ISOLATION AND BIOCHEMICALCHARACTERIZATION OF SALMONELLA & E.COLI FROM BOVINE MILK COLLECTED FROM SALE SHOPS, GOVERNAMENTAL & PRIVATE DAIRY FARMS AT QUETTA PAKISTAN

Abdul Wahid Baloch[1], Muhammad Gohram Khan Malghani [1], Mohammad Shafee Khan[2]

[1]Department of Environmental Management and Policy, Balochistan University of Information Technology Engineering & Management Sciences Quetta Balochistan,
[2]CASVAB, University of the Balochistan, Quetta

Abstract

A total of 100 raw milk samples of bovine were collected in sterile screw capped tubes(each 5ml) from Government Dairy Farm(45 samples) , Private Dairy Farms (45 samples) and Private Milk Shops(10 samples) from different locations in Quetta city to isolate milk borne microbial pathogens like Salmonella and E Coli to estimate their share in the environmental pollution. All these samples were subjected to grow on Salmonella Shigella agar (SS Agar) and Xylose Lysine Deoxycholate (XLD) agar. The isolated colonies were further identified biochemically through triple sugar iron test (TSI) and Gram's staining. From Government Dairy Farm out of forty five (45) raw milk samples six (6) samples (13%) were found positive for E Coli whereas no salmonella (0%) colony was detected. While from Private Dairy Farms out of Forty Five (45) samples ten(10) were positive for E Coli(22%) and only one(1) sample was positive(2%) for Salmonella. From private milk shops out of ten (10) samples four (4) were positive for (40%) E coli and three (3) for salmonella (30%). It is concluded from the above study that there is 4% Salmonella and 20% E.coli contamination in raw milk samples being supplied to the people in Quetta city. The high proportion of contamination (30%) of Salmonella and (40%) of E.coli was detected from Private Milk Shops due to Exposure of milk to open Environment where these both pathogens are present. which is a serious public health issue and need to create a public awareness to use treated (Pasteurized) milk and proper disposal of the waste from dairy farms as well as shops and as well as to avoid exposure of milk to open contaminated Environment of the city.

Key words: Salmonella, E.coli, Milk born diseases, Environment

INTRODUCTION

Salmonellosis and collibacillosis are two important milk borne diseases in the world. E. coli is a normal inhabitant of the micro flora of the intestinal tract and it is always useful but few strains of E.coli are harmful and pathogenic which causes diarrhea and coli O157:H7 is commonly isolated from milk and milk products due to contamination from fecal materials (Armstrong et. al., 1996). The presence of E.coli 0157 in milk represents that udder of animals are shedding this organism in their milk as a source of infection (Wells et. al., 1991). E. coli O157:H7 has been detected from raw milk in bulk tanks in milk shops (William et. al., 1997. Soomro et al (2002) Recovered E.coli in 67% of raw milk and milk product samples out of 160 samples collected in Tandojam city and concluded that the reason of contamination was due to has commonly observed in summer season than winter. The main cause of this seasonal difference is due to high temperature which favors the growth and multiplication of salmonella in raw milk and also due to consumer behavior (Edwards and Ewing, 1972).

