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A Survey on Drinking Water Contamination to Indicator Bacteria in Dairy Farms of Mashhad Suburb

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Abstract

BACKGROUND: Microbial quality of drinking water is very important in animal health.

OBJECTIVES: The present survey was conducted to evaluate the microbial quality of cows' drinking water in dairy farms in Mashhad suburb, Iran, to find out defects in this field.

METHODS: Water samples were taken from 30 farms and 4 sites including: Water tanks, inlet and outlet of bovine drinking troughs and calves' water buckets in fall, 2018. The samples were put in sterile falcons, with keeping of the cold chain and immediately sent to the microbiological laboratory. Counting of fecal *Streptococcus* and *Coliform* in the specimens were performed by using pour plate and most probable number (MPN) methods, respectively.The contamination frequency of the samples to fecal *Coliform* and *Escherichia coli* were determined by using specific biochemical tests. The positive specimens in terms of *E. coli* were also detected for the presence of serotype O_{157}: H_7 by using PCR technique.

RESULTS: According to the sampling sites, the contamination frequency with fecal *Coliform* and *Streptococcus* were recorded 30-100% and 20- 96.67%, respectively. The most frequency of contamination were observed in outlet of bovine drinking troughs. In 3.33% of samples, serotype O_{157} : H_7 and in 6.67% of samples, *undefined* H_7 serotype were diagnosed. There was no statistical significant difference in the level of bacterial contamination of drinking water due to the geographical location of farms (P> 0.05).

CONCLUSIONS: It was concluded that except for a limited number of farms, water tanks are relatively safe, while it is necessary to pay particular attention to the high contamination of outlet of bovine drinking troughs and water buckets of calves.

KEYWORDS: Contamination, Dairy farm, Drinking water, Indicator bacteria, Microbial quality

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Introduction

Livestock drinking water is one of the most important sources of bacterial contamination in farm animals (Khan *et al*., 2016). Determination of water contamination to fecal contaminants helps us to prevent the water-borne diseases. Microorganisms do not always affect the appearance or taste of water but affect animal health and milk quality, therefore, continuous microbiological investigation of drinking water of animals is necessary (Din *et al*., 2014; Ramona, 2015).

Raw water contains two classes of microorganisms, including permanent microorganisms that are naturally inhabited by water and have low nutritional requirements and transient microorganisms that are transmitted from the environment by soil, humans, and animals. Pathogenic types fall in the second category (Shaghaghi, 2019). Total microbial analysis of water is ideal for determining its health quality, but since it is not easy and inexpensive, so agents that are known as indicators, such as bacteria in the *Coliform* group, are used to measure microbial contamination of water (Shaghaghi, 2019; Shimie and Yousefi, 1997). *Escherichia coli* O_{157}: H_7 is one of the most dangerous and pathogenic serotypes in humans, while in cattle, it does not cause any clinical disease other than diarrhea, and animals mainly act as carriers of the bacterium to humans (Bindu *et al*., 2010). Some other bacteria such as *Streptococcus* (a non-*Coliform* indicator of fecal contamination in water) are also considered as a microbial contaminant of drinking water (El Emam and El Jalii, 2010). Bacteria enter the drinking water from various sites such as oral and nasal discharge, feces and urine (Van Emon, 2015). The risk of transmission of diseases through stagnant waters is much higher than that of running water (Pooyanmehr *et al*., 2008).

Most dairy farmers consider that microbial contamination of drinking water is inevitable and cattle are resistant to it, so, less attention have been paid to it and its result will be a reduction in livestock production (Pooyanmehr *et al*., 2008). There is some documentation about physical and chemical status of drinking water in farms, but little is known about the microbial quality of livestock water in Iran (El Emam and El Jalii, 2010). This study was performed to evaluate the microbial quality of drinking water in cows and compare it with existing standards to eliminate deficiencies in this field.

Materials and methods

In late fall and early winter, 2018, from 30 industrial and semi-industrial dairy farms located in 4 districts of Mashhad suburb, Iran (north, northwest, east, southeast), samples of drinking water were taken from four parts including water tanks, beginning (inlet) and end (outlet) of drinking water troughs of cows and water buckets of calves, placed in sterile falcons and transferred to the lab with cold chain preservation.

