Could the Miraculous Springs in the Pardubice Region Be Used as Drinking Water Sources?

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Abstract

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Selected springs in the Pardubice Region were repeatedly examined for analytical and microbiological indicators. Microbiological analyses included the determination of intestinal enterococci, coliform bacteria, *E. coli*, and the number of colonies cultivated at temperature form 22°C to 36°C. The analyses were carried out according to the relevant CSN ISO norms using the membrane filtration technique and also the alternative cultivation method Colilert®/Quanti-Tray 2000® quantification method using the defined substrate technology. Out of 11 wells and springs examined, only two satisfied the requirements for potable water with all indicators observed.

Keywords: coliform bacteria; *E. coli*; membrane filtration; Colilert®; potable water

Regardless of whether it arises from the underground or from the surface, water is a system which always supports the life of certain ubiquitous microorganisms. If it is to serve for human consumption, the assurance must be given that it poses no microbiological health risk. The basis of microbiological safety is identical for all waters they must not contain any indicators of faecal contamination. The hygienic requirements for water are stated in Act No. 258/2000 Coll., on the protection of public health. Hygienic requirements for the safety and purity of potable water are established by hygienic limits, which are governed by the implementing rules for the Act, regulation number 252/2004 Coll.

The Pardubice Region (Pardubický kraj) is an administrative unit of the Czech Republic, located mainly in the eastern part of its historical region of Bohemia with a small part in north-western Moravia. The regional central offices are in Pardubice, the Pardubice Region occupies an area of 4519 km^2 with a population of more than 505 000. The lowest point is the Elbe River near Kojice (200 m a.s.l.), the highest point is Králický Sněžník (1423 m a.s.l.)(http://www.pardubickykraj.cz). The goal of the study was to monitor the selected springs in the Pardubice Region and attempt to determine their microbiological and chemical parameters. There are legends about the miraculous strength and healing power of the majority of the monitored springs. In the past, chapels or columns with statues were built near them, most often dedicated to the Virgin Mary. At several locations, spas were built during the 18th and 19th centuries (Svatý Mikuláš, Klopoty, Končiny, Svatá Anna). To this day, the local

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inhabitants are accustomed to collect water from the springs and take it home, because they hope to consume high-quality water not subjected to the technological treatment that is necessary before it is distributed into the water supply network.

MATERIAL AND METHODS

Water was sampled in sterile glass tubes, transported to the laboratory within 12 h, and immediately subjected to microbiological and other analyses. The microbiological analysis included the determination of intestinal enterococci using membrane filtration technique on Slanetz-Bartley agar (Himedia, Mumbai, India) (CSN EN ISO 7899-2), and that of coliform bacteria and *E. coli* using the membrane filtration technique on Modified Tergitol 7 Agar (HiMedia, Mumbai, India) (CSN EN ISO 9308-1:2001). For the determination of coliform microorganisms, the CSN ISO 75 7837:2010 method was used as well which included the cultivation of membrane filters on Endo Agar with basic fuchsin (Himedia, Mumbai, India). Furthermore, *Escherichia coli* were determined by membrane filtration technique on the chromogenic medium Rapid' *E.coli* 2 Agar (Bio-Rad, Paris, France). Ten ml of the water sample was processed using the the membrane filtration technique and the filters were simultaneously placed on two agar plates in each method and incubated for 24 h at a temperature of 36°C.

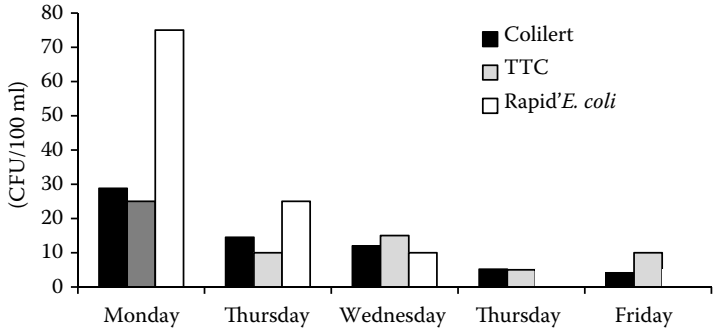
The samples were also processed using the Colilert®/Quanti-Tray 2000® (Idexx, Westbrook, USA) quantification method using defined substrate technology.

