WASHING OF ANIMAL BODY COAT EFFECTS ON SALMONELLA LOAD ON HIDE AND IN POSTSLAUGHTER CARCASSES

Abdur Rahman*[1], M. Y. Khan[1], M. S. Qureshi[1], N. Ahmad[2], N. Shaheen[3], S. Din[4], T. Ali[5],

1. Department of Livestock Management, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, postal code 25130 Pakistan
2. Chairman Animal Nutrition, Faculty of Animal Husbandry and Veterinary sciences The University of Agriculture Peshawar
3. Assistant Professor, Psychology, Jinnah College for Woman, University of Peshawar
4. Veterinary officer FATA Livestock and Dairy Development Department, government of Khyber Pakhtunkhwa Pakistan
5. Microbiology department, China University of Agriculture, Beijing, China

Author for Correspondence: (Office phone) 0092919221097 Cell. No. 00923339617919 Fax no 0092919216520*Email address; abdur_rahman@aup.edu.pk

ABSTRACT

Present study was carried out to find reduction in the level of *Salmonella* contamination in meat form public health point of view. Reduction in load of *Salmonella* on cattle body coats (hides) and in carcasses against the use of various washing treatments of the animals before slaughtering e.g. cleaning with; i. water, ii. Water plus soap iii. Water plus soap plus Dettol, iv. No washing (control). These treatments were applied on the cattle body coats of live cattle prior to slaughter, concurrently in following three groups i. fresh water, ii. 50 ^{0}C heated water and iii. 70 ^{0}C heated water. Samples were also collected after slaughtering from these groups which were treated against different treatments. In second part of the experiment, meat transporting vehicles were also washed to check the reduction in *Salmonella* prevalence. Total samples (n= 88) comprised of washed samples (n= 59) and unwashed (n=29). Data was transformed into log (LNTVC) for clearer understanding. All collected samples were positive for *Salmonella* contamination. A large number of colonies were observed and recorded as total viable count (TVC). *Salmonella* on the surface of unwashed (controlled) live animal coat (26.07± 0.03) was significantly (P <0.01) higher (24%) than *Salmonella* in carcasses (21.26± 0.04). Samples of treatment i.e. water plus soap plus Dettol showed highest 65% reduction in the level of *Salmonella* against group which used 70 ^{0}C heated water during washing of animal body coat respectively. Similarly level of *Salmonella in carcasses reduced maximum* in the group washed with 70 ^{0}C heated water. Mean Salmonella contamination on body coat decreased as samples moved controlled animals body coat (unwashed) to soap washed and soap plus Dettol wash 26.07± 0.03, 20.78±0.03, 13.37±

0.11 and 10.49±0.13 respectively. It is concluded that the washing with water, soap and Dettol at 70 ^{0}C Heat Water has significantly lesser load of *salmonella.* However the sanitizer application did not completely vanish the *salmonella in the carcasses.* This suggested that abattoir management at Peshawar should focus on the improvement of hygiene in all aspect of slaughterhouse.

Key words: Salmonella, Beef, Contamination, Peshawar abattoir, zoonotic

Introduction:

Meat is considered as a derivative of dairy industry in Pakistan, But it is one of the balance diet and a good source of essential amino acids, vitamins like A, B, E and important minerals such as copper, iron and zinc. These nutrients are important for the growth, repair of body cells and metabolic reactions (*11*). Currently the 3.33 million tons of meat produced in Pakistan (*8*). The demand for meat consumption is tremendously increased in Pakistan due to increase in population and secondly change in their life style. Meat consumption is especially higher in Khyber Pakhtunkhwa (*2*).

Domestic cattle are effective source of zoonotic diseases and their byproducts also play a major role in disseminating infectious diseases to human. The situation in Peshawar abattoir is worse and possessed serious threat to human health due to traditional slaughtering practices and unawareness of butchers about hygienic and safe meat production. The unproductive and uneconomical dairy animals are used as source of meat in Pakistan. Male dairy calves and surplus dairy bulls are slaughtered for meat. Poor hygienic practices during slaughtering and meat processing are one of the biggest problems

being faced by the Pakistani meat industry. Generally raw meat is sold in openly in butchers shops further deteriorate hygiene condition and resulted into meat spoilage. Presently slaughtering is usually performed in abattoir but under poor hygienic condition, contaminated tools and open transportation of carcasses in vehicles (3). Among meat contaminating factors, dust, sunlight, ambient temperature, relative humidity and flies are key ones. Consumption of contaminated meat causes a zoonotic diseases or infections in humans such as *Salmonellosis, listeria, monocytogenes and Clostridium perfrings.*

Salmonella is mostly present in GI tract and easily transfer to body coat and carcass and cause severe threats to public (*6*). Being Zoonotic, *Salmonellosis* transmitted directly or indirectly between animal and animals to human. *Salmonella* is found worldwide in cold and warm blooded animals including humans and in the environment causes food borne diseases. *Salmonella* found in food such as raw meat, chickens, turkeys etc. *Salmonella* infected animal's exhibit signs of severe diarrhea, prostration and sometimes abortion. It causes in human beings such as illness, typhoid fever, paratyphoid, food borne illness and infects blood stream which causes death in humans. Infected individual who has recovered from infection commonly become carrier and may further transmit the disease.

