Epidemiol. Infect. (2015), **143**, 608–617. © Cambridge University Press 2014
doi:10.1017/S0950268814000971

Seroepidemiology of *Toxoplasma gondii* infection in free-range chickens (*Gallus domesticus*) of Central Ethiopia

E. Z. GEBREMEDHIN[1]*, G. TESFAMARYAM[2], H. A. YUNUS[3], R. DUGUMA[4], G. TILAHUN[5], V. DI MARCO[6] AND M. VITALE[6]

[1] *Department of Veterinary Laboratory Technology, Faculty of Agriculture and Veterinary Science, Ambo University, Ambo, Ethiopia*
[2] *College of Veterinary Medicine, Jigjiga University, Jijiga, Ethiopia*
[3] *Department of Animal Sciences, Faculty of Agriculture, Mizan Tepi University, Mizan, Ethiopia*
[4] *Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University, Debre Zeit, Ethiopia*
[5] *Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia*
[6] *Italian National Reference Centre for Toxoplasmosis at Istituto Zooprofilattico Sperimentale della Sicilia A. Mirri, Italy*

Received 16 January 2014; Final revision 4 March 2014; Accepted 29 March 2014; first published online 24 April 2014

SUMMARY

We performed a seroepidemiological study of *Toxoplasma gondii* infection in free-range chickens from October 2012 to May 2013. We used cross-sectional two-stage cluster sampling to collect blood samples from wing veins of 601 chickens from central Ethiopia. *T. gondii*-specific antibodies were assayed by modified agglutination test (MAT). We collected information about risk factors by questionnaire and used univariable and multivariable logistic regression to assess risk factors. An overall seroprevalence of 30·5% [95% confidence interval (CI) 26·27–34·14] and 54·2% (95% CI 47·06–61·36) was found at animal- and flock-level, respectively. The MAT end titre of seropositive chickens ($n=183$) were 1 : 60 in 46, 1 : 180 in 28, 1 : 540 in 29, $\geqslant$1 : 1620 in 48, 1 : 6000 in 22, 1 : 18 000 in five, 1 : 54 000 in one, and $\geqslant$1 : 162 000 in four. Animal-level risk factors identified using multivariable logistic regression model were: midland altitude [odds ratio (OR) 2·53, 95% CI 1·12–5·72], cross and exotic breeds (OR 3·17, 95% CI 1·39–7·23), increased age of chickens (OR 2·32, 95% CI 1·19–4·49), extensive management (OR 6·92, 95% CI 1·34–35·86) and the presence of cats (OR 2·08, 95% CI 1·20–3·61). Similarly, flock-level risk factors were midland altitude (OR 3·62, 95% CI 1·31–9·99) and the presence of cats (OR 1·19–4·94). The knowledge of the local people about the health risk of cats to humans and animals is poor. Housing and management of cats and chickens are also poor. The widespread presence of *T. gondii* infection in free-range chickens of Central Ethiopia provides suggestive evidence for the high level of contamination of the living environment of people with *T. gondii* oocysts. Meat from free-range chickens might be an important source of infection for humans. Altitude, breed, age, management and presence of cats are independent predictors of seropositivity. Education of farmers about toxoplasmosis and further studies to elucidate the burden of toxoplasmosis in animals and humans warrants consideration.

Key words: Chicken, logistic regression, MAT, seroepidemiology, *Toxoplasma gondii*.

* Author for correspondence: Dr E. Z. Gebremedhin, Department of Veterinary Laboratory Technology, Faculty of Agriculture and Veterinary Science, Ambo University, PO Box 19, Ambo, Ethiopia.
(Email: endrias.zewdu@gmail.com)

INTRODUCTION

Ethiopia is estimated to have a chicken population of 42 million [1] of which over 95% are kept under a traditional backyard scavenging system [2]. Backyard poultry represents an important part of the national economy and provides about 98·5% and 99·2% of national egg and poultry meat production, respectively [2]. Lack of knowledge about poultry production, limitations of feed resources and prevalence of diseases (Newcastle, coccidiosis, etc.) are among the constraints of backyard poultry production in Ethiopia [3].

Toxoplasma gondii is an obligate intracellular protozoan that infects humans and a wide range of mammals and birds [4]. Felines are the only definitive hosts that shed oocysts in their faeces, although intermediate hosts can harbour infective tissue cysts. Most feline infections occur post-natally through ingestion of infected tissue cysts or rarely oocysts, although congenital infections can occur [5].

Humans acquire *T. gondi* infections from ingestion of oocyst-contaminated soil and water, from tissue cysts in undercooked meat, by transplantation, blood transfusion, laboratory accidents, or congenitally [4, 6, 7]. The prevalence of *Toxoplasma* infection is estimated to range from 30% to 50% worldwide, but the majority of infected humans remain asymptomatic [6]. However, it should be noted that *T. gondii* infection may cause severe illness in immunodeficient people and pregnant women [4].

