**Microbiological quality of cattle’s milk obtained from different areas of district Muzaffargarh**

Rizwan Javeid**1**, Muhammad Safdar**\*2,3**, Yasmeen Junejo**4**, Masroor Ellahi Babar**1,** Mehmet Ozaslan3, Mushtaq Ahmad Gondal5

1. Department of Biotechnology, Virtual University of Pakistan, Lahore, Pakistan

2. Department of Breeding and Genetics, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

3. Department Biology, Division of Molecular Biology and Genetics, Gaziantep University, Turkey

4. Department of Physiology and Biochemistry, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

5. Institute of Continuing Education and Extension, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

**\***Corresponding should be addressed at safdar@cuvas.edu.pk

**Abstract:**

The aim of this study was to check out the cattle milk quality in district Muzaffargarh, Pakistan by biochemical tests, the standard plate counting and direct microscopic counting. 200 samples of cattle milk were obtained from different areas of district Muzaffargarh. Different parameters were chosen to check the cattle milk quality through biochemical tests, the standard plate counting and direct microscopic counting. It was concluded that alcohol perception and clot on boiling gave 34% and 24.5% positives results. On the other hand in organoleptic test, smell, appearance, colour, consistency and temperature gave 7%, 14.5%, 23%, 38% and 20% positive results. *S.aureus*, *E.coli, B.cereus, K.pneumoniea* and *S.typhi* generated the bar at 8%, 14%, 17%, 21% and 5% positive results respectively. It was also concluded that standard plate counting is much precise method as compared to the direct microscopic counting method for determining the microbiological quality standards for cattle’s milk in Pakistan.

**Keywords:** Cattle s’ milk samples, Bacteria identification, Milk quality, Muzaffargarh

**Introduction**

Cattle milk is nutritionally rich which is available in all over the world and its demand is high because of great value of nutrition and vitamins (Getaneh *et al.,* 2016; Park *et al.,* 1991). Increase in number of pathogenic bacteria lead the economical loss to milkman and milk farmer (bonfoh *et al.,* 2003; Metz *et al.,* 2009; Suguna *et al.,* 2011). Some researchers were identified different bacterial species such as *Camphylobacter, Bacillus aureus, Streptococcus, Listeria monocytogenes and Staphylococcus* in cattle s’ milk (Adesiyun *et al.,* 2007; Kagkli *et al.,* 2007). Number of bacteria increases up to 100 times more when milk is stored for long duration at normal temperature (Chye *et al.*, 2004; Suguna *et al.,* 2011]. Suguna et al., were investigated that favorable nutrient in milk induces the selective microorganism attack (Seifu *et al.,* 2004; Suguna *et al.,* 2011] and bacterial load was differed from milk to milk because of hygieneic condition, handling, processing, location and geographical representation of the dairy farm (Millogo *et al.,* 2010; Oilver *et al.,* 2005). Therefore it is important to investigate the quality of cattle’s milk at different cattle s’ milk distribution levels in Pakistan especially in cattle dairy farming at district Muzaffargarh.

Technology and physical treatment of milk as such under passing of milk is responsible for the quality and hygienic condition of cattle milk (Kyozaire *et al.,* 2005). Quality of milk could be controlled by managing the endogeneous and exogeneous infection of cattle’s milk (Kyozaire *et al.,* 2005). Salmonellas, Brucellosis, Q.fever, Tuberculosis, Listeriosis, Toxoplasmic, Staphylococcal, Streptococcal and campylobacter infections are some mentioned diseases which could be found in human beings because of the low quality milk (Kyozaire *et al.,* 2005). Also some researcher worked on endogenous contamination in cattle milk and this contamination was occurred due to presence of *Staphylococcus aureus*, *streptococcus* *agalactiae, preudomas* and *E.coli* species (Ryan et al., 1990).

