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Level of Pathogenic Escherichia Coli on Animal'S Body Coat and in Meat under Slaughter House Environment

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Abstract
BACKGROUND Cattle slaughtering is performed in unhygienic conditions in local abattoirs. Therefore this study was conducted with the objective to investigate the level of *Escherichia coli* (*E. coli*) in various regions of live animal body coat, carcass and meat at the various stages of slaughtering processed.RESULTS Mean values of log Colony Forming Units (CFU) per square inch^{-1} of pathogenic *E. coli* was noted significantly higher on the body coat, carcass, meat and processing tools in untreated group of cattle. With application of hot water treatment, *E. coli* counts dropped both in body coat and on meat carcasses. In meat processing tools, chopper axe contained higher counts, while the washing water has slightly lesser count. In abattoir environment, lairage ground has high infection of pathogenic *E. coli,* and air samples also found positive with *E coli*. Conclusion: This study reveals that the level of pathogenic *E. coli* was high on animal's body coat, meat carcasses, processing tools and abattoir environment. Presently hot water (65.56^{o}C) application has significantly reduced the level of pathogen. However washing with some sanitizer will eliminate the pathogen from body coat thus resulting in little chances of meat contamination.
Keywords: *E. coli*, Slaughterhouse, Environment, Meat, Body Coat, Cattle

INTRODUCTION

In Pakistan there is no specific breed for beef production. The farmers keep cattle and buffaloes merely for milk production and when its production vanishes due to old age or disease or any other problem, it is then presented to meet industry for slaughtering. Meat industry also depends on males of cattle and buffaloes. In Pakistan scientists have made efforts to develop beef breed. They were successful to develop Narimaster, Charolais and Simmental crosses with Sahiwal, Dajal or Thari but their production and reproduction traits were not up to the standards of well-known exotic beef breeds.[1]

In urban areas slaughtering is done in government and private slaughter houses whereas in most of the rural areas, it is in private small butcheries owned by butchers themselves. Furthermore, slaughtering is done in absolutely unhygienic conditions. Animal slaughtering operation is performed under same shed right from restraining of animal till splitting the carcass. These meat carcasses are then transported to retailer meat shops in open trucks or pickups where displayed in open air or stored without adopting hygienic measures and proper storage facility. Chances of microbial contamination in meat cuts are much more under these conditions[2].

Microbes have the ability to multiply rapidly, show physiological differences and withstand with unfavorable environmental conditions thereby found everywhere in the universe and can contaminate the processed and stored food products. These contaminated items causes food borne diseases in humans. In addition some microbes can produce potent chemicals and toxins in food items which may lead to food born poisoning. One of such type of most important bacteria is *Escherichia coli O157:H7* which is the most prevalent serotype in food born outbreaks. The other most important serotypes of *E. coli* are *O26,O111* which contribute in spread of diseases through contaminated food[3]. Shiga toxin (Stx) produced by *E. coli(STEC)* is an emergent pathogen associated with food borne diseases, especially foodstuffs of animal origin[4].

Although *E. coli* is one of the main inhabitants of the intestinal tract of most mammalian species, including humans and birds but still Verocytotoxin producing *E. coli(VTEC) O157* also known as *Entero Hemorrhagic E. coli (EHEC)* may cause watery diarrhea, hemorrhagic colitis and hemolytic uremic syndrome in humen[5]. According to Blanco,[6]*EHEC* causes hemorrhagic colitis by the consumption of uncooked minced meat. Meat and meat products, drinking water and vegetables contaminated with animal feces are probably major sources of *E. coli O157* infection[7].

Cattle are main reservoir for *VTEC O157*[8]and bacteria can survive on cattle farms for years[9]. Arthur[10]stated that cattle hides become contaminated with *E. coli O157:H7*through pathogen transmission in feedlot, during transport and in lairage environment and bacteria can be shifted into beef carcasses at processing site.

The main objectives of the present study were to determine the concentration of *E. coli* in meat at various stages animal slaughtering and processing through contaminated hair coat, water, processing tools and

hygienic status of slaughterhouse environment and to identify critical *E. coli* load on meat carcasses.

MATERIALS AND METHODS

Study was conducted in local slaughterhouse at Peshawar. Cattle are the selected specie and among the all livestock species, cattle are 30% slaughtered in local abattoirs. All samples were collected from prevailing breeds of cattle brought for slaughtering to the said abattoir.

