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Hygiene Aspects of Drinking Water Sources Used in Primary Milk Production

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Abstract: Five sources of drinking water (2 for mass supply, 3 for individual supply) located on agricultural farms in potentially contaminated area were examined throughout one year for quality and suitability for use for primary milk production. Chemical investigation showed that limits for nitrates and chlorides set by the relevant legislation were exceeded in sources for individual supply. Coliform bacteria as an indicator of faecal contamination were present only in sources for individual supply and the limits were exceeded particularly in spring, summer and fall. Limits for bacteria cultivated at 37°C (BC37) were exceeded only in source No. 4 in autumn. The samples from individual supply showed the highest bacteriological contamination. In sources for mass supply limits for *E.coli* were not exceeded, but they were exceeded throughout investigations in sources for individual supply.

Key words: drinking water, sources, quality, coliform bacteria, *E.coli*

1. Introduction

Protection of water is currently a priority and a basic factor of environmental management. Today we commonly find territories where drinking water sources are contaminated by anthropogenic activities to such degree that the water from them is neither suitable for drinking and watering of animals nor for food processing and other purposes related to our everyday life [1].

Water is a vital nutrient needed for sustaining life and to optimize the milk production, growth rate and reproduction in livestock. Research has shown that if animals are provided with clean drinking water, the resistance against diseases is increased and performance becomes better.

The available sources of water for livestock are surface water, i.e., streams, ponds, lakes, and ground water, i.e., wells. The quality of water will be influenced by its source of contamination either biotic or abiotic as a result of dissolved nutrients, pathogens and other pollutants. The water available to livestock for drinking may be affected by a number of contaminating determinants including minerals, manure, microorganism, chemicals and algae. The effect of these contaminants is either direct on health or may cause decrease in overall water intake indirectly lowering the growth and production of animals [2].

The Directive 2000/60/EC of the European Parliament and of the Council, establishing a framework for the Community action in the field of water policy (in short, the EU Water Framework

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Directive) adopted in 2000, establishes legislative frame for introduction of uniform water policy in EU countries. It is based on integrated control of water sources within drainage basins that consists in coordination of strategic targets in sectors such as agriculture, forestry, industry and similar. It is expected that the EU member states ensure good quality of surface and ground water by the year 2015.

In the Slovak Republic, the EU Water Framework Directive is implemented through the Act. No. 364/2004 Coll. on water which takes into consideration also the requirements set by Council Directive 91/676/EEC of 12th December, 1991, on protection of water against contamination with nitrates from agricultural sources.

The ground water it may be contaminated by dissolved salts, depending upon the geology of area, rainfall, vegetation and topography. Natural and human activities may influence on both ground as well as surface water [2]. The quality of ground water is frequently reduced by intensive agriculture and the use of nitrogen fertilizers and pesticides. In order to ensure sufficient quantity of safe drinking water we must prevent penetration of nitrates originating from agriculture into water sources and eliminate all interventions and operations that could affect negatively the quality of water supplies [3, 4].

The Regulation No. 199/2008 Coll. establishes Programme of agricultural activities in declared vulnerable areas where the level of nitrates in surface and ground water or in lakes exceeds 50 $mg.l^{-1}$. Hygiene rules apply to wells as sources of drinking water for individual and mass supply [3]. The surroundings of wells must be checked regularly for any sources of potential contamination, such as septic tanks, sewage pipelines, liquid fuel tanks, animal houses, manure heaps and similar and measures must be taken to reduce the risk of groundwater contamination [5].

Good quality of water on dairy farms is crucial for normal performance of dairy cattle and for obtaining good quality milk. Intake of contaminated water by cattle can cause diarrhoea, loss of appetite and hepatotoxic disorders with fatal consequences [6]. Insufficient intake of water decreases productivity and has adverse health consequences. High-yield cows drink daily approx. 75 l of water. Water in drinkers should be adequate quality and not contaminated with feed residues or excrements. The drinking system should be frequently cleaned [7].

Anti-quality factors (constituents in excess or unwanted compounds) that may affect water intake and animal performance include total dissolved solids, sulphur, sulphate, iron, manganese, nitrate, heavy metals, pesticides, free chlorine and deleterious micro-organisms [8].

