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Seroprevalence and risk factors of *Toxoplasma gondii* infection in humans in East Hararghe Zone, Ethiopia

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SUMMARY

A cross-sectional study was conducted from April 2013 to September 2013 to determine the seroprevalence and possible risk factors for human *Toxplasma gondii* infection in East Hararghe Zone, Ethiopia. Serum samples were analysed using direct agglutination test, and immunosorbent agglutination assay for detecting IgG ($n = 354$) and IgM ($n = 167$) *T. gondii* antibodies. The *T. gondii* IgG and IgM seroprevalences were 65·8% [95% confidence interval (CI) 60·62–70·75] and 8·98% (95% CI 5·11–14·38), respectively. Gender difference in IgG seroprevalence was not significant ($P > 0·05$), but 69·5% of adults exhibited an IgG seroresponse to *T. gondii*. Pregnant women showed 76·4% and 9·3% seropositivity to IgG and IgM antibodies, respectively. Multivariable logistic regression analysis identified the risk factors significantly associated with *T. gondii* seropositivity were district [odds ratio (OR) 2·24, 95% CI 1·25–4·01, $P = 0·007$], pipe water source (OR 6·70, 95% CI 2·70–16·64, $P < 0·001$), age, with adults (OR 4·32, 95% CI 1·91–9·75, $P < 0·001$), and keeping cats in the home (OR 2·01, 95% CI 1·11–3·65, $P = 0·021$). The high seroprevalence of toxoplasmosis in the human population in the study area and the corresponding level of IgM seropositivity may be indicative of reactivation or recent infection and further studies on the status of congenital toxoplasmosis in the study area merit consideration.

Key words: Direct agglutination test, Ethiopia, ISAGA, risk factor, seroprevalence, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasmosis is caused by *Toxoplama gondii* and is widespread in humans and animals throughout the world. Domestic cats and other feline species play a key role in the life cycle of the parasite by excreting oocysts into the environment. Many animals serve as an intermediate host and maintain the infection by containing the viable tissue cyst. The most common sources for acquisition of *T. gondii* infection by humans are ingestion of contaminated raw or undercooked meat, or water containing infective oocysts, or congenitally from mother to foetus during pregnancy [1–4].

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Consumption of non-pasteurized goat's milk is also associated with human toxoplasmosis [5, 6] and exposure of women to primary *T. gondii* infection during pregnancy can result in abortion, and severe disease of the foetus [7, 8].

Estimates of seroprevalence rates for *T. gondii* infection vary with geographical area and range from 16% to 40% in England and USA, 50–80% in Europe, and parts of Africa [1], and 30–65% in the general population elsewhere in the world [9, 10]. Likewise, reported seroprevalence rates in women of child-bearing age range from 4% to 77% [7] and when pregnant, this group as well as immunocompromised individuals are at higher risk of developing serious disease [7, 11]. In Ethiopia, seroprevalence rates of 60–96·7% have been documented in different groups of people [11, 12–16], but the epidemiology of toxoplasmosis in the East Hararghe Zone of Ethiopia remains unknown. In this paper we present the results of a seroprevalence survey of *T. gondii* infection in people in three districts of this study area and report statistical analyses of associated risk factors.

MATERIALS AND METHODS

Study area

The study was carried out in Gursum, Babile and Haramaya districts of East Hararghe Zone, Ethiopia. Gursum district (9° 7′–32° N latitude, 42° 17′–42° 38′ E longitude) has an altitude ranging from 1200 to 2950 metres above sea level (masl); Babile district (8° 9′–9° 23′ N latitude, 41° 16′–41° 46′ E longitude) has an altitude ranging from 950 to 2000 masl; and Haramaya district (9° 9′–9° 32′ N latitude, 41° 50′–42° 05′ E longitude) has an altitude ranging from 1600 to 2140 masl. Three health centres were selected based on their accessibility to urban and rural people and provision of various health services to large numbers of the population.

