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Physico-chemical and bacteriological assessment of drinking water of small dairy farms of Indore district (M.P.)

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Abstract
In the present investigation, a total of 120 dairy animal drinking water samples were collected from all four administrative blocks of the Indore district (M.P.). The Study was conducted in the department of Veterinary Public Health, College of Veterinary Sciences and Animal Husbandry Mhow, Indore (M.P.) from June 2022 to November 2022. On physicochemical analysis of water, the mean values for temperature, pH, colour, TDS, turbidity, residual chlorine, hardness, chloride, nitrate, fluoride, and iron were found to be between 26.09±0.16 ºC to 26.83±0.09 ºC, 7.20±0.06 to 7.36±0.09, 9.66±1.15 to 12.00±1.49 Hazen unit, 376.93±17.98 to 459.63±27.97 ppm, 13.83±0.95 NTU to 16.33±1.19 NTU, 0.016±0.01 to 0.012±0.01 mg/l, 225.33±08.23 to 305±30.89 mg/l, 104.33±12.71 to 249.66±31.88 mg/l, 50.33±03.48 to 90.83±11.21 mg/l, 0.21±0.04 to 0.39±0.03 mg/l and 0.03±0.01 to 0.14±0.03 mg/l, respectively. The results showed that mean values of water samples for parameters such as temperature, pH, colour, TDS, residual chlorine, nitrate, fluoride and iron were found within the allowable limits whereas turbidity, total hardness and chloride were found above the allowable limits set by United States Environment Protection Agency (USEPA) and Bureau of Indian Standard (BIS) for animal water. Comparative appraisal among all blocks revealed that a high percentage of water samples were found to be odourless in Sanwer and Mhow block and 66.67% of water samples from Mhow and Sanwer block were found to be favourable in taste whereas 26.67% of water samples from the Indore block were found very unfavorable in taste. On bacteriological analysis, the mean values of the coliform count were found to be between 111.23±60.32 to 340.50±94.79 coliforms/100ml, indicated a significant difference between all four blocks (p<0.05) of the studied area.

Keywords: Water, farms, physico-chemical, bacteriological, coli-form

1. Introduction
Water is the essential tonic of life and abounds on the earth. It is true said that "No water No life''. For sustain of life both ground and surface water are essential natural resources, which is a complimentary reward of nature (Patel *et al*., 2016) [26] but this vast natural resource has been depleted and turned into scarce commodity with increased usage catering to the needs of ever-expanding livestock and human population (Barua *et al*., 2021) [9]. The rivers provide water for industries, agriculture, aquaculture, commercial and domestic purpose. But these rivers are being polluted by indiscriminate disposal of sewage, industrial wastes and plenty of human activities. These activities affect physicochemical characteristics and microbiological quality of water (Sharma *et al*., 2017) [29]. Water quality is a very important aspect of a livestock farming system since it can affect both the health of the animals and its total water consumption (Higgins *et al*., 2008) [18]. Poor quality drinking water is often a factor limiting feed intake in animals. The animal has to drink water at least every day to be productive and highly producing animals need large amounts of clean and fresh water daily (Umar *et al*., 2014) [37]. High or low temperature may affect the water intake, feed intake, respiration rate, rectal temperature, rumen fermentation, plasma thyroid hormone concentration, milk yield, weight gain and performance of the animals (Barik and Thorat, 2015) [8]. Salinity or total dissolved solid (TDS) is one of the basic chemical parameters which causes excessive salivation, diarrhoea, vomiting, blindness, seizures, ataxia, disorientation and paralysis in the acute phase of excessive salinity (Anonymous, 2006) [3]. The bicarbonates, sulphates and chlorides of calcium and magnesium may cause the hardness of water. Hard water is responsible for the development of goitre, renal calculi, dyspepsia and other gastric disturbance in animals (Sherikar *et al*., 2005) [32].

