of participants were infected with intestinal protozoa, among which prevalence of *Blastocystis hominis*, *Entamoeba* spp. and *Trichomonas hominis* were 6.0%, 1.1% and 0.3%, respectively; 7.0% were infected with intestinal helminths, among which prevalence of hookworm, *Trichuris trichiura*, *Ascaris lumbricoides* and *Clonorchis sinensis* were 4.3%, 1.9%, 0.5% and 0.3%, respectively; and 0.5% were simultaneously infected with intestinal protozoa and helminths. Among patients with PTB, body mass index (BMI) ≤ 18 (OR = 3.30, 95% CI = 1.44–7.54) and raised poultry or livestock (e.g., chicken, duck, pig) (OR = 3.96, 95% CI = 1.32–11.89) were significantly associated with harboring intestinal protozoan infection, while BMI ≤ 18 (OR = 3.32, 95% CI = 1.39–7.91), anemia (OR = 3.40, 95% CI = 1.44–8.02) and laboring barefoot in farmlands (OR = 4.54, 95% CI = 1.88–10.92) were significantly associated with having intestinal helminth infection. Additionally, there was no significant relationship between duration of anti-MTB treatment and infection rates of intestinal parasites including protozoa and helminths. Therefore, preventing malnutrition, avoiding unprotected contact with reservoirs of protozoa, and improving health education for good hygiene habits, particularly wearing shoes while outdoors, are beneficial in the prevention of intestinal protozoan and helminth infection among patients with PTB.

© 2015 Published by Elsevier B.V.

1. Introduction

Q2 In recent years, co-infection of tuberculosis (TB) and intestinal parasites, including protozoa and helminths, in humans has gradually emerged to be a major public health problem in poor regions and countries. The majority of intestinal protozoa, such as *Trichomonas hominis* and *Dientamoeba fragilis*, that inhabit the

* Corresponding author at: National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, 207 Rui Jin Er Road, Huangpu, Shanghai 200025, PR China. Tel.: +86 21 64738058; fax: +86 21 64332670.
** Corresponding author at: National Center for Tuberculosis Control and Prevention, Chinese Center for Disease Control and Prevention, 155 Changbai Road, Changping, Beijing 102206, PR China. Tel.: +86 10 58900535; fax: +86 10 58900535.
E-mail addresses: lixinxu@chinatb.org (X.-X. Li), xiaonongzhou1962@gmail.com (X.-N. Zhou).

http://dx.doi.org/10.1016/j.actatropica.2015.05.001
0001-706X/© 2015 Published by Elsevier B.V.

Please cite this article in press as: Li, X.-X., et al., Prevalence and risk factors of intestinal protozoan and helminth infections among pulmonary tuberculosis patients without HIV infection in a rural county in P. R. China. Acta Trop. (2015), http://dx.doi.org/10.1016/j.actatropica.2015.05.001

gastro-intestinal tract of humans are non-pathogenic commensals or only result in mild diseases (Ruttgers, 1983); however, some of these protozoa, namely *Entamoeba histolytica* (Ralston and Petri, 2011) and *Blastocystis hominis* (Ustun and Turgay, 2006) can cause severe diseases under favorable conditions. Intestinal protozoa most commonly cause diseases in immunocompromised individuals, as seen with *Cryptosporidium parvum*, *Isospora belli* and *Cyclospora cayetanensis* (Ferreira, 2000; Marcos and Gotuzzo, 2013). More people in the developing world are contracting TB due to a poor immune system (Lawn and Zumla, 2011), so they should also be susceptible to infections with intestinal protozoa. However, there is a paucity of investigations for association between TB and intestinal protozoan infection (IPI). To date, there is only one study that showed a statistically significant inverse linear dose-response relationship between *B. hominis* infection and TB among children (Franke et al., 2014).

Compared with mono-infection, co-infection of TB and intestinal helminths causes the host's immune system to deal with a more complex internal environment. For example, with an increase in echinococcosis chronicity, the immune profile in patients with TB displayed an elevated Th2 immune reponse with subsequent suppression of the Th1 immune response (Yang et al., 2009). Filarial infections clearly altered the magnitude and quality of the *Mycobacterium*-specific cytokine response (Metenou et al., 2012). The investigation for co-infection of TB and intestinal helminths suggested that the absolute frequencies of $CD3^{+}$, $CD4^{+}$, $CD8^{+}$, natural killer T cell and $CD4^{+}CD25^{high}$ T cell decreased in co-infected patients, as compared with TB patients or healthy controls (Resende Co et al., 2007). When the course of TB was aggravated by opisthorchiasis invasion, clinical signs of TB became more pronounced, disorders in the functions of the liver and pancreas became more frequent, antibacterial therapy intolerance increased and prognosis of the disease deteriorated (Kashuba and Rusakova, 1992; Vasil'ev et al., 1989).

Moreover, epidemiological studies of intestinal helminth infection (IHI) among patients with TB have also been conducted in many developing countries where TB and IHI were prevalent. In Ethiopia, it was found that 29% of patients with TB from the community had intestinal helminths (Abate et al., 2012). A study in Tanzania suggested that 32.4–38.2% of patients with TB from hospitals were infected with Schistosomes (Range et al., 2007). The differentials of co-infection prevalence were observed in Korea, where the infection rates of *Trichuris trichiura* and *Clonorchis sinensis* were 20.7% and 17.6% respectively among patients with TB in one hospital, and 6.5% and 6.0% respectively in other hospital (Choi et al., 1984). These findings indicate that IHI among patients with TB is common in poor areas around the world and the diagnosis and treatment of many patients with TB are under the influence of IHI.

In P. R. China, the second national survey for major human parasitic diseases in 2004 (the second national survey) reported that overall prevalence of IPI was 3.1% in Shandong Province and 6.6% in Henan Province, both in central China, where the main intestinal protozoa consisted of *Giardia lamblia*, *Entamoeba* spp., *B. hominis* and *T. hominis*. It also reported that a total of 26 helminth species were detected and overall rate of IHI was 21.7%(Disease Control Bureau of the Ministry of Health and National Institute of Parasitic Diseases of the Chinese Center for Disease Control and Prevention, 2008). In 2010, the fifth national survey for TB showed that the nationwide active pulmonary TB (PTB) prevalence was 459/100,000 among those who were aged more than 15 years old (Disease Control Bureau of the Ministry of Health and Chinese Center for Disease Control and Prevention, 2011). Although both surveys showed that TB, IPI and IHI are important infectious diseases in P. R. China, occurrence of IPI and IHI among patients with PTB is poorly understood.

