doi: 10.17221/8818-VETMED

Disinfection of potable water sources on animal farms and their microbiological safety

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ABSTRACT: The aim of this study was to examine drinking water on three farms in eastern Slovakia and to determine experimentally the optimum dose for adequate disinfection in terms of devitalisation of potential pathogens while observing the limit for residual active chlorine (0.3 mg/l) in drinking water. Our investigations included bacteriological examination focused on general contamination and indicator bacteria (bacteria cultivated at 22 and 37 °C, total coliforms, *E. coli*, enterococci), physico-chemical examination (pH, ammonium ions, nitrites, nitrates, chlorides, free chlorine, chemical oxygen demand COD_{Mn} and Ca + Mg), and EEM (excitation emission matrix) fluorescence spectroscopy which focused on the presence of natural organic matter (NOM). After determining the optimum single dose of Chloramine T for disinfection of water used for watering of animals, the bacteriological quality of water was checked on the 5th day after the disinfection. The results showed that water quality was better on Farm No. 3 than on Farms No. 1 and No. 2. The weather (precipitation) evidently affected the quality of water on all three farms and was associated with some risk to animals consuming this water. The experimentally determined doses of Chloramine T appeared relatively efficient on Farm No. 1 and Farm No. 3, while the Chloramine T dose estimated for adequate disinfection on Farm No. 2 had to be increased considerably but was still much lower than the dose recommended by the manufacturer of this preparation. It appeared effective to adjust the intervals between individual chlorine treatments according to weather conditions (heavy rain) instead of increasing the active chlorine dose.

Keywords: drinking water safety; farm animal watering; agriculture; microbiological examination; chlorination; physico-chemical examination, contamination

Water is essential for life, and a satisfactory (adequate, safe and accessible) supply must be available to all. Improving access to safe drinking-water can result in tangible benefits to health. Therefore, every effort should be made to achieve a drinking-water quality that is as high as possible (WHO 2008).

Drinking water safety is judged on the basis of national standards or international guidelines. The most important of these are the WHO Guidelines for Drinking-Water Quality. On the basis of the Regulation of the Government of the SR No. 368/2007 Coll., which amends and supplements the Regulation of the Government of the SR No. 322/2003 Coll. on protection of farm animals, all sources of water used for watering of animals must comply with requirements for water intended for human consumption. Requirements for the quality of water used for human consumption are set out in the Regulation of the Government of the SR No. 496/2010 Coll., amending No. 354/2006 Coll., which specifies methods for the control of the quality of water used for human consumption and meets the criteria set by European Union regulations and WHO recommendations.

Supported by the Science Grant Agency (VEGA), Slovak Republic (Project No. 1/0950/12) and KEGA (Project No. 008UVLF-4/2014).

doi: 10.17221/8818-VETMED

There are a number of possible sources of man-made contaminants, some of which are more important than others. These fall into the categories of point and diffuse sources. Discharges from industrial premises and sewage treatment works are point sources and as such are more readily identifiable and controlled; run-offs from agricultural land and from hard surfaces, such as roads, are not so obvious, or easily controlled. Such sources can give rise to significant variation in the contaminant load over time (Cho et al. 2000; Fawell and Nieuwenhuijsen 2003).

Many infectious diseases of animals and humans are transmitted by water contaminated with human and animal excrement, which becomes a source of pathogenic bacteria, viruses and parasites (protozoa, parasite eggs) capable of surviving for different periods, and raise the health risk for many people throughout the world. In order to eliminate the risk related to disease transfer, water intended for mass consumption is treated and disinfected before use. Monitoring of water sources involves the determination of important microbiological and physico-chemical parameters which indicate first of all potential organic pollution, particularly pollution originating from animal excrement, storage of waste, natural and artificial fertilisers, and others (Sasakova et al. 2013; Fridrich et al. 2014). On the basis of the results, adequate measures can be taken that include prevention of contamination and systemic disinfection.

The heterotrophic plate count includes all of the microorganisms that are capable of growing in or on a nutrient-rich solid agar. Two incubation temperatures and times are used: 37 °C for 24 h (bacteria cultivated at 37 °C, BC37) to encourage the growth of bacteria of mammalian origin, and 22 °C for 72 h (bacteria cultivated at 22 °C, BC22) to enumerate bacteria that are derived principally from environmental sources. If levels are substantially increased relative to normal values, there may be cause for concern.

Faecal streptococci represent evidence of faecal contamination and tend to persist for longer in the environment than thermotolerant or total coliforms. They are highly resistant to drying. Faecal streptococci grow in or on a medium containing sodium azide, at a temperature of 37–44 °C (WHO 1996).

According to the WHO (2011) *Escherichia coli* are the only true indicator of faecal contamination; they are exclusively of intestinal origin and are found in faeces. Their presence indicates mostly fresh faecal contamination and thus points to serious shortcomings in protection of the specific water source, treatment of water and its hygienic safety.

The physico-chemical properties of water, particularly pH, temperature, presence of organic material (chemical oxygen demand, COD), dissolved oxygen (DO), electric conductivity (EC), NH_4^+, and others, affect the properties of drinking water and some of these properties also bear an impact on the health of consumers (Pitter 2009). Moreover, they also affect the survival of potential microbiological contaminants and the germicidal effectiveness of chlorine disinfectants (Block 2001).

Groundwater, although filtered by natural processes, is often susceptible to microbial contamination and may need disinfection. A major groundwater pathogen occurrence study supported by the American Water Works Association (AWWA) Research Foundation and the U.S. Environmental Protection Agency (EPA), found that about 60% of vulnerable wells and about half of wells initially considered not vulnerable were positive for one or more indicators of faecal contamination in tests for total coliform bacteria, *E. coli,* coliphage and human viruses. Chlorine-based compounds are the only major disinfectants harbouring lasting residual properties that ensure continual protection against microbial regrowth (Macler et al. 1997).

