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Water quality on cattle farms in the northwest Croatia

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Abstract

In this study, quality of well water used for cattle watering on farms in the northwest Croatia was investigated. The following microbiological parameters were analysed: total coliforms, Escherichia coli, intestinal enterococci, the number of microorganism colonies at 37 ° C and 22 ° C, Clostridium perfringens and Pseudomonas aeruginosa. Physico-chemical analysis included following parameters: colour, taste, odour, pH, chlorides, sulphates, nitrites, nitrates, sodium, potassium, ammonium, free chlorine, iron and the consumption of $KMnO_4$. Among 34 samples analysed, 79.4% were microbiologically, 61.8% were chemically and 94.1% of the samples were in total unsuitable according to the Croatian water quality regulations (OJ 125/2013, 141/2013, 128/2015).

Keywords*: water quality, cattle, northwest Croatia, watering*

Sažetak

U ovom radu ispitivana je kvaliteta bunarske vode koja se koristi za napajanje goveda na farmama sjeverozapadne Hrvatske. Analizirani su slijedeći mikrobiološki parametri: ukupni koliformi, Escherichia coli, crijevni enterokoki, broj kolonija mikroorganizama na 37 °C i 22 °C, Clostridium perfringens i Pseudomonas aeruginosa. Od fizikalno-kemijskih parametara analizirani su: boja, okus, miris, pH, kloridi, sulfati, nitriti, nitrati, natrij, kalij, amonijak, slobodni klor, željezo i utrošak $KMnO_4$. Od 34 pretražena uzorka, prema Pravilniku o parametrima sukladnosti i metodama analize vode za ljudsku potrošnju (NN 125/2013, 141/2013, 128/2015) mikrobiološki nije bilo sukladno 79,4%, kemijski 61,8% uzoraka, odnosno ukupno 94,1% uzoraka.

Ključne riječi*: kvaliteta vode, goveda, sjeverozapadna Hrvatska, napajanje*

Introduction

The existence of water is certainly one of the conditions of life on our planet because it is necessary for all vital processes in the biosphere. The water accounts for about 70% of the mass of all living organisms, including animals. It is a vital body fluid essential for the transport of nutrients and removal of waste, metabolic reactions, regulation of the body temperature, etc. In most cases animals satisfy the need for water from watering and water quantity depends on their species and category, nutrition, physiological condition, activities, productivity and environmental conditions. Domestic animals, such as cows must have 4.5 L of water *per* 50 kg of body weight *per* day and 3 L for each liter of milk. Yearling cattle require on an average 20-30 L of water *per* day, and calves up to 1 month of age 8-10 L of water (Naletilić et al., 2013).

In the Republic of Croatia, the quality of water for animals must correspond to that for human consumption whose control is carried out in accordance with the criteria of the Croatian water quality regulation (OJ 125/2013, 141/2013, 128/2015). However, the water quality for the animals may often be overlooked. By investigating the quality of water used as drinking water for turkeys in Dalmatian hinterland, Ostović et al. (2011) found only water from the water supply system to be safe, whereas water from all other sources investigated (well, cistern and barrel) did not meet health safety criteria. Moreover, our previous study on water quality for broiler chickens and laying hens on farms in the northwest Croatia showed that 25.0% of samples were chemically and/or microbiologically unsuitable and all of the unsuitable samples come from wells (Kiš et al., 2017). Therefore, more emphasis should be placed on a systematic study of drinking water for animals with a goal of achieving its health and ecological acceptability.

The aim of this study was to investigate the quality of well water used for supplying cattle on Croatian farms in the northwest since, according to our knowledge, no such studies have been done yet.

Materials and methods

Water sampling

Water was sampled on dairy and beef cattle farms in the northwest Croatia from the wells used for their watering. The samples were collected by qualified employees, in clean, sterile bottles and transported in portable refrigerators to laboratories within 6 hours of sampling. Immediately after coming to the lab, they underwent microbiological and physical-chemical analysis. A total of 34 samples of well water from different farms collected during the autumn of 2016 were analysed.

Physico-chemical analysis

Colour

Colour of samples was determined according to the norm HRN EN ISO 7887:2012. Principle of method is based on using optical apparatus for comparison with hexachloroplatinate concentration at wavelength, λ=410 nm.