Increased content of calcium and magnesium (hardness of water) is not believed to affect intake of water and animal performance but may require increased detergent concentration or increased contact time with the cleaning agent and disinfectant solution. Any deposits or precipitations on surfaces of milking or milk storage equipment present a risk of disease agents accumulating on equipment surfaces and even growing and multiplying.

Water corresponding to requirements on the quality of water for drinking (Regulation of the Government of SR No. 496/2010 Coll.) must be used in the milking process and must be regularly monitored. Complete examination of water for its suitability for drinking is carried out less frequently, depending on the capacity of source, or the number of inhabitants in the area supplied this water. It is very demanding and includes more than 80 parameters divided into several groups. More frequently a mimimum examination is performed (29 parameters) which focuses on parameters indicating not only quality but also potential risk of contamination. It includes determination of plate counts of *E.coli*, total coliform bacteria, enterococci, bacteria cultivated at 22°C (BC22) and 37°C (BC 37), ammonium ions, nitrates, nitrites, free chlorine, pH, iron, chemical oxygen demand and other. Any

potential contamination of water source, particularly with human or animal wastes, will be reflected in the level of these parameters.

The aim of this study was to investigate some chemical parameters and bacteriological) quality of water sources on the selected 5 farms throughout a year and to evaluate their suitability with focus on safety of produced milk.

2. Materials and Methods

Investigations were carried out on 5 farms, three were supplied with water from sources intended for individual supply (IS – individual wells, sources 2, 3, 4) and two were connected to mass public supply (MS, sources 1, 5). The wells were covered and surrounded by a fenced protected area (min. 10 m round the well). All farms were located in a relatively flat agricultural area with only low hills with some potential of contamination of ground water (farms, villages, grazing, manure spreading and storage.

Individual water samples were collected from the 5 sources once per month from January to December (3 samplings per season). Samples were collected according to STN EN ISO 5667:1, 3 and STN EN ISO 19458. Water for chemical analysis was sampled into clean glass bottles after rinsing with the water to be tested and for microbiological analysis to sterile bottles. All samples were examined in duplicate.

Chemical examination focused on indicators of faecal contamination of water sources (ammonium ions, nitrates, nitrites, chlorides) and residual chlorine related to disinfection of water.

The testing was done first qualitatively and all positive samples were tested quantitatively.

Quantitative determination ammonium ions (NH_4^+) was carried out by distillation according to STN ISO 7150-1. Nitrites (NO_2^-) were determined colorimetrically using HACH DR 2800 analyser and procedure recommended by HACH. Nitrates (NO_3^-) were determined directly in samples with ion-selective nitrate electrode WTW (InoLab ph/ION 735P, Germany), according to manufacturer's instructions.

Nitrates (NO_3) are not common in drinking water and they are less toxic while nitrite (NO_2) is highly toxic and carcinogenic; nitrogen fertilizer and livestock operations may elevate their level [9].

Chlorides (Cl^-) and residual active chlorine (Cl_2) were determined titrimetrically, according to STN ISO 9297 and EN ISO 7393-3, respectively. Chloride the biologically active anions have potential to negatively influence digestion, acid-base/electrolyte balance, and milk production [10].

Environmental concerns are related to phosphorus due to its ability to cause eutrophication in water which makes it unpalatable and may have toxins from algae [11]. Orthophosphates were determined colorimetrically using HACH DR 2800 analyser and procedure recommended by HACH.

Excess of iron can cause toxicity in livestock. Recommended iron level in drinking water is 0.3 ppm. Iron in drinking water is more absorbable than in feed [10].

Low water quality causes health problems that result in retarded growth and decreased performance.

Manure is a usual contaminant of livestock drinking water. Large numbers of bacteria are found in watering facilities of livestock. Coliform illness results in outbreaks of *E.coli Campylobacter jejuni*, *Klebsiella*, *E. aerogenes*, *Salmonella* spp., *shigellae* spp. and *Vibrio cholera* are the common causes of coliform illness outbreaks; these can lead to diarrhea, urinary tract infections, mastitis and many other unappealing and usually deadly infections. *Listeria, Coxiella, Brucella*, and *Mycoplasma* infections are transmitted through water [8].