Prior to slaughtering, cattle were selected randomly and were divided into two groups. One group was given treatment with hot water spray having a temperature of 65.56^{o}C and the other group was kept untreated. Swab sampling was done from the rump, flank and brisket regions of cattle body coat and meat from carcasses in both the groups with a total of 60 numbers of samples. 30 samples were collected from processing tools (cutting knife, Chopper axe), water used for washing and abattoir environment (slaughtering floor, lairage ground and abattoir air). One passed swab technique[11]was used to sample a measured area on each region of body coat and meat carcasses, processing tools, slaughtering floor and lairage ground in sterile tubes containing 10ml normal saline solution (0.85% sodium chloride and 0.1% buffered peptone water). Water was collected in empty sterile, screw capped tubes while air pathogens were sampling by exposing sterilized media plates for 30-minutes at various placesunder the slaughtering shed. All the tubes and media plates of air sample were handled and transported to Microbiology laboratory according to standard method prescribed by Church and Wood[12] and analyzed following standard protocols as prescribed by Holth[13]and *E. coli* both pathogenic and non-pathogenic were isolated on Sorbitol Mac Conkey agar with pre-added Cefixime @ 0.05mg liter^{-1} or 0.05µgml^{-1} in order to inhibit the sorbitol non-fermenting *Proteus Spp.*[14]. Identification was performed by study basic morphological characteristics and microscopic features of the subject pathogen. The conformation was done by conducting biochemical tests (IMViC pattern) like Indole production test, Voges Prausker's test, Methyl Red test and Citrate utilization test. The IMViC pattern was (+, +, -, -) which conformed the *E. coli*. Further confirmation was done through PCR. The pathogenicity was determined by culturing the isolated bacteria on Tryptic Soy agar containing Congo red dye with production brick red color colonies for pathogenic *E. coli*[15,16 &17].

Statistical Analysis

Data was recorded in Microsoft Excel Sheet. Prior to execute descriptive summary statistic, the data of total viable count which is in million for all categories and seem less different because all values are very high. So the data were transformed into Natural Log values of Total Viable Count (L_{TVC}) of the isolated *E. coli* bacteria and analyzed through statistical software Genstat Discovery Edition-3 and SPSS, 18-version. ANOVA was used to determine the significance level of E.coli in the both groups at ($P < 0.005$).

RESULTS AND DISCUSSION

Prevalence of Non-pathogenic *E. coli* were isolated in the forms pink colonies on sorbitol macconkey again petri dishes, while pathogenic *E. coli*forms colorless colonies on sorbitol macconkey agar. The respective results in term of means of L_{TVC} of *E. coli*of washed and unwashed groups of animals are presented in Table 1. The level of pathogenic and non-pathogenic *E. coli* (on both body coat and meat carcasses)in unwashed group was significantly higher at the local slaughterhouse, Peshawar. The reduction in the level of *E.coli* was observed up to 50% for both pathogenic and non-pathogenic *E. coli just by simply spraying and washing the body coat with hot water* having a temperature of 65.56^{o}C (150^{o}F) with a low pressure pump for 15-20 seconds on both of animal's body coat before slaughtering and meat carcasses after slaughtering. The result of Castillo[18]revealed 3.7 log reduction of pathogenic *E. coli*by hot water spray on meat carcasses while Kalchayanand[19] received a 2.99 log reduction of microbes by the application of hot water having a temperature of 74±2^{o}C for 12 seconds on bovine heads. After washing with hot water, author still observed the pathogens on the meat carcasses and body coat of slaughtered cattle at local slaughterhouse, Peshawar. This might be due to the fact, that processing tools, butchers / workers and slaughtering floor were not washed accordingly, which might lead to contamination of the subject pathogen into the animal's body coat and meat carcasses during processing.

The level of Pathogenic and non-pathogenic *E. coli* found on the body coat and meat carcasses of slaughtered cattle at the local slaughter house; Peshawar is given in terms of mean log value in Table 1. Pathogenic and non- pathogenic *E. coli* count was found almost similar, however *E.coli* of on body coat was significantly ($P < 0.05$) higher than of *E. coli* found in the meat of carcasses. According to Dayna,[20] the aerobic plate count on beef cattle hides in the range of 6.17-8.19 log CFU 100^{-1} cm2 and that on meat carcasses in the range of 4.24-6.47 log CFU 100^{-1}cm^{2}.The counts revealed in this study are higher than that of Dayna[20]. This higher level might be due to poor sanitary conditions of our target slaughterhouse and improper transportation procedures currently practiced.

Counts of pathogenic and non-pathogenic *E. coli* found on different regions (i.e; Rump, Flank and Brisket) of body coat and meat carcasses (both collectively) of slaughtered cattle at the local slaughterhouse, Peshawar are also presented in the Table 1.A significant higher log TVC values was observed for brisket and lowest count was noted on rump region of slaughtered cattle at said abattoir. Similarly, for non-pathogenic *E.*

coli, highest and lowest were observed on brisket and rump regions of the subject cattle, respectively. Reid *et al*. (2001) also observed highest prevalence (22.2%) of pathogenic *E. coli* on brisket region of slaughtered cattle and lowest prevalence (3.3%) on rump region of slaughtered cattle. This reveals that meat carcasses are more likely to contaminate from the brisket region of slaughtered cattle during processing. Present results also revealed that level of prevalence of pathogenic and non-pathogenic *E. coli on body coat and meat of the carcasses were almost same within the washed group of cattle.*Arthur[10] observed that the aerobic plate count (APC) of *E. coliO157*:*H7* on cattle hides ranged from 6.17 to 8.19 log CFU $100^{-1}cm^2$ while on pre-eviscerated and post-intervention carcasses were in the range of 4.24 to 6.47 and 1.46 to 1.96, respectively. Another scientist, Chahed[21]found that the *E. coli* counts were444.75 CFU square cm^{-1}on meat samples of the carcasses. It was repeatedly observed that present study showed higher count of pathogenic and non-pathogenic *E. coli*. This might be because of the poor hygienic condition, processing tools and contaminated abattoir environment in the subject slaughterhouse and thereby leads to cross contamination into the meat carcasses during processing.

