Epidemiol. Infect. (2016), **144**, 567–575. © Cambridge University Press 2015
doi:10.1017/S0950268815001429

Changing risk of environmental *Campylobacter* exposure with emerging poultry production systems in Ethiopia

M. C. BRENA[1]†, Y. MEKONNEN[2]†, J. M. BETTRIDGE[1]†, N. J. WILLIAMS[1], P. WIGLEY[1], T. SISAY TESSEMA[3] AND R. M. CHRISTLEY[1,4]*

[1] *Institute of Infection and Global Health, University of Liverpool, Leahurst Campus, Cheshire, UK*
[2] *College of Veterinary Medicine and Agriculture, Addis Ababa University, Debre Zeit, Ethiopia*
[3] *Institute of Biotechnology, Addis Ababa University, Addis Ababa, Ethiopia*
[4] *NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, Liverpool, UK*

Received 21 November 2014; Final revision 14 May 2015; Accepted 5 June 2015; first published online 10 July 2015

SUMMARY

Campylobacter is a leading cause of diarrhoea, and its presence in chickens is a significant risk for zoonotic infection. Poultry production is becoming increasingly intensive in Ethiopia and is incorporating more high-producing breeds into traditionally managed smallholdings, especially in peri-urban areas. This cross-sectional study sampled 219 household environments in one peri-urban and two rural areas of Ethiopia, and an additional 20 semi-intensive farms in the peri-urban district. *Campylobacter* was detected by polymerase chain reaction (PCR)-specific assays in 44 samples; 16 of which could be identified as *C. jejuni*. Flocks in the peri-urban area were at significantly greater odds of detection, including those which only kept indigenous birds under a scavenging system. It was also noted that scavenging flocks of exotic high-production birds (Rhode Island Red) were at slightly greater risk, perhaps as exotic birds are under more stress when kept under traditional management systems. We suggest that changes to the system of chicken production may alter the ecology and epidemiology of *Campylobacter* in the environment, chickens and people, which may drive emergence of new epidemiological patterns of disease. Further research is needed to determine the extent to which the current management intensification and the distribution programmes of exotic and/or improved indigenous birds may alter *Campylobacter* epidemiology, ecology and public health risk, before their widespread adoption.

Key words: Animal husbandry, *Campylobacter*, chickens, Ethiopia, social change, zoonoses.

INTRODUCTION

Globally, diarrhoea is the second greatest cause of mortality in children aged <5 years after pneumonia [1]. Children in low- to middle-income countries are at risk of frequent diarrhoea episodes [2]; the 2011 national survey in Ethiopia estimated that 13·4% of children in the <5 years age group had suffered an episode of diarrhoea in the preceding 2 weeks, although prevalence was >20% in some regions [3]. Numerous infectious agents cause diarrhoea; many of which are zoonotic, with *Campylobacter* being one of the main bacterial causes, especially in children aged <2 years [4–6]. While the organism is also frequently isolated from healthy children and adults, and morbidity

* Author for correspondence: Dr R. M. Christley, Institute of Infection and Global Health, University of Liverpool, Leahurst Campus, Cheshire, CH64 7TE, UK.
(Email: robc@liv.ac.uk)
† These authors contributed equally to this work.

appears to decline with age, adults with HIV infection are also at increased risk of *Campylobacter*-associated diarrhoea and bacteraemia [4]. Different routes by which humans are exposed to *Campylobacter* include ingestion of contaminated food and water, direct contact with infected animals or carcasses, from environments contaminated with animal faeces or sewage, or direct person-to-person transmission [4, 7]. In countries with a highly developed intensive poultry industry, there is considerable epidemiological evidence that chickens are the main source of human *Campylobacter* infection [8–10]. In developing countries, far less is known about the epidemiology of *Campylobacter*, but it has been found at high levels in retail poultry meat [11], and strains isolated from chickens have been observed to correlate with human isolates in terms of biotype and serotype [12].

Around 83% of Ethiopia's population, currently estimated at 96 million, live in rural households [13] where sharing space with domestic livestock is common practice. Chicken production is an integral part of most rural families' livelihoods (an estimated two-thirds of Ethiopian villagers keep poultry) and birds are commonly kept at night on perches within the family dwelling, frequently in the kitchen [14, 15]. In Ethiopia, it has been reported that chickens and poultry meat have a higher *Campylobacter* prevalence compared to other farm animal and meat products [16, 17], and exposure to pets and livestock, including chickens, has been associated with increased odds of *Campylobacter* isolation in diarrhoeal children aged <5 years [18]. Furthermore, in a study from Egypt, *Campylobacter* diarrhoea was more common in households where animals were present in food preparation areas [19]. It might, therefore, be anticipated that an important route by which rural families are exposed to *Campylobacter* is from the faeces of infected chickens, which may contaminate the shared living environment.