Using the most probable number (MPN) or the multiple tubes method (9 tubes method), total and fecal *Coliform*s were counted. For this purpose, by using sterile pipette, 10 ml of water sample was inoculated in to the three tubes containing 20 ml of selective enrichment medium (lauryl sulfate) with double strength, and in the second and third three tubes, containing single strength concentration of lauryl sulfate (according to the manufacturer instructions), were inoculated with 1 ml and 0.1 ml of water sample, respectively.

All test tubes contained durham tubes in an inverted position. The test tubes were incubated at 37 ° C for 48 h. The tubes containing

gas in the durum tube with obvious turbidity, were considered positive. From each positive tube, one loopful of medium was inoculated into confirmatory culture medium (brilliant green lactose bile broth) containing a durham tube, and incubated for 24 ±2 h at 37 ±1°C. If no gas or turbidity was observed during this period, incubation was continued for another 24±2 h. Then, the tubes containing turbidity and gas were considered positive and fecal *Coliform* counts were performed based on MPN table.

To count *E.coli*, a loopful from culture medium in the MPN method, which produced gas and turbidity were inoculated into tubes containing *Escherichia coli (EC)* broth whit durum tubes, and incubated at 44°C for 48 h, if gas or turbidity was observed, a loopful was inoculated into peptone water medium and incubated at 44° C for another 48 h. For all EC tubes which produced gas and turbidity, the IMViC tests consisting of indole, methyl red, Voges-Proskauer (VP) and Simon citrate tests were performed.

Pour plate method was used to count fecal *streptococci*. Amount of 1 ml of the water sample were transferred to each Petri dish and 15 ml of sterilized KF (Kenner Fecal) medium at 45-50°C were added to each plate and mixed thoroughly, after solidifying the agar medium, plates were incubated at 37°C for 48 h, pink and purple to red colonies were counted as presumably fecal *Streptococci*. Number of 10 colonies were selected for the confirmatory tests. Catalase and gram staining tests were used to confirm fecal *Streptococci*. The bacterial count was calculated in CFU (colony- forming unit) per ml of sample.

In order to identify the *E. coli* O_{157}: H_7 serotype, linear culture method was used in Sorbitol MacConkey agar and nutrient agar mediums as well as PCR technique by using specific primer for O_{157}: H_7 antigen genes.

Statistical analysis

Statistical data analysis was performed by using SPSS software, v.22. Both normality test (Kolmogorov-Smirnov and Shapiro-Wilk) revealed the nonparametric data, therefore, the median was used as a valid statistical index to interpret the results. Friedman and Wilcoxon signed- rank tests were used to compare the rate of water contamination in different sample sites with each other (overall and pairwise, respectively), also, One sample Wilcoxon signed rank test was used to compare the data with existing standard. $P \leq 0.05$ was considered statistically significant.

Results

This survey showed that the most frequent bacterial contamination (total and fecal *Coliform*) were in the outlet of bovine drinking troughs, next up, in the water buckets of calves, inlet of bovine drinking troughs and water tanks. For fecal *Streptococcus*, the lowest and highest frequency were in the water tanks and outlet of bovine drinking troughs, respectively. Another finding was the observation of *E.coli* O_{157}: H_7 serotype only at the outlet of drinking trough in one farm and serotype *H7* Oumdifimd in two other dairy units (Table 1).

The highest contamination with indicator bacteria was observed at the outlet of bovine drinking troughs (Table 2). Pairwise comparison between sampling sites revealed no significant difference for total *Coliform* and fecal *Streptococcal* contamination between buckets of calves with inlet of bovine drinking troughs and for fecal *Coliform* and *streptococci*, between buckets of calves with outlet of bovine drinking troughs. In other cases, significant difference were observed ($P \leq 0.05$) (Table 3).Comparison between the

Table 1. Frequency of indicator bacteria contamination at the sampling sites of livestock drinking water