Animal are not washed prior to slaughtering and the carcass (meat) get contamination due to poor abattoir condition and lack of hygienic measures during slaughtering that ultimately hold serious threats to human beings in Khyber Pakhtunkhwa. To assess the impact of pre-slaughtering washing of animal body coat at different heated water on the *Salmonella* contamination load on the body coat of the live cattle and subsequently on their carcasses after slaughtering were studies.

MATERIALS AND METHODS

Study was designed to find out the effect of pre-slaughter washing (treatments at three levels i.e. fresh water, 50 ^{0}C and 70 ^{0}C heated water and sanitizers (i.e. Dettol and soap) in reducing the *Salmonella* contamination load on cattle body coat and carcasses obtained after slaughtering of theses selected animals. The study was conducted in the government abattoirs at Peshawar, Khyber Pakhtunkhwa. In routine normally, cattle were not washed prior to slaughter. Cattle were laid down on floor and slaughtered without taking care about the hygienic measures. Hides removal were practiced on dirty floors. Conditions of transport carrying carcasses were filthy and meat transported openly to various meat shops. Butchers cuts the carcasses in small pieces and hung it in open environment.

Earlier studies of Rahman *et al*., (2012) suggested that more *Dhanni and Lohani* cattle were slaughtered in Peshawar. Therefore these two breeds' cattle were selected as a species of choice for the present study. Samples were collected from washed and unwashed (controlled) animals. A total of 88 samples were collected from the government slaughter houses. A total of 12 samples each from cattle body coat and carcasses were collected from the unwashed cattle and five samples from the carriage section of the unwashed vehicles. A total of 27 samples each from body coat and carcasses were collected from the washed pre-slaughtered cattle washed animal carcasses and five samples from the carriage section of the washed vehicles. A similar amount of samples were also collected from body coat to carcasses. Samples were collected from the animal body coat using one pass swab technique in one square inch area. Samples were placed in clean sterilize bottles and transported under refrigeration to microbiology laboratory, The University of Agriculture

Peshawar and stored in freezer till use. Sterile pipits tips were used in preparation of decimal dilution ($10^{-1}…10^{-10}$). The enteric bacteria count, tenfold serial dilution was carried out. One ml from the previous sample solution and were placed in 9.0 ml of normal saline solution and homogenized the sample in a vortex (Vortex-genie-2, Scientific Industries., Inc., USA). One ml from the sample and added to test tubes and subsequently transferred to the next tube dilutions and so on and until the tenth test tube. Took 1ml from the each dilutions and were inoculated to the Petri plates which were filled with Brilliant Green Agar solution and incubated at 37 ^{o}C for 24 hours, after the number of colonies or colony forming per gram (CFU/g) were observed and recorded. For the enteric bacteria count, the Minimum Detection Limit (MDL) was 1 x 10. Took the media (i.e. Brilliant Green Agar which is a favorable media for the growth of *salmonella*) about 50g Agar were added to 1000 ml of distilled water and mix it thoroughly. The media was autoclaved at 121^{o} C for 15 minutes and 15.0 Ib pressure. After one hour the culture media was poured 10-15 ml into Petri plates and added 1 ml decimal sample solution. Culture was solidified for 5-10 minutes and then incubated at temperature 37 ^{o}C for 18-24 hours for the growth of microorganisms. Specific number of colonies was selected such as pink and opaque with a smooth appearance and entire edges are surrounded by a red color in the medium. For further confirmation of *Salmonella* contamination in body coat and carcasses, the API strips 20e were used for bio chemical tests and followed the standards and procedures of using API STRIPs 20e of *(4*)

Raw data were recorded in MS Excel 2007 sheet. Since the microorganism values were in hundred thousand and it seemed difficult to draw the conclusive difference in the values.

Therefore all the values of *Salmonella* load in the washed body coat and in their carcass were transformed to nature log and square root values to get better understanding of the situation. The descriptive statistics and ANOVA were performed using the Statistical software Genstat Discovery Edition 3. Dependent and independent variables effects were analyzed by using three into three factorial designs. Controlled and treated groups were compared for the salmonella prevalence on the body coat and cattle carcasses. Findings on *Salmonella* load on live cattle body coats were tested against different pre-slaughter washing treatments such as i. Control (not washed) ii. Soap and Water iii. Dettol and water iv. Soap+ water+ Dettol washed. Furthermore the washing water of three different types i.e. i. Fresh water, ii. 50^0 C water and iii. 70^0 C.