The national poultry population in Ethiopia is estimated at 49·3 million [20], and is constituted of about 97% indigenous chicken ecotypes [21]. These birds are predominantly maintained under a traditional free-range, scavenging or semi-scavenging system with minimal inputs for housing, feeding or healthcare. Despite their low production performances in terms of egg and meat output, indigenous chickens are well adapted to the local environmental conditions and fulfil a variety of social and cultural functions, in addition to being both nutritional and economic assets [14].

In the last 20 years, poultry production in Ethiopia has started to be seen as a profitable venture, and more families in urban and peri-urban areas have started to keep small- to medium-sized flocks (about 50–1000 birds) under semi-intensive management [21]. Entrepreneurs have also started to invest in the poultry industry by setting up larger flocks of exotic breeds kept under semi-intensive/intensive management, particularly in the cities of Dukem, Debre Zeit and Nazeret, close to the capital, Addis Ababa [22]. Efforts have also been made by the Government of Ethiopia since the early 1990s to boost the productivity of indigenous birds kept by poor rural families through its genetic improvement programme, based on the introduction and distribution of exotic breeds by poultry multiplication centres throughout the country [23]. Several non-governmental organizations are also involved in the distribution of intensively reared chickens to smallholder farmers. Although still accounting for a small proportion of Ethiopia's poultry, this ongoing shift to more intensive production using commercial breeds may alter the dynamics of zoonotic infections such as campylobacteriosis. While housing chickens may limit their exposure to environmental pathogens and reduce direct and indirect contacts between people and chickens, *Campylobacter* is known to spread very rapidly in housed flocks and interventions to limit exposure and transmission are likely to be ineffective, impractical or unaffordable in village poultry production settings. Stresses, such as heat stress, or food and water restriction, is known to be detrimental to the chicken's immune function, altering tissue invasion of intestinal bacteria [24, 25]. However, different types or lines of chickens show variation in both the effect of heat stress on their immune response [26] and their ability to regulate the immune response to *Campylobacter* [27]. The links between management system, chicken welfare, stress and the biology of *Campylobacter* infections remain poorly understood, but have raised concerns about how farming systems may impact on public health risk [28–30].

As yet in Ethiopia there has been little research examining how the changing dynamics of chicken production may have consequences for public health. The aim of this study was to investigate the impact of production settings and systems in Ethiopia on the epidemiology of *Campylobacter*, and to consider the potential implications of this evolving production system for human health in the country. Therefore, the main objectives were (i) to determine the environmental prevalence of *Campylobacter* in different geographical areas, including

rural and peri-urban areas; and (ii) to investigate whether *Campylobacter* prevalence varied in chickens from the same area reared under different production systems.

MATERIAL AND METHODS

Study design

To investigate differences in *Campylobacter* prevalence in village chickens between geographical areas, a cross-sectional study was conducted between October 2012 and April 2013 in three *woredas* (administrative regions) in the Oromia region of Ethiopia. *Woredas* are further subdivided into *kebeles*, which are the lowest administrative unit in Ethiopia, and may contain several villages or sub-areas. *Woredas* were purposively selected based on known characteristics of their poultry production systems and bird ecotypes and, for the two rural sites, knowledge that there had been no recent poultry development programmes in the areas.

Study area

Horro *woreda* is located about 310 km west of the capital, Addis Ababa, and Jarso is about 560 km east. Over 95% of the population live in rural households in both *woredas* [31]. Horro is considered an area of high agricultural potential and produces an annual food surplus [32], whereas, in Jarso, agricultural land is limited and the area tends to have a food deficit every year, with the poor reliant on food aid to cover the food gap [33]. Debre Zeit (also called Bishoftu) is an urban centre located 47 km east of Addis Ababa within the Ada'a *woreda* in the East Shewa zone. This *woreda* contains a number of towns and is food secure [34].