Odour and taste

Determination of odour and taste were carried out according norm HRN EN 1622:2008 using short method which is applicable when either a sample has no odour and taste or for compliance of odour and taste with specified level. Only microbiologically suitable samples were subjected to analysis.

pH value

The concentration of hydrogen ions or pH value of water was determined potentiometrically, i.e. by measuring the pH value using the Meter Toledo Meter SevenCompact S220 instrument. The procedure with the pH meter and the pH measurement procedure itself were carried out according to the norm HRN ISO 10523:2012.

Dissolved anions

Dissolved anions (chlorides, nitrites, nitrates, suphates) were determined by ion-liquid chromatography according to the norm HRN EN ISO 10304-1:2009. The method was set up on the DIONEX ion chromatography assay and the detection was performed with a conductometric detector with suppression. As eluent, a solution of 4.5 mmol L^{-1} Na_2CO_3 and 1.4 mmol L^{-1} $NaHCO_3$ was used. Dionex IonPac AS22 column was used, 4 × 250 mm, thermostated at 30 ° C. The flow rate was 1.2 mL min^{-1} The sample was filtered through a membrane filter Ø 45 µm prior to injection.

Dissolved cations

Dissolved cations (sodium, potassium and ammonium) were determined by ion-liquid chromatography according to the norm HRN EN ISO 14911:2001. The method was set up on the DIONEX ion chromatography assay and the detection was performed with a conductometric detector with a suppression. A solution of 20 mM methanesulfonic acid was used as eluent at a flow rate of 1 mL min^{-1}.. Dionex IonPac CS12 column, 4 × 250 mm, thermostated at 22 ° C was used. The sample was filtered through a membrane filter Ø 45 µm prior to injection.

Free chlorine

The free chlorine determination method is based on a direct reaction with N, N-diethyl-1,4-phenylenediamine (DPD) and the formation of a pink coloured compound at pH 6.2 to 6.5. The measurement of colour intensity is achieved by a chlorine pocket meter, HACH colorimeter.

Iron

The method for determining iron in water was carried out according to the norm HRN ISO 6332:1998 and is based on the measurement of absorbance at 510 nm of an orange-red complex formed by the reaction of iron (II) ions with a solution of 1,10-phenanthroline. Iron (III) ions are reduced to iron (II) ions by adding ascorbic acid before forming the complex.

Consumption of $KMnO_4$

The method for determining consumption of $KMnO_4$, i.e. the permanganate index, is based on determining the amount of oxygen required for oxidation of dissolved organic matter in water with some strong oxidizing agent ($KMnO_4$). The consumption of $KMnO_4$ was determined in accordance with the norm HRN EN ISO 8467:2001. The method is based on heating the sample in a boiling water bath with a known amount of potassium permanganate and sulfuric acid at a fixed time period (10 min). Reduction of part of the permanganate by oxidizing substances in the sample and the determination of the consumed permanganate by addition of an excess of oxalate solution, followed by titration with permanganate.

Microbiological analysis

For the purpose of confirming and determining the total number of coliform bacteria and *Escherichia coli* bacteria in water, the membrane filtration method was used according to the standard procedure HRN EN ISO 9308-1:2014, while the procedure HRN EN ISO 7899-2:2000 was used to confirm and determine the number of intestinal enterococci. For the detection of bacterial species of *Pseudomonas aeruginosa*, a procedure was used according to the norm HRN EN ISO 16266:2008. The detection and number of *Clostridium perfringens* spores after membrane filtration and anaerobic incubation at 44 °C and 37 °C for 24 hours was determined on m-CP agar (Clostridium Perfringens agar base, Merck, Germany) and TSC agar (Tripton sulfit cycloser agar, Biokar, France). The method HRN EN ISO 6222:2000 was used to determine the number of microorganism colonies colonizing on a nutrient agar.

The sample search procedure was carried out in accordance with the requirements of the above mentioned standards by membrane filtration of 100 mL of water sample through 0.45 µm pore size filters and incubated on solid selective agar (Lactose TTC agar with Tergitol 7, Merck, Germany) for total coliforms, TTC and TBX (Tryptone Bille X-gluconide, Merck, Germany) agar for *Escherichia coli* and Slanetz and Bartley agar for intestinal enterococci. Morphological and biochemical properties were used to confirm and identify the grown microorganisms. Coliform bacteria were confirmed by the production of indole from tryptophane and by negative oxidase. The bacterial species of *Escherichia coli* grows as blue-green colonies on TBX agar, indole is positive and oxidase negative. The intestinal enterococci hydrolyse aesculin on bile-aesculin-azide agar. The end-product, 6,7-dihydroxycumarin, combines with iron(III) ions to give a tan coloured to black compound which diffuses into the medium. *Pseudomonas aeruginosa* is a microorganism that grows on a selective medium with cetrimide and produces pyocyanin, it is the oxidase positive, fluoresces under UV light (360±20 nm) and is capable of producing ammonia from acetamide.