Building on previous evidences that parasitic co-infections negatively influence the outcome of TB treatment and that TB patients are more likely to be co-infected with parasites in poor areas, we previously conducted a study that identified differences of overall parasitic infections between patients with PTB and the general population in a rural county of central China (Li et al., 2014). Such an investigation has allowed for the evaluation of the co-infection situation in P. R. China. Based on that study, the present study is conducted using a cross-sectional survey to further evaluate the prevalence and risk factors of IPI and IHI among patients with PTB. The ultimate purpose of this study is to find ways to control and prevent IPI and IHI among patients with PTB in central China where both TB and intestinal parasites including protozoa and helminths are transmitted.

2. Materials and methods

2.1. Participants' recruitment

This study was conducted from July to September, 2012 in Gushi County of Henan Province, which is an agricultural county and lies in central China. Firstly, all the patients with PTB who were detected from February to July, 2012 and undergoing anti-*Mycobacterium tuerculosis* (MTB) treatment from July to September, 2012, were considered as the potential participants, and a list of the potential participants was obtained from the TB surveillance system. Secondly, the local Center for Disease Control and Prevention (CDC), responsible for the recruitment of participants, arranged for authorities of township hospitals to inform health workers at clinics of villages about the list and study procedures. Thirdly, health workers informed all the potential participants according to the list and carefully explained the objectives, procedures and potential risks of the study. Lastly, the potential participants were included in this study if they agreed to attend the study and were confirmed with no organic disease, no severe disease of immune system and no pregnancy for females through a general medical checkup. If the participant did not meet the above mentioned inclusion criteria, he/she was then excluded.

2.2. Study procedures

All the participants included in the study were given two feces collection containers beforehand for their feces specimens (at least 30 g every day) collected on two consecutive mornings at home. On the first morning when they delivered the first feces specimen to their nearest township hospitals, they were administered questionnaires about their characteristics for the past year, and blood (10 ml) was drawn for routine blood examination and human immunodeficiency virus (HIV) antibody detection. Participants' characteristics included socio-demographic conditions, concomitant physical health, hygienic habits when cooking, eating, walking, washing hands, raising animals, etc., labor in farmlands, and duration of anti-MTB treatment. On the second morning, only the second feces specimen was delivered. All the investigation and the specimen collection were conducted by the staff of the local CDC in the township hospitals and all the specimens were sent to the laboratory of the local CDC for examination as soon as possible after they were collected every morning.

Blood specimens were tested by the staff of the local CDC within 2 h of collection for HIV antibody detection by using the diagnostic kit for antibody to HIV (colloidal gold) (Zhu Hai Livzon Diagnostics Inc., P. R. China) and routine blood examination by using a MC-600 hematology analyzer (Shenzhen Maxcom Electronic Co., LTD., P. R. China). The diagnostic threshold for anemia was set at hemoglobin < 120 g/L for adult males and hemoglobin < 110 g/L for non-pregnant adult females and children in P. R. China (Lu and Zhong, 2008).

The feces specimens were processed within 8 h post-collection by using four standard stool examination methods: simple saline smear for intestinal protozoa trophozoites, iodine-stained smear for protozoal intestinal cysts, an in vitro cultivation for detection of *B. hominis* (Leelayoova et al., 2002), and the modified Kato-Katz thick smear (a semi-quantitative feces examination technique) for detection of helminthic ova (Katz et al., 1972). Three smears of each feces specimen were prepared for each method. Every smear was initially read by two separate examiners and reviewed by a third examiner if there was disagreement. Feces specimen examinations were conducted by staff from the National Institute of Parasitic Diseases of China CDC together with staff from Henan Province CDC and Anhui Province Institute of Parasitic Diseases Control.

2.3. Statistical analysis

Data were double-entered and crosschecked by using the EpiData software (version 3.1; The EpiData Association, Odense, Denmark). Infection rates with 95% confidence intervals (CIs) of intestinal protozoa and helminths were calculated by binomial distribution. Descriptive summary measures of frequency and central tendency of participants' characteristics were computed as appropriate. Enrolled participants were divided into four groups: only IPI, only IHI, simultaneously infected with intestinal protozoa and helminths and no infection. In the univariate analysis, the Pearson χ^2 tests were used to identify associations between participants' characteristics and only IPI or IHI by computing crude odds ratios (ORs) with 95% CIs. Multivariate logistic regression model was subsequently employed through stepwise elimination to adjust for confounders (adjusted ORs with 95% CIs) of those risk factors that were found to be statistically significant by the univariate analysis. A two-sided P value of 0.05 or less was regarded as significant. Statistical analyses were done with the SAS statistical package (version 9.2; SAS Institute, Inc., Cary, NC, USA).

2.4. Ethical statement

This study was evaluated and approved by the Ethics Review Committee of the National Institute of Parasitic Diseases of China CDC. The potential participants who agreed to attend this study were asked to sign the written informed consent by the staff of the local CDC, and then they were finally included in this study if they met the inclusion criteria. If participants were less than 18 years of age, their parents were asked to sign the written parental permission. All the participants were offered professional counseling by the staff of the local CDC before and during the study, and all the diagnostic results were kept strictly confidential. At the completion of the study and in accordance with the local treatment policies, anti-protozoal treatment and anti-helminthic treatment were freely offered to all the participants found to be infected with intestinal protozoa and/or helminths through the local CDC.

3. Results

There were 575 potential participants, among whom 165 worked away from hometown, 16 declined to attend the study, 3 had lung cancer and 2 died. Therefore, 389 participants were included in the study. After the exclusion of participants whose feces or blood specimens were not collected or who did not complete the questionnaires, 369 participants were finally included in the analysis. Of 575 potential participants, all those excluded (median age = 53 years, interquartile range [IQR] = 31–66) were significantly younger (P < 0.0001) than those finally included in the analysis (median age = 62 years, IQR = 50–70).

Table 1
Characteristics of the study population.