The prevalence of *T. gondii* infection in free-range chickens is a good indicator of the prevalence of *T. gondii* oocysts in the environment, due to the habits of chickens that feed by scratching the earth, which facilitates greater access to hidden faeces of cats [7]. Thus, free-range chickens play an important role in the epidemiology of *T. gondii* in the rural environment, perhaps more so than rodents, because chickens are clinically resistant to *T. gondii* and live longer than rodents. Moreover, *T. gondii*-infected chickens are an efficient source of infection for cats, because cats fed naturally infected chicken tissues may shed millions of oocysts [7, 8].

A thorough knowledge of the extent of contamination of the environment or areas surrounding human habitation by *T. gondii* oocysts is important for control purposes. In the developing world *T. gondii* seroprevalence in free-range chickens may be up to 65% and tissue cysts have been found in 81% of seropositive birds [9]. In Africa, little research has been conducted on *T. gondii* infection of free-range chickens. Lindstrom *et al.* [10] from Uganda, and Dubey *et al.* [11] from Ghana, report prevalences of 47% and 64% in chickens, respectively. The epidemiology of *T. gondii* infection in humans and animals is poorly understood in Ethiopia. Chickens are a good source of protein for humans and very much associated with food, economy, culture and religion [12], yet they pose a strong disease risk for humans via *T. gondii*. However, there seems to be only one previous study available regarding *T. gondii* status in Ethiopian chickens. That study was conducted in one area only (Addis Ababa) on free-range chickens and reported a prevalence of 38·2% from 125 chicken sera examined by modified agglutination test (MAT) and viable *T. gondii* was isolated from only 1/43 (2·3%) bioassayed seropositive chickens [13]. Therefore, the present study was conducted with the objective of estimating animal- and flock-level seroprevalence and assessing the potential risk factors associated with *T. gondii* infection in free-range chickens (*Gallus domesticus*) of East and West Shewa zones, Central Ethiopia.

MATERIALS AND METHODS

Description of the study area

The study was conducted in three selected districts (Ambo, Adea, Fentale) in East and West Shewa zones of Oromia Regional State, Central Ethiopia (Fig. 1). The study areas are separated from each other by 150–289 km and represent the highland [⩾2300 m above sea level (masl)], midland (1500–2300 masl) and lowland (<1500 masl) agro-ecologies of the region.

Ambo district (longitude 37° 32′ to 38° 3′ E; latitude 8° 47′ to 9° 20′ N) is located in Western Shewa Zone of Oromia Regional State. The altitude within the district ranges from 1400 to 3045 masl. The majority of the locations in Ambo district are highland. The range of annual rainfall and temperature is 800–1000 mm and 15–29 °C, respectively. The mean temperature is 18·6 °C.

Adea district (longitude 38° 58′ E to 39° 22′ E; latitude 08° 22′ N to 8° 56′ N) and Fentale district (longitude 39·93° E to 39° 56′ 0″ E; latitude 8·975° N to 8·58′ 30″ N) are located in East Shewa Zone at a distance of 45 km and 190 km from Addis Ababa, respectively. The altitude of Adea district ranges from 1500 to >2000 masl, although the majority of the

https://doi.org/10.1017/S0950268814000971 Published online by Cambridge University Press

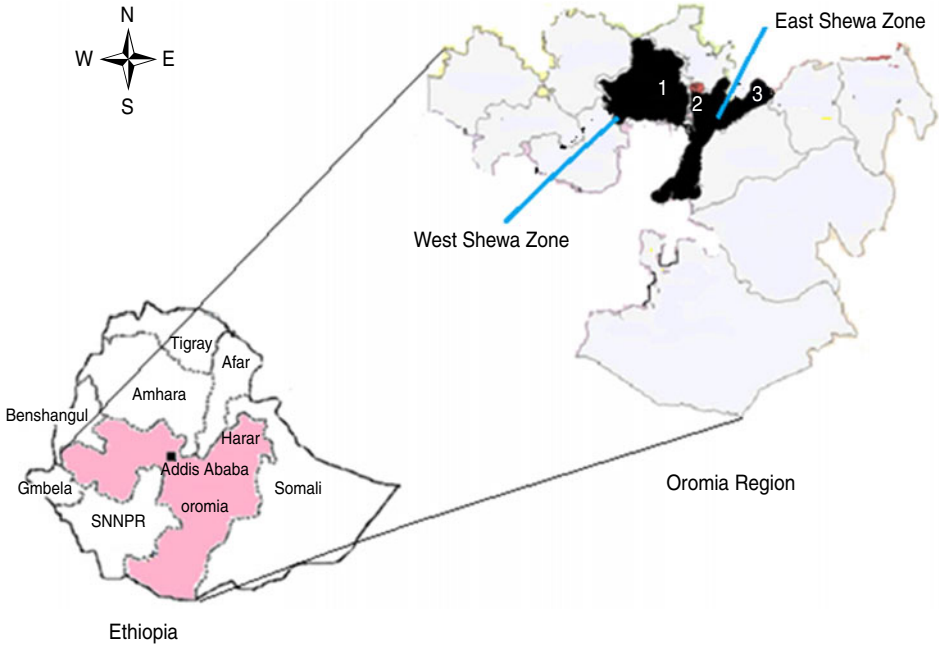

Fig. 1. Map showing the study districts highlighted in black. 1, Ambo; 2, Adea; 3, Fentale.

territory is midland. Its average rainfall is ~839 mm with temperature ranging from 7·9 °C to 28 °C.