Sampling was conducted in four *kebeles* in each of the two rural districts, where over 70% of households were estimated to own chickens, but where there were no known semi-intensive or intensive poultry farms. Twenty households were recruited to participate from each *kebele*; names of potential participants were randomly selected from a list of farmers in each *kebele*, provided by local development agents. Development agents are employees of the local agricultural extension service, whose role is to provide farmers with access to training, research and technologies (http://www.moa.gov.et/policies-and-strategies). Households were excluded from the study if they did not own at least two chickens: 30% extra additional names of potential participants were included in the random selection to allow for exclusions or non-participation. In the peri-urban area of Debre Zeit, five *kebeles* were selected for inclusion in the study, all of which contained at least one intensive chicken farm. As chicken ownership was believed to be markedly lower in this *woreda*, random selection of households was not considered feasible; instead, convenience sampling of households was undertaken based on transect walks through the *kebeles* and identification of households which kept free-ranging scavenging chickens. A total of 60 households across the five *kebeles* were recruited in this manner. In addition, in order to investigate differences in *Campylobacter* prevalence between different production systems, 20 intensive/semi-intensive farms within the same Debre Zeit *kebeles* were purposively selected for sampling during the same time period as the cross-sectional study. Sample sizes were determined based on available logistic and laboratory resources (i.e. DNA extraction kits) between the three study sites.

Sample collection

The unit of sampling was defined as either a backyard chicken-producing household (i.e. a household that kept at least two chickens under free-ranging scavenging conditions, with or without feed supplementation) or an intensive poultry farm. For the purposes of this study, an intensive farm was defined as one which kept at least 50 birds under confined conditions and provided all feed. On the day of sampling, flock information such as flock size and type was collected by interviewing the householder/farmer. Flock type was broadly grouped into four categories: indigenous ecotypes; Rhode Island Red (RIR), an established multi-purpose breed, or RIR hybrids (normally RIR crossed with local ecotypes, which are difficult to distinguish morphologically from pure RIR birds); Cobb 500, a commercial fast-growing broiler breed; and mixed flocks, which contained both local ecotypes and pure or hybrid RIR birds.

For each unit, a single sample was collected from the ground by walking through the household environment where chicken faeces were concentrated in the case of the extensive system or inside the chicken shed in the more intensive system, using one disposable fabric overshoe (boot sock) worn over the footwear. Boot socks were pre-moistened with sterile physiological saline before use, in order to allow maximum uptake of *Campylobacter*. To avoid cross-contamination between different flocks, for each sampling a clean disposable plastic overshoe was worn to separate the footwear from the fabric boot sock. After

Table 1. *Primers used in the 16S rRNA PCR assay for identification of the genus* Campylobacter *(product size 857-bp DNA)*

Primer	Sequence 5′–3′
C412 F	GGA TGA CAC TTT TCG GAG C
CampR2	GGC TTC ATG CTC TCG AGT T

collection, each boot-sock sample was carefully placed into a clean 'ziplock' sterile plastic bag and kept under refrigerated conditions until processing. Samples were processed within 6 h to 30 days depending on the district of collection.

Campylobacter detection

Boot socks were processed individually in the Microbiology Laboratory of Addis Ababa University College of Veterinary Medicine and Agriculture. Each boot sock was placed in a sterile plastic stomacher bag with 200 ml sterile saline water, and the mixture was treated in a stomacher at medium speed for 1 min to release detached matter. The boot sock was then left for about 10 min at room temperature to allow the solid material to settle. Without disturbing the sediment on the bottom, 1 ml of the suspension was then transferred to a 1·5 ml sterile Eppendorf tube. The suspension was centrifuged for 7 min at ~12 000 ***g***. The supernatant was discarded and the bacterial DNA was extracted from the pellet using a commercial DNA extraction kit (Promega Wizard Genomic DNA purification kit, USA). After DNA extraction, 50 μl of the supernatant, containing the suspended DNA, was added to 450 μl sterile distilled water, and stored at 4 °C. Samples were then exported under license to the University of Liverpool, where they were analysed with a PCR assay specific for the genus *Campylobacter* on the basis of 16S rRNA gene sequences (16S rDNA; Table 1) [35, 36].

Isolates were confirmed and identified to species level (*C. jejuni or C. coli*) using a multiplex PCR as described by Klena *et al*. based on differences in the *lpxA* gene (Table 2) [37]. All primers used in this work were obtained from Eurofins MWG Operon (Germany) and all PCR constituents were supplied by Thermo Scientific (UK).