Likewise, heterotrophic plate count was also determined in the water samples (CSN EN ISO 6222:2000).

For the semi-quantitative determination of the selected chemical indicators, the following kits were used applying colorimetric methods or, if relevant, test strips and appropriate agents (Aquaquant kits, Merck KGaA, Darmstadt, Germany): determination of chlorides (catalogue number 114401), iron (cat. No. 114403), phosphates (cat. No. 114445), manganese (cat. No. 114406), ammonia (cat. No. 114428), sulphates (cat. No. 114411), nitrites (cat. no. 108025), nitrates (cat. No. 111170), and arsenic (cat. No. 117927). Calcium and magnesium contents (overall water hardness) were determined by titration with EDTA indicating eriochrome black T (CSN ISO 6059:1996). Some anions were also estimated quantitatively in order to make the analysis more precise: chlorides by volumetric analysis with $AgNO_3$ (CSN ISO 9297:1996), and nitrates spectrophotometrically after reaction with salicylic acid in an acidic environment (CSN ISO 7890-3:1995). In addition, CHOD (CSN EN ISO 8467:1997), pH (CSN ISO 10523:2010), and conductivity (CSN EN 27888:1996) (Table 2) were determined in the samples.

RESULTS AND DISCUSSION

The Čívice spring was selected to compare the capture of coliform bacteria using various methods, since on the basis of previous sampling the occurrence of coliform bacteria or *E. coli* was to be expected. The sampling was carried out over 5 working days during March 7–11, 2011, always at the same hour of the morning. The samples were processed using the membrane filtration technique and the filters were incubated on Modified Tergitol 7 Agar and Rapid'*E coli* 2 Agar media. Simultaneously, the samples were also processed using Colilert®/Quanti-Tray 2000® (Idexx, Westbrook, USA) quantification method employing a defined substrate technology (Figure 1). On some days, the number of coliform bacteria on the chromogenic medium Rapid' *E.coli* 2 Agar exceeded the number determined on Modified



The Čívice spring, 5 working days during 7 to 11 March 2011

Figure 1. Capture of coliform bacteria in particular detection systems

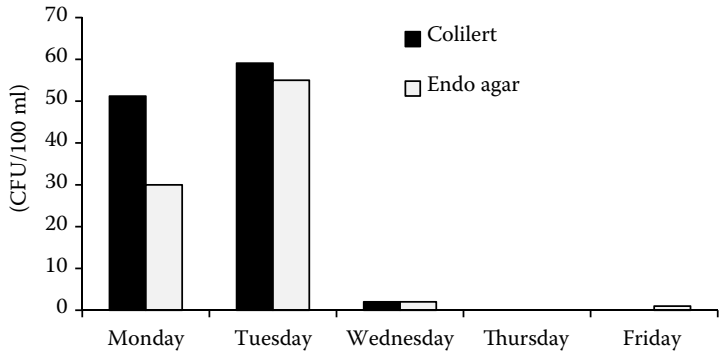


Figure 2. Comparison of coliform bacteria capture using Colilert system and Endo Agar

The Čívice spring, 5 working days during 4 to 8 April 2011

Tergitol 7 Agar and using the Colilert®/Quanti-Tray 2000® (Idexx, Westbrook, USA) method. Coliform bacteria form blue colonies on Rapid' *E.coli* 2 Agar. Reading is impeded by the growth of accompanying microflora, and hence it is necessary to examine every presumed colony. For the reasons of time saving and economy, however, it is not possible to isolate every suspicious colony and test it separately for the quantitative determination of *E. coli* in the surface waters with higher levels of faecal contamination (Baudišová 1998). One sample was thus subjected to detailed analysis, which resulted in the conclusion that blue-green and green colonies with blue centres were G+ catalase positive rods of the *Bacillus* family. Nonetheless, an undeniable advantage of these chromogenic media is the easy differentiation of *E. coli* (purple colonies) from the coliform bacteria (blue colonies). Byamukama *et al.* (2000) recommend Rapid' *E.coli* 2 Agar as a suitable alternative for the determination of coliform bacteria and *E. coli*. On the selectively diagnostic medium Modified Tergitol 7 Agar the accompanying microflora is also transformed, and furthermore the presumed yellow *E. coli* colonies and the orange coliform bacteria are difficult to differentiate. In addition, it is also necessary to assess the yellow sediment of the colony. Other studies have come to the same conclusions (Schets *et al.* 2002; Pitkänen *et al.* 2007; Maheux *et al.* 2008).