Nitrogen fertilizer and various livestock operations may elevate nitrates (NO_3) and nitrites (NO_2) levels in water. Nitrites are absorbed into the bloodstream ultimately affecting oxygen transport ability of red blood cells (RBCs), by developing methemoglobinemia (Schutz, 2012) [30]. Fluoride concentration in water should not be very high or low than their prescribed maximum permissible limit (MPL) because the deficiency of fluorine in drinking water causes the dental caries and in higher levels sometimes leads to dental or skeletal fluorosis, mottled teeth, constipation and many types of skin ailments (Patil *et al*., 2012) [27]. Low microbiological quality of water is associated with contamination of water sources with human or animal excreta (Kebede *et al*., 2013) [20]. High concentrations of minerals, nitrates, nitrites, bacterial contaminations and chemical pollution associated with agricultural and industrial activities are the major factors affecting its quality (Kumaravelu, 2019) [22]. Any deviation of physico-chemical and bacteriological contaminants from their normal ranges can cause hazardous health impacts on animals as well as humans (Pfost *et al*., 2001) [28]. Drinking water can be the vector of viral, bacterial and parasitic diseases. Livestock parasites such as protozoa, flukes, flatworms and round worms may spend part of their life cycle in or near water (Duguma *et al*., 2012) [11]. Water quality testing is very important to check the quality of drinking water to avoid waterborne diseases and improve health (Kate *et al*., 2020) [19].

The Malwa region was the seat of the Great Ujjain King-Raja Vikramaditya- the Julius Caesar of India, who gave the Vikrami Calendar to India, not unlike the Julian calendar given by Caesar. Indore is the largest city, urban area in the Malwa region and even Madhya Pradesh state (Gupta, 2013) [17]. This region is a famous milk-shed area. Screening and analysis of drinking water samples from dairy farms in this region would be helpful to make baseline data and to find out the actual status of the quality of the water supplied to the animals in the studied area. There are a number of physical, chemical and bacteriological parameters which are essential to define the water quality attributes; these are needs to be assessed.

2. Materials and Methods

A total of 120 animal drinking water samples were collected from small-scale dairy farms, situated in Mhow, Indore, Sanwer and Depalpur blocks of Indore district (M.P.), using a random sampling technique. Water samples were collected between the time periods of June 2022 to November 2022. All the water samples were collected in polypropylene bottles for physicochemical analysis and for the bacteriological analysis water samples were collected under aseptic conditions into sterilized bottles. All samples were immediately transported to the laboratory under low-temperature conditions and analysed for indicator microorganisms within 24 hours (Park, 2011) [25]. The collected water samples were analyzed in the department of Veterinary Public Health, College of Veterinary Sciences and Animal Husbandry Mhow, Indore, for various parameters.

2.1 Physico-chemical parameters

Physico-chemical parameters such as temperature, pH, colour, odour, taste, turbidity, total dissolved solids (TDS), residual chlorine, total hardness (TH), chloride, nitrate, fluoride and iron, were analysed by using standard procedures (Table 1).

2.2 Bacteriological analysis

Bacteriological analysis was also performed; coli form indicator bacteria in water samples were estimated using most probable number method (Table 1). It was conducted in three steps:

2.2.1 Presumptive test (Multiple Tube Technique)

For this purpose, three sets of test tubes containing five tubes in each set were arranged. One set with 10 ml of double strength (DS) and the other two containing 10 ml of single strength (SS) MacConkey lactose bile broth were prepared. Inverted Durham's tubes were inserted in each tube and autoclaved. Then, 10 ml of water sample was transferred to each of the DS broth tubes using a sterile pipette. Further, 1 ml of water sample to each of the five tubes of one set of SS broth and 0.1 ml water to five tubes of the remaining last set of SS broth tubes were transferred. The tubes were incubated at 37 °C for 24 hours and then observed for the gas production in the Durham's tube and colour change of the media. The number of positive results from each set was recorded and compared with the standard chart/ Mac crady's table to give a presumptive coliform count per 100 ml water sample.

2.2.2 Confirmed test

The samples from the positive tube of the presumptive test were streaked on the Eosin Methylene Blue (EMB) agar plate and were incubated at 37 °C for 24 hours.

2.2.3 Completed test: The typical colonies of coliform (with metallic sheen) were selected, then inoculated on lactose broth and incubated at 37 °C for 24 hours.

Table 1: Water quality attributes and their detection methods

S. No.	Physicochemical parameters	Methods	References
1.	Temperature (ºC)	Thermometer	APHA (1995)
2.	pH	Electrometric	Gupta (2009) [16]
3.	Colour (Hazen unit)	Visual comparison	FSSAI (2016)
4.	Odour	Sensory assessment	FSSAI (2016)
5.	Taste	Sensory assessment	FSSAI (2016)
6.	Turbidity (NTU)	Nephelometric test	FSSAI (2016)
7.	TDS (ppm)	Gravimetric	FSSAI (2016)
8.	Residual chlorine (mg/l)	DPD colorimetric method	FSSAI (2016)
9.	Total Hardness (mg/l)	EDTA method	APHA (1995)
10.	Chloride (mg/l)	Argentometric method	APHA (1995)
11.	Nitrate (mg/l)	Water testing kit	Sharma (2016) [31]
12.	Fluoride (mg/l)	Water testing kit	Sharma (2016) [31]

13.	Iron (mg/l)	Water testing kit	Sharma (2016) [31]
14.	Coliform Count/100ml	MPN method	APHA (1998)

The obtained data of physicochemical and bacteriological parameters were statistically analyzed through one way ANOVA.