The level of pathogenic and non-pathogenic *E. coli* were investigated on different processing tools like cutting knife, chopper axe and water used for washing meat carcasses in the target abattoir. Results of the mean log values are given in the Table 2. They were all contaminated with the subject pathogens. The chopper axe being highly contaminated for both pathogenic and non-pathogenic *E. coli*. The water used for washing of the carcasses was contaminated at slightly lesser level with pathogenic and non-pathogenic *E. coli. In present study* water was stored in small water tanks (top uncovered) present at different site of the slaughtering floor and workers often goes to wash their hands and cutting knife etc. In that water after slaughtering of each animal, thereby contaminated it regularly.Guyon[22] studied the critical hazard points with respect to *E. coli O157*:*H7* cross contamination into meat carcasses during processing. He isolated the said bacteria from work tops, worker's hands and beef carcasses and declared that these points contributes in the contamination of beef carcasses during processing. Another researcher, Gun[23] isolated *E. coliO157*:*H7* from Knife, worker's hands and apron used in the meat processing facility, However Gun didn't isolate the organism from the water.

Similarly, samples from slaughterhouse environment including Slaughtering floor, lairage ground and abattoir air were also studied for the prevalence level of the subject pathogens. There counts are shown in Table2. The lairage ground contained high level of both the pathogenic and non-pathogenic *E. coli.* The lowest levels for pathogenic and non-pathogenic *E. coli* were observed for abattoir air. Gun[23] found the *E. coli O157*:*H7* in abattoir environment and slaughtering floor. Authors observed that generally the environmental condition of the local slaughterhouses was very poor and that's might be the reason for high number of both pathogenic and non-pathogenic *E. coli* in Peshawar Pakistan (see the photos in slides).

There were no check and balance for the persons visiting the slaughtering area. All operations including restraining of animal, slaughtering, skinning and washing of carcasses were performed on the same floor. There was no disinfection procedures adopted for the slaughtering floor, processing tools, washing water, worker's hands and meat carcasses before transportation to retailer meat shops. All these factors may increase the chances of *E. coli* cross contamination into meat carcasses during processing. Presently warm water (65.56^{o}C) spray application has significantly reduced the level of pathogen. However washing of live animal body coat with some sanitizer will eliminate the pathogen from body coat thus resulting in little chances of meat contamination and also adopting complete hygienic practices for the floor and tools used during the slaughtering of cattle will definitely reduce the level of contamination.

Table 1 Level of Pathogenic and Non-pathogenic *E. coli* on washed and unwashed groups, Body coat &**Regions** of Slaughtered Cattle at the Local Slaughterhouse, Peshawar Pakistan

Treatments	L_{TVC} Mean ± **S.E.**	
	Pathogenic	Non-pathogenic
Unwashed (UW)	24.34 ± 0.99^{a}	25.15 ± 0.98^{a}
Washed (WA)	11.32 ± 0.62^{b}	12.14 ± 0.60^{b}
Body Coat (BC)	21.77 ± 1.44^{a}	22.52 ± 1.45^{a}
Meat Carcass (MC)	13.90 ± 1.07^{b}	14.78 ± 1.06^{b}
Rump (RM)	16.18 ± 1.70^{a}	17.01 ± 1.69^{a}
Flank (FL)	17.49 ± 1.76^{b}	18.29 ± 1.76^{b}
Brisket (BR)	19.83 ± 1.83^{c}	20.65 ± 1.85^{c}

Total viable count values of *E.coli* were transformed to natural log and mean values are presented in the above table**.**

Means in the same column not followed by the same superscript are significantly different (P <0.05).

Table 2 Level of Pathogenic & Non-pathogenic *E. coli* on Meat Processing Tools and Abattoir Environment of the Local **Slaughterhouse, Peshawar:**

	L_{TVC} Mean ± **S.E.**			
Meat Processing Tools	Pathogenic	$P_{<0.05}$	Non-pathogenic	$P_{<0.05}$
Cutting Knife	13.34 ± 0.60	0.001	14.22 ± 0.60	0.001
Chopper Axe	13.88 ± 0.58		14.81 ± 0.60	
Washing Water	6.99 ± 0.19		8.04 ± 0.04	
Abattoir Environment				
Slaughtering Floor	29.09 ± 0.89	0.001	34.03 ± 0.94	0.001
Lairage Ground	31.02 ± 0.91		37.21 ± 0.49	
Abattoir Air	1.74 ± 0.73		4.42 ± 0.20	

Photo 1.Workers busy in skinning cattle while laying on unhygienic floor

Photo 2. Slaughtering operation alongside offals & eviscerated carcasses on unhygienic abattoir floor

Photo 3. Carcasses being loaded in open pickup for transportation to local market

Photo 4. Lairage environment where animals are kept before slaughtering

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