Water quality on farms in northwest Croatia

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Abstract

This study shows the results of research on the chemical and microbiological quality of drinking water samples from farms in the area of northwest Croatia. A total of 120 samples of water from different farms (65 chicken broiler and laying hen farms, 45 cattle farms, and 10 swine farms) that were collected throughout 2016 and spring 2017 were analyzed. A basic microbiological analysis was performed using the following parameters: total coliforms, *Escherichia coli*, intestinal enterococci, colony count at 36 °C and 22 °C, *Clostridium perfringens* and *Pseudomonas aeruginosa*. A physicochemical analysis was conducted using the following parameters: taste, odor, color, pH, hardness, free chlorine, chlorides, sulfates, nitrites, nitrates, sodium, ammonium, potassium, magnesium, calcium, iron and consumption of $KMnO_4$. Of the 120 analyzed samples, 40.0% were microbiologically unsuitable and 37.5% were chemically unsuitable under Croatian water quality regulations (Official Gazette (OG) 125/2013, 141/2013, 128/2015). The analysis of the water supply on farms in northwest Croatia reveals a high percentage (58.3%) of chemically and/or microbiologically unsuitable water samples. All of the unsuitable samples came from wells, and it may be concluded that it is necessary to conduct more water quality controls, appropriate surveillance and disinfection on a regular basis.

Key words: *water quality; farms; northwest Croatia; watering*

Introduction

Water is one of the fundamental prerequisites for life. In the veterinary field, water has an important place both in terms of its quantity and quality. In most cases, animals' water needs are met by watering, with the quantity depending on the type and category of animals and their nutrition, physiological condition, activity, 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 an average of 20-30 L of water per day, and calves up to 1 month of age 8-10 L of water (Naletilić et al., 2013). Daily water needs for poultry depend on whether they are laying hens or broilers. Generally speaking, poultry drink about twice as much water compared to the

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amount of food they consume (0.2 to 0.5 L), although during high temperatures their water needs can be quadrupled (Pavičić et al., 2005). Poor water quality can negatively affect animal health as well as their productive abilities, and can have an adverse effect on the evenness and quality of eggs (Ostović et al., 2011). Sows with piglets need 30 to 50 L of water per day, sows and boars 30 to 40 L, and piglets, regardless of age, 0.5 to 2.5 L per day (Vučemilo et al., 2003). Drinking water for animals should meet the standards for animal welfare in two key points: the quantity available and the level of organoleptic, physicochemical and microbiological parameters.

According to the regulations in force in our country, drinking water for animals should be of identical quality to that for humans, and therefore is evaluated according to Croatian water quality regulations (OG 125/2017). However, water quality for animals may often be overlooked. By investigating the quality of drinking water for turkeys in the Dalmatian hinterland, Ostović et al. (2011) found only water from the water supply system to be safe, whereas water from all the other sources investigated (wells, cisterns and barrels) did not meet health and safety criteria.

Moreover, our previous study on broiler chickens and laying hens on farms in northwest Croatia showed that 25.0% of samples were chemically and/or microbiologically unsuitable (Kiš et al., 2017). A study of cattle farms showed an even worse situation, with more than 60% being unsuitable (Denžić Lugomer et al., 2017). Therefore, more emphasis should be placed on a systematic study of drinking water for animals, with the goal of improving its healthiness and ecological suitability.

The aim of this paper is to investigate the quality of water used for supplying domestic animals on northwestern Croatia farms by analyzing its physicochemical and microbiological parameters, and checking the compliance of the values obtained for certain parameters with the maximum permissible concentrations (MPC) under the regulations. According to our knowledge, no studies on water quality for different domestic animals in Croatia have been conducted yet. Some studies include water quality for cattle and horses on a family farm (Nalelitić et al., 2013), turkey farms in the Dalmatian hinterland (Ostović et al., 2011), and cattle farms (Marjanović and Tofant, 2008). The weakness of these studies is the relatively small number of water samples analyzed. This study has expanded on our earlier studies, with a larger number of farms, including swine farms, and a more detailed chemical analysis.

Materials and methods

Water sampling

Water used for watering animals was sampled on dairy and beef cattle, broiler chicken, laying hen and swine farms in northwest Croatia. 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 arriving at the lab, they underwent microbiological and physicochemical analysis. A total of 120 samples of water from different farms (65 chicken broiler and laying hen farms, 45 cattle farms, 10 swine farms) that were collected throughout 2016 and spring 2017 were analyzed. In this study, 72 samples were collected from wells, and the rest of the samples were from distribution systems.