RESULTS AND DISCUSSIONS

Salmonella load on unwashed live cattle body coat

Salmonella colony count (SCC) on the cattle body coat showed significant difference ($P < 0.01$) than the cattle carcasses in the control group (unwashed). Results revealed that cattle carcasses had around 18 % lesser *SCC* presence than on the body coat in the unwashed group (Table 1). In normal routine husbandry practices, the cattle are not washed on regular intervals. Therefore their body coats are especially belly areas and hind quarters are dirty and filthy. Since the cattle GIT is reservoir of *E. coli and Salmonella* (*6 et al*., 1993), therefore it was found in higher rate in the hides of slaughtered cattle (Danya *et al*, 2008). The present study findings were also higher then research conducted elsewhere (Miens, *et al*., 2004 and *12, et al*., 2004). Higher values in the present study might be due to the overall filthy and dirty conditioned prevailed in this part of the province. However, the

salmonella level on the body coat in the present study was found similar to those revealed by earlier study of (*1*).

Salmonella load on animal body in different washing treatments

Washing of animal body coat was effective significantly ($P<0.01$) in reducing the mean SCC values on body coats (Table 2). *Salmonella* colony counts of fresh water group were treated against i. soap, ii. dettol and iii. soap plus dettol. The result revealed that *SCC* decrease to 33.98 %) on animal body coat when washing of animal coat was done alone with fresh water (16.48 log TVC) and washed using soap and dettol (10.88log TVC) (Table 2). Similarly in the second group (Column 2 Table 2) warm (50 ^{0}C) water was used against three treatments and result illustrated that contamination of *Salmonella* decrease (41.6%) on the cattle body coats. In treatment soap plus water, when fresh water cleaned animals were compared with the animals washed with 50 and 70 ^{0}C heated water demonstrated that contamination of *Salmonella* reduced to 1.5 and 34.5 % respectively (row comparison in Table 2). In the third group (i.e. hot water 70 ^{0}C) were treated against three treatments and result explained that contamination of *Salmonella* decrease (48%) on the body coats, when animal body was washed in soap plus Dettol and water was 70 ^{0}C hot. Simultaneously in row comparison, the result of different groups revealed that the *Salmonella* frequency decrease almost 50% when animals' body coat was washed from fresh water to 70 ^{0}C heated water in the third row where soap and Dettol both are applied (Table 4). *Salmonella* has the ability to multiply rapidly, show physiological differences and withstand with unfavorable environmental conditions. Therefore it is easily found everywhere in the universe and can contaminate the processed and stored meat products. Scientific research

suggested that animal coat contains *Salmonella.* Scientists have tried how to reduce the pathogens load on animal body. In this regard (5) used the different types of sanitizers to reduce the number of the microbes on the animal coat and carcass. They found that use of acid washed after the hot water washing of animal at room temperature significantly reduced the number of microbes as the present study indicated that washing with sanitizer (Dettol) at 70 0 C heat water has shown similar results in the reduction of SCC. *Salmonellosis* mainly caused by the animal origin foods, which were raised in intensive farming system. The following factors spread *Salmonellosis* in meat; use of contaminated feeds, water, and cross-contamination of carcasses during slaughtering operations. Moreover , stress associated with prolonged deprivation of feed and water, transport of animals from rearing farms to abattoirs, crowding and prolonged stay in abattoir pens, predispose animals to infection (*7*).

***Salmonella* occurrence on Carcass**

Various treatments used in study revealed highly significant effect ($P<0.01$) on mean values of SCC on cattle carcasses. *Salmonella* colony count in fresh water group when treated against i. soap, ii. dettol and iii. soap plus dettol demonstrated that SCC decrease (34%) in treatment third. Similarly in the second group (i.e. water heated at 50 ^{0}C) were treated against three treatments and result showed that decrease (45.1%) in the contamination of *Salmonella* on the body carcass. In treatment soap plus water when washed with fresh water, 50 ^{0}C heated water and 70 ^{0}C heated water reduced *Salmonella* prevalence on the carcass 22.5, 25.0 and 49% respectively (Table 3 row comparisons). In the third group (i.e. water heated at 70 ^{0}C) were treated against three treatments and result