Variables	No. participants	%
Total	369	100.0
Sex		
Male	249	67.5
Female	120	32.5
Age (years)[a]		62 (50–70)
Han nationality	364	98.6
Education		
Illiteracy	185	50.1
Elementary school and above	184	49.9
Married	288	78.0
Body mass index (BMI)[a]		19 (18–20)
Anemia	82	22.2
Only 1 cutting board in kitchen	349	94.6
Only 1 cooking knife in kitchen	253	68.6
Never or occasionally washed fruits and vegetables before eating raw	188	51.0
Ever walked barefoot	207	56.1
Never or occasionally washed hands before meals	147	39.8
Never or occasionally washed hands after defecating	168	45.5
Raised pets (e.g., cat, dog)	224	60.7
Raised poultry or livestock (e.g., chicken, duck, pig)	285	77.2
Annual labor time in farmlands (months)[a]		2 (0–3)
Ever labored barefoot in farmlands	128	34.7
Human immunodeficiency virus (HIV) negative	369	100.0
Only intestinal protozoan infection[b]	27	7.3 (4.9–10.5)
Entamoeba spp.[b]	4	1.1 (0.3–2.8)
Blastocystis hominis[b]	22	6.0 (3.8–8.9)
Trichomonas hominis[b]	1	0.3 (0.3–1.5)
Only intestinal helminth infection[b]	26	7.0 (4.6–10.2)
Clonorchis sinensis[b]	1	0.3 (0.3–1.5)
Ascaris lumbricoides[b]	2	0.5 (0.1–1.9)
Trichuris trichiura[b]	7	1.9 (0.8–3.9)
Hookworm[b]	16	4.3 (2.5–6.9)
Co-infection of intestinal protozoan and helminth[b]	2	0.5 (0.1–1.9)
Entamoeba spp. and Hookworm[b]	1	0.3 (0.3–1.5)
Blastocystis hominis and *Trichuris trichiura*[b]	1	0.3 (0.3–1.5)

[a] Values are median (interquartile range).
[b] Values are infection rate (95% confidence interval).

Table 1 shows the characteristics of the participants. Among 369 participants, 67.5% were male, 98.6% were of Han nationality, 50.1%were illiterate and 78.0% were married. Among the 369 participants who were HIV negative, only 7.3% (95% CI = 4.9–10.5%) were infected with intestinal protozoa, among which infection rates of *B. hominis*, *Entamoeba* spp. and *T. hominis* were 6.0% (95% CI = 3.8–8.9%), 1.1% (95% CI = 0.3–2.8%) and 0.3% (95% CI = 0.3–1.5%), respectively; 7.0% (95% CI = 4.6–10.2%) were only infected with intestinal helminths, among which infection rates of hookworm, *T. trichiura*, *Ascaris lumbricoides* and *C. sinensis* were 4.3% (95% CI = 2.5–6.9%), 1.9% (95% CI = 0.8–3.9%), 0.5% (95% CI = 0.1–1.9%) and 0.3% (95% CI = 0.3–1.5%), respectively; 0.5% (95% CI = 0.1–1.9%) were simultaneously infected with intestinal protozoa and helminths.

The results of the univariate and multivariate analysis for risk factors associated with only IPI were summarized in Table 2. Risk factors associated with only IPI in the univariate analysis were BMI ≤ 18 (OR = 2.90, 95% CI = 1.28–6.55), raised pets (e.g., cat, dog) (OR = 2.50, 95% CI = 1.01–6.38) and raised poultry or livestock (e.g., chicken, duck, pig) (OR = 3.42, 95% CI = 1.15–10.12). The results of

Table 2
Univariate and multivariate analysis for risk factors associated with only intestinal protozoan infection of the study population.

Variables	No. participants	No. infections	Infection rate (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Sex					
Male	233	15	6.4% (3.6–10.4%)	1.00	
Female	108	12	11.1% (5.9–18.6%)	1.82 (0.82–4.03)	
Age (years)					
≤60 years old	153	9	5.9% (2.7–10.9%)	1.00	
>60 years old	188	18	9.6% (5.8–14.7%)	1.69 (0.74–3.89)	
Education					
Elementary school and above	174	15	8.6% (4.9–13.8%)	1.00	
Illiteracy	167	12	7.2% (3.8–12.2%)	0.82 (0.37–1.81)	
Marital status					
Other	76	3	3.9% (0.8–11.1%)	1.00	
Married	265	24	9.0% (5.9–13.2%)	2.42 (0.71–8.28)	
Body mass index (BMI)					
>18	208	10	4.8% (2.3–8.7%)	1.00	1.00
≤18	133	17	12.8% (7.6–19.7%)	2.90 (1.28–6.55)	3.30 (1.44–7.54)
Anemia					
No	272	18	6.6% (4.0–10.2%)	1.00	
Yes	69	9	13.0% (6.1–23.3%)	2.12 (0.91–4.94)	
No. of cutting board in kitchen					
>1	20	2	10.0% (1.2–31.7%)	1.00	
Only one	321	25	7.8% (5.1–11.3%)	0.76 (0.17–3.46)	
No. of cooking knife in kitchen					
>1	108	8	7.4% (3.2–14.1%)	1.00	
Only one	233	19	8.2% (5.0–12.4%)	1.11 (0.47–2.62)	
Washed fruits and vegetables before eating raw					
Often or always	173	12	6.9% (3.6–11.8%)	1.00	
Never or occasionally	168	15	8.9% (5.1–14.3%)	1.32 (0.60–2.90)	
Ever walked barefoot					
No	154	12	7.8% (4.1–13.2%)	1.00	
Yes	187	15	8.0% (4.6–12.9%)	1.03 (0.47–2.28)	
Washed hands before meals					
Often or always	210	14	6.7% (3.7–10.9%)	1.00	
Never or occasionally	131	13	9.9% (5.4–16.4%)	1.54 (0.70–3.39)	
Washed hands after defecating					
Often or always	190	13	6.8% (3.7–11.4%)	1.00	
Never or occasionally	151	14	9.3% (5.2–15.1%)	1.39 (0.63–3.06)	
Raised pets (e.g., cat, dog)					
No	137	6	4.4% (1.6–9.3%)	1.00	
Yes	204	21	10.3% (6.5–15.3%)	2.50 (1.01–6.38)	
Raised poultry or livestock (e.g., chicken, duck, pig)					
No	121	4	3.3% (0.9–8.2%)	1.00	1.00
Yes	220	23	10.4% (6.7–15.3%)	3.42 (1.15–10.12)	3.96 (1.32–11.89)
Annual labor time in farmlands					
≤2 months	254	18	7.1% (4.2–11.0%)	1.00	
>2 months	87	9	10.3% (4.8–18.7%)	1.51 (0.65–3.50)	
Ever labored barefoot in farmlands					
No	232	17	7.3% (4.3–11.5%)	1.00	
Yes	109	10	9.2% (4.5–16.2%)	1.28 (0.56–2.89)	
Duration of anti-MTB treatment					
1 month	42	3	7.1% (1.5–19.5%)	1.00	
2 months	41	5	12.2% (4.1–26.2%)	1.81 (0.40–8.10)	
3 months	35	4	11.4% (3.2–26.7%)	1.68 (0.35–8.06)	
4 months	58	0	0.0% (0.0–6.2%)	NA	
5 months	72	5	6.9% (2.3–15.5%)	0.97 (0.22–4.28)	
6 months	93	10	10.8% (5.3–18.9%)	1.57 (0.41–6.01)	

CI, confidence interval; OR, odds ratio; MTB, *Mycobacterium tuberculosis*; NA, not applicable.