Data analysis

Basic descriptive statistics, graphs and multilevel logistic regression modelling were performed using R software, using the core functions, the *plotrix* library, the *mgcv* library and the *lme4* library (*R* Foundation for Statistical Computing, Austria). A multilevel multivariable model was constructed to investigate whether area, production system or breed kept had any effect on the odds of a flock being detected as *Campylobacter* positive. As Cobb 500 chickens were only kept under intensive systems, and all other types were only kept under backyard scavenging systems, the variables 'breed' and 'production system' were combined to create a variable called 'type of flock', with four categories: 'intensive-Cobb 500', 'scavenging-RIR', 'scavenging-mixed', and 'scavenging-indigenous'. The functional form of the relationship between flock size (the only continuous explanatory variable) and the outcome was assessed using generalized additive models and suggested inclusion of a linear relationship was appropriate. Backward stepwise model selection was used to identify fixed effects to be retained in the model. The full model included the presence or absence of *Campylobacter* (at the genus level, as detected by PCR) as the outcome variable and the type of area (urban or rural), the type of flock and the flock size variables as fixed effects. Two-way interaction terms were also considered. *Kebele* was included as a random variable to account for clustering within geographical locations. Variables with a likelihood ratio test statistic P value $\leqslant 0{\cdot}05$ were retained in the model.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

RESULTS

A total of 239 boot-sock samples were collected from the three districts: 79 from Horro, 80 from Jarso and 80 from Debre Zeit. The overall *Campylobacter* prevalence detected across the three geographical areas using PCR diagnostic techniques was 18·4%. PCR assay for assignment of *Campylobacter* species was performed on all 44 *Campylobacter*-positive samples. In total, only 16 (36·4%) samples gave positive results using this multiplex PCR assay, and for all 16 samples a positive result was obtained for *C. jejuni*.

In the rural Horro region, all flocks sampled consisted purely of indigenous bird ecotypes, while in Jarso, despite a reported absence of current distribution

Table 2. *Primers used in the* lpx *gene PCR assay for* Campylobacter *isolates' speciation (product size:* C. coli *391 bp and C. jejuni 331 bp)*

Primer	Sequence 5′–3′
lpxAC. Coli	AGACAAATAAGAGAGAATCAG
lpxAC. Jejuni	ACAACTTGGTGACGATGTTGTA
lpxARKK2 m	CAATCATGDGCDATATGASAATAHGCCAT

programmes for exotic birds in this rural region, 12 (15%) of the 80 flocks sampled were made up either partially ($n = 9$), or entirely ($n = 3$) of RIR or RIR hybrids. In the peri-urban *kebeles* around Debre Zeit, 28% of the scavenging flocks consisted partly ($n = 10$), or exclusively ($n = 7$) of RIR or RIR hybrids. All 20 of the intensive farms sampled reared Cobb 500 broilers.

Higher *Campylobacter* prevalence was found in the peri-urban (46·3%) compared to the rural (4·4%) environments (Fig. 1; $\chi^2 = 105$, $P < 0{\cdot}001$). The prevalence was still significantly higher in the peri-urban flocks (41·7%), even when the intensive flocks were excluded ($\chi^2 = 45{\cdot}5$, $P < 0{\cdot}001$). Of the two rural areas, Horro had the higher prevalence (8·9%) of positive flocks, while no flocks were detected as positive by PCR in the Jarso region.

The final multi-level multivariable model (Table 3) suggested that flocks in the peri-urban area were at considerably greater odds of being detected *Campylobacter* positive [odds ratio (OR) 21·9, 95% confidence interval (CI) 2·0–235·0], even after controlling for the type of flock. After controlling for type of area, there was insufficient evidence to reject the null hypothesis of no difference in risk between mixed exotic/indigenous and indigenous-only backyard flocks. By contrast, backyard flocks which consisted only of RIR or RIR hybrids and housed flocks of Cobb 500 chickens were at increased risk compared to the backyard mixed flocks (OR 18·1, 95% CI 1·8–183·9 and OR 6·5, 95% CI 1·1–38·7, respectively). Inclusion of flock size or any two-way interaction terms did not significantly improve the model (likelihood ratio test statistic P values >0·05); hence these were not included in the final model.

DISCUSSION

The greatest risk factor identified in this study for detection of *Campylobacter* in the environment of the sampled chicken flocks was location in the peri-urban area, where many farms are starting to intensify their production systems. *Campylobacter* is highly prevalent in intensive production systems in developed countries, with birds often having high levels of infection, posing a public health risk. While it is therefore perhaps unsurprising that the more intensively farmed flocks in the peri-urban areas were at increased risk, it is of particular concern that the greater risk in the peri-urban area persisted despite controlling for the type of flock, suggesting increased risk in all flock types in the peri-urban area, irrespective of breed and production type. Although the high levels of *Campylobacter* in intensive and semi-intensive flocks are clearly of concern for farm and abattoir workers and for public exposure through contaminated meat (as is the case in more economically developed countries), this study suggests that attention should also be paid to other potential routes of transmission in situations where scavenging poultry remain commonplace. The backyard flocks, which live closely with people, can potentially contaminate the human living and food preparation environment, and infections may be shared with livestock and other animals (including peri-domestic pests) in the household.