Different techniques of chlorination can be used, including breakpoint chlorination, marginal chlorination and superchlorination/dechlorination. Breakpoint chlorination is a method in which the chlorine dose is sufficient to rapidly oxidise all the ammonia nitrogen in the water and to leave suitable free residual chlorine available to protect the water against reinfection from the point of chlorination to the point of use. Superchlorination/dechlorination denotes the addition of a large dose of chlorine to effect rapid disinfection and chemical reactions, followed by a reduction of excess residual free chlorine. Removing excess chlorine is important to prevent taste problems. It is used mainly when the bacterial load is variable or the detention time in a tank is not enough. Marginal chlorination is used where water supplies are of high quality and represents the simple dosing of chlorine to produce a desired level of free residual chlorine. The chlorine demand in such water is very low, and a breakpoint might not even occur (WHO 2008).

Chlorination denotes the application of substances with different concentrations of active chlorine ranging from gaseous chlorine, through sodium or

doi: 10.17221/8818-VETMED

calcium hypochlorite and chloramines, up to chlorine dioxide. The dose of chlorine depends on the quality of treated water and the form of the preparation used. Both these factors affect the level of residual active chlorine that reaches the consumer. Disinfection with active chlorine preparations is considered to be the most suitable way of disinfection of on-farm groundwater because it is cost effective, reliable, relatively simple, measurable and provides a residual (Macler and Pontius 1997; Kijovska 2013).

According to the period of action, concentration, and frequency of exposure, chlorine disinfectants can affect the health of consumers of the disinfected water or induce various responses (Gunten 2003). Chlorine dioxide is frequently used and is effective, but is associated with some by-products, such as chlorites and chlorates (Sorlini et al. 2014). The main drawback of chlorination is that chlorine can react with natural organic matter (NOM) to generate various disinfection by-products (DBPs), such as trihalomethane and haloacetic acid, which are linked to an increased risk of cancer (Chowdhury 2013; Lyon et al. 2013). Chloro- and bromo-benzochinones are mentioned as additional by-products of chlorination (Zhao et al. 2012).

Excitation emission matrix (EEM) fluorescence spectroscopy is a powerful tool for investigating the presence of NOM and their chemical and physical characteristics (Lyon et al. 2013).

The presence of NOM can also have an impact on the effectiveness of chlorination (residual active chlorine). Because the content of NOM in water from natural sources varies considerably, the optimum dose of chlorine disinfectants is sometimes determined by experimental chlorination (Horakova et al. 2003) in order to comply with legislative requirements on residual active chlorine.

The negative effects of gaseous chlorine and more strict legislation have generated a desire to seek new methods and technological procedures for achieving hygienic safety of drinking water. With regard to physical methods, we can mention the use of electrolytic methods (Jirotkova et al. 2012), or a combination of adsorption and electrochemical disinfection (Hussain et al. 2014). Currently, UV technologies with online fluorescence detection are also used in the disinfection of secondary water sources (Li et al. 2014); for example, a combination of mechanical filtration and disinfection by solar radiation (Sila et al. 2013), or the combined action of UV radiation and chlorine (Liu et al. 2012). These new approaches result in lower levels of undesirable by-products and minimise the negative effects on the physical properties of water, such as occur after disinfection with ozone (Raudales et al. 2014). However, most of these methods lack residual disinfection power.

The aim of this study was to examine drinking water on three farms in eastern Slovakia that used Chloramine T (commercial preparation) for disinfection of water, and to determine experimentally the optimum dose needed for adequate disinfection that could ensure hygienic safety of water in terms of devitalisation of potential pathogens, on the one hand, while observing the limit for residual active chlorine (0.3 mg/l) in drinking water on the other.

MATERIAL AND METHODS

The study was carried out on three farms, two keeping only cattle and one with both cattle and sheep. The farms are located in a hilly area in the Presov region (eastern Slovakia), about 4 km apart. Samples of drinking water were collected from January to May in intervals specified below.

Microbiological, physico-chemical and fluorescence analyses were performed to determine the quality of water on the investigated farms and the potential for the production of disinfectant by-products. Due to unfavourable bacteriological results obtained after preliminary sampling in January and February, we carried out experimental chlorination of water and checked its effectiveness under practical conditions on each farm.

The experimental chlorination was conducted using a single dose of Chloramine T (sodium tosylchloramid; sodium salt of N-chloro-4-methylbenzene-1-sulfonamide), to determine optimum conditions/intervals and the optimum dose of Chloramine T, so that the water could be used for watering of animals (complying with the national limit for residual active chlorine of 0.3 mg/l) and for other related processes. A further criterion was that the microbiological quality of the water would prevent transmission of water-borne diseases (Michalus and Bratska 2000; Ashbolt 2004).

Description of animal farms

Farm No. 1. The farm is located 13 km from Presov (eastern Slovakia). It focuses on the rear-

doi: 10.17221/8818-VETMED

ing and fattening of cattle (230 Slovak-spotted cattle), including calf section and milk-producing dairy cows. It is known also abroad as one of the biggest producers of fattening bulls in Slovakia (Varchola et al. 2013-2014). There are five water wells on this farm in close proximity, each with a capacity of about 8000 l/day. The depth of wells ranges between 6 m to 11 m. Water from these wells is pumped to a common tank from which it is supplied to animals and used for other related operations.

Farm No. 2. The farm is situated 15 km northeast of Presov and is involved in the breeding of sheep and Tsigai and Slovak-spotted breed cattle. It uses two water storage reservoirs for water, one old (OR: supplied by a well 8 m deep) and one new (NR: supplied by two new wells of depths of 21 m and 23 m), each with a capacity of about 150 000 l/day. The new reservoir was put into use in March. Both reservoirs are located on a hill above the farm.

Farm No. 3. The farm is situated 12.5 km north of Presov. There are 100 dairy cows on this farm together with other categories of young cattle, in total coming to 700 animals. Samples of water were taken from a tap. The water originates from a well located on this farm, about 20 m deep, with a capacity of about 90 000 l/day.