Study design and population

A cross-sectional study on human toxoplasmosis in people from rural and urban areas, visiting the selected health centres was conducted from April 2013 to September 2013. The sample size was determined by random sampling [17] based on the expected prevalence of 74·4%, 95% confidence interval (CI) with 5% precision [12]. The calculated sample size ($n = 296$) was increased by 20% ($n = 354$) for better precision and the study subjects were identified by systematic random sampling with the total number based proportionally on the number of people visiting the centres (39 000 in 2012). The study was approved by the Ethical Review Board of Oromia Regional State Health Bureau (ref. no. BEFO/HBTFH/1-8/2297). A questionnaire survey was used to collect data from study participants during blood sample collection and covered socio-demographic characteristics such as age, sex, residence and possible risk factors known to be associated with *T. gondii* infection (Table 1).

Serological tests

Venous blood samples were collected from consenting study participants and sera were stored at −20 °C. *T. gondii* specific IgG antibodies were detected using a direct agglutination test (DAT; Toxo-Screen DA, bioMérieux SA, France) according to the manufacturer's instructions at screening dilutions of 1/40 and 1/4000; a positive reaction at either dilution was taken as evidence of infection. Serum *Toxoplasma* IgM antibodies were determined with the immunosorbent agglutination assay (ISAGA; Toxo-screen ISAGA, bioMérieux) according to the manufacturer's protocol and reactions were scored by the ISAGA index of 0–5 (negative); 6–8 (borderline) and 9–12 (positive).

Statistical analysis

Data were recorded in a Microsoft Excel spreadsheet and analysed using Stata v. 11·0 for Windows (Stata Corp., USA). Seroprevalence was expressed as the number of seropositive samples in the total number of samples tested. A χ^2 test was used to determine associations between risk factors and seropositivity; and the strength of these associations were measured by logistic regression analysis; the first level was taken as a reference to determine levels of significance. Non-collinear variables of $P \leqslant 0{\cdot}20$ in univariable analysis were entered into a multivariable regression model and goodness-of-fit was assessed using the Hosmer–Lemeshow test [18]. The level of statistical significance was set at $P \leqslant 0{\cdot}05$.

RESULTS

A total of 354 people participated in the study (age range 5–70 years, median 30 years) with a higher

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Table 1. *Seroprevalence of* T. gondii *antibodies in relation to demography and risk factors in study districts, East Hararghe Zone, Ethiopia*

		IgG ($n = 354$)			IgM ($n = 167$)		
Factor	Category	Frequency (%)	No. positive (%)	*P* value	Frequency (%)	No. positive (%)	*P* value
District	Haremaya	120 (33·9)	70 (58·3)	0·071	49 (29·3)	4 (8·2)	0·813
	Gursum	105 (29·7)	70 (66·7)		44 (26·4)	5 (11·4)	
	Babile	129 (36·4)	93 (65·8)		74 (44·3)	6 (8·1)	
Residence	Rural	284 (80·2)	174 (61·3)	<0·001*	133 (79·6)	12 (9·0)	0·971
	Urban	70 (19·8)	59 (84·3)		34 (20·4)	3 (8·8)	
Sex	Male	164 (46·3)	107 (65·2)	0·832	—	—	—
	Female	190 (53·7)	126 (66·3)		—	—	—
Age, years	5–18	36 (10·2)	14 (38·9)	0·002*	10 (6·0)	2 (20·0)	0·189
	19–35	206 (58·2)	141 (68·5)		118 (70·7)	12 (10·2)	
	⩾36	112 (31·6)	78 (69·5)		39 (23·4)	1 (2·6)	
Meat consumption	Cooked	259 (73·2)	167 (64·5)	0·380	140 (83·8)	12 (8·6)	0·673
	Raw	95 (26·8)	66 (69·5)		27 (16·2)	3 (11·1)	
Source of meat	Shoats meat†	29 (8·2)	16 (55·2)	0·010*	8 (4·8)	1 (12·5)	0·629
	Mixed‡	300 (84·7)	194 (64·7)		151 (90·4)	14 (9·3)	
	Beef	25 (7·1)	23 (92·0)		8 (4·8)	0 (0·00)	
Milk consumption	Boiled	105 (29·7)	66 (62·9)	0·453	65 (38·9)	6 (9·1)	0·965
	Both§	178 (50·3)	116 (65·2)		64 (38·3)	6 (9·5)	
	Raw	71 (20·1)	51 (71·8)		38 (22·8)	3 (7·9)	
Vegetable consumption	Cooked	146 (41·2)	94 (64·4)	0·633	83 (49·7)	5 (6·0)	0·184
	Raw	208 (58·8)	139 (66·8)		84 (50·3)	10 (11·9)	
Cat kept at home	No	258 (72·9)	160 (62·0)	0·013*	131 (78·4)	9 (6·9)	0·069
	Yes	96 (27·1)	73 (76·0)		36 (21·6)	6 (16·7)	
Presence of feral cats	No	160 (45·2)	95 (59·4)	0·020*	76 (45·5)	5 (6·6)	0·321
	Yes	194 (54·8)	138 (71·1)		91 (54·5)	10 (11·9)	
Water source	Stream	63 (17·8)	33 (52·4)	<0·001*	27 (16·2)	2 (7·4)	0·947
	Mixed‖	221 (62·4)	141 (63·8)		106 (63·5)	10 (9·4)	
	Pipe water	70 (19·8)	59 (84·3)		34 (20·4)	3 (8·8)	
Status of pregnancy	No	124 (77·0)	77 (62·1)	0·081	124 (73·1)	11 (8·9)	0·932
	Yes	43 (23·0)	33 (76·4)		43 (25·8)	4 (9·3)	
Stage of pregnancy ($n = 43$)	2nd trimester	19 (44·2)	12 (63·2)	0·117	19 (44·2)	3 (15·8)	0·677
	3rd trimester	14 (32·6)	12 (85·7)		14 (32·6)	1 (7·1)	
	1st trimester	10 (23·2)	9 (90·0)		10 (23·3)	0 (0·00)	