Further work is needed to identify the reasons for the increased detection of *Campylobacter* in flocks in the peri-urban area, compared to the rural areas. Key questions to be addressed should include the impact of the close proximity of backyard production to large intensive farms, the potential effect of the distribution of exotic or improved birds from poultry multiplication centres to smallholder producers, the effect of movement of birds through trade, and other factors which may be associated with differences in the ecology of *Campylobacter* between peri-urban and rural areas. Furthermore, where there is close inter-species contact, such as was evident in many households in this study, the potential role of transmission from humans or other peri-domestic animals and livestock to chickens should also be considered.

An alternative explanation for the higher prevalence of detection in the peri-urban area, independent of the type of flock, is that there is some other local factor in this region which increases the risk of

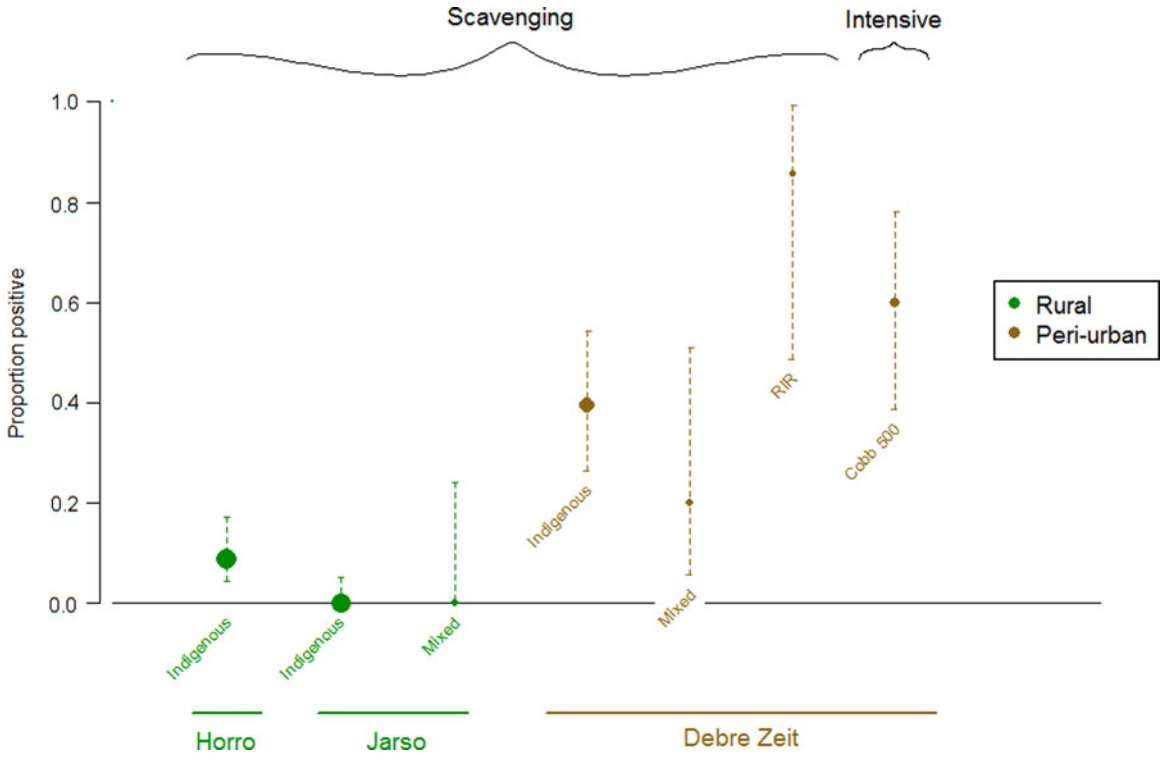

Fig. 1. Proportion of flocks tested positive by PCR for *Campylobacter*, showing location, bird and farm type. Points are scaled relative to the number of flocks of each type tested.