Sample site	Frequency: No (%)			
	Total *Coliform*	Fecal *Coliform*	Fecal *Streptococcus*	*E.coli* O_{157}:H_7
Tank	10 (33.33)	9 (30)	6(20)	0 (0)
Inlet of drinking drought	27 (90)	27 (90)	24 (80)	0 (0)
outlet of drinking drought	30 (100)	30 (100)	29 (96.67)	1-3 (3.33- 10)
Calves'drinking buckets	29 (96.67)	29 (96.67)	28 (93.33)	0 (0)

Table 2. Statistical indices of contamination with indicator bacteria at the sampling sites of livestock drinking water (Cfu/ml)

Bacteria	Site / index	Tank	Inlet of drinking drought	Outlet of drinking drought	Calves'drinking buckets
Total *Coliform*	Median	0	93	1100	350
	Min- Max	0- 1100	0- 1100	4- 1100	2- 1100
Fecal *Coliform*	Median	0	43	121.5	68
	Min- Max	0- 240	0- 1100	4- 1100	0- 1100
Fecal *Streptococcus*	Median	0	12	27.5	10.50
	Min- Max	0- 10	0- 36	0- 48	0- 06
E.Coli O_{157}:H_7	Median	0	0	0	0

Table 3. Statistical comparison of drinking water sampling sites for indicator bacterial contamination

Comparison of two samples	Total *Coliform* (*P*- value)	Fecal *Coliform* (*P*- value)	Fecal *Streptococcus* (*P*- value)	*E.coli* O_{157}:H_7 (*P*- value)
Tank- inlet	0.001	0.001	0.001	1.00
Tank- outlet	0.001	0.001	0.001	0.317
Tank- calves' bucket	0.001	0.001	0.001	1.00
Inlet- outlet	0.001	0.001	0.001	0.317
Inlet- calves' bucket	0.581	0.029	0.088	1.00
Outlet- calves'bucket	0.023	0.153	0.234	0.317

rate of contamination of water samples with indicator bacteria and existing standards (Van Emon, 2015; Beede, 2006) revealed that, for total and fecal *Coliform*, values due to inlet and outlet of bovine drinking troughs and water buckets of calves were significantly higher than expected values. Whereas the rate of contamination with above bacteria in water tanks were significantly lower than the highest standard values (< 50 and < 10, respectively) and in other cases no significant difference were found.

For fecal *streptococci*, no significant difference was observed for the outlet of bovine drinking troughs compared to the highest expected value (<30), but in others were observed ($P \leq 0.05$).

Also, the rate of contamination with the bacterium at water tanks was significantly lower and at intlet of bovine drinking troughs

and water buckets of calves were higher than expected values (Tables 2 and 4).

There was no statistically significant difference between different geographical locations (North: 14 units, Northwest: 6 units, East: 5 units, Southeast: 5 Unit) in terms of contamination of water tanks with indicator bacteria.

Table 4. Frequency of contamination rate and statistical comparison of drinking water sampling sites in terms of contamination with indicator bacteria by existing standards (colony per 100 ml).

Bacteria	Standard (Expected)	Index	Tank	Inlet	outlet	calves' bucket
Total *Coliform*	< 1	(*P*- value)	0.631	0.001	0.001	0.001
		No (%)	0 (0)	0 (0)	0 (0)	0 (0)
	< 15	(*P*- value)	0.175	0.001	0.001	0.001
		No (%)	23 (76.67)	5 (16.67)	1 (3.33)	2 (6.67)
	< 50	(*P*- value)	0.048	0.004	0.001	0.001
		No (%)	25 (83.33)	10 (33.33)	5 (16.67)	9 (30)
Fecal *Coliform*	< 1	(*P*- value)	0.974	0.001	0.001	0.001
		No (%)	0 (0)	0 (0)	0 (0)	0 (0)
	< 10	(*P*- value)	0.047	0.001	0.001	0.001
		No (%)	25 (83.33)	5 (16.67)	1 (3.33)	5 (16.67)
Fecal *Streptococcus*	< 1	(*P*- value)	0.026	0.001	0.001	0.001
		No (%)	24 (80)	6 (20)	1 (3.33)	2 (6.67)
	< 3 (calves)	(*P*- value)	0.001	-	-	0.001
		No (%)	26 (86.67)	-	-	7 (23.33)
	< 30	(*P*- value)	0.001	0.001	0.141	0.009
		No (%)	30 (100)	28 (93.33)	15 (50)	20 (66.67)

For all three indicator bacteria, the expected value is less than one colony per 100 ml. In the case of total *Coliform*, fecal *Coliform* and fecal *streptococci* for calves, the values >1, >1 and> 3, and for cows the values >15-50, >10, and >30 are problematic and unsafe, respectively.