Escherichia coli was first time discovered by a German scientist named Theodor Escherich in 1885 and this organism was placed in Enterobacteracea family as a gram negative rod shaped facultative anaerobe having a size of 1 – 3m in length with a flagella due to which it is motile and it is localized in the small intestine of human beings and animals and it may survive in open environment for some time, It is a useful bacteria but some strains are pathogenic which causes Hemolytic Uremic syndrome (HUS) and diarrhea (EC). Milk is protein, vitamin and minerals rich source for public health. As the milk hygiene is a basic factor to safe guard the public health from milk borne diseases, the consumption of untreated /un pasteurized milk may causes many severe type of bacterial diseases including salmonella because salmonella is found abundantly in untreated cattle milk, which is a gram negative rod shaped non spore forming bacteria, there are two groups of this bacteria S.enterica, S. typhimurium. Five sub species of enterica which are salmae, arizonea. diarizonae, houtenae, and indica and there are 2400 serovars .(Modern food Microbiology). The intestine is the primary habitat for salmonella where it ,s growth and multiplication takes place it damages the mucosa of the intestine and results as a bloody dysentery and then it enters in the blood stream of the patient and causes typhoid fever after treatment the patient recovers and becomes a carrier which sheds the organism in feaces without showing any sign. Milk is a white nutritious liquid secreted from the mammary glands of milch animals and utilized as a food source for human beings and offspring of human and animals and the milk is white in color due to presence of a substance called casein. Basically there are two school of thoughts regarding the milk a group thinks that milk is an energetic source but another group thinks that milk is a poison the reason is that if milk is properly treated(Pasteurized or boiled) then it is an energetic source of diet if not processed and consumed in raw condition then it is a poison due presence of high concentration of micro organism and their toxins in milk which causes food poisoning and sometimes death. The milk is rich in protein, fat and vitamins like vitamins A,D,E K and C when milk is squeezed from the teats of human•and•animals•it's•temperature•is•101•F and•it's•PH•is•from 6.4 to 6.8. Pakistan is the Second largest milk producer in the world by producing 20,372,000 liters in the year of 2007 (Official figure budget June 2007-8).

MATERIAL AND METHODS

For the sample collection and Laboratory Techniques FAO Protocols for Food Hygiene (1992), and N. S. Abd El-Atty,et al (2007) Procedures were adopted.

Sample Collection:

A total of one hundred 100 fresh raw milk samples (each of 5 ml) Figure: 1 of cows were collected from Government owned Holstein Friesian cows dairy farm and private dairy Farms in addition to Private Milk Shops at different locations in Quetta city. Forty five (45) milk samples were collected from Government Dairy Farm (GDF) and Private Dairy Farms

(PDF) while ten (10) untreated milk samples from different Milk shops at Quetta city. All the samples were collected freshly in sterile seal- capped tubes and processed at Center for Advance Studies Vaccinology & Biotechnology (CASVAB) university of Balochistan, Quetta. All the possible hygienic measures were adapted during collection and transportation of theses samples. A pre designed questionnaire was developed before collection of samples indicating all the essential description of the animals to be identified in case of positive to these milk borne pathogens.

Figure: 1 Raw Milk Samples.

LABORATORY PROCEDURES

The following procedure was adopted for the isolation and Biochemical Characterization of Salmonella and E.coli from these raw milk samples at CASVAB Laboratory.

Media preparation forculture inoculation

The XLD (Xylose Lysine Deoxycholate) and SS Agar (Salmonella Shigella Agar) media were prepared in distilled water according to the manufacturer's rule, and only XLD was autoclaved for 15 min at 121 °C and the pH was adjusted at 7.2 ± 0.2 at room temperature. After overnight incubation the sterile plates were inoculated with 2-3 drops of milk samples and equally distributed on the plate surface with the help of sterile culture swab. All the inoculated culture plates were kept for 24-48 hours at 37 ^{0}c in incubators in inverted positions.

Growth On Xld And Ss Agar

A loop full culture was taken aseptically from each bottles and streaked on Xylose lysine deoxycholate (XLD) agar and Salmonella Shigella (SS) Agar. The plates were incubated at 37 ^{O}C for 24 hours. In the presence of Salmonella and E.coli suspected colonies. On XLD agar, salmonella appeared as pink-red colonies without black centers, while E coli as yellow colonies (Matofari J.W 2007, Similarly on SS agar plate, salmonella appeared as colorless colonies with black centers and E coli as yellow colonies with black centers as shown I Figures: 3(N. S. Abd El-Atilt al 2007), Salmonella on XLD Agar E.coli on SS Agar

IDENTIICATION

For the biochemical characterization of Salmonella and E.coli 2 Biochemical tests (Gram's Staining & TSI) were conducted for further confirmation and cross examination.