Many micro-organisms found the access to milk and its products (Khatib *et al.*, 2009 ; Sadek *et al.,* 2009). So the presence of such micro-organisms in milk could cause serious and hazardous diseases in milk users. As some scientists investigated that *staphylococcal enterotoxins* was produced in milk which was kept at 37°-42°C and caused various dieases such as Nausea, abdominal cramps, vomiting and diarrhea in human beings (Kyozaire *et al.,* 2005).

Bacteria can be killed at normal heating temperature but toxic agents persists their activity (Kyozaire *et al.*, 2005). *S.aureus* produced entereotoxins which were causative agents to lead gastro intestinal in infants and adults. Entereotoxins were produced by 50% of the staphylococcal strains (Payne & Wood, 1974). *S.aureus* became reason of many small diseases to fatal diseases such as pneumonia, endocarditic, meningitis and specticaemia (Soomro *et al.,* 2003). Owing to consumer preferences and lack of technology, almost 95 percent of the milk in Pakistan is marketed raw through informal marketing chains; the remaining 5 percent is processed by the formal processing industry and marketed through the formal chain. While, there are no standards for the evaluation of cattle s’ milk has developed yet.

The aim of this study was to check out the cattle s’ milk quality in district Muzaffargarh, Pakistan by biochemical tests (organoleptic evaluation, alcohol perception, clot on boiling test, coagulase test and gram staining test), the standard plate counting and direct microscopic counting to form microbiological quality standards for cattle’s milk in Pakistan.

**Material and methods**

Different cattle dairy farms, middleman (Gawala) and milk shops etc. of district Muzaffargarh were searched to include in our studies. These farms were located in district Muzaffargarh, Punjab, Pakistan.

**Questionnaire survey**

A questionnaire survey was conducted for the general interview of cattle dairy farmers to till consumers to obtain information about handling of cattle milk to the supply of cattle milk. Cattle diet was also observed. It was observed that cattle was served with free grazing in village and field and no proper dietary supplements were provided to the cattle for consumption of human beings.

**Sample collection**

Field study for 200 samples collection was performed in different areas (Sinawan, Shahjamal, Ruhillanwali, Shehr sultan, Kotadu, Jatoi, Alipur) of district Muzaffargarh. Samples were collected in Syringes, for the prevention of any type of bacterial attack. These samples were then kept in the ice box and transported to the laboratory of Virtual University of Pakistan Multan Campus for processing and screening of microbiological analysis.

**Milk sample analysis**

Milk samples were analyzed for microbiological quality clot on boiling, organoleptic test and alcohol test were carried to check the physical examination of milk. Coagualse test and gram staining test were performed as biochemical testing of milk. Bacteria were counted by standard plate count and direct microscopic count method.

**Clot on boiling**

According to the Marshal who described the method of clot on boiling was employed. It was performed to analyze the acidity of milk either it was more than 5.8% or less than 5.8%.Test tubes were filled with 5ml of milk sample. These test tubes were boiled in water bath and then observe the appearance of the milk in test tubes (Marshall *et al.,* 1992).

**Alcohol test**

According to the Marshal description the alcohol test was performed to detect mineral disturbance of cattle milk. It was also carried out to recognize the unhealthy milk like late lactation and mastitis patients (Marshall *et al.,* 1992). Test tubes were filled with 5ml of milk and then treated with equal amount of 68% ethyl alcohol. The inversion step was taken to mix the items.

**Organoleptic test**

Organoleptic tests were performed for appearance, smell, temperature, colour and consistency features.

**SPC and DMC**

Milk samples were undergone with the coagulase and gram staining test to identify and detect the bacteria such as *S.aureus* and *E.coli*. After that SPC and DMC were carried out to count the bacterial number in milk samples with the specific serial dilution of samples.

**Results and Discussion**

The present study recruited 200 samples of cattle milk taken from a spread out group of cattle dairy farmers and associated persons. Out of these 200 samples, 16 samples (8 percent of total subjects) depicted a positive growth of *staphylococcus aureus*. Different parameters were studied to assess the growth of bacteria in these samples. Coagulase and Gram staining tests resulted in positive values as mentioned in table 1.

