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LEVEL OF *SALMONELLA* IN BEEF OF SLAUGHTERED CATTLE AT PESHAWAR

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ABSTRACT

A research study was designed to find out the level of *Salmonella* contamination on cattle body coat, fresh carcasses, beef after transportation and display at butcher shops. The animals were divided into two groups i.e. washed and unwashed animals. *Salmonella* was found in 100 % samples. A log total viable count was significantly ($P < 0.001$) higher in unwashed samples as compared with washed (22.33±0.088 and 19.54±0.09) respectively. Carcasses samples from unwashed animals had significantly ($P < 0.001$) higher log total viable count of *Salmonella* (24.45± 0.06) as compared with washed animals (21.77± 0.05). Results suggested that samples collection after 6-7 hours display at shop showed higher degree of *Salmonella* contamination and the isolation was significantly affected by duration and washing of the samples. Abattoir floor has higher (30.36±0.08) *Salmonella* contamination, followed by chopper axe (22.14± 0.05) and knives (15.14± 0.09). It is concluded from the result that *Salmonella* prevalence was reduced in all stages of the slaughtering operations by washing with Luke warm water. However, it is recommended that washing with some sanitizer will eliminate the pathogen from body coat thus resulting in little chances of beef contamination.

Key words: Meat, *Salmonella*, Contamination, Cattle, Abattoir, Khyber Pakhtun Khwa.

INTRODUCTION

In Pakistan, Meat is considered as by product of dairy industry in Pakistan (Bhatti & Khan, 1999). In many diets/dishes, meat is considered to be the vital part. Meat improves the nutrition of diet and provides a significant contribution to the intakes of 10 key nutrients: energy, protein, vitamin A, vitamin B1, vitamin B_2, Niacin, vitamin B_6, vitamin B_{12}, Iron and Zinc (Romans & Ziegler, 1994). In Pakistan, more than 37 million animals are annually slaughtered. Total meat production includes 1.711, 0.616 million tons and 0.767 million tons beef, mutton and poultry meat, respectively (GOP, 2010 -11).

Undesirable dairy cattle and buffaloes, those are no longer able to work or produce milk, usually sold for slaughter. Male calves are generally not reared up to their maturity and presented for slaughter at an early age (Islam, 2011). To fulfill the meat production gap between supply and demand for Khyber Pakhtunkhwa province, large numbers of animals were brought from Punjab. Slaughtering was performed at specified places called abattoir. These abattoirs are functioning under the administrative authority of Municipalities of each district. Modern slaughterhouses are not available in any city and towns of Khyber Pakhtunkhwa. In rural areas, animals are slaughtered near bank of river or open yard of butchers' houses.

Animals are mostly slaughtered in unhygienic conditions with unclean knives and other tools. Dehiding was performed on the unclean floor, thus carcasses got contaminated. These carcasses are transported openly to the various parts of the city without proper care and mostly unhygienic ways and displayed openly in meat vendors shop without any protection. Dust, Sun light, Ambient Temperature, Relative Humidity and flies are major factors which further deteriorate the quality of fresh meat and cause early onset of meat spoilage.

Due to chemical composition and biological characteristics, meat and meat products are highly perishable foods. Meat an excellent source for growth of many notorious microorganisms such as mesophilic and psychrophilic bacteria those can cause infection in human, spoilage of meat and economic loss (Kalalou and Ahami, 2004). Cattle are a major reservoir for *Salmonella* which is carried in the intestinal tract of healthy animals and excreted in feces (Chapman *et al,* 1993). Local Slaughter house environment is observed conducive for the growth of microorganisms, which can rapidly render the meat unsafe for human consumption. The poor hygiene and sanitation prevailing in the abattoirs as well as the shops encourage microbial contaminations and growth. The higher microbial load in the shops further enhances the chances of early meat spoilage (Sudhakar *et al,* 2007).

Amongst the microbes, *Salmonella* most frequently present on animal body coat and feces and transferred to carcasses during slaughtering and cause severe damages to human health if consumed. *Salmonella* are gram negative bacteria which belong to genus *Salmonella*, the family *enterobacteriaceae* (Yan *et al,* 2003) and it causes food poisoning in the world.