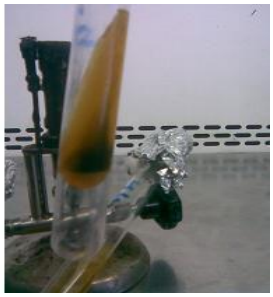
Gram staining

The proper procedure for the Gram's Staining was used in which both the organisms were found Gram's•negative and slides were examined under 100 X oil immersion lens of Electron Microscope both the organisms appeared as pink rods in which the salmonella were of 0.7 to 1.5µm in length and smaller in size than E.coli which were 1 – 3m in length only as shown in Figures below:

Salmonella E.coli

TRIPLE SUGAR IRON TEST (TSI)

TSI Agar is very useful in the presumptive identification of enteric particularly in screening for intestinal pathogens. It contains sucrose in addition to glucose and lactose. TSI agar is useful in detecting the ability of the microorganism to produce gas from the fermentation of sugars and change in the colors of slant and BUT. The solution from the TSI powder of Acumedia Company was prepared and autoclaved at 121C for 15 minutes then poured the solutions in the Screw caped Tubes incubated at 37C for 24 hours then the positive colonies of of both organisms were inoculated with sterile inoculating needle in to the tubes by streaking the slant and stabbing the butt. The tubes were recorded in which the salmonella did not produced gas and the color of solution was not changed but the E.coli fermented the lactose and produced gas in the BUT and changed the color of Slant into yellow as shown below in Figures



RESULTS

A total of one hundred (100) raw milk samples were collected from cattle of the Government Dairy farm, Quetta, Private dairy farm and milk sale shops at different locations of the District Quetta. All the samples were inoculated on Salmonella Shigella Agar (S S Agar) and Xylose, lysine Deoxycholate Agar (XLD). On S S Agar Pink colored colony were observed for Salmonella and yellow were for the E coli while on XLD agar red colony with black centers were indicating salmonella and colorless colony with black centers were for the E.coli.

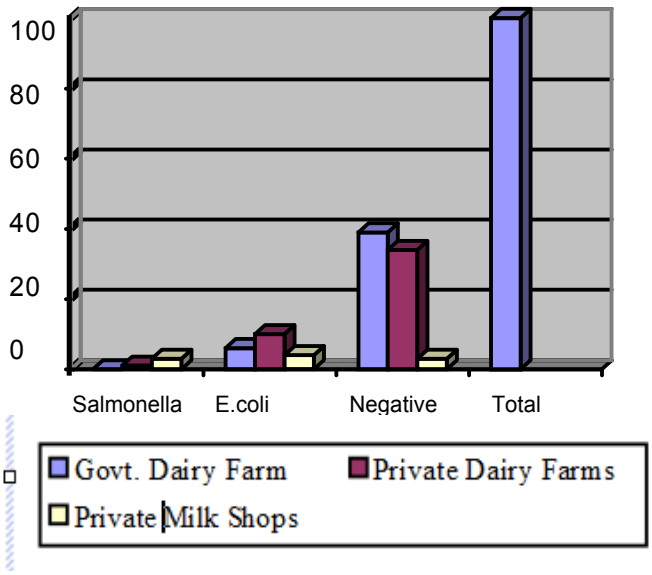
The positive samples were further characterized biochemically by staining through Gram' Staining and growth on Tipple sugar Iron (TSI). Both the organisms were Gram negative pink colored rods. On TSI slantsand Butt were acidic for E Coli (yellow) without gas production.

While for Salmonella Slants were Alkaline (pink) and Butt were Acidic (yellow) with Gas Production. At Govt. Dairy Farms (GDF) out of total of 45 milk samples no sample were found positive for Salmonella (0%) while only six(6) samples (13%) were found positive for E Coli. (Table: 8) Similarly at private.