UNCORRECTED PROOF

the multivariate logistic regression analysis showed that BMI ≤ 18 (OR = 3.30, 95% CI = 1.44–7.54) and raised poultry or livestock (e.g., chicken, duck, pig) (OR = 3.96, 95% CI = 1.32–11.89) were significantly associated with only IPI. The prevalence of only IPI during the first month of treatment (7.1% [95% CI = 1.5–19.5%]) was separately compared with those during second through sixth month of treatment (12.2% [95% CI = 4.1–26.2%], 11.4% [95% CI = 3.2–26.7%], 0.0% [95% CI = 0.0–6.2%], 6.9% [95% CI = 2.3–15.5%], 10.8% [95% CI = 5.3–18.9%], respectively), which showed that there was no significant relationship between prevalence of IPIs and duration of treatment.

The results of the univariate and multivariate analysis for risk factors associated with only IHI were summarized in Table 3. Risk factors associated with only IHI were BMI ≤ 18 (OR = 2.73, 95% CI = 1.20–6.22), anemia (OR = 3.63, 95% CI = 1.60–8.24), never or occasionally washed fruits and vegetables before eating raw (OR = 2.86, 95% CI = 1.17–6.98), never or occasionally washed hands before meals (OR = 2.26, 95% CI = 1.01–5.10), annual labor time in farmlands > 2 months (OR = 3.53, 95% CI = 1.57–7.95) and ever labored barefoot in farmlands (OR = 4.10, 95% CI = 1.77–9.52). The results of the multivariate logistic regression analysis showed that BMI ≤ 18 (OR = 3.32, 95% CI = 1.39–7.91), anemia (OR = 3.40, 95% CI = 1.44–8.02) and ever labored barefoot in farmlands (OR = 4.54, 95% CI = 1.88–10.92) were significantly associated with IHI. The prevalence of only IHI during first month of treatment (7.1% [95% CI = 1.5–19.5%]) was separately compared with those during second through sixth month of treatment (5.3% [95% CI = 0.6–17.7%], 8.8% [95% CI = 1.8–23.7%], 7.9% [95% CI = 2.6–17.6%], 9.5% [95%

Please cite this article in press as: Li, X.-X., et al., Prevalence and risk factors of intestinal protozoan and helminth infections among pulmonary tuberculosis patients without HIV infection in a rural county in P. R. China. Acta Trop. (2015), http://dx.doi.org/10.1016/j.actatropica.2015.05.001

Table 3
Univariate and multivariate analysis for risk factors associated with only intestinal helminth infection of the study population.

Variables	No. participants	No. infections	Infection rate (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Sex					
Male	233	15	6.4% (3.6–10.4%)	1.00	
Female	107	11	10.3% (5.2–17.6%)	1.66 (0.74–3.76)	
Age (years)					
≤60 years old	154	10	6.5% (3.2–11.6%)	1.00	
>60 years old	186	16	8.6% (5.0–13.6%)	1.36 (0.60–3.08)	
Education					
Elementary school and above	169	10	5.9% (2.9–10.6%)	1.00	
Illiteracy	171	16	9.4% (5.4–14.7%)	1.64 (0.72–3.73)	
Marital status					
Other	78	5	6.4% (2.1–14.3%)	1.00	
Married	262	21	8.0% (5.0–12.0%)	1.27 (0.46–3.49)	
Body mass index (BMI)					
>18	208	10	4.8% (2.3–8.7%)	1.00	1.00
≤18	132	16	12.1% (7.1–18.9%)	2.73 (1.20–6.22)	3.32 (1.39–7.91)
Anemia					
No	268	14	5.2% (2.9–8.6%)	1.00	1.00
Yes	72	12	16.7% (8.9–27.3%)	3.63 (1.60–8.24)	3.40 (1.44–8.02)
No. of cutting board in kitchen					
>1	18	0	0.0% (0.0–18.5%)	1.00	
Only one	322	26	8.1% (5.3–11.6%)	NA	
No. of cooking knife in kitchen					
>1	106	6	5.7% (2.1–11.9%)	1.00	
Only one	234	20	8.6% (5.3–12.9%)	1.56 (0.61–4.00)	
Washed fruits and vegetables before eating raw					
Often or always	168	7	4.2% (1.7–8.4%)	1.00	
Never or occasionally	172	19	11.0% (6.8–16.7%)	2.86 (1.17–6.98)	
Ever walked barefoot					
No	149	7	4.7% (1.9–9.4%)	1.00	
Yes	191	19	9.9% (6.1–15.1%)	2.24 (0.92–5.48)	
Washed hands before meals					
Often or always	207	11	5.3% (2.7–9.3%)	1.00	
Never or occasionally	133	15	11.3% (6.4–17.9%)	2.26 (1.01–5.10)	
Washed hands after defecating					
Often or always	187	10	5.3% (2.6–9.6%)	1.00	
Never or occasionally	153	16	10.5% (6.1–16.4%)	2.07 (0.91–4.70)	
Raised pets (e.g., cat, dog)					
No	139	8	5.8% (2.5–11.0%)	1.00	
Yes	201	18	9.0% (5.4–13.8%)	1.61 (0.68–3.82)	
Raised poultry or livestock (e.g., chicken, duck, pig)					
No	124	7	5.6% (2.3–11.3%)	1.00	
Yes	216	19	8.8% (5.4–13.4%)	1.61 (0.66–3.95)	
Annual labor time in farmlands					
≤2 months	248	12	4.8% (2.5–8.3%)	1.00	
>2 months	92	14	15.2% (8.6–24.2%)	3.53 (1.57–7.95)	
Ever labored barefoot in farmlands					
No	224	9	4.0% (1.8–7.5%)	1.00	1.00
Yes	116	17	14.7% (8.8–22.4%)	4.10 (1.77–9.52)	4.54 (1.88–10.92)
Duration of anti-MTB treatment					
1 month	42	3	7.1% (1.5–19.5%)	1.00	
2 months	38	2	5.3% (0.6–17.7%)	0.72 (0.11–4.57)	
3 months	34	3	8.8% (1.8–23.7%)	1.26 (0.24–6.67)	
4 months	63	5	7.9% (2.6–17.6%)	1.12 (0.25–4.96)	
5 months	74	7	9.5% (3.9–18.5%)	1.36 (0.33–5.56)	
6 months	89	6	6.7% (2.5–14.1%)	0.94 (0.22–3.96)	

CI, confidence interval; OR, odds ratio; NA, not applicable; MTB, *Mycobacterium tuberculosis*.