showed that 49, 51, and 65% decreased the contamination of *Salmonella* on the carcass in the third column of Table 3. . In treatment soap plus dettol plus water when washed with fresh water, 50 ^{0}C heated water and 70 ^{0}C heated water reduced *SCC* on the carcass 48, 58 and 65% respectively (Table 3 row third comparisons). Present study findings revealed that washing with water, dettol and soap at 70^{o}C reduced the level of *salmonella* to 65%, but still 35% of the germs are not cleaned which is in contrast to other studies conducted elsewhere where the microbes level decreased to less or equal to 7% and 0.3% (*10*). It's a matter of concerned that salmonella occurrence did not vanished completely as it might be due to the washing area of the live animal was very for away from the slaughtered location. During shifting the animal from place to slaughtering area in the abattoir got decontaminated from the air, butchers clothes and tools. However if the carcass/ primer cuts might be sprayed with Phosphoric acid-acidified sodium chloride solution (PASC) of 5% concentration further decrease the level of salmonella to another 41% in the carcasses (*5*). The microbial growth replicate in 20 minutes if environment is conducive, therefore even if very few germs are on the carcass, it would reached to millions In 7-8hours. Therefore all the attempts might be made to reduce the contamination level to zero. For this purpose standard protocols and procedures for hygienic animal slaughtering and processing might be adopted. It is suggested that on the basis of (*9*) study that identified hazards points and critical entry points in slaughtering and processing of beef animals in Khyber Pakhtun khwa. This will control contamination of meat and will improving the overall shelf life of meat.

Conclusion and Recommendation:

Animal washing before slaughter with fresh water reduce the *Salmonella* load on the live cattle body coat by 12%. The Pre-slaughter washing with soap alone declined the *Salmonella* load by 41 to 48% at different temperatures. Pre-slaughter washing with Dettol, soap and water at 70 ^{0}C has lessened S*almonella load* in those samples by 65%. However the sanitizer application did not completely vanished the *salmonella* load on the body coat and in cattle carcasses.

On the basis of above conclusion, it is recommend that local slaughter houses needed to renovate as these were built four to five decades earlier. All of them needed severe repair. Dusty pathways from stocking yard to slaughtering shed, poor waste disposal of water and fecal content, absence of washing and cleaning animal facilities in abattoirs enhances meat contamination. Therefore livestock policy makers to need to revise the existing slaughtered laws and declare the washing of animals compulsory to reduce contamination entry in meat.

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Table No.1: *Salmonella* Colony count on Cattle Body Coat and Cattle Carcasses in unwashed (control) groups

Parameter	**Log TVC**	**Square root TVC**	**P-value**
Body coat	26.07± 0.03	458170.91± 50167	<0.01
Carcass	21.26± 0.04	41386.20 ± 4879	

In each cell first value represents the square root & second value in the parenthesis represents Log TVC of *Salmonella* total viable count on cattle body coat

Table No. 2. Mean *Salmonella* Colony Count on cattle Body Coats against different Treatments and groups slaughtered at Govt. slaughterhouse Peshawar

Treatments	**Mean ±*SE* Fresh water**	**Mean ±*SE* Water at 50°C**	**Mean ±*SE* Water at 70°C**	**P value**
Water+soap	109376.19 (23.68[a]±0.01)	92549.61 (22.90 [a] ±0.04)	2308.41 (15.52 [a] ±0.07)	
Water+dettol	111029.82 (23.23 [a] ±0.03)	27426.64 (20.43 [a] ±0.11)	2207.99 (15.39 [a] ±0.10)	< 0.01
Water+soap+ dettol	32500.29 (20.78 [a] ±0.03)	792.36 (13.37[b]±0.11)	170.14 (10.49[b] ±0.13)	

In each cell first value represents the square root & second value in the parenthesis represents Log TVC of *Salmonella* total viable count on cattle body coat. Superscripts are compared within column and different superscript revealed significantly different at $\alpha = 0.05$

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Table.3. Mean *Salmonella* Colony Count on cattle Carcasses against different Treatments and groups slaughtered at Govt. slaughterhouse Peshawar

Treatments	**Mean± SE Fresh water**	**Mean ±*SE* Water at 50ºC**	**Mean ±*SE* Water at 70ºC**	**P value**
Water+soap	3785.69 (16.48[a]±0.07)	2926.42 (15.96 [a] ±0.04)	225.78 (10.84[a] ±0.07)	< 0.01
Water+dettol	3286.17 (15.92 [a] ±0.03)	2482.51 (15.63 [a] ±0.05)	181.60 (10.40[a] ±0.19)	
Water+soap+dettol	2464.47 (10.88 [b] ±0.73)	80.60 (8.77 [b]±0.11)	38.20 (7.28 [b]±0.21)	

In each cell first value represents the square root & second value in the parenthesis represents Log TVC of *Salmonella* total viable count on cattle carcasses. Superscripts are compared within column and different superscript revealed significantly different at $\alpha = 0.05$