Microbiological examination

Microbiological examination was carried out according to the Regulation of the Government of the SR No. 496/2010 Coll. This included determination of colony forming units (CFU) of bacteria cultivated at 22 °C (BC22) and 37 °C (BC37) (heterotrophic count) according to STN EN ISO 6222, coliform bacteria (CB) and *E. coli* according to STN EN ISO 9308-1 and enterococci (EC) according to STN EN ISO 7899-2.

A pour-plate method was used for determination of the number of BC22 and BC37 bacterial colonies growing in nutrient agar medium after aerobic incubation. The number of colony forming units (CFU) per ml of sample was counted after incubation at 22 °C and 37 °C, respectively. The limit value for BC22 in Slovakia is 200 CFU/ml and for BC37 the limit is 20 CFU/ml (STN EN ISO 6222).

Coliform bacteria (CB) and *E. coli*. Endo agar (HiMedia, India) was used as cultivating medium. After incubation for 24 h at 37 °C or 43 °C, respectively, the characteristic colonies were counted. When no colonies were present, the incubation was prolonged for another 24 h (Grant 1997). The lactose test was used for confirmation of coliform bacteria.

According to the 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; STN EN ISO 9308-1).

Enterococci (EC). Determination of enterococci was based on the filtering of 100 ml or 10 ml of water sample through a membrane filter (filter size 0.45 μm) placed onto a solid selective medium containing sodium azide (to suppress the growth of Gram-negative bacteria) and colourless 2,3,5-trifenyltetrazolium chloride, which is reduced by intestinal enterococci to red formazan. Similar to CB and *E. coli*, enterococci must not be detectable in any 100 ml sample of water (EC Regulations 2007).

Experimental chlorination of water

After initial bacteriological examination of water on the farms in January and February we carried out experimental chlorination of water from all three and on the basis of the results we estimated appropriate doses of Chloramine T for disinfection of water sources on the respective farms. These doses were used to disinfect water from March to May (five times on Farm No. 1 in about two-week intervals; three times on Farm No. 2 in the old and new reservoir in monthly intervals; three times on Farm No. 3 in monthly intervals) and bacteriological examination of previously disinfected water was performed on the 5th day after disinfection. The dose of Chloramine T determined according to experimental chlorination (20 g per well) of water on Farm No. 1 was doubled after heavy rain in April to 40 g (five water wells, each with a capacity of about 8000l/day). On Farm No. 2 the dose was doubled after the chlorination in March from 180 g to 360 g in both reservoirs, and to 1200 g in May in the NR (each with a capacity of about 150 000 l/day) and 180 g (9000 l/day) on Farm No. 3.

Experimental chlorination was carried out using a 0.1% solution of Chloramine T with the active substance tosylchloramide sodium, 81% active chlorine (manufactured by Bochemie – http://www.bochemie.cz/en-US/contact). The dosage recommended

doi: 10.17221/8818-VETMED

by the manufacturer is 10 g per 1000 litres of water (this presumes maximum pollution of water). We disinfected equal volumes of water measured into individual flasks with increasing doses of a 0.1% solution of Chloramine T and allowed it to act for the prescribed time. The optimum dose of Chloramine T (g/l) was obtained by recalculation on the basis of the volume of 0.1% Chloramine T added to the flask in which the residual free chlorine corresponded to the range set (0.05–0.3 mg/l) by the relevant legislation (STN EN ISO 7393-3) using the method of Horakova et al. (2003).

Physico-chemical examination of water

Sensory evaluation of water (colour, odour, turbidity) was carried out on site and was verified after transfer of water to a laboratory. No detectable changes were observed and the results corresponded to the requirements set by the legislation for drinking water. The temperature of water was measured at sampling and ranged between 7 °C and 10.5 °C.

Chemical examination included determination of pH, electrical conductivity, dissolved oxygen (saturation) and qualitative examination for the presence of ammonium ions, nitrites, nitrates, free chlorine and chlorides by colour reactions. In the case of positive results, the respective parameters were determined quantitatively, together with quantitative determination of chemical oxygen demand (COD_{Mn}) and the sum of calcium and magnesium. Examinations were carried out monthly starting on the 28th January and ending on the 27th May.

The pH was determined according to STN EN ISO 10523 by means of a pH-meter HACH and a WATERPROF pH Tester 30. Conductivity was determined using a WTW InoLab Cond 720 conductometer (Germany).

Quantitative determination of nitrates was carried out directly in samples with an ion-selective nitrate electrode WTW (InoLab pH/ION 735P, Germany). Chlorides were determined according to STN ISO 9297 by titration, active chlorine according to STN EN ISO 7393-3 by titration, and Ca^{2+}+Mg^{2+} by titration according to Horakova et al. (2003). Dissolved oxygen was determined electrochemically using an LDO HQ Series Portable Meters oxygen probe supplied by HACH and chemical oxygen demand by oxidation with $KMnO_4$ according to STN EN ISO 8467.

Fluorescence excitation emission matrix (EEM) spectroscopy

February samples of water from water sources and from drinkers were taken for EEM spectroscopy together with samples for microbiological and physico-chemical determinations and were examined using a Perkin Elmer LS 55 luminescence spectrophotometer (USA) (Institute of Medical and Clinical biochemistry of Faculty of Medicine of Pavol Jozef Safarik University in Kosice and LABMED, Inc.) at the following settings: excitation wavelength in the range 250–450 nm with gradual incremental increase (10 nm), range λ = 250–600 nm (excitation/emission slit: 5/10 nm, quartz cuvette of 1 cm width, scanning rate of emission monochromator: 20 nm/s). Excitation – emission matrices – EEM were obtained using the FIW Inlab programme (Dubayova et al. 2008).

Statistical analysis

The results are given as mean ± SD of three different farms: Farm No. 1: n = 7, Farm No. 2 and 3: n = 5.

Statistical analysis was performed by calculating one-way analysis of variance (ANOVA) with the *post hoc* Tukey multiple comparison test using the Prism 3 software program.