To determine the number of microorganism colonies in a 1 mL of water sample, yeast extract agar was applied and the plates were incubated at 22 °C for 68 hours and 37 °C for 44 hours.

Results and discussion

The percentage of microbiologically and chemically unsuitable samples among 34 analysed samples regarding the Maximum Permissible Concentration (MPC) set out by the Croatian water quality regulations (OJ 125/2013, 141/2013, 128/2015) are presented in Table 1 and the results of unsuitable samples according to a individually analysed parameter are shown in Table 2.

Table 1. *Microbiologically and chemically unsuitable samples pursuant to the exposure levels set out by the Croatian water quality regulations (OJ 125/2013, 141/2013, 128/2015)*

Type of analysis	Analysed samples (n)	Unsuitable samples (n)	%
Microbiological	34	27	79.4
Chemical		21	61.8

Table 2. *Unsuitable samples pursuant to the exposure levels set out by the Croatian water quality regulations (OJ 125/2013, 141/2013, 128/2015) per individually analysed parameter with minimum and maximum obtained values among 34 analyzed samples*

Parameter	Unsuitable samples (n)	%	Min-max values among 34 samples
Total coliforms	25	73.5	0-890 cfu/100 mL
Microorganism count at 37 °C	24	70.6	0-2000 cfu/mL
Microorganism count at 22 °C	23	67.6	0-990 cfu/mL
Intestinal enterococci	23	67.6	0-300 cfu/100 mL
Escherichia coli	21	61.8	0-400 cfu/100 mL
Nitrates	13	38.2	1.2-212.7 mg L^{-1}
Clostridium perfringens	9	26.5	0-47 cfu/100 mL
Ammonium	8	23.5	<0.03-1.31 mg L^{-1}
Pseudomonas aeruginosa	3	8.8	0-61 cfu/100 mL
Iron	2	5.9	<20 -819 µg L^{-1}
Potassium	2	5.9	0.21- 101.9 mg L^{-1}
Free chlorine	1	2.9	<0.02-1.03 mg L^{-1}

Apart from housing and nutrition, cattle breeding also depends on water supply. Unsatisfactory, poor quality of water, either organoleptic, physico-chemical or microbiological, affects the animal health, welfare and productivity. Problems related to the quality of water for livestock watering, in terms of impaired health and productivity, are caused by the qualitative composition of water but may also be the result of water disinfection due to the resulting harmful by-products (Marjanović and Tofant, 2008).

Water for animals should be colourless, odourless and tasteless. The colour of water comes from dissolved and suspended matter. The water colour does not have a hygienic meaning, but it gives to water an unappealing look. In this study, the colour of all water samples was within the MPC of 20 mg L^{-1} PtCo scale. Odour and taste have sanitary significance because they are often the first obvious indicators of water contamination. The present study showed that all of the analysed microbiologically suitable samples were odourless and tasteless.

Determining the pH value is very important because of the influence on the chemical and biological properties of water. Water with low pH is corrosive to equipment and has a negative impact on animal acceptability, because it is acidic, while water with high pH is also unacceptable because it is unsavoury. No sample was found to be unsuitable with this parameter and the average pH value was 7.4.

The consumption of KMnO4 is a parameter that points to content of dissolved organic substances in water. In natural waters, increased amounts of organic matter are usually due to secondary sources of pollution (e.g. the rupture of agricultural surfaces, human or animal waste substances or industrial waste products) and that is why the determination of this parameter in water is an important indicator of a potential water source contamination with an organic matter (Asaj, 1974). All the samples tested were suitable with this parameter, respecting the MPC of 5.0 mg O_2 L^{-1}.