Present study was designed to determine the prevalence and level of *Salmonella* species on body coat

of the cattle, beef at various stages during the meat supply chain such as; on body coat pre and post slaughter, post transportation to the butchers shops, prevalence of *Salmonella* on the butcher's tools and on the floor of the butchers' shops.

MATERIALS AND METHODS

In total meat production, share of beef from cattle is quite considerable therefore cattle beef is selected for present study. Samples were collected randomly from the private and Government slaughter houses in Peshawar City of Khyber PakhtunKhwa province. The microbiology Laboratory of Veterinary Research Institute (VRI) Peshawar was used for conformation of meat contamination. Cattle were divided into two groups for sample collection i.e. washed animals and unwashed animals before slaughtering. Samples were obtained from the body coat of the randomly selected washed and non-washed cattle for the prevalence of microbes on animal's body coat. The collected samples were placed in sterilized test tubes and transferred to laboratory for further analysis. A total of hundred samples were collected, out of which 25 were from the washed group while 75 samples were from the unwashed cattle group. Samples collected at fourth different stages i.e. body coat of live animals, post slaughter soon after skinning, post flaying after transportation and six to seven hour display at meat shops.

Prior to slaughtering, cattle were selected randomly and were divided into two groups. One group was given treatment with Luke warm water spray having a temperature of 65.56^{o}C and the other group was introduced to conventional method of slaughtering. Swab sampling was done from the neck, rump, and thigh regions of cattle body coat and meat from carcasses in both the groups with a total of 15 numbers of samples. Tool 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 was used to sample a measured area on each region of body coat and meat carcasses, processing tools, slaughtering floor in sterile tubes containing 10ml normal saline solution (0.85% sodium chloride and 0.1% buffered peptone water) (Reid *et al,* 2001).

Samples from body coat and cattle beef was obtained from the carcass (neck, rump and thigh) at the slaughter house and was immediately transported in ice box to the Veterinary Research Institute (VRI) Peshawar. These samples were stored in refrigerator at 04^{0}C until tested. The following procedures and diagnostic tests were conducted for Isolation and Identification of *Salmonella* at VRI Peshawar; Preparation of culture media, Peptone water, MacConkey's agar, Tetrathionate Broth Base Tetrathionate Broth Base, Brilliant Green Agar, Preparation of sample inoculum from raw meat Colonies Picking, Enteric bacteria count, Microscopic Examination: Gram's staining using Khushi guidelines (2002), Methyl Red Test, Indole Production Test and Hydrogen Sulphide Production Test.

Data Collection and Analysis: Data on the prevalence of *Salmonella* on body coat, fresh carcass, meat cuts, and tools of the butcher and slaughter house floor was recorded and entered in MS Excel program, and initial descriptive statistic performed in Genstat Discovery Edition 3. Data was log transformed for better understanding of differences and analyzed by above mention software for statistical analysis by using CRD (complete randomized design). All the experimental data was analyzed through CRD using statistical model as below;

$$y_{ij} = \mu + \alpha j + G_{ij}$$

Where y_{ij} = yield / output subjected to i*th* observation and j*th* stage, μ = over all true population mean, αj = effect of j*th* stage, G_{ij} = residual from which follow a normal distribution with zero mean and constant variance

RESULTS AND DISCUSSION

Prevalence of *Salmonella* on cattle body coat, carcasses, slaughtering floor and tools of the butchers were investigated in Table 1. Level of *Salmonella* on body coat in washed and non-washed groups were studied during spring, 2010 at slaughterhouse Peshawar. It is revealed that level of *Salmonella* on live animal body coat was significantly ($P<0.001$) higher in unwashed samples. Washing of body coat has reduced the level of *Salmonella* by 12.50 %. It showed that just simple washing is not sufficient to remove the entire microorganisms on the body coat. The present findings are in line with the study of Danya *et al*, 2008 conducted an experiment in United States and samples were taken from 4 different regions from hide and carcass. The samples were checked for the presence of *Salmonella* and *E coli*. The colony forming unit level on hides ranges from 6.17 to 8.19, while it was higher in summer. The occurrence of *Salmonella* was 89.6% on hides. Our findings justify the study of Danya *et al,* 2008. Present findings are also similar with the experimental study of Puyalto *et al,* (1997), carried out a research on cattle in farm and slaughterhouse. The coat and meat were tested for *Salmonella* contamination. Eleven various types of *Salmonella* were isolated from the samples and 67% of the results were positive for *typhimurium* serotype. Hairs contamination with *Salmonella* was 8% while leaving farm. The rate of contamination with microbes was 25% and this is by hair coat contamination during shipping to slaughterhouse.

