

## Detection of *Escherichia coli* from the udder of the dairy farm buffaloes in Phagwara region, Punjab, India

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Received: 29-01-2012, Accepted: 02-03-2012, Published Online: 10-06-2012

doi: 10.5455/vetworld.2012.522-525

### Abstract

**Aim:** To know the presence of *Escherichia coli* on the udder skin of the dairy farm buffaloes in the Phagwara region, Punjab, India.

**Materials and Methods:** A total of 135 swabbed samples were collected randomly from the udder of buffaloes in ten dairy farms over the period of three months from August to October 2011 without concern to their breed with the prior approval of the farm owners. The sterilized cotton swabs were examined by Gram's staining for the morphology of the culture, culture characteristics was confirmed by growth on different media and by performing the different biochemical tests like Indole production, Voges-Proskauer test, Urease Production, Nitrate Reduction, Methyl red and Presumptive test.

**Results:** Out of 135 samples were examined, 23(17.03%) were positive for *E. coli*. Most Probable Number (MPN) results confirmed the one possibility of the bacteria from the contaminated water.

**Conclusion:** The results of the present study suggest that *E. coli* isolates are present on the udder skin of the dairy farm buffaloes in the Phagwara region, pose a serious threat to the animal as well as consumer health. Thus, more hygienic preventive measures are required to inhibit the bacterial growth, so as to improve the health of the animals as well as the wholesomeness of the milk.

**Key words:** Buffaloes, *Escherichia coli*, Milk, Mastitis, Phagwara, Udder.

### To cite this article:

Palaha R, Chaudhary N, Kumar H (2012) Detection of *Escherichia coli* from the udder of the dairy farm buffaloes in Phagwara region, Punjab, India, *Vet World*, 5(9): 522-525, doi: 10.5455/vetworld.2012.522-525

### Introduction

*Escherichia coli* is one of the most intensively studied living species. *Escherichia coli* is a normal part of the microbiota of the lower gastrointestinal tract of mammals, including humans, and usually exist as a harmless commensal. However, there also exist many pathogenic strains of *E. coli* that can cause a variety of diseases in both humans and animals [1]. The mastitis which is caused by *E. coli* is commonly called Environmental mastitis [2]. This pathogen infects the udder generally through the ducts papillaris, which is the only opening of the udder to the outside world. In the buffalo species, mastitis is the most costly disease even though buffaloes have been traditionally considered less susceptible to mastitis than cattle [3].

However, in comparison with cattle, buffaloes have some characteristics that may contribute to greater risk to mastitis such as more pendulous udder and longer teats [4]. Higher incidence of *E. coli*

mastitis may be due to poor hygienic conditions or intensive use of antimicrobials targeted against Gram positives for mastitis control [5]. Mastitis, inflammation of udder, results in Rs 7,165 crore annual losses to the dairy industry in India and Rs 503 crore in Punjab [6]. The percentage distribution of Gram-negative bacteria causing clinical mastitis is herd dependent, but studies in the United States and Europe consistently report that approximately 40% of clinical cases are the result of Gram-negative bacteria [7-10].

In terms of milk contamination, the quality of milk is determined by aspects of composition and hygiene. Due to its complex biochemical composition and high water activity milk serve as an excellent culture medium for the growth and multiplication of many kinds of microorganisms [11]. Among all microorganisms *E. coli* is frequently contaminating organism and is reliable indicator of fecal pollution generally in insanitary conditions of milk [12]. Two

Table- 1. Culture Characteristics of *Escherichia coli* on different media

Media Used	Culture Character
Eosin Methylene Blue Agar	Blue- Black colonies with green metallic sheen growth
Endo DEV Agar	Green metallic sheen colonies with deep pink growth
Nutrient Agar	Colourless and Yellowish white, circular, smooth colonies with entire edge
Nutrient Broth	Grayish Uniform turbidity having no Pellicle
Gelatin Agar	Opaque, moist, grayish white, entire colonies

cases of hemolytic uraemic syndrome have been reported which provide evidence that raw milk may be a vehicle of transmission of *E. coli* O157: H7, both affected person consumed raw milk [13]. Recovery of *E. coli* from food is an indicative of possible presence of enteropathogenic and/or toxigenic micro-organism which could constitute a public health hazard. Enteropathogenic *E. coli* (EEC) can cause severe diarrhea and vomiting in infants and young children [14].