Microbiological examination included parameters indicating general contamination (bacteria cultivated at 22°C–BC22 and 37°C– BC37) potential contamination with faeces or sewage (coliform bacteria CB), and presence of micro-organisms that are part of the

digestive tract of man and animals (*E. coli*) and enterococci (EC).

Plate counts of BC22 and BC37 were determined by pour-plate method according to STN EN ISO 6222: 1 ml sample was pipetted onto a sterile Petri dish, specific warmed-up culturing medium was poured over, the content was mixed gently by a circular motion and allowed to solidify before incubation. The number of colony forming units (CFU) per ml of sample was counted after the incubation at 22°C and 37°C, resp. In Slovakia the limit values for BC22 and BC37 are 200 CFU/ml and 20 CFU/ml, respectively.

Coliform bacteria and *E. coli* were determined according to STN EN ISO 9308-1: 100 ml (Ms) or 10 ml (IS) of water sample was filtered through a pre-sterilised 0.45 μm pore size sterile membrane filter and the filter was placed face up on the Petri plate containing Endo agar (HiMedia, India) and incubated for 24 hours at 37°C or 43°C, resp. After incubation the characteristic colonies (total coliforms – dark red; *E.coli* – dark red with metallic sheen) were counted. In case of absence of colonies the incubation was prolonged for another 24 hours. The lactose test (fermentation of lactose) was used for confirmation of coliform bacteria. According to WHO (2008) *E.coli* or thermotolerant coliform bacteria must not be detected in any 100-ml sample. Also total coliform bacteria must not be detectable in any 100-ml sample (WHO, 1996).

Plate counts of enterococci were determined according to STN EN ISO 7899-2.

The method is based on filtering of 100 ml (MS) or 10 ml (IS) of water sample through a membrane filter (filter size 0.45 μm) capable of retaining these bacteria. After filtration, the filter was placed onto a solid selective medium containing sodium azide (to suppress growth of Gram-negative bacteria) and colourless 2, 3, 5-trifenyltetrazolium chloride which is reduced by intestinal enterococci to red formazan. Typical colonies are convex, red, chestnut-brown or pink in colour either in the centre or throughout the colony surface. Similar to CB and *E.coli*, enterococci must not be detectable in any 100 ml sample of water (EC Regulations, 2007).

Results of all analyses were evaluated according to the Regulation of the Government of the SR 496/2010 Coll. on requirements for drinking water and control of quality of drinking water [1].

3. Results

Ammonium ions (NH_4^+) were detected only in samples from source 2 (IS) and reached the highest concentration in spring (0.65 mg/l) which could be related to melting of snow or extensive rainfall.

Nitrites (NO_2^-) were present in this source in autumn (0.59 mg/l).

Nitrates (NO_3^-) were found in all sources at every sampling in spring and at lower concentrations in the remaining seasons with the exception of source 3 in winter (50.26 mg/l) and source 4 in autumn (78.3 mg/l) when the maximum level set by legislation (50 mg/l) was exceeded.

The highest chloride (Cl^-) levels were detected in winter in sources 2 and 3 and in other sources only in low concentrations. The limit for chlorides (250 mg/l) was exceeded in source 3 (475.03 mg/l) in autumn.

The content of iron varied only in source 2 during the sampling and the higher content was detected in winter.

The orthophosphate (PO_4^{3-}) were detected only in source 2 in minimally content during each sampling.

Results of microbiological examination are shown in Figs. 1-3. The maximum limit value (MLV = 0/100 ml) for *E. coli* (EC) for mass supply sources was not exceeded. In sources for individual supply (MLV = 0/10 ml) the MLV for EC was exceeded in sources 3 and 4 throughout the sampling, in the source 3 in winter (1 CFU/10 ml), summer (6 CFU/10 ml) and autumn (13 CFU/10 ml) and in source 4 in winter (8 CFU/10 ml), summer (66 CFU/10 ml) and autumn (130 CFU/10 ml).