Animals were slaughtered and skinned. Samples were collected from the carcasses and checked for prevalence of *Salmonella* and found that non-washed animals after skinning has significantly higher ($P < 0.001$) log TVC of *Salmonella* (Table 1). Our findings are likely similar with Reid *et al*, (2001) study, who carried out an experiment on pathogens contamination with carcass. The tests of the brisket muscle meat 10% were positive for *Salmonella spp*. The rump muscle meat was less contaminated i.e.2.2% for *Salmonella spp*. This is may be due to the contact of the brisket with the slaughter house floor which is the main source of contamination.

Levels of *Salmonella* at butcher shops (after transportation from slaughter house to market) in meat samples of non-washed animals after transportation were significantly ($P<0.001$) affected by transportation. The results of the present research study were confirmed by the study of Barham *et al,* (2000) that the concentration of *Salmonella* from carcass of cattle was increased from 6% to 89% after slaughtering and transportation. Similarly the levels of meat contamination were 33% higher after 6-7 hrs of display at meat shops because the number of microbes increases with the passage of time.

Level of *Salmonella* on meat processing tools of butchers & slaughterhouse floor are reported in Table 2. The study results revealed that the slaughter house floor has significantly ($P < 0.05$) higher degree of *Salmonella* contamination. The floor of slaughter house is the main source of microbial contamination and provide environment to the bacterial growth. The main source of bacterial contamination was animal body coat and subsequently it can contaminate the deep tissues when it is cut. Sofos *et al,* (2000) and Padungtod and Kaneene (2006) enlightened that live animals and the environment serve as sources for pathogenic microorganisms, which contaminate carcasses during the slaughtering process and meat products during processing, storage and handling. The decontamination processes, include animal cleaning, chemical de-hairing at slaughter, spot-cleaning of carcasses before evisceration by knife-trimming or steam and vacum, spraying, rinsing, or deluging of carcasses before evisceration and/or before chilling with water or chemical solutions (e.g. organic acids, tri-sodium phosphate, etc.) or steam (Jocelyn & Alison, 2006). The processes applied at various concentrations or intensities, pressures (2-20bar), temperatures (15-80^{o}C) and for different lengths of time (5-20 sec), individually or in sequential combinations and under hygienic conditions. The other important causes of bacterial contamination were butcher hands, dress and slaughtering equipments. These results were confirmed by the study of Whyte et al, (2002). It is postulated that the control of pathogens on carcasses could be reduced by adopting standard dressing procedures. The presence of *Salmonella* in slaughter house and carcasses have confirmed by McEvoy *et al*, (2000); Bell and Hathaway (1996) and Bell (1997).

Table 1: Average level of *Salmonella* prevalence on various stages of cattle slaughtering and processing of both groups of cattle slaughtered at road slaughter house Peshawar during spring, 2010.

Description (*Salmonella* prevalence on)	Controlled Group LN TVC Mean±SE	un washed Group LN TVC Mean±SE	P-Value
Body coat of cattle	15.54±0.09	22.33±0.088	0.001
Fresh carcass	19.77±0.05	24.45± 0.06	0.001
Immediate after arrival to the shop	23.46±0.10	27.63± 0.06	0.001
After six to seven hours display	30.18±0.03	36.87 ± 0.06	0.001

LNTVC means log total viable count

Table 2: Average log TVC of *Salmonella* at the slaughter house floor and tools of the butcher in Peshawar in spring 2010.

Group	LN TVC Mean±SE	P-Value
Slaughter floor	30.36± 0.08	
Chopper	22.14± 0.05	0.001
Knife	15.14± 0.09	

LNTVC means log total viable count

Conclusion: *Salmonella* was found present in 100 percent samples. Washing with luke warm water has 30.5% reduced the level of pathogens on the live animal body coat. Studied results have confirmed that *Salmonella* prevalence increased by 52% from the slaughtering stage to meat shops phase. It is postulated that if washing carried out with some sanitizer, it can eliminate almost completely the pathogens from body coat, thus resultantly chances of meat contamination will reduce significantly in abattoirs.

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