During April 4–8, 2011, the Čívice spring was again subjected to a week-long analysis. The numbers of coliform microbes determined on Endo Agar and by the Colilert®/Quanti-Tray 2000® method (Figure 2) were compared as well. Despite the fact that the results of the comparison of the two methods did not differ very much, it can be said that Endo Agar, like Modified Tergitol 7 Agar and the chromogenic medium Rapid'*E. coli* 2 Agar, struggles with deficiencies occurring in the problematic visual reading. The coliform bacteria and *E. coli* cannot be easily differentiated from the accompanying microflora without confirming tests.

Colilert®/Quanti-Tray 2000® method is suitable especially for quantitative determination. The advantage of this system is the reading of the results after as few as 18 h, and also the differentiation

Table 1. Geographic location of the studied springs

No.	Name of spring	GPS	District
1	Čívická studánka, Kokešov	50°1'20"N, 15°39'21.999"E	Pardubice
2	Pramen Svatý Mikuláš, Vraclav	49°58'15.998"N, 16°5'33.394"E	Ústí n. Orlicí
3	Studánka Sv. Anny, Nová Ves	49°52'14"N, 15°59'31.999"E	Chrudim
4	Hlubočská studánka, Hlubočice	49°51'47.39"N, 16°4'47.441"E	Chrudim
5	Pramen u kaple P. Marie v Končinách	49°54'11.369"N, 16°18'18.409"E	Svitavy
6	Pramen v Klopotech	49°59'46.155"N, 16°21'10.211"E	Ústí n. Orlicí
7	Javorka, Česká Třebová	49°53'50"N, 16°26'11"E	Ústí n. Orlicí
8	Pramen na Horách, Česká Třebová	49°53'38"N, 16°25'11"E	Ústí n. Orlicí
9	Pramen u Horákovy kaple, Dobrouč	49°58'52.999"N, 16°29'9.999"E	Ústí n. Orlicí
10	Mariánský pramen, Dolní Hedeč	50°4'26.999"N, 16°46'23"E	Ústí n. Orlicí
11	Pramen Panny Marie Pomocné, Horní Orlice	50°3'59.115"N, 16°46'45.386"E	Ústí n. Orlicí

between coliform bacteria and *Escherichia coli* without the necessity of further confirmation. This makes the, it is a method suitable especially for quick quality screening of individual water sources. Valente *et al*. (2010) established in their study that the Colilert®/Quanti-Tray 2000® method can show a higher value of coliform bacteria in comparison to lactose TTC agar. This was not confirmed by our study.

The water samples from 11 selected springs in the Pardubice Region (Table 1) were collected in autumn 2010 and spring 2011. The water from Čívice was also examined in April 2010.

From the 11 springs studied, only two satisfied the requirements for potable water with all indicators observed – the spring of Panna Maria na Horách chapel in Česká Třebová and the spring of Panny Marie Pomocné in Horní Orlice.

No other springs fulfilled the requirements due to the microbiological properties. In nine cases, the occurrence of coliform microorganisms that were considered indicative of faecal contamination were recorded together with *E. coli*. Nonetheless, several types of coliform microorganisms can occur saprophytically in these water types and their discovery does not necessarily represent an elevated risk of infection (Baudišová 2007). Říhová Ambrožová (2008) argues that coliform bacteria are not the final indicators of faecal contamination, since a number of them commonly occur in the environment. They are more suitable for assessing disinfection efficacy and are of little significance for non-disinfected water sources.

