

OCCURRENCE OF AEROMONAS IN RAW BOVINE MILK SAMPLES IN SRINAGAR, KASHMIRMALIHA GULZAR*, MUDASIR ALI RATHER, SYED AKRAM HUSSAIN, SABIA QURESHI¹, ISHRAQ HUSSAIN², MUJEEB UR REHMAN FAZILI³ and SHOWKAT AHMAD SHAH⁴Division of Veterinary Public Health, ¹Division of Veterinary Microbiology and Immunology,²Division of Veterinary Biochemistry, ³Division of Veterinary Clinical Service Complex,⁴Division of Veterinary Pathology, F.V.Sc. & A.H., Sher-e-Kashmir University of Agricultural Sciences and Technology (SKUAST), Kashmir-190 001, India

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SUMMARY

The present study was undertaken to find out the occurrence of *Aeromonas* in raw bovine milk samples in Srinagar district. A total of 240 milk samples were collected from unorganized farms (n=240) from different areas under study. The samples were screened for the presence of aeromonads. Out of the 240 milk samples, 194 (80.83%) were found positive for *Aeromonas* spp. on biochemical testing. Of all the tested isolates, *A. hydrophila* predominated with a total occurrence of 27.31% (n=53). Further, the isolation rate of *A. caviae* and *A. veronii* was 14.43% (n=28) and 6.18% (n=12), respectively, while 101 (52.06%) isolates were identified upto genus level. The presence of *Aeromonas* species in the milk samples is a concern for public health as the pathogen uses the milk as vehicle for its transmission.

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Aeromonas belong to the family Aeromonadaceae, order Aeromonadales, class Gamma Proteobacteria. The bacteria *Aeromonas* is gram-negative, facultatively anaerobic, oxidase and catalase positive (Garrity *et al.*, 2001). *Aeromonas* species are autochthonous in the aquatic system (Abulhamd, 2010). The bacteria are considered as a common contaminant in the variety of foods, i.e., vegetables, sea foods, raw and cooked meats, eggs, milk and milk products (Sharma and Kumar, 2011; Dallal *et al.*, 2012; Hussain *et al.*, 2014). Gastroenteritis caused due to the bacteria *Aeromonas* is reported to be common in India. The presence of *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas veronii* in milk has been reported in many studies and implicated to pose a serious human health risk in Indian subcontinent (Yucel *et al.*, 2005; Porteen *et al.*, 2006; Subashkumar *et al.*, 2010; Manna *et al.*, 2013; Alhazmi, 2015; Tahoun *et al.*, 2016).

Raw bovine milk samples (n = 240) were collected randomly from 240 different unorganized farms of different areas of Srinagar city, Kashmir. The samples were processed as per the protocol described by Janda and Abbott (2010) with slight modifications. Briefly, five ml of milk samples were enriched in 20 ml of alkaline peptone water (Himedia, Mumbai) and then it was incubated at 35°C for 24 hours which was followed by cold enrichment at 4°C for 5 hours. A loopful of inoculum from the enriched broth was streaked on to Ampicillin Dextrin Agar (ADA) (Himedia, Mumbai) and incubated at 35°C for 24 hours.

The presumptive colonies were designated as genus *Aeromonas* on the basis of oxidase, catalase and nitrate

production, resistance to O/129 (2, 4-diamino 6, 7 diisopropylpteridine), production of acid from D-trehalose, inability to utilize malonate, and fermentation of inositol, D-xylose and dulcitol (Ottaviani *et al.*, 1998). For speciation of aeromonads, a battery of biochemical tests i.e., hemolysis, H₂S from cysteine, esculin hydrolysis, Voges-Proskauer, gluconate oxidation, arginine dihydrolase lysine, ornithine decarboxylase, gas from glucose, acid from arabinose, sucrose, D-rhamnose, mannitol, D-sorbitol, lactose, salicin, glycerol, D-mannose, cellobiose, elastase, citrate and acid phenylpyruvic utilization, pyrazinamidase, cephalotin and ampicillin susceptibility was performed as per the standard procedures (Abbott *et al.*, 1992; Janda *et al.*, 1996).

The samples streaked on the Ampicillin Dextrin Agar showed typical convex yellow-colored colonies. Biochemical testing revealed that 80.83% (194/240) of collected milk samples were positive for *Aeromonas* spp. Out of these, 101 isolates (52.06%) *Aeromonas* isolates were identified up to genus level only, whereas 53 (27.31%) isolates were found positive for *A. hydrophila* on biochemical testing. However, *A. caviae* and *A. veronii* were confirmed in 28 (14.43%) and 12 (6.18%) isolates, respectively. The presence of *Aeromonas* in raw milk (Kirov *et al.*, 1993; Manna *et al.*, 2013) as well as in pasteurized milk (Manna *et al.*, 2013) has been reported in many studies. The results obtained in our study conclude that mesophilic strains of *Aeromonas* i.e. *A. hydrophila*, *A. caviae* and *A. veronii* can contaminate raw milk. Presence of these bacteria in raw milk decreases its shelf life and may result in human gastrointestinal infections because of

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their capacity of gut colonization and production of toxins (Kirov *et al.*, 1993). This necessitates source tracking of such bacteria in milk besides taking precautions and following hygienic practice at the farm level to avoid bacterial contamination of milk.

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