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ISOLATION OF CAMPYLOBACTER SPPAND ESCHERICHIA COLI 0157: H7 FROM FREE-RANGE INDIGENOUS CHICKEN VALUE CHAIN IN KENYA

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ABSTRACT

Objectives: **To determine the biosafety of a free range indigenous chicken value chain with reference to zoonotic bacteria, *Campylobacter spp* and *Escherichia coli* 0157: H7**
Design: **cross-sectional sampling of chickens and chicken meat carcasses at farm and market level**
Setting: **Makueni and Nairobi Counties**
Subjects: **Cloacal swabs were collected, 280 in farms and 390 in live bird market. Forty dressed carcasses were obtained from the market's slaughter facility and rinse- wash fluid prepared from each carcass. Cloacal swabs and rinse-wash fluid samples were cultured in selective media to isolate the specific organism. *Campylobacter spp* was confirmed at genus level by biochemical tests and PCR analysis for 16S rRNA gene, and at species level by multiplex PCR. *Escherichia coli* O157:H7 was confirmed by biochemical and serological tests.**
Results: **The prevalence of *Campylobacter spp* in farm, live bird and in dressed carcasses was 50.87%, 9.49% and 27.5% respectively. *C. jejuni* and *C. coli* had a prevalence of 36.78% and 6.42%; 3.85% and 0.77%; and 7.5% and 0% at the three value chain levels respectively. *E. coli* O157:H7 had a prevalence of 1.42%, 5.92% and 11.42 % in the three levels respectively.**
Conclusion: **Free-range chicken value chain may carry zoonotic organisms such as *Campylobacter spp* and *E.coli* 0157:H7. There is need therefore to sensitise consumers in proper handling and cooking of meat carcasses to minimize threat to human health**

INTRODUCTION

Worldwide, poultry and poultry products have been identified as a source of food borne diseases (1, 2). Poultry meat is believed to be the main source of *Campylobacter spp* infections which cause human gastro-enteritis more frequently than other enteric pathogens in developed countries (3). The main species involved are *Campylobacter jejuni* and *C. coli* (4). *Escherichia coli* O157:H7 is an important food pathogen in the developed world (5, 6) and ruminants, particularly cattle, are considered the most important reservoir (6,7). In Africa, the importance of *E.coli* O157:H7 in foodborne illnesses has recently emerged but little is known about the reservoir (8) although the organism has been isolated in chicken and processed poultry in Nigeria and Senegal (9, 10,11). The role of poultry in the transmission of *E.coli* O157:H7 to humans has however not been clearly established. Production, marketing and processing of free-range indigenous chicken in Kenya is practiced under minimal biosecurity measures, which may expose the chicken to pathogenic zoonotic microorganism and pose a health risk to consumers (12,13). Studies on value chain biosafety have been limited mainly to intensive production systems in developed countries (14,15, 16, 17, 18). This study aimed to assess the biosafety of a free range indigenous chicken value chain in Kenya by estimating the prevalence of *Campylobacter spp* and *E.coli* 0157:H7

MATERIALS AND METHODS

Study site: The study comprised of collection and laboratory analysis of cloacal swabs and dressed chicken carcasses. Sampling was as carried out in a rural County, Makueni and in the capital City County, Nairobi. The rural County is a major supplier of indigenous chicken to Nairobi County. A live chicken market, Burma Maziwa, is one of several unstructured markets in Nairobi. It receives chicken from several parts of the country but the traders' organizational structure facilitates identification of the origin of the chicken. A non-regulated poultry slaughter facility in the market is a significant outlet of dressed carcasses for supermarkets, butcheries, and homes within Nairobi city.

Samples: A cross-sectional sampling was carried out in the two study areas between October 2012 and September 2013. Cloacal swabs were collected using cotton swabs in casings containing Cary-Blair transport medium (Zhejiang Gongdong Medical Technology Co., Ltd. China). A total of 280 swabs were collected from 25 farms randomly selected from a list of 76 in four of the six sub-counties, 12 per farm. At the market, swabs were collected randomly from all chicken clusters originating from Makueni County in 8 sampling days. A total of 390 swabs were collected. The swabs were labelled, and placed in a cool box. Forty (40) dressed carcasses of the birds swabbed for cloacal materials were obtained. Every 7th bird swabbed was selected for carcass collection. The carcasses were put in sterile double polythene bags, labelled, and placed in a cool box. The swabs and carcasses were transported to laboratory within 6hrs. In the laboratory, the carcasses were rinse-washed with 400mls of buffered peptone water (pH 7.2) following the method described by NACMCF (19). The rinse fluid was then analysed for the organisms.