In raw milk samples 9, 20%, isolates of the *E. coli* were detected, whereas in case of mastitis infected buffaloes raw milk 30.12% isolates of the *E. coli* were detected [12, 15, 16]. The detection of *E. coli* from the udder indicates a possible contamination of the surroundings like fecal matter in the soil, contaminated milking equipment's, water uses for the washing of the udder during milking. So by keeping these views in mind a study was carried out to detect the *E. coli* from the udder of the dairy farm buffaloes in the Phagwara region, Punjab, India.

#### Materials and Methods

Collection of sample: A total of 135 swabbed samples were collected randomly from the udder of buffaloes in ten dairy farms over the period of three months from August to October 2011 without concern to their breed with the prior approval of the farm owners. The entire swab samples were collected as per the guidelines of the International Animal Ethics Committee. Animals were not used in this experiment in anyway either experimental animals or control. The sterilized cotton swabs were swabbed onto the overall outer skin surface area of the udder. Simultaneously 100ml water sample were also collected for the same dairy farms to check the MPN level (most probable number). The samples were collected in the sterile sampling bottles that were kept in a cold box and were immediately shifted to the laboratory of Microbiology, Department of Biotechnology, Lovely Professional University, India.

Isolation and identification of the *Escherichia coli*: Each sample was inoculated on the Eosin

Table-2. Biochemical reactions of *Escherichia coli*

No. of Isolates	Biochemical Test	Reaction
23	Catalase Test	+ve
	Simmons Citrate	-ve
	Indole Production	+ve
	Methyl Red	+ve
	Voges- Proskauer	-ve
	Starch Test	-ve
	Urease	-ve
	Presumptive Test	+ve
	Nitrate Reduction	+ve

Methylene Blue Agar (CDH Pvt. LTD.) and incubated at 37°C for 24 hrs. The plates were observed for the growth of *E. coli*. A single Colony was picked and sub cultured on Eosin Methylene Blue Agar for the purification of the isolate. Gram's staining technique was used for the study of the morphology of the isolates. The culture characteristics were confirmed by inoculating the pure colonies on Endo DEV Agar (Titan Biotech LTD.), Nutrient Agar (CDH Pvt. LTD), Gelatin Agar (Titan Biotech LTD.) and Nutrient Broth (Titan Biotech LTD. Biochemical tests were performed to confirm the *E. coli* using Catalase test, Simmons Citrate Agar (Titan Biotech LTD.), Starch Agar (Titan Biotech LTD.), Indole production, Voges-Proskauer test, Urease Production, Nitrate Reduction, Methyl red and Presumptive test [17, 18].

#### Results

The presence of the *E. coli* on the skin of the udder was confirmed by the growth pattern on different media and by the biochemical tests whose result were given in the tables 1 and 2 respectively.

The results of the Most Probable Number (MPN) showed in table-3, which shows the level of contamination in per hundred milliliters of water sample collected from the dairy farms [19]. The water was used for drinking and washing of buffaloes udder, cleaning the utensils (used for the storage of milk) was same, hence there is always probability of contamination of *E. coli*, was very high, which was already there in the water, which ultimately reached the skin of the udder and utensils through water. This activity brought the bacteria directly into the milk and made it unfit for human consumption. The results of the present study were summarized in the table-4. According to these results the 23 samples out of 135 showed the presence of *E. coli* (17.03%), although the percentage was less, but normal flora of the udder restricts the growth of *E. coli* on the udder skin [20].