RESULTS AND DISCUSSION

The availability of high-quality drinking water has become a key issue around the world. The survival of human populations depends on natural water sources, either surface or ground, and on their protection and treatment.

The good quality of water intended for human consumption and for the watering of animals is essential for their health and for the prevention of food chain contamination.

Groundwater treatment is the process of converting raw water from a sub-surface source into a potable form that is suitable for drinking and other domestic uses. The method of treatment will depend on the pollution or contaminants involved (Ojo et al. 2012). The disinfection of water serves as the final measure against the spread of disease and should be carried out when necessary. All as-

doi: 10.17221/8818-VETMED

sociated risks should be borne in mind and should be minimised in so far as is possible.

Although the harmful by-products associated with some disinfection methods have stimulated scientists to look for new methods, or to combine several technologies in order to resolve this problem (Badawy et al. 2012), disinfection with active chlorine remains the most frequently used method.

Microbiological examination of chlorinated groundwater

Because our evaluations concerned water that should comply with the limits for drinking water, we compared our results with the benchmark set by the relevant legislation (WHO 1996, 2008; EC Regulations 2007; Regulation of the Government of the SR No. 496/2010 Coll.).

Farm No. 1. After the first two bacteriological examinations (January, February) and experimental chlorination of water taken from the common tank, we disinfected water in the tank five times between the 25th March and the 27th May. Samples were bacteriologically examined on the 5th day after chlorination. The results are presented in Table 1.

Calculations based on experimental chlorination set a dose of Chloramine T of 20 g per well. Application of this dose did not ensure acceptable results and was doubled in April to 40 g per well (Table 1).

The disinfection appeared ineffective and no free chlorine was detected in water on the 5th day after chlorination, probably due to the intensive rain in the first half of May. The situation improved at the end of May during dry weather. Still, total coliforms were detected in water. This would indicate faecal pollution and water containing members of this group in any 100 ml sample cannot be considered safe unless their source is identified without doubt and is not related to animal or human waste. Precipitation could have contributed to the contamination of wells with some run-off, as the wells were situated in an agricultural area potentially polluted with animal manure and fertilisers, the levels of which may peak in spring.

The samples were taken from a common tank where water from five relatively shallow wells (6–11 m deep) was collected. Bonton et al. (2010) reported variable bacteriological pollution of groundwater in an agricultural area in space and time with higher contamination during the summer months. Only 2% of the raw water samples exhibited contamination exceeding the drinking water standard for treated water. Further, total coliforms seemed to be a good indicator of *E. coli* or enterococci contamination.

Cho et al. (2000) observed that heavy rainfall facilitates the transport of pathogenic bacteria, and such pathogens introduced into groundwater can survive in a culturable state or in a viable but non-culturable state. Most bacteriological data for groundwater ecosystems have concerned conventional heterotrophic bacteria, total microscopic counts and functional groups such as nitrifying, denitrifying, and sulphate-reducing bacteria. In pollution studies, usually two to three indicator bacteria (such as total coliforms, faecal coliforms, and faecal streptococci) have been measured for the evaluation of water quality. The combined use

Table 1. Microbiological examination and the level of free chlorine on Farm No. 1 before and after disinfection with Chloramine T

Parameter	Before disinfection		5th day after disinfection					Limit (CFU)/(ml)
	28 January	25 February	25 March (20 g)	15 April (40 g)	29 April (40 g)	13 May (40 g)	27 May (40 g)	
CB	> 300	8	3	0	0	300	21	0/100
E. coli	0	1	0	0	0	0	0	0/100
BC37	8	18	12	0	0	42	6	20/1
BC22	3	23	15	2	3	0	8	200/1
EC	5	1	0	2	1	5	0	0/100
Cl_2 (mg/l)	ND	ND	0.15	0.1	0.1	ND	0.1	

CB = coliform bacteria; BC37 or BC22 = bacteria cultivated at 37 °C or 22 °C, EC = enterococci; ND = not detected; *E. coli* = *Escherichia coli*; Cl_2 = free chlorine

doi: 10.17221/8818-VETMED

of different kinds of pollution indicator bacteria provides more precise information on faecal contamination in a given environment. This approach was also used in our study as we determined the heterotrophic counts as well as indicator bacteria.

Farm No. 2. In January only one old reservoir (OR) was used on this farm. Two new wells were made and started to supply water for the new reservoir (NR) in March when we carried out experimental chlorination of water from both reservoirs, performed disinfection of water with Chloramine T in both from March to May, before evaluating its efficiency five days after disinfection by bacteriological examination.

On the basis of experimental chlorination a single dose of 180 g was recommended per each reservoir. After disinfection and subsequent examination in April this dose was increased to 360 g which appeared sufficient for OR but was increased again to 1200 g for NR. These doses appeared sufficient to keep the water samples (100 ml) free of *E. coli*, and enterococci (Table 2) but total coliforms were present in increased numbers in May, probably related to rainy weather. The quality of water in the new reservoir was influenced by the fact that both wells supplying the water were newly drilled, and it takes some time before the quality of water stabilises from both a chemical and microbiological point of view.

On this farm we recommend that the interval between subsequent applications of disinfectant during periods of intensive rain be shortened. Although the physico-chemical results (Table 4) obtained by examination of water from the OR were worse than those obtained for the NR, the microbiological results for OR were better and the quality of water in this reservoir showed lower variations and lower demands on disinfection. However, this may change in the future for the reasons mentioned above.

Farm No. 3. According to the experimental chlorination of water on Farm No. 3, the optimum single dose of Chloramine T was calculated to be 180 g. Repeated chlorination and subsequent analyses showed that this dose was sufficient as it ensured the absence of *E. coli* and enterococci (Table 3). Plate counts of coliform bacteria from this well increased due to heavy rains, similar to the other two farms. This confirmed the presumption that in periods of heavy rains chlorination should be performed at higher frequency.

The better quality of water on this farm may be related to the fact that the well was about 20 m deep which can result in better filtration of water, but there are many other factors that could affect the situation and more detailed investigation is required.