Isolation of Campylobacter spp: *Campylobacter* blood-free medium containing antibiotics and supplement (mCCDA, Oxoid CM739, UK) was used for isolation of thermophilic *Campylobacter* species. All samples were cultured directly on the media (20,3) within 6 hrs of collection. Samples collected in farms were cultured in a local hospital laboratory while those from the market were cultured at the Faculty of

Veterinary Medicine, University of Nairobi. Swabs were streaked directly on the media. Tenfold serial dilutions of the rinse-wash fluid were prepared in peptone water and 0.5mls of 4 consecutive serial dilutions, 100 to10-3, inoculated into the media using the spread plate method. Inoculated plates were incubated at 42^{0}C, (21), for 48 hours in candle extinction jar (22,23). Suspect *Campylobacter* colonies were then selected for further analysis by Gram stain, catalase and oxidase biochemical tests. The Gram stain was performed using reagents prepared according to WHO (24) method. Suspect isolates were confirmed by DNA analysis.

Campylobacter DNA analysis: Confirmation of *Campylobacter* genus was done using PCR analysis for 16S rRNA gene (Linton et al., 1997) and identification of C. jejuni and C. coliby multiplex PCR using species specific primers. The primers were based on nucleotides sequences of monospecific probes from DNA fragments library (25). Primers for *Campylobacter* genus (C412F and C1228R) generated amplicons of 812bp. *Campylobacter jejuni* primers (ENg03F and ENg04R) and *C. coli* primers (ENg01F and ENg02R) generated amplicons of 773bp and 364bp respectively.

DNA extraction: A loopful of suspect *Campylobacter* colonies was harvested and suspended in 200µl of sterile distilled water in labelled 0.5ml Eppendorf tubes. The tubes were then heated in boiling a water bath at 100^{0}C for 10 minutes, cooled immediately on ice for 5-10 minutes and then centrifuged (Eppendorf Gerãtebau; West Germany) at 11,000×g for 5 minutes. The supernatant was stored at -20^{0}C and used as DNA templates.

DNA amplification: Amplification for the genus DNA was performed in a 25µl reaction volume per sample. Briefly, aliquots of 12.5µl of Taq Master Mix (Qiagen GmbH, Limburg, Netherlands), 10pmol of each primer (Bioneer, Inc. USA), 5µl of DNA template and 7.3µl of molecular grade water (Qiagen GmbH, Limburg, Netherlands) were put in labelled sterile PCR tubes, and placed in a thermocycler(MJ Research, Watertown, MA, USA).. The samples were subjected to initial denaturation temperature of 95^{0}C for 10 minutes followed by 35 cycles of denaturation at 95^{0}C for 30 seconds, annealing at 59^{0}C for 90 seconds, extension at 72^{0}C for 60 seconds and a final extension of 72^{0}C for 10 minutes (25). Amplification of *C. jejuni* and *C. coli* species DNA was performed in a 50µl multiplex reaction volume per sample as follows. Briefly, 25µl Taq PCR Master Mix (Qiagen GmbH, Limburg, Netherlands), 5µl of DNA template, 60pmol of *C. coli* primers (Bioneer, Inc. USA), 25pmol of *C. jejuni* primers (Bioneer, Inc. USA) and 18.3µl of molecular grade water (Qiagen GmbH, Limburg, Netherlands) were put into labelled PCR tubes. The PCR protocol included initial denaturation temperature of 94^{0}C for 5 minutes; 2 cycles of 1 minute at 94^{0}C, 1 min at 64^{0}C, and 1 minute at 72^{0}C; 2 cycles of 1 minute at 94^{0}C, 1 minute at 62^{0}C, and 1 minute at 72^{0}C; 2 cycles of 1 minute at 94^{0}C, 1 minute at 60^{0}C, and 1 minute at 72^{0}C; 2 cycles of 1 minute at 94^{0}C, 1 minute at 58^{0}C, and 1 minute at 72^{0}C; 2 cycles of 1 minute at 94^{0}C, 1 minute at 56^{0}C, and 1 minute at 72^{0}C; 30 cycles of 1 minute at 94^{0}C, 1 minute at 54^{0}C, and 1 minute at 72^{0}C; and a final extension step of 10 min at 72^{0}C (25)