Table 3. *Final multi-level multivariable logistic regression model of factors associated with detection of* Campylobacter *by PCR from environmental samples collected from chicken production sites in three regions of Ethiopia*

Variable	Levels	Parameter estimate	S.E.	OR	95% CI	*P* value
Fixed effects						
Intercept		−4·5	1·1			
Type of area	Rural	Reference				0·04*
	Peri-urban	3·1	1·2	21·9	2·0–235·0	0·01
Type of flock	Backyard: mixed	Reference				0·03*
	Backyard: indigenous	1·1	0·8	2·9	0·6–15·2	0·2
	Backyard: RIR/hybrid	2·9	1·2	18·1	1·8–183·9	0·01
	Housed: Cobb 500	1·9	0·9	6·5	1·1–38·7	0·04
Random effect		Variance estimate				
Kebele		0·8				

S.E., Standard error; OR, odds ratio; CI, confidence interval; RIR, Rhode Island Red.
* Likelihood ratio statistic *P* value for overall variable.

detection of *Campylobacter* in this area, such as a more favourable environment, climate or increased presence of wild bird, insect or rodent vectors. One of the features of Debre Zeit is the presence of numerous permanent and seasonal lakes, which provide habitat for birds and insects. The positive flocks in Horro were also located in *kebeles* where bodies of water are a feature, whereas Jarso, where no positive flocks were identified, has a much more arid climate and no substantial water bodies were present in the *kebeles* where sampling was carried out. Only a single peri-urban region was sampled in this study, therefore these results may not be generalizable to other areas. However, it is of note that other major urban centres

of poultry production and distribution in Ethiopia, such as Awassa and Bahir Dar, are also located close to large permanent lakes.

Of the backyard flocks, the odds of *Campylobacter* detection in the environmental samples was greatest for those flocks consisting of only RIR birds, or their hybrids. The cause of this is unknown, but factors underpinning this finding may include a genetic susceptibility of this breed, that this breed type is subjected to particular stress when kept under backyard management, or differences in the *Campylobacter*-exposure risk between these and indigenous flocks, such as bird sources, contact patterns or behaviours. It has previously been reported that survival of RIR-type birds in backyard conditions is poor, compared to indigenous breeds, and they are poorly adapted to scavenging and highly susceptible to disease [38]. It should be considered that such stress might have implications beyond the health and welfare of the bird, if it alters carriage of zoonotic pathogens such as *Campylobacter*.

A potential limitation of this study was that samples collected from the two rural areas had, of necessity, a longer gap between collection and processing than samples collected in the peri-urban area, which was in close proximity to the laboratory facility. It has not been possible, in this study, to analyse whether this time lag had any impact on the detection rate of *Campylobacter* from the study samples. While viable *Campylobacter* decline rapidly in stored samples, especially at temperatures >4 °C, it has been shown that DNA-based PCR methods continue to detect *Campylobacter* in manure for at least 30 days even in samples stored at temperatures up to 52 °C [39]. However, this also highlights that the PCR-based method used in this study detected *Campylobacter* DNA, not viable bacteria, so further work is required to better evaluate the public health risk of these findings.

Only just over one-third of *Campylobacter*-positive samples could be identified to the species level. This is not surprising, as the multiplex PCR [37] was not designed for speciation on DNA extracted from bootsock samples and, given the amount of DNA present in such samples, sensitivity may be an issue. That all 16 samples identified to the species level were found to be *C. jejuni* may suggest that this is the most prevalent species. However, the possibility that other species are present should not be discounted, particularly as these may include some species less pathogenic to people. Further investigation with more sensitive methods is needed in order to clarify the relative importance of different *Campylobacter* spp. and molecular epidemiological approaches are required to determine the extent to which the *Campylobacter* identified in each area and flock-type are similar.

There is currently much interest in Ethiopia in developing the poultry industry and enhancing the productivity of backyard chickens, and current programmes often advocate the adoption of high-producing breeds and changing from scavenging to semi-intensive management. This can be readily observed in the peri-urban areas, such as Debre Zeit, where exotic chickens such as the RIR have been incorporated into the scavenging flocks, with the aim of enhancing egg production. There is also ongoing research to retain the desirable characteristics of the indigenous poultry types while raising the output of eggs and meat from the scavenging system by selective and cross-breeding of the local ecotypes with exotic breeds. Since this study was performed, 'improved' indigenous birds selected over seven generations for increased productivity traits (body weight at age 16 weeks and cumulative egg number at week 45 of lay) in Debre Zeit have been placed on farms in the Horro and Debre Zeit regions to evaluate their performance under semi-intensive conditions. While higher productivity is desirable to increase food availability, it is important that measures to enhance food security are also evaluated in terms of their ability to produce safe food.