#### Discussion

*Escherichia coli* is significant from the point of

Table-3. MPN Results of the Water Samples

Serial No.	Sample Name	MPN*/100ml
1	DF1	49
2	DF2	63
3	DF3	1600
4	DF4	4
5	DF5	2
6	DF6	13
7	DF7	< 2
8	DF8	6
9	DF9	< 2
10	DF10	14

\* - with 95% confidence limit

view of public as well as animal health, as they have been responsible for diarrheal disease in human and environmental mastitis disease in buffaloes [21,22]. There are about twelve strains associated with the bovine mastitis and five pathotypes are recognized for diarrheal in man [21,23]. As the *E. coli* do not normally live on the skin or in the udder but which enter the teat canal when the buffaloes comes in contact with a contaminated environment, contagious mastitis is transmitted from buffalo- cow to cow by pathogen for which the udder is the primary reservoir; it tends to be sub-clinical in nature [2]. Subclinical mastitis was found more important in India (varying from 10-50% in cows and 5-20% in buffaloes) than clinical mastitis (1-10%) [24]. Common contagious pathogens have been reported to infect 7 to 40% of all buffaloes- cow [25]. Frequency of contagious pathogens among mastitis cases is greater [26].

In Faisalabad, 200 milk samples were collected from mastitis quarters of buffaloes in which the rate of contamination of the *E. coli* was (1.40%), whereas 2400 milk samples were collected for screening of the sub-clinical mastitis form the six hundred lactating dairy buffaloes from the four districts (Lahore, Sialkot, Narowal and Okara) in Pakistan, in which the percentage of *E. coli* was (16.18%) [27,28]. In Jharkhand, India, (8.95%) isolates of the *E. coli* were detected from the raw milk of clinical cases of the bovine mastitis [29]. Whereas 43.8% cases of *E. coli* mastitis were detected in dairy farm buffaloes in Alexandria Desert road, Egypt, as the organism were present in high level in the water tank as they act as primary reservoir for this environmental pathogen [30, 31]. Environmental factors such as poor hygiene, poor husbandry, and poor milking technique results the environmental mastitis as well as milk contamination [4, 32]. Due to rapid urbanization, dairy animals are kept in closed areas within the boundary walls of the house and animal get with very less open and covered area. In this type of housing, overcrowding of animals results in spreading of pathogenic bacteria. The prevalence of sub-clinical mastitis was highest in

Table-4. Occurrence of *Escherichia coli* growth obtained from the udder

Animal Species	No. of samples cultured	No. of positive that yielded bacterial growth	% of positive samples
Buffaloes	135	23	17.03

animals kept as individual holding at backyards followed by small holdings in periurban area (42%) and the lowest at organized farms with reasonable good management conditions (32%) [28].

While environmental mastitis cannot be totally eliminated from a herd, the incidence can be held to a minimum. The key elements in the control of mastitis include: sound husbandry practices and sanitation [33]. The result of the present study showed that *E. coli* still present on the udder skin pose a serious threat to the animal as well as consumer health. Thus, more hygienic preventive measures are required to inhibit the bacterial growth, so as to improve the health of the animals as well as the wholesomeness of the milk. Training and guidance programs should be started in order to develop awareness among farmers emphasizing the need for hygienic practice at farm level, as well as farm environment should be improved in order to decrease the breeding ground for pathogenic bacteria. Wash hands with soap and water, wash teats and udder in sanitizing solution, thoroughly dry teats and udder with individual towels, dip teats in an effective germicidal teat dip reduced the number of pathogenic bacteria from the udder skin [34].

#### Author's contribution

Rajdeep Palaha carried out the field study (Sample Collection), Participated in scientific discussion and sample analysis. Narendra Chaudhary participated in the sample analysis, scientific discussion. Harsh Kumar participated in the design of the study and coordination, scientific discussion and wrote the final draft. All authors read and approved the final manuscript.

#### Acknowledgments

Authors are pleased to thank the university authorities for providing the laboratory during this research work.

#### Competing interest

Authors declare that they have no conflict of interest.

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