Agar gel electrophoresis: Amplicons were analysed by gel electrophoresis in agarose (Ultra PURETM, BRL, and Gaithersburg, MD) containing ethidium bromide (77µl/100mls) and submerged in 1x Tris-acetate buffer solution. Electrophoresis of *Campylobacter* genus and species amplicons was performed in1.3% and 1% agarose gel respectively. The PCR products were mixed with the loading dye (4:1) and loaded into the gel wells. A 100bp DNA molecular ladder was used as size reference. Genomic DNA from *C. jejuni* (Kenya Medical Research Institute (KEMRI) 4529 and 4478)

and *C. coli* (KEMRI 4443 and 4543) were used as positive control in all the PCR assays. Electrophoresis was conducted at 100V for 1.5hrs after which the amplicons were viewed and photographed under UV-transilluminator (VilberLourmat).

Isolation of E.coli 0157: H7:Isolation of E.coli: 0157: H7 was carried out, first by incubating the cloacal swabs dipped into tubes containing 4mls Lauryl broth (HimediaM091, India) , and 10mls aliquots of the rinse- wash fluid , at 37^{0}C for 2hrs for pre-enrichment. Loopfuls of the cultures were then streaked onto sorbitol MacConkey agar plates (Oxoid CM 813, UK). The plates were incubated at 37^{0}C for 18-24 hours. Suspect sorbitol negative colonies were then streaked onto MacConkey agar (Oxoid- CM 0507 UK) plates and incubated at 37^{0}C for 18-24hrs. Lactose positive colonies were then subjected to indole, methyl red, Voges – Proskauer and citrate (IMViC) tests. Colonies positive formethyl red and indole tests were re-streaked onto sorbitol MacConkeyagar and incubated at 37^{0}C for 24 hrs. Sorbitol negative colonies were stored at 10% skimmed milk (OxoidLP 0031, UK) at -20^{0}C before serotyping.

Serotyping of E.coli O157:H7:E.coli: O157:H7 suspect isolates were confirmed by serotyping using Wellcolex® *E.coli* O157:H7 latex kit (OxoidR30959601, UK,). Isolates stored in 10% skimmed milk were inoculated into tryptonesoya Agar slants (OxoidCM 0131, UK) and incubated at 37^{0}C for 20hrs. The bacterial suspension at the bottom of the slants was then used as the antigen. Equal volumes of 20µl of the bacterial broth and latex reagent were tested. Negative control tests were performed according to kit instructions. All cultures positive for O157 antigen were further tested for the H7 antigen.

RESULTS

The prevalence of the two zoonotic microorganisms in the three value chain levels is summarized in Table 1. Two morphological types of *Campylobacter* colonies were observed as described (26, 27). They were large, flat, irregular, greyish, and watery with a tendency to spread, or greyish, raised convex, with discrete margins. Occasionally, more than one colony type was isolated from the same sample. Most presumptive isolates were positive for catalase and oxidase tests, but a few were positive only in either test.

Table 1
The prevalence of Campylobacter spp, and Escherichia coli 0157:H7 in free-range indigenous chicken value chain in Kenya

Organism	Value chain level and prevalence					
	Farm level		Market level		Dressed carcasses	
	Presumptive	Confirmed	Presumptive	Confirmed	Presum ptive	Confirmed
Total *Campylobacters*	178/280 (63.57%)	142/289 (50.87%)	111/390 (28.4%)	37/390 (9.49%)	20/40 (50%)	11/40 (27.5%)
C. jejuni	-	103/280 (36.78%)	-	15/390 (3.85%)	-	3/40 (7.5%)
C. coli	-	18/280 (6.43%)	-	3/390 (0.77%)	-	0%
C. jejuni and *C. coli*	-	5/280 (1.78%)	-	0%	-	0%
Other *Campylobacters*	-	26/142 (9.28%)		19/390 (4.87%)	-	8/390 (20%)
E.coli 0157	37/280 (13.2%)	4/280 (1.42%)	106/338 (31.36%)	29/338 (8.58%)	4/35 (1.42%)	4/35(11.42%)
E.coli 0157:H7	-	3/280 (1.07%)	-	20/338 (5.9%)	-	4/35(11.42%)

