

## Evaluation of Safety of Beef Sold in and around Mekelle with Special Reference to Enterohemorrhagic *Escherichia coli* O157:H7

Endale Balcha, Ashwani Kumar and Habtamu Tasew

Mekelle University, College of Veterinary Medicine, P.O. Box: 2084, Mekelle, Ethiopia

**Abstract:** A study was undertaken to evaluate the safety of beef sold in and around Mekelle, Ethiopia. A total of 80 meat samples were collected from 40 butcher shops: 60 samples from 30 butcher shops in Mekelle, 12 samples from 6 butcher shops in Wukro and 8 samples from 4 butcher shops in Quiha. The meat samples collected were analyzed for presence of *Escherichia coli*. Isolates of *E. coli* were characterized by Omni Log to detect the presence of Enterohemorrhagic *E. coli* (EHEC). A total of 50 samples (62.5 %) were positive for *E. coli* with prevalence of 62.5, 66.7 and 61.7 % in Wukro, Quiha and Mekelle, respectively. From the isolates of *E. coli*, nine isolates (18 %) were found to be EHEC (*E. coli* O157:H7): 3 (5%) Mekelle, 5 (12.5%) Quiha and 1 (41.7 %) Wukro. There is significant difference ( $p < 0.05$ ) in the prevalence of EHEC among the study sites. The result indicated the risk of food borne diseases due to EHEC in the study areas. Hence butchers in the study areas should be given training on hygienic practices in order to prevent secondary contamination of meat by EHEC from feces or the environment.

**Key words:** Beef • Mekelle • Wukro • Quiha • Enterohemorrhagic *E. coli* • Food Borne Disease

### INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) is an important food-borne pathogen [1]. Cattle frequently excrete these bacteria in their feces [2, 3]. The food borne illness is often linked to the consumption of contaminated and undercooked ground beef. Most of the outbreaks of infection have been directly associated with the consumption of foods of bovine origin. Over 150 serotypes of EHEC have commonly been shared by human and animals indicating the potential of animals and foods of animal origin in the transmission of pathogen to human beings [4].

Enterohemorrhagic *E. coli* strains belonging to serogroup EHEC O157 are most frequently associated with human disease [5]. *E. coli* O157:H7 is a food borne pathogen that has been associated with meat products particularly associated with the consumption of undercooked ground beef [6].

In Ethiopia, beef is a commonly consumed meat and on many occasions may be eaten as raw or undercooked [7]. This could increase the occurrence of food borne illness due to EHEC [8]. However, little is known about the

prevalence of this serogroup in Ethiopia, either in humans or in the animal population or in foods. Even though there is information about isolation of EHEC in Ethiopia [9], there is no available information regarding the prevalence of the organism in Tigray region particularly in and around Mekelle. The present study was conducted to address the lack of information regarding *E. coli* O157:H7 in meat sold in and around Mekelle.

### MATERIALS AND METHODS

**Study Area:** The study was undertaken from January 2013 to May 2013 to determine the safety of beef sold in Mekelle, Quiha and Wukro towns in the Tigray region, Ethiopia. The areas were selected based on their convenience to undertake the study. Mekelle is the capital city of Tigray and is 783 km from Addis Ababa, whereas Wukro and Quiha are 42 km and 8 km away from Mekelle, respectively.

**Sampling and Sample Size:** Beef meat samples were collected from the carcass area commonly used for consuming raw meat (gluteal muscle). From the selected

areas 50 % of butcher shops were considered for collecting meat samples. The butcher shops in each study area were selected using simple random sampling technique. From each butcher shop two samples were collected on two randomly selected days. Accordingly, 60 samples from 30 butcher shops in Mekelle, 12 samples from 6 butcher shops in Wukro and 8 samples from 4 butcher shops in Quiha were collected. Samples were collected in sterile plastic containers, labeled and transported using ice box to the laboratory for immediate analysis.

**Isolation of *E. coli*:** Each gram of the meat sample was homogenized in a sterile mortar. The homogenate was suspended in 9 ml peptone water. The suspensions were then plated on Eosin methylene blue (EMB) agar and incubated at 37 °C for 24 h. Colonies showing typical dark red to purple red with metallic sheen were taken for confirmation by biochemical tests as per [10].