**Table 1.** Biochemical test for identified bacteria.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sr. no. | Identified bacteria | Gram staining | Coagulase | Indole test | Methyl Red test | Voges-Proskauer test | Citrate test |
| 1 | *S.aureus* | +ve | +ve | -ve | +ve | +ve | +ve |
| 2 | *E.coli* | -ve | -ve | +ve | +ve | -ve | +ve |
| 3 | *B.cereus* | +ve | -ve | -ve | -ve | +ve | +ve |
| 4 | *K.pneumoniea* | -ve | -ve | -ve | -ve | +ve | +ve |
| 5 | *S.typhi* | -ve | -ve | -ve | +ve | -ve | +ve |

The samples were further classified on basis of five parameters covered under organoleptic test evaluation. These parameters were smell, color, consistency, appearance and temperature. 83.5% (167 samples) gave milky smell, 9.5% (19 samples) gave cowey smell, while 7% (14 samples) had a feedy smell. 51.5% (103 samples) had a white colour, 25.5% (51 samples) were yellowish, while, 23% (46 samples) were reddish in colour. 62% (124 samples) were normal in layer appearance, 20% (40 samples) were thick in consistency and remaining 18% (36 samples) were watery. 85.5% (171 samples) were clear in appearance while remaining 14.5% (29 samples) were dirty in appearance. 80% (160 samples) had higher than 500fahrenheit temperature, while 20% (40 samples) had less than 500fahrenheit temperature.Value of clot on boiling showed positive value of clot in 24.5% (49) samples while negative value of clot was seen in 75.5 %( 151) samples as described. It was elaborated by (Bashir *et al*., 2013) that 38% of milkman samples gave positive result and remaining 62% of the milk samples yielded negative results.

*E.coli* had standard plate count values of 28 positive cultures ranged from 3.0 x 10(lowest value) to 9.7 x 103 (highest value). The direct microscopic count values ranged from 2.2 x 102 (lowest value) to 3 x107 (highest value) (Table 2). The positive results of *E.coli* had showed different percentage as compared to the results of Vahedi *et al.*, 2013 that resulted 42% of *E.coli* were positive in her research work.

According to *S.aureus* standard plate count values of 16 positive cultures were ranged from 2.30 x 10(lowest value) to 7.52 x 102 (highest value). The direct microscopic count values were ranged from 3.2 x 102 (lowest value) to 1.02 x106 (highest value) (Table 3).

Our results of milk contamination from cattle milk could be statistically associated to those of Freitas *et al*., 2005 when raw milk was analyzed in Belem, finding a reasonable sample contamination with *Staphylococcus aureus*, varying from < 1x10¹ to 1.25x106 CFU/mL. Less than 30 colonies of *S.aureus* were also observed in milk samples collected by Kousta*et al.,*2010. The ratio of *S.aureus* positive samples was closely related to the ratio of 10% by Thaker. It distantly seemed to the 7.3% by Fagundes. Quintana*et al.,* 2006 assessed raw milk contamination with *S.aureus* finding 28.5% of the samples having values of *S. aureus* above 104 CFU/mL. Our results suggest a strong co-relation with previous studies.

The optimum range of the bacterial count is different in all over the world. As Cempirkova2002 investigated that 4.5 log CFU/ml of bacterial count is the optimum range for the Europe countries while on the other hand Zwefiel et al., 2005 indicated the optimum range of bacterial count in United States is 5.0 CFU/ml.

Contamination in milk is a serious concern, both for farmer’s economical perspective and health aspects of general public. Dairy professionals and farmers at large need to take corrective measures while milking their animals in order to prevent or minimize the contamination risks. Health workers need to carry educational programs for farmers and dairy people in order to aware them on the proper handling of milk throughout the milking process.