Very significant microbiological contamination with coliform microorganisms was found in the Hlubočice spring in the Chrudim District. The spring rises from a canyon-shaped argillite valley, with climatic factors influencing the quality of its water (e.g. during heavy rains and local floods, there is run-off to the valley of agricultural soil from the fields located further up). In three springs – the spring in Klopoty, the Čívice spring in Kokešov, and the Svatý Mikuláš spring in Vraclav – the occurrence of *Escherichia coli* was recorded in addition to coliform microorganisms. And in another three springs – the spring by Horák chapel in Dobrouč, Our Lady's spring in Dolní Hedeč, and the spring by Panna Maria chapel in Končiny – enterococci were recorded in addition to coliform bacteria. Without disinfection or technological modifications, this microbially contaminated water can be a source for a number of infectious diseases.

Table 2. Determined bacteriological and chemical indicators

Indicator (according to 252/2004 Sb.)	Limit	Type of limit
Sulphates (mg/l)	250	LV
Manganese (mg/l)	0.05	LV
Chloride (mg/l)	100	LV
Iron (mg/l)	0.2	LV
Ammonium (mg/l)	0.5	LV
Nitrites (mg/l)	0.5	MLV
Nitrates (mg/l)	50	MLV
Arsenic (mg/l)	0.01	MLV
Calcium and magnesium (mmol/l)	2–3.5	RV
Conductivity (mS/m)	125	LV
pH	6.5–9.5	LV
Chemical oxygen demand (CHOD) (mg/l)	3	LV
Intestinal enterococci (CFU/100 ml)	0	MLV
Escherichia coli (CFU/100 ml)	0	MLV
Coliform bacteria (CFU/100 ml)	0	LV
Heterothrophic plate count at 22°C (CFU/100 ml)	100 (500)	LV
Heterothropic plate count at 36°C (CFU/100 ml)	20 (100)	LV

CFU – colony forming units; LV – limit value; MLV – maximum limit value; RV – recommended value

From the chemical viewpoint, all wells and springs fulfilled the requirements of the regulation. Only in Svatá Anna spring in Štěpánov was registred a higher overall water hardness. This is connected with the location of the spring source, which is geologically part of the east Bohemian cretaceous plateau. In the case of this indicator, neither a higher values nor a lower one presents any risk. Conversely, for Our Lady's spring in Dolní Hedeč and Panny Marie Pomocné spring in Horní Orlice, a lower total hardness and lower pH values were established. This is connected with the character of water springing from the bedrock of the Orlice Mountains, and the lower pH value is applicable rather for controlling the treatment of potable water from the operational and technical points of view (Olmer & Kessl 1990).

However, not even the satisfactory results of the analyses conducted makes it possible to say with certainty that the water is potable in the long-term period. Water quality can change due to negative anthropogenic activities. The Čívice spring in Kokešov showed an above-limit quantity of coliform bacteria in the autumn and spring sampling. This is directly related to the extensive reconstruction of Pardubice-Opočínek warehouse centre, which was carried out in the period from September 2010 until January 2011. The centre is located in the immediate vicinity of the spring. As this spring is the one located closest to Pardubice, the greatest attention was given to it and the largest numbers of samplings and analyses were carried out on it. It was interesting to see that the number of coliform bacteria fluctuates depending on the day of the week. The highest numbers were repeatedly measured at the beginning of the week, the lowest oned on Fridays. As the warehouse centre is also supplied with water from this spring, the coliform bacteria can proliferate during the weekend when the consumption is minimal.

There exist a relatively very large number of springs in the Pardubice Region. The legends connected with the individual wells and springs arose at a time when people worked outdoors for the whole day in the fields and forests, and there were no other possibilities to refresh oneself but with water. Nowadays many miraculous springs no longer serve their original purposes and are not used anymore as sources of potable water.

Unless a spring is the only public source of potable water for a community (a public well), it is monitored neither by the community nor by hygienic services, and no one can guarantee the safety of its water. It is evident from this study that untreated drinking water from such springs can be very risky. Microbially contaminated water can cause a number of infectious diseases, especially diarrhoeal illnesses. Hence, only the consumption of potable water from the public water supply network can be recommended with certainty.

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