3. Results and Discussion

3.1 Physico-chemical parameters

The physico-chemical characteristics of animal drinking water samples were analyzed with respect to temperature, pH, colour, odour, taste, turbidity, TDS, residual chlorine, total hardness, chloride, nitrate, fluoride and iron. The mean values for temperature were found to be between ranges of 26.09±0.16 ºC to 26.83±0.09 ºC. The results of the present study are in line with the findings of Yirga *et al.* (2019) [38] who also found the temperature ranged from 23.10-29.00 ºC. The minor variations in the temperature of animal drinking water might be due to the various redox reactions because of the contamination. The mean values for pH were found to be between ranges of 7.20±0.06 to 7.36±0.09. The results of the present findings are comparable to the findings of Duguma *et al.* (2012) [11] and Ehiowemwenguan *et al.* (2014) [13]. They observed the pH of the water ranges between 7.8-8.2 and 6.8-7.3, respectively. The mean values for colour were found to be in ranges of 9.66±1.15 to 12.00±1.49 Hazen unit. The present study is comparable with Lugomer *et al.* (2017) [23] who studied the quality of well water used for livestock watering on farms. They found that the colour of all water samples was found to be within the ranges of 5.0-20.0 Hazen units. Among water samples of all blocks, a high percentage of water samples were found to odourless in Sanwer as 73.30% and in Mhow as 66.67%. The results of our study are partially in accordance with the findings of Lugomer *et al.* (2017) [23] which showed that most of the microbiologically suitable samples tested were found to be odourless. Upon a comparison of 4 blocks, 66.67% of water samples from Mhow and Sanwer block were found to be favourable in taste whereas 26.67% of water samples from the Indore block were found very unfavorable in taste. The results of our study are comparable to the findings of Sharma (2016) [31]. She investigated that the taste of all RO water samples and packaged water was sweet and agreeable whereas 93.30% of tap water samples, 63.30% of water samples from restaurants/pyaus and 73.33% of school water samples were found to be salty.

In the present study, the range of turbidity was found between 13.83±0.95 NTU to 16.33±1.19 NTU. These results are corroborated by the findings of Kpowulu (2015) [21] and Garg *et al.* (2009) [15]. They found a comparable result related to the turbidity of water which was found to be ranged from 10.25-12.34 NTU and 2.17-16.72 NTU, respectively. An increase in turbidity of water may be due to the presence of particulate organic and inorganic matter such as clay, silt, colloidal particles and microscopic organisms. The TDS values were falling between 376.93±17.98 to 459.63±27.97 ppm. The results of the present findings are comparable with Ehiagbonare and Ogunrinde (2010) [12]. In this study, the TDS values of water were found to be ranged from 22.0-906.0 ppm. The results of the present study are also in line with the findings of Mobin *et al.* (2014) [24] who reported highest TDS of all collected water samples was found as 902 ppm and the lowest was found as 203 ppm with an average value of 340.86 ppm (SD±62.28).

In this investigation, the mean values of residual chlorine (mg/l) were found to be ranged between 0.016±0.01 to 0.012±0.01. The results of the present finding are comparable with Shrestha *et al.* (2017) [33] who evaluated the quality of drinking water samples. They reported that free and total residual chlorine was found to be ranged from 0.1 to 0.5 mg/l in all the water samples. The mean values of the hardness of water were found to be ranged between 225.33±08.23 to 305±30.89 mg/l. It may be due to the geology of the area such as the richness of the sedimentary rocks and calcium-bearing minerals. Excessive application of lime to the soil in agricultural areas may be one of the major causes of hardness in water. The mean values of chloride of water were found to be ranged between 104.33±12.71 to 249.66±31.88 mg/l. The results of the present investigation are comparable to the findings of Kumaravelu *et al.* (2019) [22] who reported that the chloride content (mg/l) in livestock drinking water samples was found to be 63.30±4.90 in tap water during summer and 66.67±3.33 in tap water during the winter season. In another study, a higher level of chloride content in water was reported by Thirunavukkarasu (1997) [36] who investigate the quality of drinking water supplied to commercial layer farms. In open wells water, the mean values for chlorides were found to be 375.75±49 mg/l and in drilled wells were found to be 326.50±37 mg/l. In the present study high concentrations of chloride in water samples may be due to pollution with anthropogenic activities and high salt concentration in water may be one of the major reasons.