There is only one water source on this farm with a capacity of approximately 90 000 litres. After the first experimental chlorination, the optimum single dose appeared to be 180 g. Because this dose provided favourable results it was not changed and was used for the disinfection of water up to May (Table 3).

Physico-chemical examination

Physico-chemical examination of water provides information on its acceptability and potential

Table 2. Microbiological examination and the level of free chlorine on Farm No. 2 before and after disinfection with Chloramine T

Parameter	Before disinfection		5th day after disinfection						Limit (CFU)/(ml)
	28 January	25 February	March		April		May		
	OR	OR	NR (180 g)	OR (180 g)	NR (360 g)	OR (360 g)	NR (1200 g)	OR (360 g)	
CB	85	2	> 300	0	15	0	8	1	0/100
E. coli	0	0	0	1	0	0	0	0	0/100
BC37	2	0	35	0	195	85	32	15	20/1
BC22	3	0	88	2	192	136	125	30	200/1
EC	0	0	20	0	2	0	0	0	0/100
Cl_2 (mg/l)	ND	0.15	ND	0.15	0.1	0.1	0.1	0.3	

CB = coliform bacteria; BC37 or 22 = bacteria cultivated at 37 °C or 22 °C, EC = enterococci; ND = not detected; NR = new reservoir; OR = old reservoir; *E. coli* = *Escherichia coli;* Cl_2 = free chlorine

doi: 10.17221/8818-VETMED

Table 3. Microbiological examination and the level of free chlorine on Farm No. 3 before and after disinfection with Chloramine T

Parameter	Before disinfection		5th day after disinfection			Limit (CFU)/(ml)
	28 January	25 February	March 180g	April 180g	May 180g	
CB	8	10	1	9	3	0/100
E. coli	0	0	0	1	0	0/100
BC37	0	2	3	0	13	20/1
BC22	0	11	8	19	38	200/1
EC	0	0	0	1	0	0/100
Cl_2 (mg/l)	ND	ND	ND	0.3	ND	

CB = coliform bacteria; BC37 or 22 = bacteria cultivated at 37 °C or 22 °C, EC = enterococci; ND = not detected; *E. coli* = *Escherichia coli*; Cl_2 = free chlorine

health risks. Some chemical parameters serve as indicators of faecal or environmental contamination of water sources and may also be useful in deciding about the measures that should be taken to protect and maintain these sources.

In water, chlorine reacts to form hypochlorous acid and hypochlorites, commonly referred to as "free" or "available" chlorine. Their relative amounts vary with the pH. Hypochlorous acid is the active disinfecting component of free chlorine. As pH increases the percentage concentration of hypochlorous acid in free chlorine decreases. At pH values of above eight free chlorine loses most of its disinfectant power (EHFS 2013).

One of the most important parameters for the monitoring of the pollution of groundwaters is ammonium nitrogen ($N\text{-}NH_4^+$), which represents an intermediate or final product of the microbiological decomposition of organic matter and unused nutrients in animal excrement, although $N\text{-}NH_4^+$ is adsorbed on soil particles. Bartel-Hunt et al. (2011) and Fridrich et al. (2014) detected increased concentrations of ammonium nitrogen in the shallow groundwater of wells downstream from pigsties and lagoons. Natural levels in groundwater and surface water are usually below 0.2 mg/l. Anaerobic groundwaters may contain up to 3 mg/l (WHO 2008).

Nitrates can reach both surface water and groundwater as a consequence of agricultural activity (including excess application of inorganic nitrogenous fertilisers and manures), from wastewater disposal and from oxidation of nitrogenous waste products in human and animal excreta, including from septic tanks. Surface water nitrate concentrations can change rapidly owing to surface run-off of fertiliser, uptake by phytoplankton and denitrification by bacteria, but groundwater concentrations generally change only slowly. Some groundwaters may also have nitrate contamination as a consequence of leaching from natural vegetation. In general, the most important source of human exposure to nitrate and nitrite is through vegetables (nitrite and nitrate) and through meat in the diet. In some circumstances, however, drinking water can make a significant contribution to nitrate and, occasionally, nitrite intake (Kroupova et al. 2005). In the case of bottle-fed infants, drinking-water can be the major external source of exposure to nitrate and nitrite.

The guideline value for the chronic effects of nitrites is considered provisional owing to the uncertainty surrounding the susceptibility of humans compared with animals (WHO 2008).

In most countries, nitrate levels in drinking water derived from surface water do not exceed 10 mg/l, although nitrate levels in well water often exceed 50 mg/l; nitrite levels are normally lower, less than a few milligrams per litre.

The assessment of groundwater quality and its variation in an agricultural area conducted by Bonton et al. (2010) indicated high spatial and temporal variations in nitrate concentrations ranging from 6 to 125 mg NO_3^-/l.

Chlorides in drinking water originate from natural sources, sewage and industrial effluents, urban run-off containing de-icing salt and saline intrusion. Values above 250 mg/l indicate pollution of water (EC Regulations 2007).

A number of ecological and analytical epidemiological studies have shown a statistically significant

doi: 10.17221/8818-VETMED

Table 4. Physico-chemical examination of water on Farms No. 1–3 in the Presov region

Parameters	Farm No. 1 (n = 7)	Farm No. 2 (n = 5)	Farm No. 3 (n = 5)
pH	7.1 ± 0.2^a	7.2 ± 0.4^a	7.2 ± 0.5^a
O_2 saturation (%)	67 ± 15^a	87 ± 6^b	89 ± 14^b
Conductivity (mS/m)	98 ± 2^a	110 ± 10^a	51 ± 15^b
Cl^- (mg/l)	18 ± 6^a	62 ± 4^b	18 ± 6^a
NO_3^- (mg/l)	15 ± 7^a	73 ± 11^b	34 ± 14^c
COD_{Mn} (mg/l)	1.1 ± 0.2^a	1.8 ± 1.1^a	0.5 ± 0.3^{ab}
Ca^{2+} + Mg^{2+} (mmol/l)	5.3 ± 0.2^a	5.6 ± 0.3^a	2.7 ± 1.0^b

ANOVA post-hoc Tukey test. Results are expressed as mean ± standard deviation, significant differences ($P < 0.05$) are indicated by different alphabetic superscripts

inverse relationship between hardness of drinking-water and cardiovascular disease. No health-based guideline value is proposed for hardness by the WHO (2008).