Dairy Farms (PDF) out of total of 45 milk samples only one sample (2.22%) were found positive for Salmonella while only ten (10) samples (22.22%) were found positive for E Coli. (Table: 9) From milk Sale shops out of ten (10) Milk Samples three (03) samples (30%) were positive for Salmonella and four (04)samples (40%) were found positive for E .Coli.(Table: 1)

Table 1. Prevalence of Salmonella & E.coli in Raw milk.

Name of Farm	Total	Salmonella	E.Coli	Negative
Govt Dairy Farm	45	0	6	39
Private Dairy Farm	45	1	10	34
Milk Shops	10	3	4	3
Total	100	4	20	76



DISCUSSION

The data collected reveals an alarming situation regarding the presence of Salmonella and E. coli in milk samples collected from Government and privately owned Dairy Farms in addition to milk sale shops in Quetta City, Balochistan, Pakistan.

Out of total of 100 raw milk samples collected from different sources only 04 (04%) samples were found positive for salmonella while E. Coli were present at 20 (20%) percentage(Table-01) indicating relatively higher prevalence of E Coli than salmonella in the milk samples of the target area. Our theses finding are in line with (Somroo A H et al 2002) who also observed much higher prevalence of E Coli in raw milk samples collected from different sources in Tandojam, Sindh Pakistan and do not agree with the findings of (N S Attay-2007) who reported 00% prevalence of E Coli and Salmonella from raw milk samples in Egypt. This may be due to use of more hygienic conditions by users or the possible be the difference in the environmental condition of two different countries. In Government Dairy farm (GDF) out of Forty Five (45) Samples no sample were found positive for salmonella but 06 samples were positive for E Coli. These results corroborate with the findings of (K Ekici, 2004) who also reported zero percent (00%) prevalence for Salmonella and (06 %) prevalence for E Coli in Turkey from sixty six samples of raw milk. Similarly (Muhammad Farhan 2007) also isolated both pathogens from milk samples in Lahore region of Pakistan.

During this study in milk sale shops out of total of ten (10) raw milk samples 03 and 04 samples were found positive for salmonella and E Coli respectively.

Theses results show relatively higher prevalence forboth pathogens in Private Milk Sale Shops as compared to Government Dairy Farm and Private Dairy Farms, which is indicating Environmental contamination.

These findings are in agreement with Aksu et al., 1999; Mustafa 2004, while Abd El-Hady, et al., 1995; Gonul, 1997; El-Kosi, 2001,reported minimum Salmonella and maximum E.coli contamination in raw milk samples and also low prevalence of both organisms in Winter than in summer season due to Environmental factors. E.coli is an important milk borne disease causing pathogen which is responsible for several outbreaks in the World due to consumption of raw milk and milk products (Martin et al., 1986; Tuttle and Gomez 1990; Griffin and Tauxe 1991; Chinen et al., 2001; Licene et al., 2001).Most E.coli infections includes bloody diarrhea, Hemorrhagic colitis, Hemolytic Uremic Syndrome (HUS) and purpura due to production of verocytotoxin (Wells et al., 1991 ; Abdul-Raouf et al., 1993). According to Table: 1 which is showing several outbreaks of milk borne diseases of Salmonellosis and Collibacillosis from the consumption of raw milk and milk products (McEwen et al., 1988; Escartin et al., 1999). It is concluded from the data from this study that the consumption of raw milk being supplied from different sources in Quetta city represents a high potential hazard for the consumers due to presence of high prevalence of Salmonella and E.coli 0157 which prior to utilization to minimize the chances of milk borne disease hazards. indicates the mishandling of fresh raw milk in a contaminated Environment. The author suggests for hygienically handling and pasteurization of raw milk prior to utilization to minimize the chances of milk borne disease hazards.