Physicochemical analysis

Color

The color of samples was determined according to the HRN EN ISO 7887:2012 norm. The principle of the method is based on using an optical apparatus for comparison with hexachloroplatinate concentration at wavelength, λ=410 nm. The MPC laid down by Croatian water quality regulations (OG 125/2013, 141/2013, 128/2015) is 20 mg/LPt/Co scale.

Odor and taste

The determination of odor and taste was carried out according to the HRN EN 1622:2008 norm by using the short method, which is applicable when either a sample has no odor or taste, or for compliance of odor and taste with specified levels. Only microbiologically suitable samples were subjected to analysis. According to the regulations, water should be odorless and tasteless.

Hardness

Total permanent water hardness was calculated with the following formula:

TOTAL PERMANENT HARDNESS = CALCIUM HARDNESS + MAGNESIUM HARDNESS

Calcium and magnesium hardness is the concentration of calcium and magnesium ions determined by ion chromatography, expressed as the calcium carbonate equivalent. Thus, total permanent water hardness, expressed as the $CaCO_3$ equivalent, was calculated with the following formula:

$$[CaCO_3] = 2{,}5\ [Ca^{2+}] + 4{,}1\ [Mg^{2+}]$$

Although there are no guidelines, water with a hardness greater than 500 mg/L (as calcium carbonate) is considered very poor quality for water distribution systems and will be prone to scaling (Olkowski, 2009). For applications where water is heated and/or used for cleaning milk tanks, hardness should be less than 200 mg/L (as calcium carbonate).

pH value

The concentration of hydrogen ions or the pH value of water was determined potentiometrically, *i.e.* by measuring the pH value using a Mettler Toledo Meter SevenCompact S220 instrument. The pH meter and pH measurement procedures were carried out according to the HRN ISO 10523:2012 norm. The pH value should be in the range from 6.5 to 9.5 pH units.

Dissolved anions

Dissolved anions (chlorides, nitrites, nitrates, sulfates) were determined by ion-liquid chromatography according to the HRN EN ISO 10304-1:2009 norm. The method was set up on the DIONEX ion chromatography system with suppressed conductiometric detection. A solution of 4.5 mmol/L Na_2CO_3 and 1.4 mmol/L $NaHCO_3$ was used as an eluent at a flow rate of 1.2 mL/min. Separation was performed on a Dionex IonPac AS22 column, 4 × 250 mm, thermostated at 30 °C. All the samples were filtered through a membrane filter (0.45 μm pore size) prior to injection. The MPC for chloride is 250.0 mg/L, for nitrates 50.0 mg/L, for nitrites 0.50 mg/L, and for sulfates 250.0 mg/L.

Dissolved cations

Dissolved cations (sodium, ammonium, potassium, calcium and magnesium) were determined by ion-liquid chromatography according to the HRN EN ISO 14911:2001 norm. The method was set up on the DIONEX ion chromatography system with suppressed conductiometric detection. A solution of 20 mM methanesulfonic acid was used as an eluent at a flow rate of 1 mL/min. Separation was performed on a Dionex IonPac CS12 column, 4 × 250 mm, thermostated at 22 °C. All the samples were filtered through a membrane filter (0.45 μm pore size) prior to injection. According to the regulations, the MPCs are as follows: sodium 200.0 mg/L, ammonium 0.50 mg/L and potassium 12 mg/L. At present, there is no guideline for magnesium and calcium in drinking water. An upper limit of 300 to 400 mg/L has been suggested for magnesium for dairy cows. In the majority of situations, livestock should tolerate concentrations of calcium in water up to 1000 mg/L if calcium is the dominant cation and dietary phosphorus levels are adequate.

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-colored compound at pH 6.2 to 6.5. The color intensity is measured with a chlorine pocket meter HACH colorimeter. The MPC for free chlorine is 0.5 mg/L.

Iron

The method for determining iron in water was carried out according to the HRN ISO 6332:1998 norm 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. The MPC for iron is 200.0 μg/L.

Consumption of $KMnO_4$

The method for determining the 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 a strong oxidizing agent ($KMnO_4$). The consumption of $KMnO_4$ was determined in accordance with the HRN EN ISO 8467:2001 norm. The principle of the method is heating a sample in a boiling water bath with a specified amount of potassium permanganate and sulfuric acid for a fixed period of time (10 minutes), reducing part of the permanganate with oxidizable material, and determining the consumed permanganate with the addition of an excess of oxalate solution, followed by titration with permanganate. The MPC set for the consumption of $KMnO_4$ is 5.0 mg O_2/L.