UNCORRECTED PROOF

CI = 3.9–18.5%], 6.7% [95% CI = 2.5–14.1%], respectively), which showed that there was no significant relationship between prevalence of IHIs and duration of treatment.

4. Discussion

The second national survey showed that *G. lamblia* was the most prevalent intestinal protozoan in five provinces, including Beijing, Shanghai, Shandong, Henan and Xinjiang, where only IPIs were reported (Disease Control Bureau of the Ministry of Health and National Institute of Parasitic Diseases of the Chinese Center for Disease Control and Prevention, 2008). Since then, there were only two studies, one in Yunnan Province in southwestern China in 2006 and the other in Anhui Province in central China in 2008, both of which reported that *B. hominis* was the most prevalent intestinal protozoan in rural areas (20.0% and 22.1%, respectively) (Steinmann et al., 2008; Tian et al., 2012). In our current study, we observed that an overall rate of only IPI to be 7.3% among patients with PTB, and *B. hominis* was also the most prevalent intestinal protozoan (6.0%). Compared with the second national survey, findings of these studies give a clue that infection spectrum of intestinal protozoa might have gradually changed over recent years in P. R. China. Similarly, infection spectrum of intestinal helminth maybe has changed in central China. The second national survey showed that *A. lumbricoides* was the most prevalent intestinal helminth (12.7%) (Disease Control Bureau of the Ministry of Health and National Institute of Parasitic Diseases of the Chinese Center for Disease Control and Prevention, 2008). However, we found that overall rate of only IHI

Please cite this article in press as: Li, X.-X., et al., Prevalence and risk factors of intestinal protozoan and helminth infections among pulmonary tuberculosis patients without HIV infection in a rural county in P. R. China. Acta Trop. (2015), http://dx.doi.org/10.1016/j.actatropica.2015.05.001

was 7.0% among patients with PTB, and hookworm was the most prevalent intestinal helminth (4.3%). Other study in Anhui Province in central China in 2008 also showed the same results that hookworm was most prevalent (3.6%) in intestinal helminths whose overall rate was 4.3% among HIV positive patients (Tian et al., 2012).

Among many possible impact factors, we found that underweight was independently associated with only IPI and underweight and anemia were independently associated with only IHI among patients with PTB. Given that *B. hominis* was the most prevalent intestinal protozoan in this study, the impact factor for IPI maybe had a closer relationship with this protozoan. As a common clinical presentation of TB (Rathman et al., 2003), weight loss not only can be caused by chronic diarrhea that is the most common symptom with stools positive only for *B. hominis* (Al-Fellani et al., 2007; El-Shazly et al., 2005; Juckett and Trivedi, 2011), but also the odds of being underweight was significantly higher in intestinal helminth infected individuals (Degarege et al., 2013). Similarly, anemia is a common hematological abnormality in patients with TB as well as IHI. A study in Korea showed that 31.9% patients with TB had anemia on diagnosis of TB (Lee et al., 2006), and other study in Ethiopia suggested that infection with multiple intestinal helminths was associated with lower hemoglobin level (Degarege et al., 2013). As indicators of physical health, underweight and anemia may suggest malnutrition or poor health. However, it is difficult to determine the chronological sequence, i.e., the causal relationship of underweight and anemia, PTB, IPI and IHI because the latter three are debilitating diseases and often widespread in poor areas with population suffering from malnutrition or poor health. Therefore, underweight and anemia cannot be identified as the etiological factors of IPI and IHI among patients with PTB, but are likely to be more severe under the conditions of co-infection of PTB and intestinal protozoa or helminths. In any case, the improvement of nutrition status and health is beneficial to prevention and inhibiting deterioration of co-infection of PTB and intestinal protozoa or helminths.

For the transmission of *B. hominis*, the fecal-oral pathway is most accepted, which involves only the cyst form of this protozoan (Yoshikawa et al., 2004). Noël et al. (2003) deemed that animals such as pigs and dogs could in fact be acting as a large reservoir of *B. hominis*, which could be capable of human infection. This notion was supported by a genomic study with the finding that subgroups of *Blastocystis* isolates from humans, pigs, and a horse were similar and *Blastocystis* isolates from a pig and a horse were monophyletic and closely related to *B. hominis* from humans with 92–94% identity (Thathaisong et al., 2003), and by an epidemiological survey with the finding that infection was more common in people living in proximity to farm animals or pets (Doyle et al., 1990). In this study, we found that patients with PTB who raised poultry or livestock (e.g., chicken, duck, pig) were more likely to be infected with *B. hominis*, which is consistent with findings of the above-mentioned studies.

We noticed that the risk (OR) of getting IHI was higher for people laboring barefoot in farmlands than those who are underweight and anemia, which may suggest that unwholesome way of laboring in farmlands has greater effect on IHI than other risk factors among patients with PTB. The central China is an agricultural area where most of the population engaged in farm work. Therefore, unwholesome way of laboring in farmlands also increases risks of IHI among general population. A study in Anhui Province in central China indicated that laboring barefoot in farmlands is one of the risk factors for *A. lumbricoides* infection among local residents (Guo et al., 2004). Moreover, as one of the poor hygiene behaviors, not wearing shoes outside or walking barefoot also were the main risk factors for IHI among local population in poor communities in Nepal, Vietnam and Ethiopia (Le Hung et al., 2005; Parajuli et al., 2014; Taye et al., 2013). An evaluation for the control program of soil-transmitted helminth (STH) infections in rural Malaysia and a systematic review for STH infection around the world showed that wearing shoes outside was associated with reduced odds of infection with any STHs (Nasr et al., 2013; Strunz et al., 2014). Hence, there is a great need for a proper health education for good hygiene habits, particularly wearing shoes outside or in farmlands, to enhance prevention of IHI in TB high-burden areas of P. R. China.

In P. R. China, the main regimen of anti-MTB treatment is the combined use of Isoniazid, Rifampicin, Pyrazinamide, Ethambutol, Streptomycin or their derivatives (Disease Control Bureau of the Ministry of Health et al., 2009). Some studies found that one or more of these drugs had significant effect in vivo only against *Leishmania* spp. (Conti and Parenti, 1983; Mendez et al., 2009), *Plasmodium* spp. (Aditya et al., 2010; Pukrittayakamee et al., 1994), *Toxoplasma gondii* (Araujo et al., 1996), *Wuchereria bancrofti* (Debrah et al., 2011), *Brugia malayi* (Rao and Well, 2002), *Onchocerca gutturosa* (Townson et al., 2006), *Onchocerca lienalis* and *Brugia pahangi* (Townson et al., 2000), but there has not been a study reporting that anti-MTB drugs have effect against intestinal parasites, which may suggest that anti-MTB drugs only have effect against parasites that can enter the circulations of blood and lymph and even the cells in humans, and have not effect against parasites that only exist in intestines. These may partly explain that the duration of anti-MTB treatment has not significant effect on IPI and IHI among patients with PTB in this study. However, it should not be neglected that anti-MTB treatment can improve host immune response (de Oliveira et al., 2014; Sai Priya et al., 2010; Zhang et al., 2014), which theoretically should affect IPI and IHI during treatment of patients with PTB.