The results of the physico-chemical monthly examinations of water (mean ± SEM) on Farms No. 1, 2 and 3 are presented in Table 4 with the exception of the level of free chlorine which is included in Tables 1–3 as it is related to the disinfection and bactericidal efficiency of Chloramine T.

Farm No. 1. In water on Farm 1 the pH ranged between 6.9 and 7.4 and therefore complied with the requirements for drinking water. Saturation with oxygen ranged from 55.4 % to 80.9 %. It was below the recommended level in May (45.4% vs. recommended min. 50%), which was most likely related to intensive precipitation in the first half of this month.

Conductivity was in the range of 94.9–100.3 mS/m and did not exceed the limit for this parameter (125 mS/m). Chemical oxygen demand ranged from 0.9–1.3 mg/l (limit 3.0 mg/l). Qualitative examination of ammonium ions and nitrites provided negative results. Nitrate levels varied from 5.0 mg/l to 24 mg/l (limit 50 mg/l) and chlorides were in the range of 18.0–24.8 mg/l (limit 250 mg/l). The recommended maximum level (5 mmol/l) for calcium and magnesium was exceeded at all samplings (5.18–5.78 mmol/l).

In contrast to the positive results for bacterial indicators the physico-chemical examination of water on this farm failed to indicate increased faecal contamination in the period of heavy precipitation.

Farm No. 2. All pH results complied with the recommendations. The level of dissolved oxygen (DO) in water is used as an indicator of pollution and its potability. At least 50% saturation of water with DO is recommended (STN EN 5814, ion-selective method). Depletion of dissolved oxygen in water supplies can encourage the microbial reduction of nitrate to nitrite and sulphate to sulphide (WHO 2008). Saturation with oxygen was in the range of 81.9–95.6% and 89.6–94.3% in the old and new reservoir, respectively, and thus well above the minimum limit (50%), indicating good quality of water.

Electrical conductivity (EC) is a measure of the capacity of water to conduct electrical current and it is directly related to the concentration of salts dissolved in water, and therefore to the Total dissolved solids (TDS). These principally include calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulphates and some small amounts of organic matter that are dissolved in water. The EC of the groundwater is a general indicator of manure pit leakage (Krapac et al. 2002).

Conductivity of water in both reservoirs (OR = 93.9–117.3 mS/m; NR = 76.0–83.1 mS/m), complied with the standard. Oxidisability (COD_{Mn}) in samples from OR ranged from 1.2 to 1.8 mg/l, i.e. below the limit (3.0 mg/l) except for the sampling in January (3.7 mg/l) while all the results in water from NR ranged between 1.2 and 1.24 mg/l, i.e. well below the maximum limit. The level of nitrates in water from OR exceeded the limit at all samplings (72–88 mg/l in OR) while in water from NR nitrates were in the range of 6–18 mg/l, i.e. well below the 50 mg/l limit. Water from Farm 2 exceeded the recommended maximum level for calcium and magnesium in the old reservoir (OR) (5.10–5.88 mmol/l) and was within the recommended range (1.10–5.00 mmol/l) in the samples from the new reservoir (NR) (3.8–3.9 mmol/l).

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Results of qualitative examination of water for the presence of ammonium ions and nitrites were negative. The level of chlorides (limit 250 mg/l) reached 56.4 mg/l–65.2 mg/l in OR and 24.8–28.9 mg/l in NR.

Overall, similar to Farm No. 1, the results of the physico-chemical examination of water on Farm 2, particularly water in the new reservoir, did not indicate significant pollution with faeces.

Farm No. 3. All pH values (6.6–7.7) corresponded to the standard, as did the saturation with oxygen (64.5–98.3%). Conductivity (40.3–77.2 mS/m) was lower than on Farms No. 1 and No. 2, while the values of COD_{Mn} oxidisability were also the lowest among the three farms and ranged between 0.16 and 0.8 mg/l, indicating very low levels of organic, chemically oxidisable pollutants, and therefore a low probability of the development of disinfection by-products.

In relation to disinfection with active chlorine, chemical oxygen demand (COD) or oxidisability, the parameter reflecting the content of organic substances is very important as it indicates a potential risk for the production of by-products (DBPs) such as trihalomethane and haloacetic acid, which are linked to an increased risk of cancer (Chowdhury 2013; Lyon et al. 2013). In groundwater vulnerability assessment, it is assumed that groundwater closer to the soil surface is of greater risk of contamination by pollutants, including N compounds. Shallow groundwater conditions also affect the proportions of N forms (Morari et al. 2012).

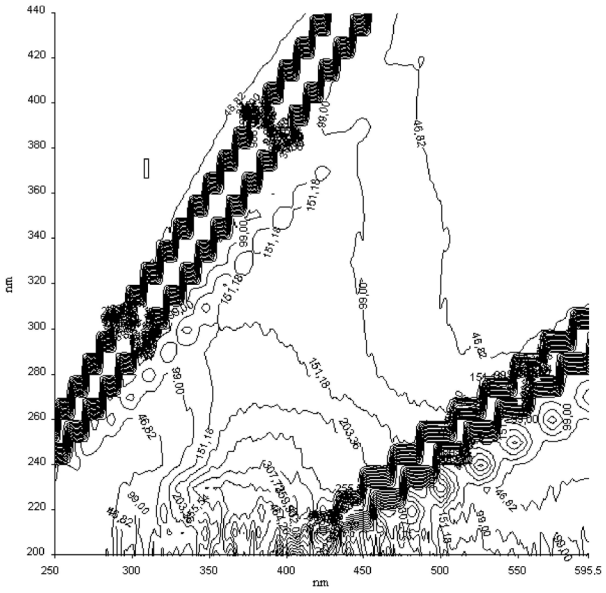

Figure 1. EEM of a sample of water from a public drinking water source (a graphic standard)

Nitrites and ammonium ions were absent in the examined water and free chlorine reached the level of 0.3 mg/l (limit set by legislative provisions) at two samplings. Nitrates ranged between 25–32 mg/l and only at one sampling exceeded the limit by 8 mg/l. Chloride levels were well below the limit of 250 mg/l (6.8–22.3 mg/l). The sum of calcium and magnesium ions in water from Farm No. 3 ranged between 2.0 and 2.4 mmol/l, and was in the recommended range.