Fentale district ranges in altitude from 955 to 2007 masl, but comprises mainly lowland. Its average rainfall and temperature are ~553 mm and 29–38 °C, respectively. Fentale district has an arid to semi-arid climate and the production system is predominantly pastoral and agro-pastoral. Sedentary farming, dominated by an extensive type of management system, is a feature of the highlands and midlands of Ambo and Adea districts. However, semi-intensive farming is practised in some urban and peri-urban areas.

Study animal population and production system

Previous reports on *T. gondii* infection in sheep [14], goats [15] and women of childbearing age [16] from the same study districts initiated this investigation into the level of *T. gondii* oocyst contamination of the environment by using free-range chickens as indicators.

The biological characteristics and management properties of village chickens in Ethiopia have previously been well described [12, 17]. The chicken production systems in the selected districts are dominated by extensive/free-range system, where main feed sources are insects, worms, cereals and plants. In this system chickens are set free during the day and spend the night on perches in or around the main house.

Study design and sample size

A cross-sectional study design was conducted from October 2012 to May 2013 to estimate the seroprevalence and to identify the potential risk factors in the epidemiology of *T. gondii* infection in free-range chickens of the three study districts.

In the absence of any well-designed previous studies on chicken toxoplasmosis in Ethiopia, a 50% expected prevalence (p) and a 95% confidence interval (Z = 1·96) with a 5% desired absolute precision (d) was considered to arrive at the required sample size, using the formula: $n = p(1-p)Z^2/d^2$) [18]. However, the calculated sample size (n = 384) was increased to 601 for better accuracy. The total chicken population of the study area was 196 644. The required sample size was distributed on the basis of the chicken population of each respective district: Ambo (n = 286), Adea (n = 209) and Fentale (n = 109). The study '*kebeles*' (*kebele* refers to the smallest administrative unit) from Ambo (n = 7), Adea (n = 5) and Fentale (n = 4) were chosen purposely based on accessibility. The average number of chickens per household in Ethiopia is 2–6 hens and 1–3 cocks [12, 17]. The lower ratio (i.e. 1 male : 2 females) was considered in order to sample three chickens per household using a simple random sampling technique. Accordingly, 88, 65 and 37 households were sampled from Ambo, Adea and Fentale districts, respectively. The households of selected *kebeles* were sampled by considering the relative

https://doi.org/10.1017/S0950268814000971 Published online by Cambridge University Press

distance from one cluster to another, since rural communities live in clusters. Finally, 2–3 households per cluster were sampled.

Questionnaire survey

A questionnaire survey was used to collect data regarding the potential risk factors including breed (local, cross, exotic), age (⩽6 months, 7–12 months, ⩾13 months), sex (male/female), flock size (small, ⩽4; medium, 5–10; large, ⩾11 chickens), management systems (extensive, semi-intensive, intensive), altitude (lowland, ⩽1400 masl; midland, 1500–2200 masl; highland ⩾2300 masl), presence of cat (yes/no), presence of dog (yes/no), presence of feral cat (yes/no), type of residence (rural/peri-urban), owner education (literate/illiterate) and water source (tap, river, pond, well). The questionnaire also included origin and housing of chickens, type of feed and housing for cat(s); and awareness and knowledge of the respondents about toxoplasmosis.

Sample collection and transportation

About 2–3 ml blood samples were collected from the wing vein of each chicken, using a disposable plain vacutainer tube and needle. Samples were labelled and placed in a slant position for a few minutes until clotting. Centrifugation at 2250 ***g*** for 5 min was performed for some sera which failed to separate overnight. The sera were collected in 1·5 ml Eppendorf tubes, labelled and transported in an ice box with ice packs to the Ethio-Belgium laboratory of the College of Veterinary Medicine and Agriculture, Addis Ababa University, Debre-Zeit and stored at −20 °C until tested.

MAT

All sera samples collected were examined for the presence of antibodies (IgG) against *T. gondii* by MAT (Toxo screen DA, bioMérieux, France) according to the manufacturer's instructions. Sera were assayed at a screening dilution of 1 : 40 and 1 : 4000. A titre of 1 : 40 or 1 : 4000 or both was considered indicative of *T. gondii* exposure. Sedimentation of antigen at the bottom of the well, and clear agglutination above half of the well at either dilution were recorded as negative and positive results, respectively. MAT-positive samples were titrated to obtain the endpoint titre. Positive and negative controls were included in each test.