At farm level, *Campylobacter* was suspected in 178 of the 280 (63.57%) samples on the basis of colonial morphology and biochemical tests. The PCR analysis for the 16S rRNA gene confirmed *Campylobacter* genus in 142 of the 178 suspects, giving a prevalence of 50.87% [95% CI: (44.87- 56.55)]. Multiplex PCR further confirmed *C. jejuni* in 108(38.57%) isolates and *C. coli* in 23, (8.21%) isolates. Concomitant *C. jejuni* and *C. coli* infections were observed in 5 (1.78%) samples. At live bird market level, *Campylobacter* genus was suspected in 111 (28.4%) of 390 samples but only 37 (9.49%) isolates were confirmed. [95% CI: (6.87- 12.71]. *Campylobacter jejuni* and *C. coli* were confirmed in 15 (3.35%) and3 (0.76%) isolates respectively. In chicken carcasses, suspect *Campylobacter* colonies were detected in 20 (50%) of the 40 samples. *Campylobacter* genus was confirmed in 11 (27.5%) isolates [95% CI: (15.39 - 42.7)]. The prevalence of *C. jejuni* was 7.5% but *C. coli* were not detected in any sample. At farm level, sorbitol negative *E. coli* was isolated from 37 (13.2%) of 280 samples. Of these, (1.42%) were confirmed as *E. coli* 0157 and 3 (1.07%) we reconfirmed as H7 [95% CI: (0.22- 3.09)]. At market level 31.36 % (106/338) isolates were sorbitol negative and 29 (8.58%) isolates were confirmed *E. coli* 0157, and 20 (5.91%) confirmed *E. coli* 0157:H7 [95% CI: (3.72- 8.80)]. In carcasses 4/35, (11.42%) were presumptive for *E.coli* 0157, and all were confirmed *E.coli* O157 and H7 [95% CI: (3.74 -25.31)]. At farm and market level, three and two samples had concomitant infections with *Campylobacter* species and *E.coli* 0157:H7 respectively.

DISCUSSION

The findings in this study indicated that free range indigenous chicken and chicken carcasses carry *Campylobacter* and *E. coli* 0157:H7 organisms. *Campylobacter* species were identified at a prevalence of 50.87%, 9.49% and 27.5% in the three value chain levels respectively. In Tanzania (28) and in Nigeria (29) a genus prevalence of 69.8% and 77.6% respectively has been reported at farm level while a prevalence of 56% has been reported in chicken carcasses in Senegal (11).

Campylobacter jejuni was dominant at the farm level with a prevalence of 36.7% and contributed 72.5% of all confirmed *Campylobacters*. Salihuet al., (29) reported a 67% *Campylobacter jejuni* prevalence in indigenous chicken in Nigeria. *Campylobacter* species other than *C. jejuni* and *C. coli* were dominant at the market and carcass levels at 4.87% and 20% respectively. In contrast, Cardinal et al., (2003) found *C. jejuni* dominant in local chicken carcasses in Senegal at 59%. Though the prevalence of *C. jejuni* was relatively low at market and carcass levels (3.5% and 7.5% respectively) in this study, the public health risk is significant considering the low infective dose for *C. jejuni* of 500 to 10,000 cells (30), and the possibility of cross contamination of other carcasses and foodstuffs. The prevalence of *C. coli* was the least in the three levels of the value chain, at 6.43%, 0.76% and 0% in the farms, live bird market and carcasses respectively, an indication that the organism may not be a significant pathogen in chickens in the study areas. The findings in this study however, concur with other studies that live poultry and poultry meat are important sources of Campylobacter infections to human (3,4, 31, 32).