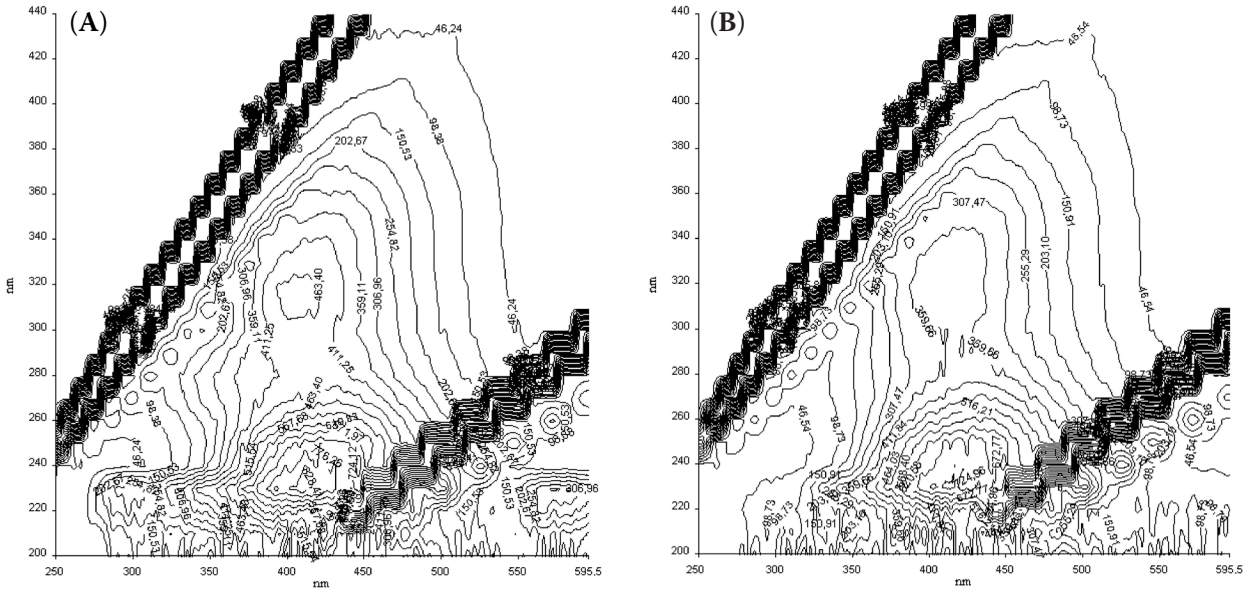

Figure 2. EEM of water from Farm No. 1 – drinker (**A**). EEM of water from Farm No. 1 – water source (**B**)

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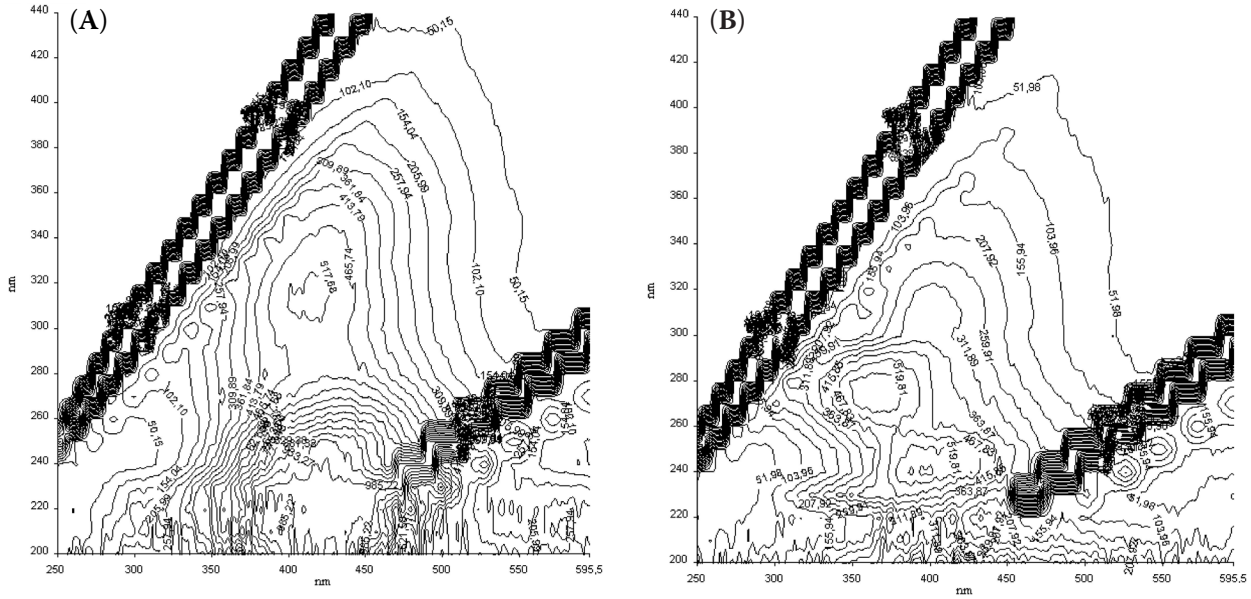

Figure 3. EEM of water from Farm No. 2 – drinker (**A**). EEM of water from Farm No. 2 – water source (**B**)

EEM fluorescence spectra of water from Farms No. 1–3

EEM detects the presence of pollutants by means of fluorescent characteristics, namely the position of the fluorophore in EEM, or excitation and emission maxima (Chen et al. 2003). Recent studies have shown that different methods of disinfection of water affect its fluorescence properties due to the development of various disinfection by-products (Markechova et al. 2013). The basis for the correct evaluation of the EEM of respective samples is the determination of a standard that can be used for the comparison of quality in the absence of previous chemical analysis. A sample of drinking-water taken from a public drinking-water supply (Figure 1) was used as a graphic standard in our study.