Data management and analyses

The data generated from the field and laboratory investigations were entered and coded using Microsoft Excel 2007 and analysed using Stata v. 11.0 for Windows (StataCorp., USA). Descriptive statistics were used to summarize the data. Flocks containing at least one seropositive animal were considered positive. Seroprevalence was calculated by dividing the total number of seropositive chickens by the total number of chickens tested. Similarly, flock level seroprevalence was calculated as the number of flocks with at least one positive animal, divided by the total number of flocks tested. The variables considered as potential risk factors for *T. gondii* seropositivity were selected based on existing literature. During analyses, categories of variables, e.g. cross-breed and exotic breed, urban and peri-urban locations, intensive and semi-intensive management were merged. Association of risk factors (independent variables) with dependent variable (*T. gondii* seropositivity) was assessed initially using cross-tabulation. Strength of association was assessed using univariable logistic regression to compute the odds ratio associated with potential risk factors. Non-collinear variables, that presented a P value ⩽0·25 in univariable analyses, were included in the multivariable logistic regression model. In line with this, the sex, flock size, presence of feral cats and source of water were not entered in the animal-level multivariable model, due to a P value >0·25 in the univariable analyses, while feed was omitted for management due to collinearity. Similarly, during flock-level analysis, district was dropped from joining the model due to collinearity with altitude. Model fitness was assessed by the Hosmer–Lemeshow goodness-of-fit test [19]. The receiver-operating characteristic curve (ROC) was used to evaluate the reliability of both individual- and flock-level models. The 95% confidence level was used and results were considered significant at $P \leqslant 0{\cdot}05$.

RESULTS

Overall seroprevalence and antibody end titre in free-range chickens

The overall seroprevalence of *T. gondii* infection in the study districts were 30·5% [95% confidence interval (CI) 26·76–34·14] and 54·2% (95% CI 47·06–61·36) at animal and flock level, respectively. The highest (animal level 41·4%, flock level 72·3%) and lowest (animal level 9·2%, flock level 21·6%) seroprevalences

https://doi.org/10.1017/S0950268814000971 Published online by Cambridge University Press

were recorded in chickens from Adea and Fentale districts, respectively. There was a statistically significant difference in seroprevalence of *T. gondii* infection between districts, both at animal ($\chi^2_2 = 34{\cdot}9632$, $P \leqslant 0{\cdot}001$) and flock ($\chi^2_2 = 24{\cdot}4104$, $P \leqslant 0{\cdot}001$) levels.

Titration was conducted for each seropositive serum to evaluate the endpoint titre at which the MAT antibody is no longer detectable. Subsequently, 183/601 chickens were seropositive with an end titre of 1 : 60 in 46, 1 : 180 in 28, 1 : 540 in 29, $\geqslant$1 : 1620 in 48, 1 : 6000 in 22, 1 : 18 000 in five, 1 : 54 000 in one, and $\geqslant$1 : 162 000 in four.

Risk factors of *T. gondii* infection in free-range chickens

Animal level

The variables altitude, age, management, and presence of cats were all significant factors associated with *T. gondii* seropositivity in both univariable and multivariable logistic regression analyses ($P \leqslant 0{\cdot}05$). Presence of dogs and breed were significantly associated with *T. gondii* seropositivity only in univariable and multivariable logistic regression model, respectively ($P \leqslant 0{\cdot}05$, Table 1).

Flock level

Univariable logistic regression analysis revealed that the variables altitude, location, and presence of cats and dogs were all significantly associated ($P \leqslant 0{\cdot}05$) with *T. gondii* seropositivity. However, only altitude and presence of cats were found to be independent predictors of seropositivity by the multivariable logistic regression model (Table 2). From the flock-level multivariable logistic regression model it was evident that not all factors were equally responsible as a risk factor for *T. gondii* seropositivity, implying that only some factors are sufficient risk factors. Table 3 shows the best-fitting model that contains all the appropriate risk factors for *T. gondii* seropositivity at both animal and flock levels.

Respondents' awareness of *T. gondii* infection and its risk factors

From a total of 190 respondents interviewed, 95 (50·0%) owned domestic cats. Table 4 shows that 90·5% of respondents reported that their cats lived completely outdoors. All (100%) respondents indicated that the cats had no separate accommodation while 85·3% reported that the cats ate raw animal products as well as household leftovers. Most respondents do not have adequate knowledge about the health risk posed by cats to animals and humans. This health risk to animals showed no significant difference between districts ($\chi^2_2 = 1{\cdot}6161$, $P = 0{\cdot}446$). However, there was a significant difference with respect to awareness about health risks of cats to humans across districts, in that awareness was considerably higher in Fentale (37·8%) than Ambo (10·2%) and Adea (15·4%) districts ($\chi^2_2 = 14{\cdot}1072$, $P = 0{\cdot}001$). The sanitation of chicken houses was judged as poor in 93·7% of cases, while 97·9% of respondents reported that they did not have a separate house for chickens (free ranging during the day and spending the night on perches in or around the owners' home). Dead chickens were simply thrown into open fields by 72·1% of the respondents (Table 4).

DISCUSSION

T. gondii occurs widely in humans and animals, including domestic poultry throughout the world. The distribution of *T. gondii* is believed to be best detected by using its levels of distribution in free-range chickens [7]. To the best of the authors' knowledge, this is the first detailed report on seroprevalence and risk factors of *T. gondii* infection in free-range chickens in different agro-ecologies of Ethiopia. The choice of MAT as a diagnostic tool for the current study was made because of its high sensitivity (96·22%) and specificity (98·8%) in chickens compared to other serological tests [7, 20].

Overall seroprevalence

An overall animal- and flock-level seroprevalence of 30·5% and 54·2%, respectively, was documented. The highest animal-level (41·4%, 95% CI 34·62–48·06) and flock-level (72·3%, 95% CI 61·27–83·34) seroprevalences were recorded from Adea district while the lowest animal-level (9·2%, 95% CI 3·72–14·63) and flock-level (21·6%, 95% CI 8·09–35·16) seroprevalences were recorded from Fentale district. The variation in seroprevalence between districts can be defined by the difference in temperature, moisture and management practices across the studied districts.