† Shoats meat (sheep and goat).
‡ Mixed (shoats meat and beef).
§ Both (boiled and raw).
‖ Mixed (stream and well water).
* Significant.

proportion of females (53·7%) to males (46·3%) (Table 1). Approximately one-quarter (27·1%) of subjects kept cats at home, and consumed raw meat (26·8%). IgG *T. gondii* seropositivity in all participants was 65·82% (233/354; 95% CI 60·62–70·75), with 66·3% females and 65·2% males. Of the 167 females tested, 8·98% (15/167; 95% CI 0·05–0·14) and 65·9% (110/167; 95% CI 0·58–0·73) were seropositive for *T. gondii* IgM and IgG, respectively, with varied distribution across the districts (Fig. 1). Likewise, of the 43 pregnant women, 33 (76·4%) and four (9·3%) were positive for specific IgG and IgM antibodies, respectively (Table 2), with a higher IgM seropositivity in the second trimester of pregnancy (Fig. 2). By χ^2 analysis, none of the investigated risk factors showed an association with IgM seropositivity (Table 1).

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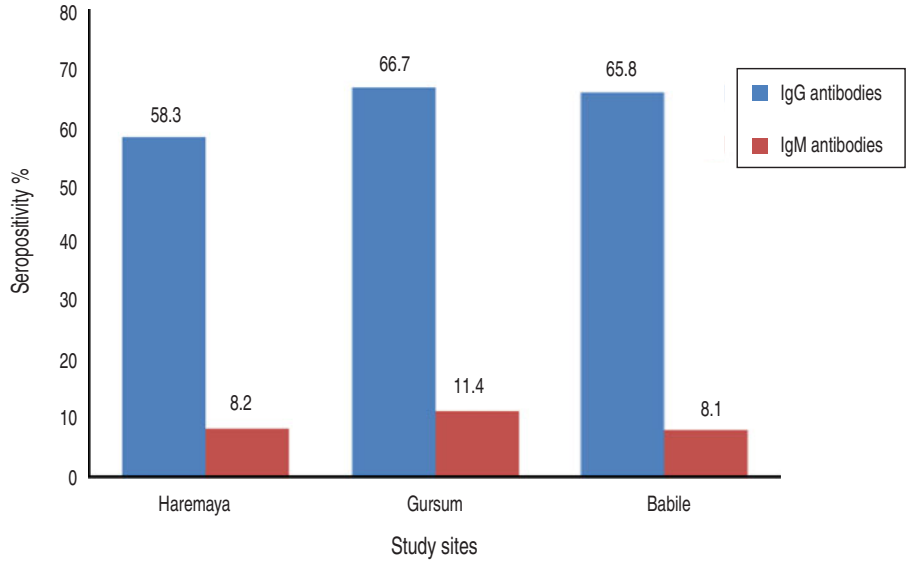

Fig. 1. Distribution of anti-*T. gondii* IgG and IgM antibodies of the examined women ($n = 167$) in the study districts.