The EEM of drinking water shows a natural fluorescence background without the presence of organic, biodegradable or other pollutants.

The EEMs of water from a drinker and a tank on Farm No. 1 are similar (Figure 2A, 2B). The graphic

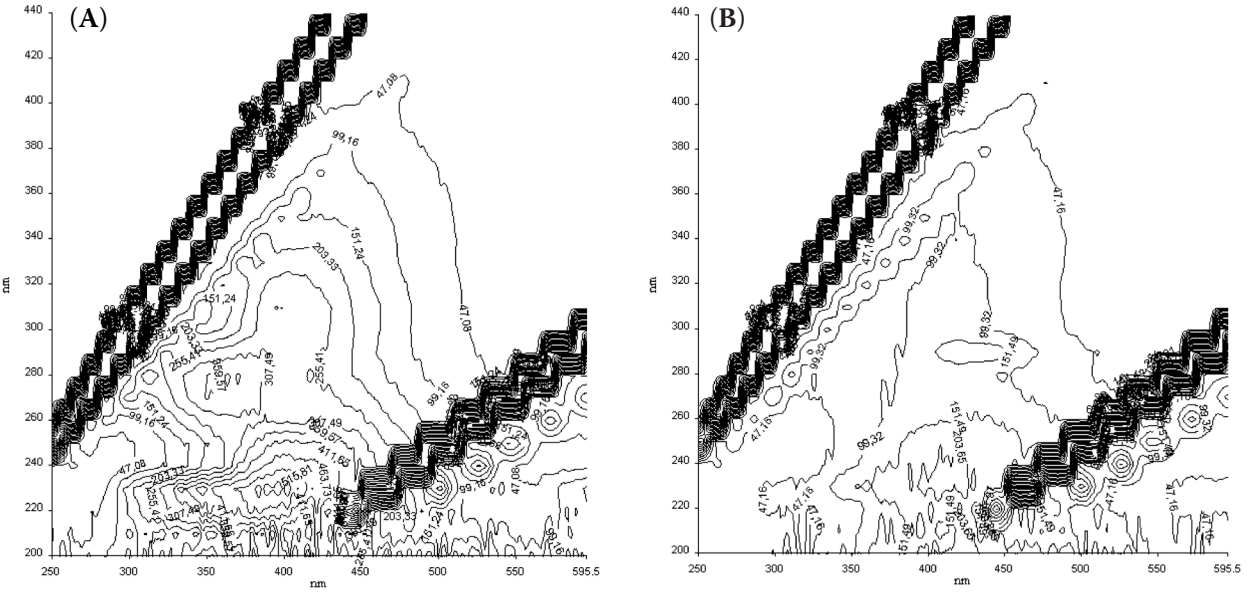

Figure 4. EEM of water from Farm No. 3 – drinker (**A**). EEM of water from Farm No. 3 – water source (**B**)

doi: 10.17221/8818-VETMED

record indicates that water from the drinker contained some organic (humic) substances and biodegradable products – proteins with l_{ex}/l_{em} = 230/300 (Dubayova et al. 2008). Also, the well water contained organic substances and its characteristics were similar to those commonly observed in surface water (river), or groundwater of lower quality. The EEM suggests that the water does not comply with the fluorescence criteria for drinking water.

The EEM of the sample from the source on Farm No. 2 (Figure 3B) corresponded to the quality of surface water with some organic (humic) substances. It indicated the presence of proteins and biodegradable products that may occur in free-form or bound to humic acids. Water from the drinker was considerably polluted and its quality, as indicated by fluorescence analysis (Figure 3A), was the lowest from all examined samples.

The EEM water from a drinker on Farm No. 3 (Figure 4A) resembled that of surface water or water of lower quality, potentially polluted with proteins and other organic biodegradable products. A higher concentration of humic acids and protein-humic acid complexes was determined in this sample. The EEM of the sample from the well (Figure 4B) is characteristic for high-quality water from well or a spring. This sample provided the best fluorescence spectrum of all examined samples.

CONCLUSION

Physico-chemical and microbiological examinations and EEM fluorescence spectroscopy performed on the three investigated farms showed that the water source on Farm No. 3 provided water of better quality than the sources on Farms No. 1 and No. 2. The results obtained did not indicate pollution of water with animal or human waste. The discrepancies between the results of EEM spectroscopy and other analyses could be ascribed to the limited number of EEM examinations and inability to identify the sources of natural organic matter detected using this method.

Our results also indicated that weather (precipitation) most likely affected the quality of water on all three farms and was associated with some risk to animals consuming this water, as indicated by the presence of total coliform bacteria in examined samples. This is again a complex issue requiring additional, more detailed investigations.

The doses of Chloramine T calculated based on experimental chlorination and used for disinfection on the investigated farms appeared relatively efficient on Farm No. 1 and Farm No. 3, while on Farm No. 2 the estimated Chloramine T dose had to be increased, particularly for the new reservoir supplied by water from two newly drilled wells. However, the dose was still much lower than that recommended by the manufacturer of this preparation. This has important implications for decreasing the production of potential by-products during water disinfection with active chlorine preparations. It may be desirable to adjust the intervals between individual treatments (disinfection) to climactic conditions (heavy rain), instead of significantly increasing the active chlorine doses.

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Received: 2015–04–20
Accepted after corrections: 2016–02–18

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