Microbiological analysis

For the purpose of confirming and determining the total number of coliform bacteria and *Escherichia coli* bacteria in the water, the membrane filtration method was used according to the HRN EN ISO 9308-1:2014 standard procedure, while the HRN EN ISO 7899-2:2000 procedure was used to confirm and determine the number of intestinal enterococci. For the detection of the bacterial species *Pseudomonas aeruginosa*, a procedure was used based on the HRN EN ISO 16266:2008 norm. The detection and determination of the number of *Clostridium perfringens* spores were carried out after membrane filtration and anaerobic incubation at 44 °C for 24 hours using TSC agar (Tryptose Sulfite Cycloserine agar, Biokar, France) according to the ISO 14189:2013 norm. The HRN EN ISO 6222:2000 method was used to determine the number of microorganism colonies on the nutrient agar.

A sample search procedure was carried out in accordance with the requirements of the abovementioned standards by means of membrane filtration of 100 mL of the water samples through 0.45 μm pore size filters and incubation 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 *E. coli* grows as blue-green colonies on TBX agar, and indole is positive and oxidase negative. The intestinal enterococci hydrolyze esculin on bile-esculin-azide agar. The end product, 6,7-dihydroxycoumarin, combines with iron (III) ions to give a tan-colored to black compound which diffuses into the medium. *P. aeruginosa* is a microorganism that grows on a selective medium with cetrimide and produces pyocyanin. It is 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 water sample, yeast extract agar was applied and the plates were incubated at 22 °C for 68 hours and 36 °C for 44 hours. Coliform bacteria, intestinal enterococci, *Cl. perfringens*, *P. aeruginos* and *E. coli* must not be present in drinking water. The permissible level for microorganisms at 22 °C is 100, while for microorganisms at 36 °C it is 20 colonies.

Results

The percentage of microbiologically and chemically unsuitable samples among the 120 analyzed samples, of which 72 samples were from wells, are presented in Table 1. The results of unsuitable samples by individually analyzed parameter are shown in Table 2. All of the unsuitable samples came from wells, while all the samples from water distribution systems were suitable. Table 3 shows

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

Type of analysis	Analysed samples (*n*)	Unsuitable samples (*n*)	%
Microbiological	120	48	40.0
Chemical		45	37.5

Table 2. Unsuitable samples according to the exposure levels set out by Croatian water quality regulations (OG 125/2013, 141/2013, 128/2015) by analyzed parameter with minimum and maximum obtained values among the 120 analyzed samples

Parameter	Unsuitable samples (*n*)	%	Min-max values among 120 samples
Total coliforms	44	36.7	0-890 cfu/100 mL
Microorganism count at 36 °C	43	35.8	0-2000 cfu/mL
Escherichia coli	40	33.3	0-400 cfu/100 mL
Microorganism count at 22 °C	38	31.7	0-990 cfu/mL
Intestinal enterococci	37	30.8	0-300 cfu/100 mL
Nitrates	26	21.7	1.2-212.7 mg/L
Ammonium	12	10.0	<0.03-1.31 mg/L
Iron	12	10.0	<20 -9860 µg/L
Clostridium perfringens	9	7.5	0-47 cfu/100 mL
Pseudomonas aeruginosa	7	5.8	0-61 cfu/100 mL
Potassium	3	2.5	0.21- 101.9 mg/L
Free chlorine	2	1.7	<0.02-1.03 mg/L

Table 3. Unsuitable samples according to the exposure levels set out by Croatian water quality regulations (OG 125/2013, 141/2013, 128/2015) at different animal farms

Farms	Analyzed samples (n)	Unsuitable samples (n)	%
Chicken broiler and laying hens	65	25	38.5
Cattle	45	37	82.2
Swine	10	8	80

the percentage of unsuitable samples at different animal farms.

The present study shows that all of the analyzed microbiologically suitable samples were odorless and tasteless. No sample was found to be unsuitable within this parameter and the average pH value was 7.2. The hardness of the water in the analyzed samples ranged from moderately hard to very hard. Only a few samples (8) were moderately hard, with a mean concentration of hardness of 91.4 mg $CaCO_3$/L. A very large number of analyzed samples (54.2%) had a hardness greater than 180 mg $CaCO_3$/L, with a mean value of 285.5. The highest measured sulfate concentration was 105 mg/L, sodium 78.1, calcium 161.7 and magnesium 63.4 mg/L.