Table 2. *Seroprevalence of anti*-T. gondii *antibodies in pregnant women*

Risk factor	Category	*n*	DAT IgG Positive (%)	ISAGA IgM Positive (%)
District	Haramaya	13	11 (84·6)	1 (7·7)
	Gursum	7	6 (85·7)	2 (28·6)
	Babile	23	16 (69·6)	1 (4·35)
Age, years	⩽30	35	25 (71·4)	3 (8·6)
	>30	8	8 (100·0)	1 (12·50)
Stage of pregnancy	2nd trimester	19	12 (63·2)	3 (15·8)
	3rd trimester	14	12 (85·7)	1 (7·1)
	1st trimester	10	9 (90·0)	0 (0·00)

DAT, Direct agglutination test; ISAGA, immunosorbent agglutination assay.

Risk factor analysis

Univariable analysis showed that age, residence, district, source of meat, keeping cats at home, presence of feral cats, and water source were significantly associated with *T. gondii* IgG seropositivity (Table 3). Accordingly, by multivariable analysis, age, sex and cats kept at home were identified as predictors of IgG seropositivity (Table 4) and variables such as residence and pregnancy in the model were dropped relative to water source and sex due to collinearity. Living in Babile district (OR 2·24, 95% CI 1·25–4·01, $P = 0·007$) increased the chance of *T. gondii* infection by twofold compared to other districts and individuals aged from 19 to 35 years (OR 4·32, 95% CI 1·91–9·75, $P < 0·001$) were more likely to be infected with *T. gondii* compared to other age groups. On the other hand, consumption of beef meat (OR 5·67, 95% CI 1·00–32·21, $P = 0·050$) resulted in a sixfold increase in the chance of an individual being infected than consumption of any other meat source. Furthermore, keeping cats at home (OR 2·01, 95% CI 1·11–3·65, $P = 0·021$) increased by twofold the likelihood of acquiring *T. gondii* infection in the household, whereas drinking pipe water was almost six times more likely to result in infection (OR 6·07, 95% CI 2·70–16·64, $P < 0·001$) compared to drinking water from other sources (Table 4). Analysis of the model fitness showed a difference between the observed and predictive value. The Hosmer–Lemeshow $\chi^2 = 2·84$, area under curve (AUC) = 0·7272 and $P = 0·9439$ indicated that the model fitted the data.

DISCUSSION

The seroprevalence of toxoplasmosis (anti-*T. gondii* IgG antibodies) in the human population in Eastern Hararghe, Ethiopia was 65·82% and anti-*T. gondii* IgM antibodies were detected in 8·98% of women. This rate is consistent with the 60% seroprevalence reported from an earlier study in Ethiopia [11] but is

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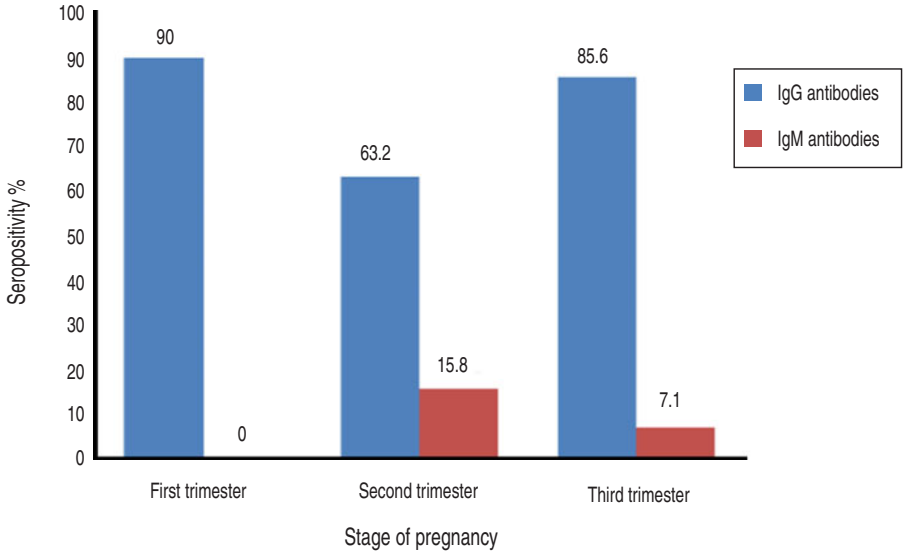