In the study, the prevalence of *E. coli* O157:H7 in cloacal samples was 1.07%, 5.91% and 11.43% at the farm, market, and carcass levels respectively. Comparative data on occurrence of *E. coli* O157:H7 in chickens in general and scavenging chicken in particular is scarce. At farm level, a prevalence of 13.6% has been reported in Norway (33) and 0.9% in United States (34). In live bird market, the findings a comparable to the 5% reported in Nigeria (9). Previous studies to detect the bacteria in poultry meat have reported lower values than in this study. In Turkey (35), Hyderabad (India) (36) and Bangkok (Thailand (37), a prevalence of 1.05%, 4% and 2% have been reported respectively. Chang et al., (38) found much higher value of 40% in organic chicken meat in Malaysia market outlets. In the current study, the higher prevalence in carcasses compared to farm and live bird market was an indication of possible cross contamination of carcasses. Ruminants' dung is considered the most important reservoirs for *E. coli* O157 (6) and human infections are mainly associated with consumption of raw vegetables contaminated with cattle manure (39).There is need to establish whether infection in chicken is related to scavenging on ruminants' dung. Although the specific role of chicken in the transmission of *E. coli* O157 and subsequent human illnesses is lacking (40), the results of this study provide an indication that free-range chicken and chicken meat is a possible source of *E. coli* O157:H7 infection. Overall, this study establishes the presence of zoonotic bacteria in free range indigenous chicken value chain from Makueni to Nairobi County, Kenya and recommends a need to sensitize consumers on proper handling of meat carcasses to avoid cross contamination of other foodstuffs as well as adequate cooking.

CONCLUSION

In conclusion, free-range indigenous chicken and chicken meat carcasses may play a role

in dissemination of Campylobacter and E.coli 0157:H7 infections to humans. This system of production is a popular livestock enterprise in Kenya but the poor biosafety production and marketing practices may expose the consumer to such zoonotic microorganisms.

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REFERENCES

1. Food and Drug Administration (FDA) of the United States of America. Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook: Escherichia coliO157:H7.http://www.fda.gov/food/foodsafety/foodborneillness/foodborneillnessfoodbornepathogensnaturaltoxins/badbugbook/ucm071284.html on 21/8/2012.
2. Hermans, D., Pasmans, F., Messens, W., Martel, A., Van Immerseel, F., Rasschaert, G., Heyndrickx, M., VanDeun, K. and Haesebrouck, F. Poultry as a host for the zoonotic pathogen Campylobacter jejuni. Vector-BorneZoonot. 2012, 12: 89-98. DOI: 10.1089/vbz.2011.0676.
3. Man, S. M. The clinical importance of emerging Campylobacter species. Nat Rev Gastroenterol Hepatol. 2011; 8: 669-685. doi:10.1038/nrgastro.2011.191, PMid:22025030
4. Wagenaar, J.A.Campylobacter: a friend of poultry but an enemy of public health. Proceedings of the XXIV World´s Poultry Congress, 5 - 9 August 2012.Salvador - Bahia – Brazil
5. Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M. and Swerdlow, D. L.Epidemiology of Escherichia coliO157:H7 outbreaks, United States, 1982–2002. EmergInfec Dis.2005, 11: 603–609. DOI: 10.3201/eid1104.040739, PMCID: PMC3320345
6. Hedican, E.B., Medus, C., Besser, J.M., Juni, B.A., Koziol, B., Taylor, C. and Smith K.E.Characteristic of O157 versus Non-O157 Shiga Toxin-Producing Escherichia coli infections in Minnesota, 2000-2006.Clin Infect Dis 2009, 49:358-364. PMID: 19548834, DOI: 10.1086/600302
7. Ferens, W. A. and Hovde, C. J. Escherichia coli O157:H7: Animal Reservoir and Sources of Human Infection. Foodborne Pathog Dis. 2011, 8:465–487. DOI: 10.1089=fpd.2010.0673
8. Okeke, I. N. DiarrheagenicEscherichia coli in sub-Saharan Africa: status, uncertainties and necessities, J Infect DevCtries. 2009, 3: 817–842. PMID: 20061678
9. Umeh, S. I. and Okpokwasili, G.S.C. Seasonal prevalence of Escherichia coli0157: H7 in ruminants and non-ruminants and the antimicrobial resistance profile of the organisms from different Sources.Nig J Microbio. 2009, 23: 1852 – 1858
10. Adetunji, V. O. and Odetokun, I. A.Contamination and Critical Control Points (CCPs) along the processing line of sale of frozen poultry foods in retail outlets of a typical market in Ibadan, Nigeria. Malays J Microbio. 2013, 9: 289-294
11. Cardinale, E., Perrier-Gros-Claude, J.D., Tall, F., Cissé, M., Guèye, E.F. &Salvat, G.Prevalence of Salmonella and Campylobacter in Retail Chicken Carcasses in Senegal.Revued'élevageet de médecinevétérinaire des paystropicaux. 2003, 56: 13-16
12. Nyaga, P. N, 2007. The structure, marketing and importance of the commercial and village poultry industry: an analysis of the poultry sector in Kenya. Food and Agriculture Organization (FAO) of the United Nations, Rome. 2007
13. King'ori, A.M., Wachira, A.M. and Tuitoek, J.K.Indigenous chicken production in Kenya: A Review.Int. J. Poult. Sci. 2010, 9: 309-316. DOI: 10.3923/ijps.2010.309.316
14. Lindblad, M., Lindmark, H., Lambertz, S.T. and Lindqvist, R. Microbiological baseline study of broiler chickens at Swedish slaughterhouses. J Food Prot. 2006, 69: 2875–2882. PMID: 17186653
15. McCrea, B. A., Tonooka, K. H., VanWorth, C., Boggs C. L., Atwill E. R. and Schrader J.