Discussion

In this survey, the high frequency of contamination with indicator bacteria at the outlet of bovine drinking troughs compared to the other sampling sites (Table 1) can be related to the stagnation of water in outlet part (the inlet areas receive fresh water regularly), continuous shedding of materials attached to the hair and body of the animals (it often occurs as a result of sitting on a bed of manure or sticking of stool to the tail and lower area of the animal) into the drinking water or even inserting contaminated muzzle into the water troughs (due to the smelling behavior of the lower part of other livestock in estrus cows). Therefore, continuous drainage and cleaning of drinking water troughs is recommended (Pooyanmehr *et al*., 2008). It seems that the outlet part of water troughs to be a more realistic representative of the water consumed by livestock, because the number of livestock that drink water from inlet part is lower than the other parts, so,it is essential to identify the causes of its pollution.

However, the comparison between the frequency of bacterial contamination in the drinking water of cows and the bucket of calves due to their different hygienic and

breeding conditions does not seem reasonable, because calves' water buckets are generally specific and washed daily while the cows' water troughs are public and rarely cleaned, but the results of this comparison are important managerially, because, in spite of the special attention paid by dairy farmer to the health of calves (due to their high sensitivity to intestinal diseases), the contamination rate of their water bucket with all indicator bacteria were approximately similar to that of cows' water troughs (Table 2).

High level of water contamination in calves' buckets especially due to fecal *Coliform* and *Streptococci* (Tables 2 and 3), indicate secondary contamination by feces or other environmental factor and poor management of neonatal drinking water. Obviously, the stagnation of water in calves' buckets during the day compared to the inlet water trough may contribute to its greater contamination than the inlet parts. In one study, the high contamination of drinking water in one goat farm were attributed to manual transportation of water to the drink trough, hand contamination, and lack of respect for workers' personal hygiene during carrying water (Ramona, 2015), so, training dairy workers to accurately wash the buckets and follow the hygienic points during their filling and transporting to the place of consumption will be effective in reducing the rate of contamination (Pooyanmehr *et al*., 2008).

High levels of contamination with fecal *streptococci*, especially at the outlet water troughs (27.5 cfu/ml), confirms the release of excreted substances into the water and indicates poor health in bovine drinking water (Tables 2 and 4). Keeping water tanks indoors and away from access to fecal and environmental pollutants such as dust, will justify lower abundance and significant reduction of fecal *streptococci* contamination in them than other sampling sites (Tables 1, 2 and 3). In a survey was performed in sudan, only 4.76% of the isolates were *Streptococcus* bacteria (El Emam and El Jalii, 2010).

If the contamination with fecal *Coliform*s is more than several times that of fecal *Streptococci*, it can be suspected to contamination by human sources and in contrary to the above condition, contamination by animal ones is considered (Beede, 2006; Looper, 2012). In present study, fecal *Coliform* contamination in calves' water buckets was more than 6 times that of fecal *Streptococci* (Table 2), So it seems that its contamination has often been caused by human sewage, therefore, more attention to human health is necessary. The presence of *E.coli O157: H7* serotype contamination in outlet of bovine drinking troughs and its absence in other sampling sites could be due to secondary contamination from human or animal sources in stagnant water (Table 1).

In a study was performed in Balochistan, Pakistan, the rate of *Coliform* contamination in buffaloes and dairy cattle was reported 17%. and attributed it to the open sewage system, decaying and rusting pipes, and use of inappropriate water troughs (Khan *et al*., 2016). In the present study, more attention should be paid to the high frequency of contamination (33.3% in tanks, up to 100% in outlet water troughs) compared to the recent study. In another study was conducted on drinking water in Quetta, Pakistan, in addition to severe water pollution (especially due to *E. coli*), reduction the susceptibility of all isolated pathogens to a wide range of antimicrobial drugs has been identified as a problematic factor in the treatment of water born diseases (Din *et al*., 2014).