The concentration of chlorides in water has no major health significance, but it is important because of their corrosive action on metal tubes of the water supply systems and because of the taste of water (Naletilić et al., 2013). Dissolved chlorides are the most dissolved anions in water. Their concentration in water sources is largely constant and any significant change may indicate to secondary pollution of the water source with faecal matter and/or wastewaters (Asaj, 1974). MPC for chlorides is 250 mg L^{-1}. All analysed water samples showed compliance with this parameter. In addition, sulphate concentration was also monitored, as their increased concentrations in water (>150 ppm) cause salty taste, diarrhoea, and in some cases copper deficiency (Higgins et al., 2008). Increased sulphate concentrations stimulate the development of polioencephaloma, a neurological disorder characterised by weakness, muscle tremors, lethargy and even paralysis and death in cattle (Higgins et al., 2008).The highest measured sulphate concentration was 105 mg L^{-1}, well below prescribed 250 mg L^{-1}.

For the purpose of testing and assessing the water quality, determining the presence of substances resulting from the decomposition of waste from plants and/or animals is of great importance. Cattle breeding affects the deterioration of water quality through different sources of contamination (e.g. farms, fertilizers on agricultural surfaces) (Nemčić-Jurec and Valda, 2010). Nitrogen compounds, such as ammonia, nitrite and nitrate, are products of the breakdown of the substances of biological origin. All these forms of nitrogen can be discharged into the water by polluting water sources with wastewaters. Presence of nitrites in the drinking water points to partially decomposed organic waste substances. Nitrites are very toxic. Nitrite concentrations in all water samples were satisfactory, below MPC, which is 0.5 mg L^{-1}.

The concentration of nitrates as nitrogen compounds, which are the highest degree of the nitrogen oxidation in its natural cycle, is small in the surface waters (usually 1 to 30 mg L^{-1}), but can be found in larger quantities in deep waters. Increased concentration of nitrate in drinking water can potentially cause health problems in humans and animals. It is known that when nitrite in the blood binds to hemoglobin, it turns into methemoglobin, reducing the oxygen transfer capacity, which can be very dangerous. Nitrate toxicity causes poor growth, slimness and poor coordination in poultry (Ostović et al., 2011). It is also known that nitrates can produce nitrosamines which can modify certain DNA components and cause tumours (Nemčić-Jurec et al., 2009). Signs of lower level of nitrate poisoning in cattle include inferior growth, infertility, abortion and vitamin A deficiency (Higgins et al., 2008). In acute nitrate poisoning there is breathing difficulty, rapid pulse, foaming, convulsion, blue muzzle and dark circles around the eyes. Signs of chronic nitrate poisoning include reduced weight gain, decreased appetite, reduced milk production and increased exposure to infection (Higgins et al., 2008). In this study, nitrate concentrations were satisfactory (below MPC which is 50 mg L^{-1}) in 61.8% of the analysed water samples. Ostović et al. (2011) found that nitrate concentrations in water supply containers for turkeys, though within the permitted limits, were almost twice as high when compared to water sources. The mean nitrate concentration in the suitable samples was 14.3 mg L^{-1}, with the highest measured value of 36.6 mg L^{-1}.

From all dissolved cations, ammonium, potassium and sodium concentrations were monitored. The presence of ammonium in water indicates a “fresh” pollution with organic matter and presents a danger to users of such water. Sometimes rainfall and very often the water of deep wells can contain traces of ammonia that is of geological origin and does not pose a threat to users. In this study 76.5% of the samples were found to be suitable with this parameter taking into account MPC of 0.5 mg L^{-1}.Monitoring the concentration of the other two cations was decided because of their role in the functioning of the organism. Studies have shown that animals can tolerate large doses of sodium if they are given sufficient amounts of water. Cattle given water containing 975 mg L^{-1} of sodium ions for 28 days increased the water intake, reduced milk production and had diarrhoea. Excessive intake of potassium salts suppresses absorption of magnesium in ruminants (Olkowski, 2009). All analysed samples were compliant with sodium ion MPC (200 mg L^{-1}), while 94.1% of samples were found to be compliant with potassium ion MPC (12 mg L^{-1}).

Iron is an important microelement, but in excessive doses it can also be harmful. Water with an increased concentration of iron has a negative influence on the organoleptic properties of the water, causes bitter, oily and sour taste, so during the processing and preparation of water for consumption it is necessary to remove iron ions. Iron in the drinking water causes cloudiness and at higher concentrations gives it a taste of ink. In the water supply system, the occurrence of iron is the most common consequence of corrosion of pipes and reservoirs. Although high levels of iron in drinking water do not have toxicological significance, secondary metabolic effects should be considered for at least two reasons: 1) iron may affect the taste of water, and consequently reduce water intake; and 2) excessive iron intake may have harmful effects on the metabolism of several essential micronutrients including copper, zinc, magnesium, manganese and calcium (Olkowski, 2009). Of the 34 examined samples, 32 (94.1%) were found to be suitable with MPC of 200 μg L^{-1}.