**Identification of Enterohemorrhagic *E. coli*:** Pure colony of *E. coli* was taken from secondary culture of EMB and sub-cultured on BUG (BiOLOG Universal Growth Media) at 37°C for 18 to 24 h as a primary and secondary culture. Isolated colonies were then inoculated into inoculation fluids incubated for 18 to 20 h to prepare bacterial suspension with turbidity of 20 % transmittance which is measured by turbidity meter. The suspension is then poured using multiple channel pipettes into micro plates. Consequently, the microplates were loaded to omnilog tray and incubated for 18 to 24 h. Finally, the plates were taken out and characterization of colonies was done as per [11].

**Statistical Analysis:** Descriptive statistics such as frequencies were used to present the findings. Tables were used to present the results and the overall prevalence. For all the analysis  $P < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

*Escherichia coli* have been recognized as a serious food-borne pathogen and have been associated in both intestinal and extra-intestinal diseases as well as with numerous outbreaks of disease [12, 13]. The contamination of meat by *E. coli* and other Gram negative bacteria occurs when an animal is slaughtered and processed by improper methods and *E. coli* bacteria in their intestines can get on the meat. The isolation of

*E. coli* from meats in the present study might be due to the contamination from hide, unhygienic surfaces and equipments, contaminated water and butchers hands. This is well supported by Clarence *et al.* [14]. In the present study *E. coli* was isolated from 62.5 % of meat samples (50/80) that depict a deplorable state of hygienic and sanitary practices employed in the slaughtering, handling and selling of fresh meats.

Meat samples of all the three different areas were having *E. coli* contamination with significantly higher rate (66.7 %) of isolation from meat samples collected from Quiha than samples collected from Wukro (62.5%) and Mekelle (61.7 %) (Table 1). The variation in the isolation rate could be due to the difference in the hygienic practices during slaughtering and subsequent handling of the meat. This claim is well supported by other studies [14-16].

Some *E. coli* strains can cause a variety of infections in consumers [17]. EHEC is one of the six types of diarrheagenic *E. coli* that produces serious illnesses like hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) [18]. A number of outbreaks or sporadic cases of HC and HUS due to EHEC O157:H7 have been reported throughout the world [19]. Ruminant animals are reservoirs of this pathogenic bacteria and approximately 30% of feedlot cattle shed *E. coli* O157:H7 [20, 21]. This study reports the contamination of 11.3% of 80 meat samples by EHEC (Table 2) that were identified as EHEC O157:H7 by BiOLOG User Guide [11]. The result is in agreement with the work of Hussein [22] who recorded *E. coli* O157: H7 prevalence rates in the range of 3 to 19.7% for beef cattle.

This study reveals that a high proportion of samples of fresh meats sold in the market are contaminated by *E. coli*. The presence of these organisms in meat foods should receive particular attention, because some of these *E. coli*, as isolated in this study, are EHEC and their presence indicate serious public health hazard and give warning signal for the possible occurrence of food borne outbreak [23]. In the present study the low level of hygienic practices coupled with lack of adequate data on the outbreaks of the disease in the country and region, the isolation of EHEC O157:H7 should not be underestimated. Since there is a habit of consuming raw meat, such habits should change and only properly cooked meats should be consumed. In order to prevent occurrence of food borne diseases as a result of EHEC particularly *E. coli* O157: H7 good manufacturing practices should be adhered strictly by butchers and those selling the meat, the water used in washing the meat

Table 1: Prevalence of *E. coli* in beef samples collected from butcher shops

Collection site	Samples collected	Samples positive for <i>E. coli</i>	Prevalence (%)	p-value
Mekelle	60	37	61.7	0.9465
Quiha	12	8	66.7	
Wukro	8	5	62.5	
Total	80	50	62.5	

Table 2: Prevalence of enterohemorrhagic *E. coli* in beef samples from butcher shops

Collection site	Number of Meat samples	enterohemorrhagic <i>E. coli</i>	Prevalence (%)	p-value
Mekelle	60	3	5.0	0.0012
Quiha	12	5	12.5	
Wukro	8	1	41.7	
Total 80	9	11.3		

should have good potable quality, also the equipment must be washed properly before use. Proper inspection of animals and carcasses before the meat is sold and education of the meat handlers and sellers are the other measures to prevent this public health hazard.

#### ACKNOWLEDGEMENTS

The authors would like to thank Mekelle University for providing the fund and facilities for the current research project.