The purpose of this survey was not to in-

vestigate the risk factors associated with bacterial contamination of drinking water, but the field observations and filling out a questionnaire revealed some issues. For example, in one farm (where the total *Coliform* count at tank was >1100 Cfu/ml and the fecal *Coliform* at the inlet and outlet of the water trough were measured: 150 and 1100 Cfu/ml, respectively), the water was first transferred to an outdoor pool, then pumped into the water trough, the designer observations also indicate that the cows' drinking water is very dirty. It is clear that the possible use of the pool for hand washing or even swimming and exposure to dust can increase the chance of *Coliform* contamination. Also, in another highly contaminated farm (fecal *Coliform*: 240 cfu/ml), the water of well was first transported to the cement pond, then to the tank and drinking trough by pipeline. Therefore, exposing the pond to animal and bird excreta and stagnating water in it will increase the chance of contamination. Other points that this questionnaire made clear were: No records of microbial water testing or doing a test many years ago in some dairy units and no use of disinfectants for washing of water troughs in any of the dairies. Despite the presence of five farms in the vicinity of the slaughterhouse, their drinking water contamination with the indicator bacteria in the tanks were very low (< 2 cfu/ml), probably due to the use of village disinfected water (which is also used by people).

In all studied farms, underground water (wells in the farm or outside of it) were used for bovine drinking. The wide range of contamination variations in tanks (minimum to maximum levels, especially due to total *Coliform*) (Table 2) may be related to the differences in health status of the tanks or environmental and physical factors in the well. Some conditions such as the presence of decaying plant or animal material are conducive to the survival or even growth of waterborne microorganisms, while other factors such as high salt, protozoans and bacteriophages can kill millions of bacteria in water. Some physical factors such as temperature, pressure, acidity, osmotic pressure (solute content) of water, penetration of air and sunlight into the water are also effective on the number and species of water-borne microorganisms. In general, deep well water often contains a small number of bacteria that are usually non-pathogenic (Shimie and Yousefi, 1997).

During the last few years, the quality of underground water in Mashhad plain has been severely reduced because of the sharp decline of water in aquifers following the drought and the inflow of various agricultural, industrial and urban pollutants in to the water, so, pay attention to the privacy of the wells is essential. It is said that the distance between the sewage and water wells should be sufficient to takes fifty days to reach the pollution to the water well (Alizadeh *et al*., 2009). One of the issues mentioned in the report of Alizadeh *et al*. (2009) is the overwhelming expansion of Mashhad city towards the adjacent villages and farms. This has caused the contamination of water wells of the farms to the household sewage or discharge of waste.

In one study, the absence of *Coliform* contamination in water wells was attributed to several factors, including: the long distance between water wells and human and animal wastewater, disinfection of water with chlorine and the proximity of water wells with the cobalt mines (due to the antimicrobial properties of cobalt compounds). The high concentration of nitrite, nitrate and chlorine

contamination has also been noted in water samples free of *Coliform* contamination (Mirsoleimani *et al*., 2015).

In a research was done on the water sources in Sistan region (in Sistan and Baluchistan province), the mean number of MPN of fecal *E. coli* in collected samples from wells and drinking water were 550 and 2.4 per 100 ml, respectively. Although all their samples were positive for fecal *Coliform*, but the researchers concluded that the drinking water in the studied area was suitable for livestock consumption according to the US standards (Nazemi *et al*., 2018).

In another survey was performed on microbial quality of drinking water in the 168 Zahedan villages, the results showed that the *Coliform* contamination in the reservoir was lower than in the distribution network (which is consistent with our findings) and the total *Coliform* contamination in the reservoir and distribution network were 3 to 9 times that of the fecal *Coliform* (RadFard *et al*., 2018). In present study, the contamination (mean value) with those indicator bacteria at the tanks were the same (0), but, the total *Coliform* contamination in the inlet and out of drinking drought and calves'drinking buckets were 2.16, 5.15 and 9.05 times that of the fecal *Coliform*, respectively (Table 2).