In current study, the mean values of nitrate were ranged between 50.33±03.48 to 90.83±11.21 mg/l. The results of the present study are comparable with the findings of Ara *et al.* (2004) who reported the values of nitrate content ranged from 88.5 - 367.5 mg/l. The findings of the present study are also comparable to Kumaravelu *et al.* (2019) [22] who observed that the nitrate content was found to be ranged from 45.10±7.10 to 150.97±16.16 mg/l. The mean values of fluoride of water were found to be ranged between 0.21±0.04 mg/l to 0.39±0.03 mg/l. The results of the present study are comparable to Fadaei and Sadeghi (2014) [14] who reported that the level of fluoride was found to be ranged from 0.19 to 0.28 mg/l with a mean value of 0.20±0.1 mg/l. The mean values of iron content of water were found to be ranged between 0.03±0.01 to 0.14±0.03 mg/l. Earlier studies conducted by Yirga *et al.* (2019) [38] reported reasonably higher iron content ranging from 3.0-10 mg/l in water samples of rift valley lakes. The results of the present study are in line with the findings of Sharma (2016) [31] who analysed the iron content of Yamuna water which was found to be between the ranged 0-0.50 mg/l. The results of current findings revealed that the mean values for physico-chemical parameters such as temperature, pH, colour, TDS, residual chlorine, nitrate, fluoride and iron were found within the allowable limits whereas turbidity, total hardness and chloride were found above the allowable limits set by USEPA (2009) and BIS (2012) for livestock water (Table 2).

Table 2: Mean values of various physico-chemical parameters of livestock drinking water samples in studied area

Parameters	Mhow	Indore	Sanwer	Depalpur
Temperature (ºC)	26.83^{b}±0.09	26.09^{a}±0.16	26.18^{a}±0.22	26.19^{a}±0.21
pH	7.20±0.06	7.24±0.05	7.28±0.08	7.36±0.09
Colour (Hazen unit)	9.66±1.15	12.00±1.49	11.66±1.38	11.33±1.45
Turbidity (NTU)	16.33±1.19	13.83±0.95	14.50±1.18	13.00±1.25
TDS (ppm)	459.63^{b}±27.97	376.93^{a}±17.98	457.17^{b}±28.44	444.73^{b}±34.53
Residual chlorine (mg/L)	0.013±0.01	0.016±0.01	0.012±0.01	0.013±0.01
Total Hardness (mg/L)	305.00^{b}±30.89	246.00^{a}±08.26	225.33^{a}±08.23	303.16^{b}±21.25
Chloride (mg/L)	178.00^{ab}±19.74	108.00^{a}±05.47	104.33^{a}±12.71	249.66^{b}±31.88
Nitrate (mg/L)	82.83^{b}±10.37	53.33^{a}±06.53	90.83^{b}±11.21	50.33^{a}±03.48
Fluoride (mg/L)	0.39^{b}±0.03	0.34^{b}±0.03	0.26^{a}±0.04	0.21^{a}±0.04
Iron (mg/L)	0.03^{a}±0.01	0.05^{a}±0.02	0.05^{a}±0.02	0.14^{b}±0.03

Means bearing different superscript (a, b) within row differ significantly (p<0.05)

3.2 Bacteriological analysis

3.2.1 Presumptive test (Multiple Tube Technique)

In the present investigation, the bacteriological quality of dairy animal drinking water samples was evaluated by the MPN method (Plate 1, 2). The overall range of MPN in all the water samples was found to be 0-1800 coliforms/100 ml. The mean values of the coliform count were found to be a maximum of 340.50±94.79 coliforms/100 ml for the Sanwer block and a minimum of 111.23±60.32 coliforms/100ml for the Mhow block (Table 3). The results of the present study are in line with Sunitha *et al.* (2013) [35] who analyzed water samples that exhibited faecal contamination and the total coliform count values ranged between 2 and 1600 organisms/100 ml. The result of the present study is also comparable with the findings of Caroline *et al.* (2018) [10] who assessed the microbial quality of water sources. The highest MPN reported in lakes as 886.66±134.92 coliforms/100ml and in ponds as 993.33±106.66 coliforms/100 ml followed by municipal water supplies as 57.83±11.12 coliforms/100 ml and the lowest in open wells as 41.66±07.44 coliforms/100 ml and in drilled wells as 29.50±05.65 coliforms/100ml. Variation in the values in our study may be due to the water samples being contaminated with feacal, manure, soil and sewage seepage to groundwater.