The high overall animal-level seroprevalence of the present study is in agreement with the report of Tilahun *et al.* [13] from Addis Ababa, Ethiopia (38·4%). The high seroprevalence of *T. gondii* in

https://doi.org/10.1017/S0950268814000971 Published online by Cambridge University Press

Table 1. *Results of logistic regression analysis of animal-level* Toxoplasma gondii *seropositivity in free-range chickens*

			Univariable		Multivariable	
Risk factors	*N* (positive)	% (prev.)	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Altitude						
Lowland	109 (10)	9·2				
Midland	406 (144)	35·5	5·44 (2·65–11·15)	0·001	2·53 (1·12–5·72)	0·026
Highland	86 (29)	33·7	5·04 (1·71–14·79)	0·003	2·69 (0·94–7·68)	0·065
Breed						
Local	567 (166)	29·3				
Cross and exotic	34 (17)	50	2·42 (0·84–6·91)	0·100	3·17 (1·39–7·23)	0·006
Sex						
Male	173 (48)	27·8				
Female	428 (135)	31·5	1·19 (0·79–1·79)	0·378		
Age						
⩽6 months	125 (27)	21·6				
7–12 months	369 (107)	29·0	1·48 (0·84–2·62)	0·175	1·34 (0·73–2·47)	0·345
⩾13 months	107 (49)	45·8	3·06 (1·59–5·89)	0·001	2·32 (1·19–4·49)	0·013
Location						
Rural	297 (80)	26·9				
Urban and peri-urban	304 (103)	33·9	1·39 (0·85–2·28)	0·193	1·39 (0·85–2·27)	0·191
Flock size						
⩽4	222 (60)	27·0				
5–10	259 (82)	31·7	1·25 (0·75–2·07)	0·384		
⩾11	120 (41)	34·2	1·40 (0·66–2·96)	0·376		
Management						
Intensive and semi-int.	31 (3)	9·7				
Extensive	570 (180)	31·6	4·30 (1·37–13·58)	0·013	6·92 (1·34–35·86)	0·021
Presence of cats						
No	292 (60)	20·6				
Yes	309 (123)	39·8	2·56 (1·58–4·14)	0·001	2·08 (1·20–3·61)	0·009
Feral cats						
No	92 (25)	27·2				
Yes	509 (158)	31·0	1·21 (0·66–2·19)	0·539		
Presence of dogs						
No	253 (57)	22·5				
Yes	348 (126)	36·2	1·95 (1·21–3·14)	0·006	1·23 (0·69–2·19)	0·471
Owner education						
Illiterate	232 (59)	25·4				
Literate	369 (124)	33·6	1·48 (0·90–2·43)	0·116	1·15 (0·70–1·90)	0·574
Feed						
Cereals	536 (161)	30·0				
WBCS	65 (22)	33·8	1·19 (0·57–2·42)	0·628		
Water source						
River	52 (13)	25·0				
Tap	357 (104)	29·1	1·23 (0·56–2·73)	0·605		
Mixed	152 (48)	31·6	1·38 (0·55–3·48)	0·490		
Pond and wells	40 (18)	45·0	2·45 (0·89–6·74)	0·081		

N, Number tested; prev., prevalence; OR, odds ratio; CI, confidence interval; WBCS, wheat bran, cereals and supplements.

free-range chickens in the present study also corroborates well with the high prevalence of oocyst shedding (19%, 7/36) previously reported in feral cats which are commonly fed on dead avian carcases in Addis Ababa [21]. Surveys conducted in different countries around the world report prevalences of *T. gondii* infection in

https://doi.org/10.1017/S0950268814000971 Published online by Cambridge University Press

Table 2. *Results of logistic regression analyses of flock-level* Toxoplasma gondii *seropositivity in free-range chickens*

			Univariable		Multivariable	
Risk factor	*N* (positive)	% (prev.)	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Altitude						
Lowland	37 (8)	21·6				
Highland	24 (11)	45·8	3·07 (0·99–9·41)	0·050	2·01 (0·58–6·94)	0·269
Midland	129 (84)	65·1	6·77 (2·86–16·03)	0·001	3·62 (1·31–9·99)	**0·013**
Flock size						
⩽4	78 (37)	47·4				
5–10	32 (18)	56·3	1·42 (0·62–3·26)	0·402		
⩾11	80 (48)	60·0	1·66 (0·88–3·12)	0·114		
Locations						
Rural	77 (32)	41·6				
Urban and peri-urban	113 (71)	62·8	2·38 (1·31–4·29)	0·004	1·67 (0·82–3·38)	0·155
Presence of cats						
No	96 (38)	39·6				
Yes	94 (65)	69·2	3·42 (1·88–6·23)	0·001	2·43 (1·19–4·94)	**0·014**
Presence feral cats						
Yes	162 (85)	52·5				
No	28 (18)	64·3	1·63 (0·71–3·75)	0·250	1·26 (0·49–3·28)	0·632
Presence of dogs						
No	84 (36)	42·9				
Yes	106 (67)	63·2	2·29 (1·28–4·11)	0·006	1·13 (0·53–2·40)	0·758
Management						
Intensive and semi-int.	9 (3)	33·3				
Extensive	181 (100)	55·3	2·47 (0·59–10·18)	0·211		
Water source						
Mixed	17 (9)	52·9				
Tap	114 (60)	52·6	0·98 (0·35–2·74)	0·981		
River	45 (23)	51·1	0·93 (0·30–2·84)	0·898		
Pond and well	14 (11)	78·6	3·26 (0·66–16·03)	0·146		
Owner education						
Illiterate	75 (36)	48·0				
Literate	115 (67)	58·3	1·51 (0·84–2·72)	0·166	1·15 (0·58–2·25)	0·679
Feed						
Cereals	169 (89)	52·7				
WBCS	21 (14)	66·7	1·79 (0·69–4·68)	0·229	1·23 (0·39–3·81)	0·718