The present survey was conducted only in the cold season (December to February) and no comparison was made between the rates of contamination in different seasons. However, the seasons have been mentioned as a potential factor affecting the microbial quality fluctuations of wells. Increasing per capita consumption, decreasing water flow and condensation of pathogens are considered as the causative agents of low microbial quality in under groundwater in summer (Pirsaheb *et al*., 2013; Musa and Abdelgadir, 2014).

In general, several causes of microbial contamination of drinking water are cited as follows: Public use of water by cows, open water system and its high contamination with urine and feed materials, drinking water trough near the floor, inappropriate water storage, prolonged water retention in its container, not regularly cleaning and using disinfectants when washing the water trough (El Emam and El Jalii, 2010; Musa and Abdelgadir, 2014).

In present study, it can be concluded that the contamination of the main sources of water with indicator bacteria was not significant, while secondary and environmental contamination of drinking water, especially in outlet of drinking drought and calves' water buckets should be considered.

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Conflict of interest

The authors declared that there is no conflict of interest.

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مجله طب دامی ایران، ۱۳۹۹، دوره ۱۴، شماره ۳، ۲۵۱-۲۶۰

بررسی آلودگی آب شرب گاوداری‌های حومه مشهد به باکتری‌های شاخص

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چکیده

زمینه مطالعه: کیفیت میکروبی آب شرب در بهداشت دام بسیار حائز اهمیت است.

هدف: بررسی حاضر جهت ارزیابی کیفیت میکروبی آب شرب گاوها در گاوداری‌های حومه مشهد به منظور شناسایی کاستی‌های موجود در این زمینه انجام شد.

روش کار: نمونه‌های آب از ۳۰ واحد گاوداری شیری و از چهار محل شامل منبع آب، ورودی و خروجی آبشخور و سطل آب گوساله‌ها در اواخر پائیز سال ۱۳۹۷ جمع آوری گردید. نمونه‌ها در فالکون‌های استریل قرار داده شد و با حفظ زنجیره سرما به آزمایشگاه میکروبیولوژی ارسال گردید. جهت شمارش کلی استرپتوکوک مدفوعی و باکتری‌های کُلی‌فرمی نمونه‌ها به ترتیب از روش‌های پورپلیت و بیشترین تعداد احتمالی (MPN) استفاده شد. میزان آلودگی نمونه‌ها به کُلی‌فرم مدفوعی و اشریشیاکُلای توسط آزمایشات بیوشیمیایی، تعیین گردید. نمونه‌های مثبت از نظر اشریشیاکلای با استفاده از آزمایش واکنش زنجیره‌ای پلیمراز(PCR) از نظر وجود سروتیپ $O_{157}:H_7$ مورد بررسی قرار گرفتند.

نتایج: بر حسب محل نمونه‌گیری، فراوانی آلودگی با کُلی‌فرم و استرپتوکوک مدفوعی به ترتیب ۳۰ تا ۱۰۰٪ و ۲۰ تا ۶۷/۹۶٪ ثبت شد. بیشترین فراوانی آلودگی در خروجی آبشخور گاوها مشاهده شد. در ۳/۳۳٪ از نمونه‌ها، سروتیپ $O_{157}:H_7$ و در ۶۷/۶٪ از نمونه‌ها سروتیپ *undefined H7* تشخیص داده شد. هیچ‌گونه اختلاف آماری معنی‌داری از نظر میزان آلودگی باکتریایی آب شرب با توجه به موقعیت جغرافیایی گاوداری‌ها وجود نداشت ($P<0/05$).

نتیجه گیری نهایی: نتیجه‌گیری شد که بجز در تعداد محدودی از گاوداری‌ها، منابع آب از نظر آلودگی به باکتری‌های شاخص نسبتاً ایمن می‌باشند ولی آلودگی بالای خروجی آبشخور گاوها و سطل آب گوساله‌ها را باید مد نظر قرار داد.

واژه‌های کلیدی:

آب شرب، آلودگی، باکتری‌های شاخص، کیفیت میکروبی، گاوداری شیری.

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