Means bearing the different superscript (a, b) within row shows a significant difference (p<0.05)

Table 3: Mean values of coliform count in livestock drinking water samples in studied area

Parameter	Mhow	Indore	Sanwer	Depalpur
Coliform Count (per100mL)	111.23^{a}±60.32	115.63^{a}±60.72	340.50^{b}±94.79	300.86^{b}±95.56

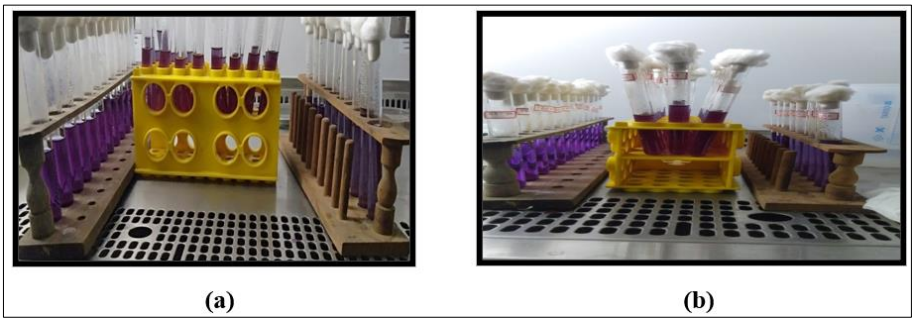

Plate 1: Water samples containing MacConkey lactose bile broth with Durham's tubes before incubation

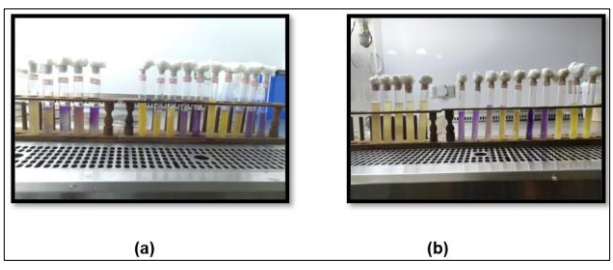

Plate 2: Tubes showing change in colour and gas formation after 24 hours of incubation at 37 °C

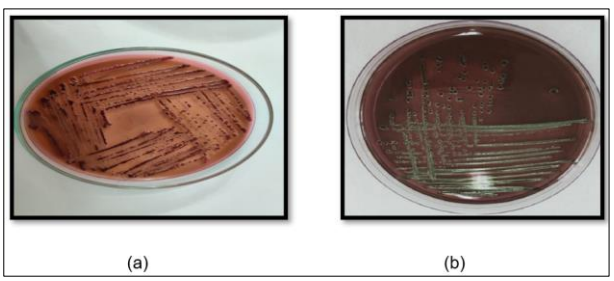

Plate 3: Growth of coliform (a) and faecal coliform (b) on EMB agar medium after incubation at 37 °C for 24 - 48 hours

3.2.2 Confirmed test

The bacterial colonies with black centers or metallic sheen were observed marking positive confirmed test (Plate 3).

3.2.3 Completed test

The production of gas shows the presence of coliform groups and the completed test was found positive (Plate 4)

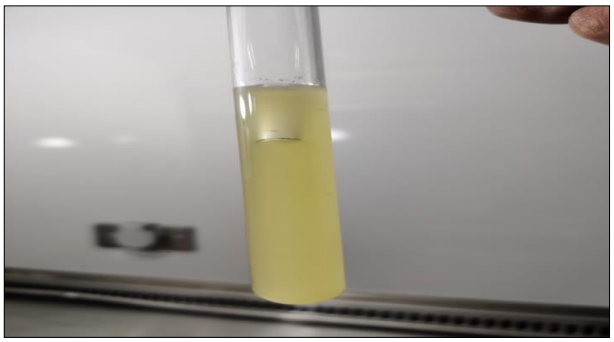

Plate 4: Completed test for coliform with acid and gas production

4. Conclusion

In our study, the results showed that mean values for physico-chemical parameters such as temperature, pH, colour, TDS, residual chlorine, nitrate, fluoride and iron were found within the allowable limits whereas turbidity, total hardness and chloride were found above the allowable limits set by USEPA and BIS for animal water. Most of the dairy animal drinking water samples contained coliforms above the permissible limit set by USEPA. So, necessary interventional measures should be adopted to resolve the issue as a whole.

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