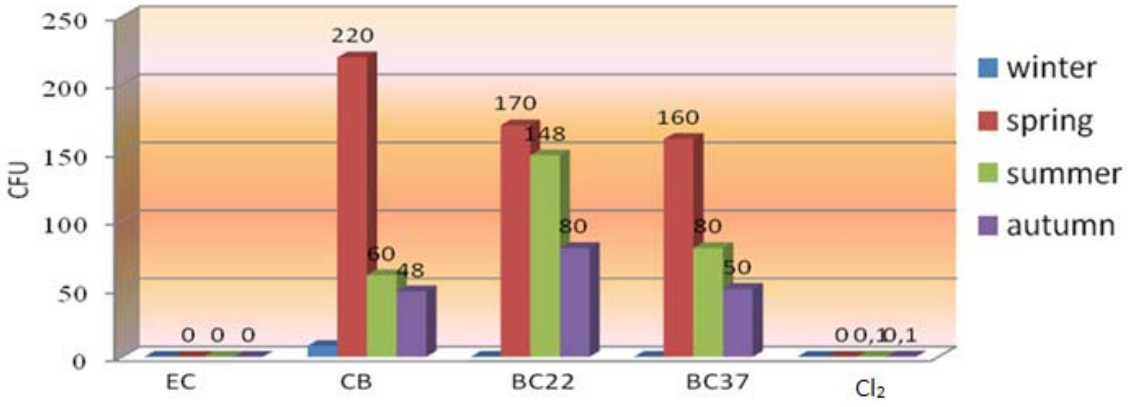

Fig. 1 Plate Counts of Investigated Bacterial Groups in Source 2 (IS)

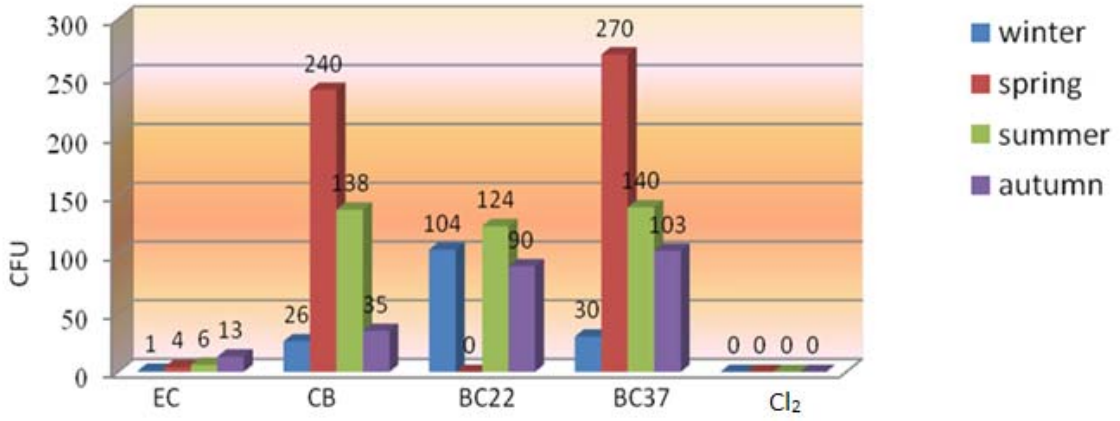

Fig. 2 Plate Counts of Investigated Bacterial Groups in Sample in Source 3 (IS)

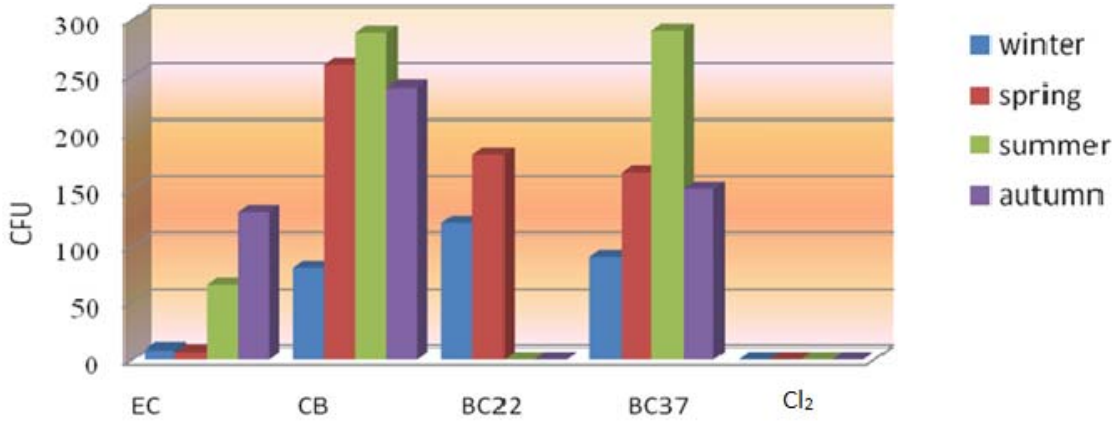

Fig. 3 Plate Counts of Investigated Bacterial Groups in Source 4 (IS)