Discussion

Animals are very sensitive and demanding as far as the odor and taste of water are concerned. Odor is of the highest sanitary importance, since it is often the first obvious sign of contamination. Taste has a similar significance. The color of water does not have a hygienic meaning but can give water an unappealing look.

Water with a pH outside of the preferred range may cause non-specific effects related to digestive upsets, diarrhea, poor feed conversion, and reduced water and feed intake (Olkowski, 2009). The pH of water may impact on health in some animals more than others. For instance, in ruminants, consumption of water with a pH below 5.5 may contribute to metabolic acidosis, whereas alkaline water with a pH greater than 8.5 may result in a higher risk of metabolic alkalosis. In dairy cattle, these conditions have been associated with reduced milk yield and milk fat, low daily gains, increased susceptibility to infectious, metabolic disorders, and reduced fertility. In addition, water with a low pH can corrode equipment.

Water hardness is more important for equipment status due to the accumulation of insoluble calcium and magnesium carbonate deposits.

In natural water, increased amounts of organic matter determined as the consumption of $KMnO_4$ 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 by organic matter (Asaj, 1974). All tested samples were suitable within this parameter. Nalelitić et al. (2013) found that concentrations of the consumption of $KMnO_4$ were greater during warmer seasons, with values above those prescribed.

A high concentration of chlorides in the water supply has a corrosive action on the metal tubes of water supply systems (Naletilić et al., 2013) and causes a greater number of shell defects in chicken hens, as well as increased mortality and less dependability (Ostović et al., 2011). All tested samples were suitable within this parameter, the same as in the studies on turkey farms of Ostović et al. (2011), cattle and horse farms of Nalelitić et al. (2013), and our previous study on cattle farms (Denžić Lugomer et al., 2017). Higher levels of sulfur in drinking water can be tolerated by animals such as pigs or poultry, whereas relatively low levels can be detrimental to health and performance in cattle or sheep (Olkowski, 2009). Increased sulfate concentrations in water (>150 mg/L) cause a salty taste, diarrhea, and in some cases copper deficiency (Higgins et al., 2008). Increased sulfate concentrations stimulate the development of polioencephalomalacia, a neurological disorder characterized by weakness, muscle tremors, lethargy and even paralysis and death in cattle. The highest measured sulfate concentration was well below the prescribed 250 mg/L.

The determination of nitrogen in the form of ammonia, nitrates and nitrites is of great sanitary importance, since these compounds occur as a result of the decomposition of organic substances containing nitrogen, or of bacterial activity, or they are induced by chemical processes. Nitrites can cause methemoglobinuria, as can nitrates that reduce to nitrites in the alimentary system. It is also known that nitrates can produce nitrosamines which can modify certain DNA components and cause tumors (Nemčić-Jurec et al., 2009). Nitrate toxicity causes poor growth, thinness and poor coordination in poultry (Ostović et al., 2011).

Signs of lower levels of nitrate poisoning in cattle include inferior growth, infertility, abortion and vitamin A deficiency. In acute nitrate poisoning, there is breathing difficulty, a rapid pulse, foaming, convulsions, a 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 in 78.3% of the analyzed 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 as those in water sources. Nitrite concentrations in all the water samples were satisfactory. In a similar study conducted during the period June-December on a cattle farm, the nitrite concentration exceeded the MPC value in water only during the month of August (Nalelitić et al., 2013).

Of all the analyzed dissolved cations, ammonium, potassium and sodium are the only with set MPCs. The presence of ammonium in water indicates "fresh" pollution with organic matter and presents a danger to the users of such water. Water with a sodium level over 50 mg/L may affect the performance of poultry if the sulfate or chloride is high (50 mg/L, respectively 14 mg/L) (German et al., 2008). Water with over 800 mg sodium/L can cause diarrhea and a drop in milk production in dairy cows. Excessive intake of potassium salts suppresses the absorption of magnesium in ruminants (Olkowski, 2009). Magnesium is an essential nutrient required for numerous biochemical and physiological functions. A concentration of 6000 mg/L reduces growth and bone mineralization in immature chickens (Olkowski, 2009). In this study, concentrations of calcium and magnesium were far below the recommended limits.

Water with an increased concentration of iron has a negative influence on the organoleptic properties of the water, causes a bitter, oily and sour taste, and consequently reduces water intake. On the other hand, excessive iron intake may have harmful effects on the metabolism of several essential micronutrients, including copper, zinc, magnesium, manganese and calcium (Olkowski, 2009).