Backyard chickens are an important component of families' livelihoods, and likely to remain so for many years to come [40], even in the face of increasing urbanization. While chicken production can contribute to improved livelihoods and nutrition, careful consideration is required of all impacts of programmes to develop the chicken production sector if negative human health effects may also occur. Here, we identify a greater risk of environmental detection of *Campylobacter* in a major peri-urban production area in Ethiopia, compared to rural areas. Further work is needed to determine whether other zoonotic pathogens may be similarly affected. If chicken production in urban/peri-urban areas increases the risk of environmental contamination with *Campylobacter* (and, potentially, other zoonotic pathogens), then managing these risks will be a vital component of veterinary public health services in these areas. Furthermore, agricultural development programmes aimed at improving chicken production in rural areas, especially where this involves distribution of intensively reared birds or major changes to the management system, need to

carefully consider the potential for perturbation to the ecology of *Campylobacter* in these areas.

ACKNOWLEDGEMENTS

We thank Biotechnology and Biological Sciences Research Council (BBSRC), the UK Department for International Development (DFID) and the Scottish Government for providing funding for the 'Reducing the impact of infectious disease on poultry production in Ethiopia' project under the Combating Infectious Diseases of Livestock for International Development (CIDLID) programme (BB/H009396/1). Further funding was provided by the Institute of Infection and Global Health at the University of Liverpool. We thank the Chicken Health for Development project team members and the study participants for their time and assistance. Funders had no involvement in study design; the collection, analysis and interpretation of data; the writing of the manuscript; or in the decision to submit the article for publication.

DECLARATION OF INTEREST

None.

REFERENCES

1. **World Health Organisation/United Nations Children's Fund.** Ending preventable child deaths from pneumonia and diarrhoea by 2025 The integrated global action plan for pneumonia and diarrhoea (GAPPD). Geneva, 2013.
2. **Fischer Walker CL, *et al.*** Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. *BMC Public Health* 2012; **12**: 220.
3. **Central Statistical Agency (Ethiopia) and ICF International.** Ethiopia Demographic and Health Survey 2011. Addis Ababa, Ethiopia and Calverton, Maryland, USA, 2012.
4. **Coker AO, *et al.*** Human campylobacteriosis in developing countries. *Emerging Infectious Diseases* 2002; **8**: 237–244.
5. **Gedlu E, Aseffa A.** *Campylobacter* enteritis among children in north-west Ethiopia: a 1-year prospective study. *Annals of Tropical Paediatrics: International Child Health* 1996; **16**: 207–212.
6. **Mitike G, *et al.*** *Campylobacter* enteritis among children in Dembia District, Northwest Ethiopia. *East African Medical Journal* 2000; **77**: 654–657.
7. **Whiley H, *et al.*** The role of environmental reservoirs in human campylobacteriosis. *International Journal of Environmental Research and Public Health* 2013; **10**: 5886–5907.
8. **Wagenaar JA, Mevius DJ, Havelaar AH.** *Campylobacter* in primary animal production and control strategies to reduce the burden of human campylobacteriosis. *Revue Scientifique et Technique (International Office of Epizootics)* 2006; **25**: 581–594.
9. **Sheppard SK, *et al.*** *Campylobacter* genotypes from food animals, environmental sources and clinical disease in Scotland 2005/6. *International Journal of Food Microbiology* 2009; **134**: 96–103.
10. **Adak GK, *et al.*** Disease risks from foods, England and Wales, 1996–2000. *Emerging Infectious Diseases* 2005; **11**: 365–372.
11. **Osano O, Arimi SM.** Retail poultry and beef as sources of *Campylobacter jejuni*. *East African Medical Journal* 1999; **76**: 141–143.
12. **Adegbola RA, *et al.*** Correlation between human and animal bio-serogroups of Campylobacter isolates in Nigeria. *Journal of Tropical Medicine and Hygiene* 1990; **93**: 280–3.
13. **CIA.** The World Factbook (https://www.cia.gov/library/publications/the-world-factbook/geos/et.html). Accessed 6 January 2014
14. **Dessie T, Ogle B.** Village poultry production systems in the central Highlands of Ethiopia. *Tropical Animal Health and Production* 2001; **33**: 521–537.
15. **Halima H, *et al.*** Village-based indigenous chicken production system in north-west Ethiopia. *Tropical Animal Health and Production* 2007; **39**: 189–197.
16. **Kassa T, Gebre-selassie S, Asrat D.** The prevalence of thermotolerant *Campylobacter* species in food animals in Jimma Zone, Southwest Ethiopia. *Ethiopian Journal of Health Development* 2005; **19**: 225–229.
17. **Dadi L, Asrat D.** Prevalence and antimicrobial susceptibility profiles of thermotolerant *Campylobacter* strains in retail raw meat products in Ethiopia. *Ethiopian Journal of Health Development* 2008; **22**: 195–200.
18. **Lengerh A, *et al.*** Prevalence, associated risk factors and antimicrobial susceptibility pattern of *Campylobacter* species among under five diarrheic children at Gondar University Hospital, Northwest Ethiopia. *BMC Pediatrics* 2013; **13**.
19. **Rao MR, *et al.*** Pathogenicity and convalescent excretion of *Campylobacter* in rural Egyptian children. *American Journal of Epidemiology* 2001; **154**: 166–173.
20. **CSA.** Report on livestock and livestock characteristics (private peasant holdings). Addis Ababa: Central Statistical Agency, 2010/11.
21. **FAO.** Poultry sector country review. Rome: Food and Agriculture Organization of the United Nations, 2008.
22. **Mammo M.** Chicken production scenarios and the headway options for improvement in Ethiopia. *World's Poultry Science Journal* 2012; **68**: 299–305.
23. **FAO.** Review of the new features of the Ethiopian poultry sector biosecurity implications. Rome: Food and Agriculture Organization of the United Nations, 2008.
24. **Leitner G, Heller ED.** Colonization of *Escherichia coli* in young turkeys and chickens. *Avian Diseases* 1992; **36**: 211–220.
25. **Mashaly MM, *et al.*** Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poultry Science* 2004; **83**: 889–894.