Coliform bacteria (CB) were present only in individual sources (2, 3 and 4). In all sources for IS coliform bacteria were present in 10 ml of water in all seasons (source 2: 8 CFU in winter, 220 in spring, 60 in summer and 48 in autumn; source 3: 26 CFU in winter, 240 in spring, 138 in summer and 35 in autumn; source 4: 80 CFU in winter, 260 in spring, 288 in summer and 240 in autumn).

BC22 were exceeded (> 300 CFU) only in source 3 in spring and in source 4 in summer.

The limit for plate counts of BC37 was exceeded in source 4 in summer (290 CFU/1 ml) and autumn (150 CFU/1 ml).

Free residual chlorine was regularly detected only in sources for mass supply (1 and 5). In sources for individual supply free chlorine was present only in source 2 (0.1 mg/l in summer and autumn).

Results of microbiological examination are presented in Figures 1 to 3 as means of three samplings for each season. Despite the fact that the sources for individual supply 3 and 4 were fenced, their surroundings were not kept up and protected adequately. These sources could be contaminated by grazing of farm animals or application of their excrements on soil. This may result in contamination of ground water in the entire location.

4. Discussion

Determination of quality of drinking water requires a complex system of evaluation and of risks resulting from exposure to chemical substances and other contaminants. Studies of toxicity in animals are the biggest source of data for risk evaluation [8]. As far as the quality of water used on animal farms is concerned, there are many factors, including the changing environment (periods of dry weather, heavy precipitations, changing structure of soil), which may contribute to variability of results and present a problem for risk evaluation.

Polluted water can affect adversely the animals that have to consume it (decreased intake of water and reduced performance, exitus of calves, ketosis or acetonaemia of cattle, chronic diarrhoea, liver damage, spreading of infections) [8].

In dairy cows more than 95% of udder infections is caused by agents spread by alimentary way (*Streptococcus agalactiae, Stahylococcus aureus, Mycoplasma spp.)* or by environmental agents such as *Streptococcus dysgalactiae, Streptococcus uberis, Escherichia coli* and similar [12].

As far as the safety of milk and milk products is concerned all activities in primary milk production, including the milking, present potential risk and one should eliminate first of all potential microbial contamination from all sources already in this phase of food chain. This includes farm environment, water, milking personnel, animals and observation of rules of good practice in animal rearing and keeping the animals healthy. Low level of hygiene during milking and treatment of milk presents a risk already in the phase of primary production [13].

Results obtained in this study showed presence of *E. coli,* an indicator of potential faecal contamination, in sources intended for individual supply (3 and 4) at all samplings and in all seasons and thus these sources failed to comply with the Regulation of the Government of the SR No. 496/2010 Coll.

Determination of free chlorine showed an effort to decrease the risk of waterborne diseases by disinfection with active chlorine which was done, however, only sporadically.

On the other hand, free chlorine was present in sources for mass supplies which are disinfected regularly. Methods for cleaning water are available, [14] described many comparative methods for treatment of water from dairy industry.

Despite the fact that sources for individual supply 3 and 4 were fenced, their wider surroundings were not kept up and protected adequately. Contamination of these sources could be associated with keeping and grazing of farm animals or application of their excrements on soil. This may produce situation which results in contamination of ground water in the entire location [15].

The presence of *E. coli* in wells on farm 3 and especially on farm 4 was the highest in autumn, the period of application of manure to soil and some rainy weather.

5. Conclusions

The results obtained imply increased need for regular monitoring of water on the investigated farms which use water from individual wells. Protection of individual water sources extending to a wider area, careful handling of wastes, prevention of flooding of

the most sensitive locations and sufficient knowledge of all processes that may present health risk are measures that could contribute to quality and safety of produced milk.

Cattle are sensitive to the water quality, water intake is closely related to feed intake and thus animal productivity.

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