CONCLUSIONS:

On the basis of this study the following conclusions may be drawn:
High level of contamination was found in milk being sold in milk shops is due to exposure of milk in Environment, where Salmonella and E.coli like biologics are present in high concentration at Quetta city. The low level of contamination of Salmonella in milk samples is due to seasonal; factor because this organism is always observed in summer because it cannot survive less than 37C.Temprature. Both the organisms were detected from raw milk collected from all sources were positive for Salmonella and E.coli, except Salmonella was negative in Government Dairy Farm Quetta which indicates unhygienic conditions in handling of milk but Government Dairy Farm is better than other sources. When milk from these sources obtained and utilized without Pasteurization that may have the potential of health hazards for consumers.

RECOMENDATIONS:

- Milk may be contaminated when exposed to open environment therefore exposure of milk to open environment during handling may be avoided.
- Consumption of raw milk is hazardous for human health so proper pasteurization may be done prior to consumption which may destroy the salmonella and E.coli along with other pathogens and safe guard the public health from milk borne diseases.
- Government is advised to ban on sale of raw milk, ISO standard may be adopted for sale of milk and legislation may be done in the matter without further delay.
- Monitoring of milk sale on sale points is advised on regular basis by city government in collaboration of livestock department.
- Public awareness may be created through mass media about pasteurization of raw milk prior to consumption.
- Proper disposal of waste of the dairy farms to limit the dissemination of pathogens in the environment.

REFERENCES:

- Abd El-atty (2007) Prevalence of Salmonella and E.coli O157 in some foods in BS. Vet. Med. J. November 2007, 5TH scientific conference, P. 73-78
- Abdul Raouf UM, Beuchat LR, Ammar MS. (1993) Survival and growth of Escherichia coli O157:H7in ground, roasted beef as affected by pH, acidulants,and temperature. Appl Environ Microbiol 59:2364-2368.
- Armstrong, et al (1996). Emerging food borne pathogens: Escherichia coli O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemiol. Rev. , 18: 29-51.
- Edwards, R. and Ewing, W.N. (1972). Identification of Enterobacteracea. 3rd edn., Burgess Publishing Co., Minnesota
- Escartin, E. F.; Castillo, A.; Hinojosa-Puga, A. and Saldana-Lozano, J. (1999): Prevalence of Salmonella in chorizo and its survival under diffe rent storage temperature. Food Microbiol., 16:479-486
- FAO. (1992) Manual of Food Quality Control 4.Rev MicrobiologicalAnalysis Food & Agriculture Organization Rome. Italy.
- Licence,K.; Oates, K.R.; Synge, B.A. And Reid, T.M.S.(2001): An outbreak of E.coli O157: H7 infections with evidence of spread from animals to man through contamination of private water supply. Epidemiol.Infect, 126:135-138.
- Martin, M.L.; Shipman, L.D.; Wells, J.G.; Potter, M.E.;Hedberg, K.; Wachsmuth, I.K.;

Tauxe, R.V.; Davis, J.P.;Arnolai, J. and Tilleli, J. (1986): Isolation of Escherichia coli O157 :H7 from dairy cattle associated with two cases of hemolytic uremic syndrome. Lancet, 1:2, 8414: 1043

- Mohammed Farhan (2007) Evaluation of Hygienic Quality of Market Milk of Khartoum State(Sudan) in International Journal of Dairy Science 2(1):pp 33-41
- Soomro et al (2002) '' Isolation of Escherichia Coli from Raw Milk and Milk Products in Relation to Public Health Sold under Market Conditions at Tandojam" in Pakistan Journal of Nutrition 1(3): 151-152, 2002
- Stender, K. et al (2001) "Rapid detection, identification, enumeration Escherichia coli by fluorescence in sit hybridization using an array scanner",•J.•Microbiol. Methods . Vol. 45, pp. 31-39, 2001.
- Wells, J.G., Shipman, C.D. and Greene, K.D. (1991). Isolation of E.coli serotype O157:H7 and other shig a like toxin producing E.coli from dairy cattle. J. Clin. Microbiol. , 29: 985-989.
- William, E.K., et. al.(1997). A prolonged outbreak of Escherichia coli O157:H7 infections caused by commercially distributed raw milk. The J. Infect. Dis.,176: 815-818.