There were evidences that protozoan pathogens such as *Plasmodium*, *Leishmania*, *Trypanosoma* and *Entamoeba* evaded the host immune system by penetrating and multiplying within cells, varying their surface antigens, eliminating their protein coat, modulating the host immune response, and so on (Sacks and Sher, 2002; Zambrano-Villa et al., 2002). Moreover, other studies confirmed that hookworm infections decreased the ability of the immune system to respond to hookworm and bystander antigens (Kalinkovich et al., 1998; Olatunde and Onyemelukwe, 1994; Onyemelukwe and Musa, 2001). Hence, it is worthwhile further investigating whether host immune response that is improved by anti-MTB treatment is evaded by intestinal protozoa or decreased by IHI among patients with co-infection of PTB and intestinal protozoa or helminths.

There were several limitations to the data used in this study. Some information such as facilities in kitchen, washing hands and labor time in farmlands might be inaccurate because they were collected by self reporting. Additionally, the excluded participants were generally younger than those included in this study because currently the main trend of population flow in China is that a large number of the middle-aged populations in rural areas drift into the cities to seek works. Actually migrant populations have little time in their hometowns and are almost not a part of rural populations. In view of these limitations, the findings should be interpreted carefully when being generalized to the larger population and compared with results from other studies.

In conclusion, 7.3% of patients with PTB were only infected with intestinal protozoa, among which infection rates of *B. hominis*, *Entamoeba* spp., and *T. hominis* were 6.0%, 1.1% and 0.3%, respectively; 7.0% were only infected with intestinal helminths, among which infection rates of hookworm, *T. trichiura*, *A. lumbricoides* and *C. sinensis* were 4.3%, 1.9%, 0.5% and 0.3%, respectively; 0.5% were simultaneously infected with intestinal protozoa and helminths. Underweight and raised poultry or livestock (e.g., chicken, duck, pig) were independently associated with IPI, and underweight, anemia and ever labored barefoot in farmlands were independently associated with IHI among patients with PTB. Anti-MTB treatment did not influence prevalence of IPI and IHI among patients with

UNCORRECTED PROOF

Please cite this article in press as: Li, X.-X., et al., Prevalence and risk factors of intestinal protozoan and helminth infections among pulmonary tuberculosis patients without HIV infection in a rural county in P. R. China. Acta Trop. (2015), http://dx.doi.org/10.1016/j.actatropica.2015.05.001

PTB. In any case, preventing malnutrition, avoiding unprotected contact with reservoirs of protozoa and improving health education for good hygiene habits, particularly wearing shoes outside, are beneficial for prevention of IPI and IHI among patients with PTB.

Authors' contribution

X-X L, J-X C, L-X W and X-N Z conceived and designed the study, X-X L, L-G T, Y-P Z, S-P D, X-G H, J L, F-F W, Y W, X-M Y, L-G H, Q-Y Y, H-W Z and B-L X performed the study, X-X L and L-G T analyzed the data, X-X L wrote the first draft of the manuscript, J-X C, L-X W and B-L X provided constructive opinions and suggestions, X-N Z provided strategic advices and assisted with editing of the manuscript. All the authors read and approved the final version of the manuscript.

Competing interest

The authors declare that they have no competing interests.

Acknowledgements

We appreciate the coordination of Tian-Ping Wang from Anhui Province Institute of Parasitic Disease Control, Xu-Dong Zhao and Yan Deng from Henan Province Center for Disease Control and Prevention. We are also grateful to the staff at Gushi County Center for Disease Control and Prevention and health workers at township hospitals and clinics of villages in Gushi County, and to all the participants sharing their experiences with us. Great thanks to Peiling Yao for her help on language improvement for the manuscript. This work is supported by National Science & Technology Major Program (grant no. 2012ZX10004-220).

References

Abate, E., Belayneh, M., Gelaw, A., Idh, J., Getachew, A., Alemu, S., Diro, E., Fikre, N., Britton, S., Elias, D., Aseffa, A., Stendahl, O., Schon, T., 2012. The impact of asymptomatic helminth co-infection in patients with newly diagnosed tuberculosis in north-west Ethiopia. PLoS ONE 7, e42901.

Aditya, N.P., Patankar, S., Madhusudhan, B., 2010. Assessment of in vivo antimalarial activity of rifampicin, isoniazide, and ethambutol combination therapy. Parasitol. Res. 106, 1481–1484.

Al-Fellani, M.A., Khan, A.H., Al-Gazoui, R.M., Zaid, M.K., Al-Ferjani, M.A., 2007. Prevalence and clinical features of *Blastocystis hominis* Infection among patients in Sebha, Libya. Sultan Qaboos Univ. Med. J. 7, 35–40.

Araujo, F.G., Khan, A.A., Remington, J.S., 1996. Rifapentine is active in vitro and in vivo against *Toxoplasma gondii*. Antimicrob. Agents Chemother. 40, 1335–1337.

Choi, W.Y., Yoo, J.E., Kim, W.G., Yun, B.H., Kim, S.G., Yoo, W.H., 1984. Prevalence of intestinal helminthic infections and skin tests for paragonimus and clonorchis in tuberculosis patients. Kisaengchunghak Chapchi 22, 209–214.

Conti, R., Parenti, F., 1983. Rifampin therapy for brucellosis, flavobacterium meningitis, and cutaneous leishmaniasis. Rev. Infect. Dis. 5 (Suppl. 3), S600–S605.

de Oliveira, L.R., Peresi, E., Golim Mde, A., Gatto, M., Araujo Junior, J.P., da Costa, E.A., Ayres, J.A., Fortes, M.R., Calvi, S.A., 2014. Analysis of Toll-like receptors, iNOS and cytokine profiles in patients with pulmonary tuberculosis during anti-tuberculosis treatment. PLOS ONE 9, e88572.

Debrah, A.Y., Mand, S., Marfo-Debrekyei, Y., Batsa, L., Albers, A., Specht, S., Klarmann, U., Pfarr, K., Adjei, O., Hoerauf, A., 2011. Macrofilaricidal activity in *Wuchereria bancrofti* after 2 weeks treatment with a combination of rifampicin plus doxycycline. J. Parasitol. Res. 2011, 201617.