#### REFERENCES

- Benjamin, M.M. and A.R. Datta, 1995. Acid tolerance of enterohemorrhagic *Escherichia coli*. Appl. Environ. Microbiol., 61: 1669-1672.
- Van Donkersgoed, J., T. Graham and V. Gannon, 1999. The prevalence of verotoxins, *Escherichia coli* O157: H7 and *Salmonella* in the feces and rumen of cattle at processing. Can. Vet. J., 40: 332-338.
- Molina, P.M., A.E. Parma and M.E. Sanz, 2003. Survival in acidic and alcoholic medium of Shiga toxin-producing *Escherichia coli* O157:H7 and non-O157:H7 isolates in Argentina. BMC Microbiol., 3: 17.
- Lior, H., 1994. *Escherichia coli* O157:H7 and verotoxigenic *Escherichia coli* (VTEC). Dairy Food Environ. Saint, 14: 378-382.
- Chapman, P.A., 1994. Isolation, identification and typing of verotoxin producing *Escherichia coli* O157. PHLS Microbiol. Digest, 11: 13-27.
- Doyle, M.P. and J.L. Schoeni, 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol., 53: 2394-2396.
- Kumar, A. and G. Tadesse, 2011. Bovine cysticercosis in Ethiopia: A review, Ethiopian Vet. J., 15: 15-35.
- Ferens, W.A. and C.J. Hovd, 2011. *Escherichia coli* O157: H7: Animal reservoir and sources of Human infection. Foodborne Pathog. Dis., 8: 465-487.
- Adem, H., A. Daniel and Z. Girma, 2008. Occurrence of *Escherichia coli* O157:H7 in retail raw meat products in Ethiopia, J. Infect. Developing Countries, 2: 389-393.
- Holt, J.G., H.S. Krevy, R.H.A. Sneathe and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology 9<sup>th</sup> Edition. Williams and Wilkins Company, Baltimore, USA.
- BiOLOG User Guide, 2008. OmniLog Data Collection Software, OmniLog Data Collection, Version 2.1. Identification System, User Guide. USA., pp: 4-111.
- Scotter, S., M. Aldridge and K. Capps, 2000. Validation of a method for the detection of *E. coli* O157: H7 in foods. Food Control, 11: 85-95.
- Karch, H., P.I. Tarr and M. Bielaszewska, 2005. Enterohaemorrhagic *Escherichia coli* in human medicine. Int. J. Med. Microbiol., 295: 405-418.
- Clarence, S.Y., C.N. Obinna and N.C. Shalom, 2009. Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. Afr. J. Microbiol. Res., 3: 390-395.
- Enabulele, S.A. and N. Uraih, 2009. Enterohaemorrhagic *Escherichia coli* O157:H7 prevalence in meat and vegetables sold in Benin City, Nigeria. Afr. J. Microbiol. Res., 3: 276-279.
- Ukut, I.O.E., I.O. Okonko, I.S. Ikpoh, A.O. Nkang, A.O. Udeze, T.A. Babalola, O.K. Mejehe and E.A. Fajobi, 2010. Assessment of bacteriological quality of fresh meats sold in Calabar metropolis, Nigeria. EJEAFCh, 9: 89-100.
- Donnenberg, M.S., G.L. Mandel, J.E. Bennett, R. John and D. Mandel, 2005. Enterobacteriaceae principles and practice of infectious Diseases 6<sup>th</sup> edition Elsevier Churchill Livingstone Publishers, Philadelphia, pp: 267-286.

18. Nataro, J.P. and J.B. Kaper, 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev., 11: 142-201.
19. Griffin, P.M., 1998. Epidemiology of Shiga-Toxin producing *Escherichia coli* infections in continental Europe. In: J.B. Kaper, A.D. O' Brien, (Eds.), *Escherichia coli* O157:47 and other Shiga-toxin producing *E. coli* strains, ASM Press, Washington, DC, pp: 15-22.
20. Zhao, T., M.P. Doyle, J. Shere and L. Garber, 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. Appl. Environ. Microbiol., 61: 1290-1293.
21. Callaway, T.R., M.A. Carr, T.S. Edrington, R.C. Anderson and D.J. Nisbet, 2009. Diet, *Escherichia coli* O157:H7 and cattle: a review after 10 years. Curr. Issues Mol. Biol., 11: 67-79.
22. Hussein, H.S., 2007. Prevalence and pathogenicity of Shiga toxin producing *Escherichia coli* in beef cattle and their products. J. Anim. Sci., 85: E63-E72.
23. Kabir, S.M.L., 2009. Effect of probiotics on broiler meat quality. Afr. J. Biotechnol., 8: 3623-3627.