Fig. 2. Distribution of anti-*T. gondii* IgG and IgM antibodies of pregnant women ($n = 43$) in the study districts.

lower than the rate range (81·4–96·7%) found in the most vulnerable groups from different parts of the country [12–16, 19]. Nevertheless, this and other studies underpin the fact that the seroprevalence of toxoplasmosis is substantially higher in Ethiopia than other parts of the world, where reported rates range from 9·3% to 43·8% [20–24]. Several factors apart from geographical area might account for such differences and include the sensitivity and specificity of the test employed, demographic characteristics and socioeconomic status of the studied populations [12, 25, 26]. The present study showed increased seropositivity with advancing age, which might reflect the longer exposure of older individuals to infection from various sources leading to lifelong elevated IgG antibody levels as similarly recorded by others [12, 16, 27, 28].

It was observed that people living in Babile district were twice as likely to be infected with the parasite as those in other districts. The rural population in Babile district are semi-pastoralists, moving frequently with animals seeking pasture and water thus possibly increasing exposure to sources of infection. The district has a relatively hot climate and is at a lower altitude compared to the other districts, these conditions better favour the sporulation and long-term survival of oocysts than colder climates at high altitude [1]. The observed high seroprevalence in people residing in urban (84·3%) compared to rural (61·3%) areas, might be connected with increased consumption of raw meat in the former (52·9%) than the latter (20·4%), which is facilitated by the availability of retail meat. By contrast, in China no significant association was evident between *T. gondii* infections and urban living; consumption of raw meat and keeping pet animals are not popular practices in China [21]. Viable *T. gondii* is rarely found in beef meat and isolation of the parasite from the tissue of cattle has not to our knowledge been documented [29]. Nevertheless, in this study, the chance of acquiring infection through consuming raw beef meat was 5·67 times (95% CI 1·00–34·21) higher than for other raw meat sources ($P = 0·05$), and is probably explained by cultural differences in different regions of Ethiopia. Similarly, several authors have noted an association between seropositivity and raw meat consumption [1, 11, 14, 20, 22].

The observed difference in seroprevalence of *T. gondii* between people having or not having domestic cats in the household (76·0% *vs.* 62·0%, respectively) reached statistical significance ($P < 0·05$) and suggests high environmental contamination with shedding of infective oocysts by cats. This finding is consistent with some previous studies [16, 28]; however, other studies have reported the absence of such an association [12, 30]. However, the contribution of feral cats to environmental contamination and subsequent exposure of people to the infection should not be discounted. We found a slightly higher IgM response in women (8·98%) compared to previous local reports (2·5–4·2%) [16, 28], which could be due to the demographic variables and degree of exposure to the source

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Table 3. *Results of univariable logistic regression analysis for predictors of* T. gondii *IgG seropositivity*