Degarege, A., Animut, A., Medhin, G., Legesse, M., Erko, B., 2013. The association between multiple intestinal helminth infections and blood group, anaemia and nutritional status in human populations from Dore Bafeno, southern Ethiopia. J. Helminthol., 1–8.

Disease Control Bureau of the Ministry of Health, Chinese Center for Disease Control and Prevention, 2011. Report on the Fifth National Tuberculosis Epidemiological Survey in China-2010. Military Medical Science Press, Beijing, China.

Disease Control Bureau of the Ministry of Health, Department of Medical Administration of the Ministry of Health, Chinese Center for Disease Control and Prevention, 2009. Guidelines for Implementing the National Tuberculosis Control Program in China (2008). Peking Union Medical College Press, Beijing, China.

Disease Control Bureau of the Ministry of Health, National Institute of Parasitic Diseases of the Chinese Center for Disease Control and Prevention, 2008. Report on the National Survey of Current Status of Major Human Parasitic Diseases in China. People's Medical Publishing House, Beijing, China.

Doyle, P.W., Helgason, M.M., Mathias, R.G., Proctor, E.M., 1990. Epidemiology and pathogenicity of *Blastocystis hominis*. J. Clin. Microbiol. 28, 116–121.

El-Shazly, A.M., Abdel-Magied, A.A., El-Beshbishi, S.N., El-Nahas, H.A., Fouad, M.A., Monib, M.S., 2005. *Blastocystis hominis* among symptomatic and asymptomatic individuals in Talkha Center, Dakahlia Governorate, Egypt. J. Egypt. Soc. Parasitol. 35, 653–666.

Ferreira, M.S., 2000. Infections by protozoa in immunocompromised hosts. Mem. Inst. Oswaldo Cruz 95 (Suppl. 1), 159–162.

Franke, M.F., Del Castillo, H., Pereda, Y., Lecca, L., Fuertes, J., Cardenas, L., Becerra, M.C., Bayona, J., Murray, M., 2014. Parasite infection and tuberculosis disease among children: a case-control study. Am. J. Trop. Med. Hyg. 90, 279–282.

Guo, J.D., Shen, G.J., Wu, W.D., Li, Q.Y., Yin, X.M., Zhou, L., Ji, H., 2004. Distribution and risk factors of *Ascaris* infection in Anhui Province. Zhongguo xue xi chong bing fang zhi za zhi 16, 456–459.

Juckett, G., Trivedi, R., 2011. Evaluation of chronic diarrhea. Am. Fam. Physician 84, 1119–1126.

Kalinkovich, A., Weisman, Z., Greenberg, Z., Nahmias, J., Eitan, S., Stein, M., Bentwich, Z., 1998. Decreased CD4 and increased CD8 counts with T cell activation is associated with chronic helminth infection. Clin. Exp. Immunol. 114, 414–421.

Kashuba, E.A., Rusakova, L.I., 1992. Anthelmintic therapy of opisthorchiasis in patients with active tuberculosis. Probl. Tuberk., 33–36.

Katz, N., Chaves, A., Pellegrino, J., 1972. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev. Inst. Med. Trop. Sao Paulo 14, 397–400.

Lawn, S.D., Zumla, A.I., 2011. Tuberculosis. Lancet 378, 57–72.

Le Hung, Q., de Vries, P.J., Giao, P.T., Binh, T.Q., Nam, N.V., Kager, P.A., 2005. Intestinal helminth infection in an ethnic minority commune in southern Vietnam. Southeast Asian J. Trop. Med. Public Health 36, 623–628.

Lee, S.W., Kang, Y.A., Yoon, Y.S., Um, S.W., Lee, S.M., Yoo, C.G., Kim, Y.W., Han, S.K., Shim, Y.S., Yim, J.J., 2006. The prevalence and evolution of anemia associated with tuberculosis. J. Korean Med. Sci. 21, 1028–1032.

Leelayoova, S., Taamasri, P., Rangsin, R., Naaglor, T., Thathaisong, U., Mungthin, M., 2002. In-vitro cultivation: a sensitive method for detecting *Blastocystis hominis*. Ann. Trop. Med. Parasitol. 96, 803–807.

Li, X.X., Chen, J.X., Wang, L.X., Tian, L.G., Zhang, Y.P., Dong, S.P., Hu, X.G., Liu, J., Wang, F.F., Wang, Y., Yin, X.M., He, L.J., Yan, Q.Y., Zhang, H.W., Xu, B.L., Zhou, X.N., 2014. Intestinal parasite co-infection among pulmonary tuberculosis cases without human immunodeficiency virus infection in a rural county in China. Am. J. Trop. Med. Hyg. 90, 106–113.

Lu, Z., Zhong, N., 2008. Internal Medicine. People's Medical Publishing House, Beijing, China.

Marcos, L.A., Gotuzzo, E., 2013. Intestinal protozoan infections in the immunocompromised host. Curr. Opin. Infect. Dis. 26, 295–301.

Mendez, S., Traslavina, R., Hinchman, M., Huang, L., Green, P., Cynamon, M.H., Welch, J.T., 2009. The antituberculosis drug pyrazinamide affects the course of cutaneous leishmaniasis in vivo and increases activation of macrophages and dendritic cells. Antimicrob. Agents Chemother. 53, 5114–5121.

Metenou, S., Babu, S., Nutman, T.B., 2012. Impact of filarial infections on coincident intracellular pathogens: *Mycobacterium tuberculosis* and *Plasmodium falciparum*. Curr. Opin. HIV AIDS 7, 231–238.

Nasr, N.A., Al-Mekhlafi, H.M., Ahmed, A., Roslan, M.A., Bulgiba, A., 2013. Towards an effective control programme of soil-transmitted helminth infections among Orang Asli in rural Malaysia. Part 2: Knowledge, attitude, and practices. Parasit. Vectors 6, 28.

Noël, C., Peyronnet, C., Gerbod, D., Edgcomb, V.P., Delgado-Viscogliosi, P., Sogin, M.L., Capron, M., Viscogliosi, E., Zenner, L., 2003. Phylogenetic analysis of Blastocystis isolates from different hosts based on the comparison of small-subunit rRNA gene sequences. Mol. Biochem. Parasitol. 126, 119–123.

Olatunde, B.O., Onyemelukwe, G.C., 1994. Immunosuppression in Nigerians with hookworm infection. Afr. J. Med. Med. Sci. 23, 221–225.

Onyemelukwe, G.C., Musa, B.O., 2001. T-lymphocyte subsets in patients with hookworm infection in Zaria, Nigeria. Afr. J. Med. Med. Sci. 30, 255–259.