The most common and most noticeable problems with drinking water are due to its microbiological composition. Not only are microorganisms considered dangerous but also are their toxins which often remain in the water when microorganisms are no longer present. As a sanitary indicator, the number of bacteria in a milliliter of water is most often determined, and as the most common causes of infection, especially of the digestive system, the total number of coliforms and intestinal enterococci is determined. Microbial contamination, besides the negative impact on animal health, is also reflected in their productivity, usually by reduced weight gain. Our previously study of health safety of drinking water which are used in the milk collection points in the Bjelovar-bilogora’s district showed that very high percentage of samples (46.3%) were microbiologically unsuitable (Denžić et al., 2016). The results of this study showed that only 7 samples (20.6%) were microbiologically suitable. In 11 (32.4%) samples, the number of colonies at 22 ° C in 1 mL of water was within the permissible level which for aerobic mesophiles is 100, while in 10 (29.4%) samples the number of colonies at 37 ° C in 1 mL of water was below the permitted 20 colonies. According to the Croatian water quality regulations (OJ 125/2013, 141/2013, 128/2015), coliform bacteria must not be present in the drinking water, and only 9 (26.5%) of the samples were found to be suitable. Water contaminated with *Escherichia coli* bacteria and intestinal enterococci is the most common evidence of faecal contamination because they are part of the normal intestinal microflora of humans and warm-blooded animals. These bacteria can survive in the environment for a long period of time and their concentration is so high that they can be easily proven even in very diluted samples. In the present study, *Escherichia coli* was detected in 21 (61.8%) and enterococci in 23 samples (67.6%). *Clostridium perfrigens* can survive for an indefinite period because it creates spores in unfavourable conditions. Therefore, the presence of *Clostridium perfrigens* and *Pseudomonas aeruginosa* indicates contamination with faecal or wastewater for a longer period of time. *Clostridium perfrigens* was detected in 9 (26.5%) samples, while *Pseudomonas aeruginosa* was detected in only 3 (8.8%) samples.. In the study of Marjanović and Tofant (2008) who investigated the quality of water for cattle watering, the water from all analysed wells was also unsuitable, and only water from water supply system was safe.

In order to destroy pathogenic microorganims, disinfection of water is necessary. However, irregular dosage of disinfectant can affect the taste of water to the extent that animals do not want to drink it; the concentrations of residual disinfectants, i.e. free chlorine, are also monitored. Residues of disinfectants with other substances present in the water can produce by-products with a consequent impact on animal health and productivity. An example being the dissolved organic carbon which, when heated with chlorine, produces high-dose trihalomethane carcinogens. Some studies show that over-chlorinated drinking water was the cause of reproducitive failure, increased number of spontaneous abortion, return to oestrus number and percentage of stillbirths, and reduced farrowing and total number of piglets in gilts and sows (Tofant et al., 2010). Also, adverse effects manifested as an increased percentage of death losses in all production categories, i.e., suckling, nursery and fattening pigs (Tofant et al., 2011). Almost all investigated samples (97.1%) were within MPC for free chlorine (0.5 mg L^{-1}), with a large number of samples with the free chlorine concentrations below or very close to the detection limit of 0.02 mg L^{-1}.

Conclusions

The analysis of water supply for cattle on the farms in northwest Croatia reveals high percentage of chemically and microbiologically unsuitable water samples. The study showed that cattle are given unsuitable water on as many as 94.1% of farms. Since only water supply sources were investigated in the study, the situation in drinkers and in water containers could be even worse. These results dictate that farmers should be informed about the importance of the drinking water hygiene. The quality of the well water is variable, as it may contain contaminants due to inadequate drainage from its own or nearby facilities (stables, septic tanks) or after rainfall when surface water flows into wells. Laboratory test results show water status only at the time when the sample was taken, so the true state of well water health safety and its changes can only be determined by more frequent repetition of the analysis and by appropriate supervision. As one of the solutions, it is recommended to use public water supply systems for livestock watering because basic microbiological, physico-chemical and organoleptic properties of water are controlled regularly thus making the water safer.

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