N, Number tested; prev., prevalence; OR, odds ratio; CI, confidence interval; WBCS, wheat bran, cereals and supplements.

free-range chickens ranging between 5·5% and 100% [10, 22–24]. The differences in the seroprevalence observed between the current and above-mentioned studies may relate to differences in relative cat densities, management, study design, the number of chickens examined, sanitation, housing of chickens, type of serological tests used and the cut-off value reported [4, 7, 25].

Risk factors

Epidemiological data on risk factors of *T. gondii* infections in chickens are generally limited across the globe. In the present study, the potential risk factors of *T. gondii* infection in free-range chickens were assessed. Accordingly, altitude, presence of cats, age, management and breed were to a large degree associated with *T. gondii* seropositivity ($P<0·05$), whereas risk factors like sex, location, presence of feral cats, education level of owner, type of feed, source of water and flock size did not show statistical association with *T. gondi* infection ($P>0·05$).

The likelihood of acquiring *T. gondii* infection in chickens reared in the midland areas is twice that of chickens reared in lowland areas ($P=0·026$). This may be due to the fact that the moist and warm

https://doi.org/10.1017/S0950268814000971 Published online by Cambridge University Press

Table 3. *Best-fitting model of risk factors for* Toxoplasma gondii *seropositivity in chickens*

	Animal level		Flock level	
Risk factors	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Breed	3·70 (1·59–8·59)	0·002		
Management	7·95 (1·24–51·08)	0·029		
Presence of cats	2·16 (1·30–3·57)	0·003	2·45 (1·29–4·63)	0·006
Altitude				
Highland	3·36 (1·19–9·44)	0·022	2·41 (0·76–7·62)	0·136
Midland	3·40 (1·55–7·45)	0·002	4·85 (1·98–11·90)	0·001

OR, Odds ratio; CI, confidence interval.
Animal level: Hosmer–Lemshow $\chi^2 = 0.34$, $P = 0.95$, receiver-operating characteristic curve = 0.68.
Flock level: Hosmer–Lemshow $\chi^2 = 0{\cdot}08$, $P = 0{\cdot}96$, receiver-operating characteristic curve = 0·71.

macro-climate of midland areas supports survival of oocysts and its higher infection rate, compared to the dry and hot climate of lowland areas which decreases the survival and infection rate of *T. gondii* [4, 26]. Relatively lower seroprevalence has previously been reported in lowland sheep and goats compared to highland and midland areas [14, 15]. The influence of the environment on the epidemiology of toxoplasmosis has been well documented [20, 22, 26, 27].

The seroprevalence (45·8%) in older chickens was significantly higher than the seroprevalence in young chickens (21·6%, $P < 0{\cdot}001$). This difference is partly due to an increase in the cumulative effect of exposure to the parasite by adult chickens, i.e. as the age of chickens increases the likelihood of encountering oocysts of *T. gondii* from the environment also increases [4]. There have been numerous reports indicating age-related increase in seroprevalence in chickens worldwide [26, 28].

The odds of acquiring *T. gondii* infection by chickens kept under an extensive management system was nearly seven times higher compared to chickens managed under semi-intensive and intensive management systems ($P = 0{\cdot}021$). This variation may be explained by the fact that chickens kept under an extensive system are exposed to *T. gondii* oocysts shed by cats in the environment since chickens scratch the soil to collect feed, whereas chickens under intensive and semi-intensive management systems receive better hygiene and have limited or no access to the contaminated environment, since they are kept in confinement [4, 29]. The influence of management on seroprevalence of chicken toxoplasmosis has previously been well documented [30].

A significantly high seroprevalence of *T. gondii* infection in free-range chickens kept in areas where cats are present ($P = 0{\cdot}009$) is obviously an indication of the dissemination of cat-originated oocysts in the soil close to the households. The frequent cohabitation of chickens with cats in the same environment was observed during sample collection. An increased risk of infection in the presence of cats has been reported by different studies [7, 26, 29].