S.Prevalence of Campylobacter and Salmonella species on farm, after transport, and at processing in specialty market poultry.PoultSci.2006, 85: 136–143. DOI: 10.1093/ps/85.1.136

16. Arsenault, J., Letellier, A., Quessy, S., Normand, V. and Boulianne, M.Prevalence and risk factors for Salmonella spp. and Campylobacter spp. Caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. Prev. Vet. Med., 2007, 81: 250–264. DOI: 10.1016/j.prevetmed.2007.04.016
17. Pointon, A., Sexton, M., Dowsett, P., Saputra, T., Klermeler, A., Lorimer, M., Holds, G., Arnold, G., Davos, D., Combs, B., Fabiansson, S., Raven, G., McKenzie, H., Chapman, A. and Summer, J. A baseline survey of the microbiological quality of chicken portions and carcasses at retail in two Australian states (2005 to 2006). JFood Prot.2008, 71: 1123-1134. PMID:18592737, RMID: 0020083540
18. Bohaychuk, V. M., Checkley, S. L., Gensler, G. E. and Barrios, P. R.Microbiological baseline study of poultry slaughtered in provincially inspected abattoirs in Alberta, Canada. Can Vet J. 2009,50: 173–178. PMID: 22467964, PMCID: PMC2629421
19. National Advisory Committee on Microbiology Criteria for Foods (NACMCF). Analytical utility of Campylobacter methodologies. J Food Prot. 2007, 70: 241 – 250
20. Office International des epizootics (OIE). Working group on animal production food safety: Animal production food safety challenges in global markets. SciTech Rev Off IntEpiz(Paris). 2008, 25: 479-492
21. Blaser, M. and Allos, B. M.Campylobacter jejuniand related species. In: Mandell, G., Bennet, J. E., Dolin, R., editors. Principles and practice of infectious diseases: 6thed. 2005. Elsevier/Churchill Livingstone, New York. 2548–57
22. Wang, W.L., Luechtefeld, N.W., Blaser, M.J., and Reller, L.B.Comparison of CampyPak II with standard 5% oxygen and candle jars for growth of Campylobacter jejuni from human feces. J. Clin. Microbiol. 1982, 16: 291–294.PMCID: PMC272347
23. El-Sherbeeny, M.R.Use of a candle jar for incubating Campylobacter jejuni.Campylobacters, Helicobacters, and related organisms. Newell, D.G., Ketley, J.M. & Feldman, R. A. (Eds) 1996, pp 89-92. Springer Science and Business media, new Yolk, ISBN: 978-1-4757-9560-8. DOI: 10.1007/978-1-4757-9558-5_16
24. World Health Organization (WHO). Global Salm-Surv- A Salmonellasurveillance and laboratory support project of the World Health Organization Level 2 Training Course: Identification of thermotolerant Campylobacter. Laboratory Protocols 5th Ed, 2003
25. World Health Organization (WHO). Global Salm-Surv- A Salmonella surveillance and laboratory support project of the World Health Organization. Level 4 Training Course: Multiplex PCR for differentiation of C. coli and C. jejuni. Laboratory Protocols 2nd Ed. 2009.
26. Skirrow, B. Campylobacters: cultural characteristics of intestinal campylobacters from man and animals. J Hyg. (Lond).1980, 85:427-442
27. Kaplan, R. L. and Weissfeld, A. S.Campylobacter, Helicobacter, and related organisms, In "Howard, B. J., Keiser, J. F., Smith, T. F., Weissfeld, A. S & Tilton, R. C., (eds.)". Clinical and pathogenic microbiology, 2nd ed., 1994, p. 453-460, Mosby, Chicago, IL
28. Mdegela, R. H., Nonga, H. E., Ngowi, H. A. and Kazwala, R. R.Prevalence of thermophilicCampylobacter infections in humans, chickens and crows in Morogoro, Tanzania. J Vet Med B. 2006, 53: 116–121. DOI: 10.1111/j.1439-0450.2006.00926.x
29. Salihu, M.D., Junaidu, A.U., Oboegbulem, S.I., Egwu, G.O., Magaji, A.A., Abubakar, M.B. and Ogbole, A.Prevalence of Campylobacter spp. in Nigerian indigenous chicken in Sokoto State Northwestern Nigeria. IJVM. 2009, 7 No 1
30. Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P. and Blaser, M. J.Experimental Campylobacter jejuni infection in humans. J Infect Dis.1988, 157: 472–479. PMID: 3343522
31. Wingstrand, A., Neimann, J., Engberg, J., Nielsen, E. M., Gerner-Smidt, P., Wegener,