Factors	Category	Tested	No positive (%)	Crude OR (95% CI)	*P* value
District	Haremaya	120	70 (58·3)	1·00 (ref.)	—
	Gursum	105	70 (66·7)	1·43 (0·83–2·46)	0·119
	Babile	129	93 (65·8)	1·85 (1·09–3·13)	0·023*
Residence	Rural	284	174 (61·3)	1·00 (ref.)	
	Urban	70	59 (84·3)	3·39 (1·71–6·74)	<0·001*
Sex	Male	164	107 (65·2)	1·00 (ref.)	—
	Female	190	126 (66·3)	1·05 (0·68–1·63)	0·832
Age, years	5–18	36	14 (38·9)	1·00	—
	19–35	206	141 (68·5)	3·41 (1·64–7·09)	0·001*
	⩾36	112	78 (69·5)	3·61 (1·65–7·88)	0·001*
Meat consumption	Cooked	259	167 (64·5)	1·00 (ref.)	—
	Raw	95	66 (69·5)	1·25 (0·76–2·08)	0·380
Source of meat	Shoats meat†	29	16 (55·2)	1·00 (ref.)	—
	Mixed‡	300	194 (64·7)	1·49 (0·69–3·21)	0·312
	Beef	25	23 (92·0)	9·34 (1·85–7·20)	0·007*
Milk consumption	Boiled	105	66 (62·9)	1·00 (ref.)	—
	Both§	178	116 (65·2)	1·11 (0·67–1·83)	0·695
	Raw	71	51 (71·8)	1·51 (0·79–2·89)	0·217
Vegetable consumption	Cooked	146	94 (64·4)	1·00 (ref.)	—
	Raw	208	139 (66·8)	1·11 (0·71–1·74)	0·633
Cat kept at home	No	258	160 (62·0)	1·00 (ref.)	—
	Yes	96	73 (76·0)	1·94 (1·14–3·31)	0·014 (ref.)
Presence of feral cats	No	160	95 (59·4)	1·00	—
	Yes	194	138 (71·1)	1·69 (1·08–2·63)	0·021 (ref.)
Water source	Stream	63	33 (52·4)	1·00 (ref.)	—
	Mixed‖	221	141 (63·8)	1·60 (0·91–2·82)	0·102
	Pipe water	70	59 (84·3)	4·88 (2·17–10·98)	0·000 (ref.)
Status of pregnancy	No	124	77 (62·1)	1·00 (ref.)	—
	Yes	43	33 (76·4)	1·87 (0·85–4·09)	0·120
Stage of pregnancy	2nd trimester	19	12 (63·2)	1·00 (ref.)	—
	3rd trimester	14	12 (85·7)	3·50 (0·60–20·4)	0·164
	1st trimester	10	9 (90·0)	5·25 (0·54–50·64)	0·152

OR, Odds ratio; CI, confidence interval.
† Shoats meat (sheep and goat).
‡ Mixed (shoats and beef meat).
§ Both (boiled and raw).
‖ Mixed (stream and well water).
* Significant.

of infection. The finding that 9·3% of pregnant women had anti-*T. gondii* IgM antibodies signifies the increasing risk of congenital transmission during pregnancy, particularly if the woman acquires the infection for the first time. Such a situation needs to be emphasized to antenatal healthcare practitioners against toxoplasmosis throughout the country. The decrease in anti-*T. gondii* IgG antibody from the first to second trimester and rise of anti-*T. gondii* IgM in that order may be attributed to recent infection or reactivation of an existing chronic infection and merits future investigation.

In conclusion, the seroprevalence of toxoplasmosis in the human population in three districts of East Hararghe zone in Ethiopia was considerably high. Water source, age, district and presence of cats at home were found to be risk factors for acquiring *T. gondii* infection. The moderately high level of IgM seropositivity indicates the presence of current infection and possible occurrence of congenital transmission during pregnancy. Epidemiological studies focusing on congenital toxoplasmosis, and increasing awareness of the disease through education of the people in the study area, are worthy of consideration in the future.

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Table 4. *Multivariable logistic regression analysis of predictors of* T. gondii *IgG seropositivity*

Factor	Category	aOR (95% CI)	*P* value
District	Haremaya	1·00 (ref.)	
	Gursum	1·20 (0·64–2·27)	0·564
	Babile	2·24 (1·25–4·01)	0·007*
Age, years	5–18	1·00 (ref.)	
	19–35	4·32 (1·91–9·75)	<0·001*
	⩾36	4·21 (1·74–9·87)	0·001*
Source of meat	Shoats meat†	1·00 (ref.)	
	Mixed‡	1·77 (0·76–4·14)	0·187
	Beef	5·67 (1·00–32·21)	0·050
Cat kept at home	No	1·00 (ref.)	
	Yes	2·01 (1·11–3·65)	0·021*
Presence of feral cats	No	1·00 (ref.)	
	Yes	1·63 (0·99–2·70)	0·055
Water source	Stream	1·00 (ref.)	
	Mixed§	2·18 (1·17–4·07)	0·014*
	Pipe water	6·70 (2·70–16·64)	<0·001*

aOR, Adjusted odds ratio; CI, confidence interval.
† Shoats meat (sheep and goat).
‡ Mixed (shoats and beef meat).
§ Mixed (stream and well water).
* Significant.

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DECLARATION OF INTEREST

None.

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