**Table 2.**

 S.P.C values of the positive culture growths against 28 samples for *E.coli*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| S. No | S.P.C cfu/ml |  D.M.C cfu/ml | S. No. | S.P.C cfu/ml | D.M.C cfu/ml |
| 1 | 3.6 x 102 | 2 x106 | 15 | 3.2 x103 | 4 x 106  |
| 2 | 4.9 x 102 | 3 x 103 | 16 | 1.2 x102 | 3 x 107  |
| 3 | 3.8 x 102 | 5 x 103 | 17 | 3.6 x103 | 5 x 105  |
| 4 | 7.7 x 10 | 3 x 102 | 18 | 2.7 x102 | 2 x 102 |
| 5 | 8.9 x 102 | 2 x 105 | 19 | 5.8 x103 | 4 x 103 |
| 6 | 3.0 x 10 | 3 x 104 | 20 | 4.3 x103 | 2 x 105 |
| 7 | 2.3 x 102 | 2 x 105 | 21 | 6.7 x102 | 3 x 104 |
| 8 | 2.5 x 102 | 3 x 103 | 22 | 9.7 x103 | 6 x 105 |
| 9 | 3.3 x 10 | 2 x 102 | 23 | 3.5 x102 | 2 x 105 |
| 10 | 3.7 x 102 | 4 x 103 | 24 | 6.8 x103 | 5 x 106 |
| 11 | 5.4 x 102 | 6 x 104 | 25 | 2.9 x102 | 4 x 105 |
| 12 | 4.7 x 102 | 3 x 105 | 26 | 3.7 x102 | 3 x 103 |
| 13 | 4.0 x 102 | 8 x 103 | 27 | 2.5 x103 | 3 x 102 |
| 14 | 5.1 x 102 | 4 x 105  | 28 | 6.9 x 103 | 5 x 106 |

\*S.P.C: standard plate count, \*D.M.C: Direct microscopic count

**Table 3.**

S.P.C values of the positive culture growths against 16 samples for *S.aureus*

|  |  |  |
| --- | --- | --- |
| S. No | S.P.C cfu/ml | D.M.C cfu/ml |
| 1 | 3.3 x 102 | 7.2 X 105 |
| 2 | 2.21 x 102 | 6.7 X 103 |
| 3 | 5.00 x 102 | 9.8 x 102 |
| 4 | 2.45 x 10 | 6.9 x 105 |
| 5 | 3.47 x 102 | 8.5 x 104 |
| 6 | 8.5 x 10 | 1.02 x 106 |
| 7 | 2.92 x 102 | 6.5 x 104 |
| 8 | 7.50 x 102 | 1.35 x 103 |
| 9 | 2.30 x 10 | 7.6 x 103 |
| 10 | 1.97 x 102 | 3.2 x 102 |
| 11 | 2.49 x 102 | 7.0 x 103 |
| 12 | 5.11 x 102 | 9.8 x 102 |
| 13 | 3.00 x 102 | 9.0 x 104 |
| 14 | 2.1 x 102 | 6.2 x 102 |
| 15 | 3.2 x 10 | 8.9 x 105 |
| 16 | 2.59 x 10 | 7.5 x 104 |

 \*S.P.C: standard plate count, \*D.M.C: Direct microscopic count

**Conclusion**

It was concluded that *S.aureus* and *E.coli* are present in cattle s’ milk samples with a high counting. This contamination occurred because of the poor hygienic conditions and the low facilities about milk handling, milking, transporting, processing and storing. These were the major reasons for the contamination of milk. The germs of *S.aureus* and *E.coli* existed in cattle s’ milk through the poor hygienicity and the animals as well as human could be suffered from serious diseases. This study suggests that precautionary measures should be taken for the germ free milk. It was also concluded that there is a great difference between the calculation of standard plate counting and direct microscopic counting of *S.aureus* and *E.coli* numbering because standard plate counting is the method which count viable and living bacteria while on the other hand direct microscopic counting methods counts both dead and living cells of the targeted bacteria. Through the findings of this study, standard plate counting should be preferred for bacterial counts and maintain the quality of cattle s’ milk in Pakistan as well as in developing countries.

**Acknowledgements**

We would like to thanks Department of Biotechnology, Virtual University of Pakistan and Diagnostic laboratory of CUVAS, Bahawalpur for their generous support to conduct this project.

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