26. **Tirawattanawanich C, *et al.*** The effects of tropical environmental conditions on the stress and immune responses of commercial broilers, Thai indigenous chickens, and crossbred chickens. *Journal of Applied Poultry Research* 2011; **20**: 409–420.
27. **Humphrey S, *et al.*** *Campylobacter jejuni* is not merely a commensal in commercial broiler chickens and affects bird welfare. *mBio* 2014; **5**, e01364–01314.
28. **Humphrey T.** Are happy chickens safer chickens? Poultry welfare and disease susceptibility. *British Poultry Science* 2006; **47**: 379–391.
29. **Rostagno M.** Can stress in farm animals increase food safety risk? *Foodborne Pathogens and Disease* 2009; **6**: 767–776.
30. **Humphrey T.** Zoonotic diseases, human health and farm animal welfare: *Campylobacter*. Compassion in World Farming/World Society for the Protection of Animals, 2013 (http://www.ciwf.org.uk/includes/documents/cm_docs/2013/c/campylobacter.pdf). Accessed 12 August 2013.
31. **CSA.** The 2007 population and housing census of Ethiopia: statistical report for Oromiya region. Addis Ababa: Central Statistical Agency, 2007.
32. **FEG.** Livelihood Profile Oromiya Region, Ethiopia Central Horo Teff, Nug and Cattle (CHT) Livelihood (http://www.feg-consulting.com/feg-shared-folder/liu/oromiya/woreda-profiles/horo-gurduru/Guduru.pdf). Accessed 20 February 2014
33. **FEG.** Livelihood Profile Oromiya Region, Ethiopia Chat Vegetable and Groundnut (CVG) Livelihood Zone (http://www.feg-consulting.com/feg-shared-folder/liu/oromiya/woreda-profiles/east-haraghe/Jarso.pdf). Accessed 20 February 2014.
34. **FEG.** Livelihood Profile Oromiya Region, Ethiopia Becho-Adea Teff and Chickpea (BAT) Livelihood Zone (http://www.feg-consulting.com/feg-shared-folder/liu/oromiya/woreda-profiles/east-shewa/Adaa.pdf). Accessed 19 March 2014.
35. **Linton D, Owen RJ, Stanley J.** Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. *Research in Microbiology* 1996; **147**: 707–718.
36. **Katzav M, *et al.*** PCR assay for the detection of *Campylobacter* in marinated and non-marinated poultry products. *Food Microbiology* 2008; **25**: 908–914.
37. **Klena JD, *et al.*** Differentiation of *Campylobacter coli, Campylobacter jejuni, Campylobacter lari*, and *Campylobacter upsaliensis* by a multiplex PCR developed from the nucleotide sequence of the lipid A gene *lpxA*. *Journal of Clinical Microbiology* 2004; **42**: 5549–5557.
38. **Dana N, *et al.*** Production objectives and trait preferences of village poultry producers of Ethiopia: implications for designing breeding schemes utilizing indigenous chicken genetic resources. *Tropical Animal Health and Production* 2010; **42**: 1519–1529.
39. **Bui XT, *et al.*** Fate and survival of *Campylobacter coli* in swine manure at various temperatures. *Frontiers in Microbiology* 2011; **2**: 262 (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3245958/pdf/fmicb-02-00262.pdf).
40. **Alders RG, Pym RAE.** Village poultry: still important to millions, eight thousand years after domestication. *World's Poultry Science Journal* 2009; **65**: 181–190.