The microbiological composition of drinking water is responsible for the most common and easily visible water-related health problems. A risk to health is presented by both microorganisms and the toxins that often remain in water after the disappearance of microorganisms. The number of bacteria in a milliliter of water is most often determined as a sanitary indicator, while the total number of coliforms and intestinal enterococci is determined as the most common cause of infection, especially of the digestive system. Microbial contamination, besides its negative impact on animal health, is also reflected in their productivity, usually as a result of reduced weight gain. Our previous study of the health and safety of drinking water used in milk collection points in Bjelovar-Bilogora County showed that a very high percentage of samples (46.3%) were microbiologically unsuitable (Denžić et al., 2016). The results of this study showed that 72 samples (60.0%) were microbiologically suitable. Water contaminated with *E.coli* bacteria and intestinal enterococci is the most common evidence of fecal contamination, because they are part of the normal intestinal microflora of humans and warm-blooded animals (Duraković et al., 2002). These bacteria can survive in the environment for a long period of time and their concentration is so high that their presence can be easily proven even in very diluted samples. *Cl. perfrigens* can survive for an indefinite period because it creates spores in unfavorable conditions. Therefore, the presence of *Clostridium perfrigens* and *Pseudomonas aeruginosa* indicates contamination with fecal or wastewater over a longer period of time.

The application of disinfection agents to drinking water reduces the microbial risk but poses a chemical risk in the form of disinfection by-products. In most cases, chlorination is the primary method of disinfection, and chlorine and its compounds are the disinfectants most commonly used for water disinfection. Effective water disinfection is achieved when the correct level of free chlorine residual is present in the water. An irregular dosage of disinfectant can affect the taste of water to the extent that animals do not want to drink it, so analyzing water for free chlorine residues is of great interest. Some studies show over-chlorinated drinking water to be the cause of reproductive failure, an increased number of spontaneous abortions, return-to-oestrus cases, and percentage of stillbirths, and reduced farrowing and total number of piglets in gilts and sows (Tofant et al., 2010). In addition, adverse effects may be manifested in an increased percentage of death losses in all production categories, i.e., suckling, nursery and fattening pigs (Tofant et al., 2011). Inside drinker pipes, certain bacteria can form a biofilm, which helps them to maintain persistent infection and to tolerate the presence of antimicrobials. In order to prevent hydric infections, disinfection of drinker pipes should also be carried out (Pavičić et al., 2008). In the present study, almost all investigated samples (98.3%) were within the MPC for free chlorine, with a large number of samples with free chlorine concentrations below or very close to the detection limit of 0.02 mg/L, suggesting that adequate chlorination had not been achieved.

Conclusion

An analysis of the water supply on farms in northwest Croatia reveals a high percentage of chemically and microbiologically unsuitable water samples. The study showed that animals are given unsuitable water on as many as 58.3% 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 drinking water hygiene. The quality of 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 the wells. The laboratory test results only show the water status at the time when the sample was taken, so the true state of well water in terms of health and safety and changes to it can only be determined by more frequent analysis and appropriate supervision. As a solution, it is recommended to use public water supply systems for livestock watering, because the basic microbiological, physicochemical and organoleptic properties of the water are controlled regularly, thus making the water safer.

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Kvaliteta vode za napajanje na farmama sjeverozapadne Hrvatske

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U ovom radu ispitivana je kvaliteta vode koja se koristi za napajanje životinja na farmama sjeverozapadne Hrvatske. Ukupno je pretraženo 120 uzoraka s različitih farma (65 kokoši nesilica i brojlera, 45 goveda, 10 svinja) sakupljenih tijekom 2016. g. i proljeća 2017. g. Analizirani su sljedeći mikrobiološki parametri: ukupni koliformi, *Escherichia coli*, crijevni enterokoki, broj kolonija mikroorganizama na 36 °C i 22 °C, *Clostridium perfringens* i *Pseudomonas aeruginosa*. Od fizikalno-kemijskih parametara analizirani su: boja, okus, miris, pH, kloridi, sulfati, nitriti, nitrati, natrij, amonijak, kalij, kalcij, magnezij, slobodni klor, željezo i utrošak $KMnO_4$. Od 120 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 40,0%, kemijski 37,5% uzoraka, odnosno ukupno 58,3% uzoraka. S obzirom da su svi nesukladni uzorci podrijetlom bunarske vode, potrebne su češće kontrole kvalitete tih voda, odgovarajući nadzor i dezinfekcija.

Ključne riječi: *kvaliteta vode; farme; sjeverozapadna Hrvatska; napajanje*