H. C. and Mølbak, K. Fresh chicken as main risk factor for Campylobacteriosis in Denmark. EmergInfec Dis.2006, 12:280-284. DOI: 10.3201/eid1202.050936, PMCID: PMC3373097

32. Edwards, D. S., Milne, L. M., Morrow, K., Sheridan, P., Verlander, N. Q., Mulla, R., Richardson, J. F., Pender, A., Lilley, M. and Reacher, M.Campylobacteriosis outbreak associated with consumption of undercooked chicken liver pâté in the East of England, September 2011: identification of a dose–response risk. Epidemiol Infect.2014, 142: 352–357. DOI: 10.1017/S0950268813001222
33. Wasteson, Y., Johannessen, G.S., Bruheim, T., Urdahl, A.M., O'Sullivan, K. and Rørvik, L.M.Fluctuations in the occurrence of Escherichia coli O157:H7 on a Norwegian farm. LettApplMicrobio. 2005, 40: 373-377. DOI: 10.1111/j.1472-765X.2005.01673.x
34. Doane, C.A., Pangloli, P., Richards, H.A., Mount, J.R., Golden, D.A. and Draughon, F.A.Occurrence of Escherichia coli O157:H7 in diverse farm environments. J Food Prot. 2007, 70:6-10. PMID: 17265852
35. Akkaya, L., Atabay, H.I., Kenar, B. and Alisarli, M.Prevalence of verocytotoxigenicEscherichia coli O157:H7 on chicken carcasses sold in Turkey. Bull Vet InstPulawy.2006, 50: 513-516
36. Kiranmayi, CB andKrishnaiah, N.Detection of Escherichia coli O157:H7 prevalence in foods of animal origin by cultural methods and PCR technique. Vet World. 2010, 3: 13-16 .DOI: 10.5455/vetworld.2010.13-16
37. Akbar, A., Sitara, U., Khan, S.A., Ali, I., Khan, M.I., Phadungchob, T. and Anal, A.K.Presence of Escherichia coli in poultry meat: A potential food safety threat. Int Food Res J. 2014, 21: 941-945
38. Chang, W.S., Afsah-Hejri, L., Rukayadi, Y., Khatib, A., Lye, Y. L., Loo, Y. Y., Mohd, S. N., Puspanadan, S., Kuan, C.H., Goh, S.G., John, Y.H.T., Nakaguchi, Y., Nishibuchi, M. and Son, R.Quantification of Escherichia coliO157:H7 in organic vegetables and chickens. Int Food Res J. 2013, 20:1023-1029
39. Linton, D., Lawson, A.J., Owen, R. J. and Stanley, J.PCR detection, identification to species level, and fingerprinting of Campylobacter jejuni and Campylobacter coli direct from diarrheic samples. J. Clin. Microbiol.1997, 35: 2568-2572. PMCID: PMC230012
40. Recep, K., Hasan, O. and Burhan, C.Isolation and molecular characterization of Escherichia coli O157 from broiler and human samples. Foodborne Pathog Dis. 2012,9: 313-318. DOI:10.1089/fpd.2011.0991