Local breeds of chickens have been claimed to have better disease resistance than exotic breeds [2]. In this study, the likelihood of getting *T. gondii* infection by cross-bred and exotic-bred chickens (50·0%) was significantly higher than for locally bred chickens (29·3%, $P = 0{\cdot}006$). A possible reason for increased risk of infection in the exotic and cross-breeds may be due to higher susceptibility to *T. gondii* infection or the preference of owners to keep these chicken breeds for a longer period as potential breeders for high production purposes, hence there is an increased chance of exposure to the possible source of infection [2, 8]. However, the role of breed in predisposing chickens to *T. gondii* infection needs further investigation.

With regard to the owners' awareness about the role cats play in the transmission of disease to humans and animals, 17·4% (33/190) of respondents reported association of cats in the transmission of diseases like asthma and parasites to humans, but none of the interviewed owners were aware of the health risk of cats to other animals. None of the respondents were aware

https://doi.org/10.1017/S0950268814000971 Published online by Cambridge University Press

Table 4. *Awareness of respondents about the risks of cats to humans and animals, management of cats and chickens in the study areas (n = 95)*

Questions	No. of respondents	%
How does cat live?		
Completely outdoor	86	90·5
Completely indoor	0	0
Mixed	9	9·5
Do you have a separate house for cat?		
Yes	0	0
No	95	100
What is the feed of your cat?		
Raw animal product	7	7·4
Household leftovers	7	7·4
Raw animal product and household leftovers	81	85·3
Do you know health risk of cats to humans?		
Yes	33	17·4
No	157	82·6
Do you know health risk of cats to other animals?		
Yes	3	1·6
No	187	98·4
Is there contamination of stored animal feed by cat faeces?		
Yes	33	17·4
No	157	82·6
Sanitation of chicken house		
Poor	178	93·7
Fair	11	5·8
Good	1	0·1
Housing of chickens		
Free range and night perch	186	97·9
Semi-fenced daytime and night perch	1	0·1
Fully confined	3	1·6
What do you do with dead chickens?		
Bury	14	7·4
Bury and throw away	3	1·6
Feed for pet and throw away	36	18·9
Throw into field	137	72·1

of toxoplasmosis. Similarly, none of the respondents kept their cats in a separate house. Cats were fed with raw animal products like viscera of ruminants and chickens slaughtered for human consumption, and meat of dead animals, which might have *T. gondii* cysts, hence boosting the spread of toxoplasmosis. Moreover, 97·9% of respondents keep their stock free range (outdoors) during day-time hours and at night used a perch housing system, where the sanitation is poor (93%). Furthermore, about 92% of respondents used unsafe methods when disposing of chicken carcases (throw away into fields or fed to pets). Poor housing, feeding and management of free-range chickens and cats together with poor sanitation and the lack of awareness of toxoplasmosis create favourable conditions for the high prevalence of the disease and successful continuation of the life-cycle of the parasite in the study areas.

In view of home slaughtering of free-range chickens, limited refrigeration facilities and poor sanitation in Ethiopia in general, and the study districts in particular, the high seroprevalence in free-range chickens indicates a high likelihood of transmission to humans, unless chicken meat is well cooked and good kitchen sanitation is practised [7].

In conclusion, *T. gondii* antibodies are widespread in free-range chickens in Central Ethiopia suggesting high contamination of the living environment with oocysts of *T. gondii* and that the meat from free-range chickens may be an important source of infection for humans. Management, age, presence of cats, altitude and breed are epidemiologically important predictors for *T. gondii* seropositivity. The general absence of awareness of toxoplasmosis may play an important role in the epidemiology of the disease. Education of farmers about toxoplasmosis and further studies to elucidate the burden of toxoplasmosis in animals and humans warrants consideration.

ACKNOWLEDGEMENTS

The financial support of Addis Ababa University (Combating HIV/AIDS and promoting maternal and child health project) as well as the partial financial support to Maria Vitale (grant no. RF 2007/RC 2009 from the Italian Ministry of Health) are greatly appreciated. The study could not have been conducted without this support. We are grateful to Margareta Langbacka Walker, advisor and trainer in the English Language Improvement Centre at Ambo University, for her valuable comments on the language aspect. The authors also extend their gratitude to the owners of chickens for their collaboration during the study.