Parajuli, R.P., Fujiwara, T., Umezaki, M., Konishi, S., Takane, E., Maharjan, M., Tachibana, K., Jiang, H.W., Pahari, K., Watanabe, C., 2014. Prevalence and risk factors of soil-transmitted helminth infection in Nepal. Trans. R. Soc. Trop. Med. Hyg. 108, 228–236.

Pukrittayakamee, S., Viravan, C., Charoenlarp, P., Yeamput, C., Wilson, R.J., White, N.J., 1994. Antimalarial effects of rifampin in Plasmodium vivax malaria. Antimicrob. Agents Chemother. 38, 511–514.

Ralston, K.S., Petri Jr., W.A., 2011. Tissue destruction and invasion by *Entamoeba histolytica*. Trends Parasitol. 27, 254–263.

Range, N., Magnussen, P., Mugomela, A., Malenganisho, W., Changalucha, J., Temu, M.M., Mngara, J., Krarup, H., Friis, H., Andersen, A.B., 2007. HIV and parasitic co-infections in tuberculosis patients: a cross-sectional study in Mwanza, Tanzania. Ann. Trop. Med. Parasitol. 101, 343–351.

Rao, R., Well, G.J., 2002. In vitro effects of antibiotics on *Brugia malayi* worm survival and reproduction. J. Parasitol. 88, 605–611.

Rathman, G., Sillah, J., Hill, P.C., Murray, J.F., Adegbola, R., Corrah, T., Lienhardt, C., McAdam, K.P., 2003. Clinical and radiological presentation of 340 adults with smear-positive tuberculosis in The Gambia. Int. J. Tuberc. Lung Dis. 7, 942–947.

Resende Co, T., Hirsch, C.S., Toossi, Z., Dietze, R., Ribeiro-Rodrigues, R., 2007. Intestinal helminth co-infection has a negative impact on both anti-*Mycobacterium tuberculosis* immunity and clinical response to tuberculosis therapy. Clin. Exp. Immunol. 147, 45–52.

UNCORRECTED PROOF

Ruttgers, H., 1983. Epidemiology and symptomatology of trichomoniasis. Gynakol. Rundsch. 23 (Suppl. 2), 3–9.

Sacks, D., Sher, A., 2002. Evasion of innate immunity by parasitic protozoa. Nat. Immunol. 3, 1041–1047.

Sai Priya, V.H., Latha, G.S., Hasnain, S.E., Murthy, K.J., Valluri, V.L., 2010. Enhanced T cell responsiveness to Mycobacterium bovis BCG r32-kDa Ag correlates with successful anti-tuberculosis treatment in humans. Cytokine 52, 190–193.

Steinmann, P., Du, Z.W., Wang, L.B., Wang, X.Z., Jiang, J.Y., Li, L.H., Marti, H., Zhou, X.N., Utzinger, J., 2008. Extensive multiparasitism in a village of Yunnan province, People's Republic of China, revealed by a suite of diagnostic methods. Am. J. Trop. Med. Hyg. 78, 760–769.

Strunz, E.C., Addiss, D.G., Stocks, M.E., Ogden, S., Utzinger, J., Freeman, M.C., 2014. Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis. PLoS Med. 11, e1001620.

Taye, B., Alemayehu, B., Birhanu, A., Desta, K., Addisu, S., Petros, B., Davey, G., Tsegaye, A., 2013. Podoconiosis and soil-transmitted helminths (STHs): double burden of neglected tropical diseases in Wolaita zone, rural Southern Ethiopia. PLoS Negl. Trop. Dis. 7, e2128.

Thathaisong, U., Worapong, J., Mungthin, M., Tan-Ariya, P., Viputtigul, K., Sudatis, A., Noonai, A., Leelayoova, S., 2003. Blastocystis isolates from a pig and a horse are closely related to *Blastocystis hominis*. J. Clin. Microbiol. 41, 967–975.

Tian, L.G., Chen, J.X., Wang, T.P., Cheng, G.J., Steinmann, P., Wang, F.F., Cai, Y.C., Yin, X.M., Guo, J., Zhou, L., Zhou, X.N., 2012. Co-infection of HIV and intestinal parasites in rural area of China. Parasit. Vectors 5, 36.

Townson, S., Hutton, D., Siemienska, J., Hollick, L., Scanlon, T., Tagboto, S.K., Taylor, M.J., 2000. Antibiotics and Wolbachia in filarial nematodes: antifilarial activity of rifampicin, oxytetracycline and chloramphenicol against *Onchocerca gutturosa*, *Onchocerca lienalis* and *Brugia pahangi*. Ann. Trop. Med. Parasitol. 94, 801–816.

Townson, S., Tagboto, S., McGarry, H.F., Egerton, G.L., Taylor, M.J., 2006. Onchocerca parasites and Wolbachia endosymbionts: evaluation of a spectrum of antibiotic types for activity against *Onchocerca gutturosa* in vitro. Filaria J. 5, 4.

Ustun, S., Turgay, N., 2006. *Blastocystis hominis* and bowel diseases. Turkiye parazitolojii dergisi 30, 72–76.

Vasil'ev, A.V., Shenderova, R.I., Ginzburg, Z.I., Vasil'ev, V.I., 1989. Tuberculosis of the lungs complicated by opisthorchiasis under conditions of the extreme north. Probl. Tuberk., 41–44.

Yang, Y.R., Gray, D.J., Ellis, M.K., Yang, S.K., Craig, P.S., McManus, D.P., 2009. Human cases of simultaneous echinococcosis and tuberculosis – significance and extent in China. Parasit. Vectors 2, 53.

Yoshikawa, H., Yoshida, K., Nakajima, A., Yamanari, K., Iwatani, S., Kimata, I., 2004. Fecal-oral transmission of the cyst form of *Blastocystis hominis* in rats. Parasitol. Res. 94, 391–396.

Zambrano-Villa, S., Rosales-Borjas, D., Carrero, J.C., Ortiz-Ortiz, L., 2002. How protozoan parasites evade the immune response. Trends Parasitol. 18, 272–278.

Zhang, M., Zeng, G., Yang, Q., Zhang, J., Zhu, X., Chen, Q., Suthakaran, P., Zhang, Y., Deng, Q., Liu, H., Zhou, B., Chen, X., 2014. Anti-tuberculosis treatment enhances the production of IL-22 through reducing the frequencies of regulatory B cell. Tuberculosis (Edinb) 94, 238–244.

UNCORRECTED PROOF

Please cite this article in press as: Li, X.-X., et al., Prevalence and risk factors of intestinal protozoan and helminth infections among pulmonary tuberculosis patients without HIV infection in a rural county in P. R. China. Acta Trop. (2015), http://dx.doi.org/10.1016/j.actatropica.2015.05.001