DECLARATION OF INTEREST

None

REFERENCES

1. **Central Statistical Authority (CSA).** Federal Democratic Republic of Ethiopia, Central Statistical Agency, Agricultural sample survey 2009/10. Report on livestock and livestock characteristics, *Statistical Bulletin* No. 468. Addis Ababa, Ethiopia, 2009.
2. **Tadelle D, Kijora C, Peters KJ.** Indigenous chicken ecotypes in Ethiopia: Growth and feed utilization potentials. *International Journal of Poultry Science* 2003; **2**: 144–152.
3. **Ashenafi H, *et al.*** Study on coccidiosis of scavenging indigenous chickens in central Ethiopia. *Tropical Animal Health and Production* 2004; **36**: 693–701.
4. **Dubey JP.** *Toxoplasmosis of Animals and Humans*, 2nd edn. Florida: CRC Press. 2010, pp.1–338.
5. **Dubey JP, Jones JL.** *Toxoplasma gondii* infection in humans and animals in the United States. *International Journal for Parasitology* 2008; **38**: 1257–1278.
6. **El-Awady MK, El-Hosseiny LA, Ismail SM.** Comparison between *Toxoplasma gondii* and specific immunoglobulin's during pregnancy. *East Mediterranean Health Journal* 2000; **6**: 888–897.
7. **Dubey JP.** *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): prevalence, clinical disease, diagnosis and public health significance. *Zoonoses and Public Health* 2010; **57**: 60–73.
8. **Dubey JP, *et al.*** Biological and genetic characterization of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from Sao Paulo, Brazil: unexpected findings. *International Journal for Parasitology* 2002; **32**: 99–105.
9. **Kijlstra A, Jongert E.** Control of the risk of human toxoplasmosis transmitted by meat. *International Journal for Parasitology* 2008; **38**: 1359–1370.
10. **Lindstrom I, *et al.*** Isolation and genotyping of *Toxoplasma gondii* from Ugandan chickens reveals frequent multiple infections. *Parasitology* 2007; **135**: 39–45.
11. **Dubey JP, *et al.*** Seroprevalence and isolation of *Toxoplasma gondii* from free range chickens in Ghana, Indonesia, Italy, Poland, and Vietnam. *Journal of Parasitology* 2008; **94**: 68–71.
12. **Reta D.** Understanding the role of indigenous chicken during the long walk to food security in Ethiopia. *Livestock Research for Rural Development* 2009; **21** (www.lrrd.org/lrrd21/8/dugu21116).
13. **Tilahun G, *et al.*** Seroprevalence of *Toxoplasma gondii* from free-range chicken (*Gallus domesticus*) from Addis Ababa, Ethiopia. *Journal of Parasitology* 2013; **99**: 740–741.
14. **Gebremedhin EZ, *et al.*** Seroepidemiological study of ovine toxoplasmosis in East and West Shewa zones of Oromia regional state, central Ethiopia. *BMC Veterinary Research* 2013; **9**: 117.
15. **Zewdu E, *et al.*** Seroepidemiological study of caprine toxoplasmosis in East and West Shewa zones, Oromia regional state, central Ethiopia. *Research in Veterinary Science* 2013; **94**: 43–48.
16. **Gebremedhin EZ, *et al.*** Seroepidemiology of *Toxoplasma gondii* infection in women of child-bearing age in central Ethiopia. *BMC Infectious Disease* 2013; **13**: 101–112.
17. **Fisseha M, Azage T, Tadelle D.** Indigenous chicken production and marketing systems in Ethiopia: characteristics and opportunities for market-oriented development. International Livestock Research Institute, Addis Ababa, Ethiopia, 2010, pp. 5–8.
18. **Thrusfield M.** *Veterinary Epidemiology*, 3rd edn. London: Blackwell Science Ltd, 2007, pp. 227–247.
19. **Dohoo I, Martin W, Stryhn H.** *Veterinary Epidemiologic Research*. Charlottetown, Canada: AVC Inc. 2003, pp. 521–539.
20. **Dubey JP, *et al.*** Serologic and parasitologic responses of domestic chickens after oral inoculation with *Toxoplasma gondii* oocysts. *American Journal of Veterinary Research* 1993; **54**: 1668–1672.
21. **Dubey JP, *et al.*** Isolation of viable *Toxoplasma gondii* from tissues and feces of cats from Addis Ababa, Ethiopia. *Journal of Parasitology* 2013; **99**: 56–58.
22. **Dubey JP.** Toxoplasmosis – a waterborne zoonosis. *Veterinary Parasitology* 2004; **126**: 57–72.
23. **Dubey JP.** Unexpected oocyst shedding by cats fed *Toxoplasma gondii* tachyzoites: in vivo stage conversion and strain variation. *Veterinary Parasitology* 2005; **133**: 289–298.
24. **Dubey JP.** The history and life cycle of *Toxoplasma gondii*. In: Weiss LM, Kim K, eds. *Toxoplasma gondii. The Model Apicomplexan: Perspectives and Methods*. New York: Academic Press, 2007, pp. 1–17.
25. **Beltrame MAV, *et al.*** Seroprevalence and isolation of *Toxoplasma gondii* from free-range chickens from Espírito Santo state, southeastern Brazil. *Veterinary Parasitology* 2012; **188**: 225–230
26. **Tenter AM, Heckeroth AR, Weiss LM.** *Toxoplasma gondii*: from animals to humans. *International Journal for Parasitology* 2000; **30**: 1217–1258.
27. **Yang N, *et al.*** Seroprevalence of *Toxoplasma gondii* infection in slaughtered chickens, ducks, and geese in Shenyang, northeastern China. *Parasites & Vectors* 2012; **5**: 237.
28. **Alvarado-Esquivel C, *et al.*** Seroprevalence of *Toxoplasma gondii* Infection in Chickens in Durango State, Mexico. *Journal of Parasitology* 2012; **98**: 431–432.
29. **Zhu J, *et al.*** A seroepidemiological survey of *Toxoplasma gondii* infection in free range and caged chickens in northeast China. *Veterinary Parasitology* 2008; **158**: 360–363.
30. **Xu P, *et al.*** Seroprevalence of *Toxoplasma gondii* infection in chickens in Jinzhou, northeastern China. *Journal of Parasitology* 2012; **98**: 1300–1301.