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Article

Drinking Water Quality and Occurrence of *Giardia* in Finnish Small Groundwater Supplies

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Abstract: The microbiological and chemical drinking water quality of 20 vulnerable Finnish small groundwater supplies was studied in relation to environmental risk factors associated with potential sources of contamination. The microbiological parameters analyzed included the following enteric pathogens: *Giardia* and *Cryptosporidium*, *Campylobacter* species, noroviruses, as well as indicator microbes (*Escherichia coli*, intestinal enterococci, coliform bacteria, *Clostridium perfringens*, *Aeromonas* spp. and heterotrophic bacteria). Chemical analyses included the determination of pH, conductivity, TOC, color, turbidity, and phosphorus, nitrate and nitrite nitrogen, iron, and manganese concentrations. *Giardia intestinalis* was detected from four of the water supplies, all of which had wastewater treatment activities in the neighborhood. Mesophilic *Aeromonas salmonicida*, coliform bacteria and *E. coli* were also detected. None of the samples were positive for both coliforms and *Giardia*. Low pH and high iron and manganese concentrations in some

samples compromised the water quality. *Giardia intestinalis* was isolated for the first time in Finland in groundwater wells of public water works. In Europe, small water supplies are of great importance since they serve a significant sector of the population. In our study, the presence of fecal indicator bacteria, *Aeromonas* and *Giardia* revealed surface water access to the wells and health risks associated with small water supplies.

Keywords: *Giardia*; groundwater quality; small water supply; surface water contamination

1. Introduction

Small drinking water supplies that provide water to small communities are an important public health issue because they are often vulnerable and may cause microbiological or chemical quality-associated health risks to the water consumers [1,2]. Even if the number of users in a supply is low, the total number of these supplies is high, and they often constitute the major water supplies, especially in rural areas. In 2001, there were 1359 water supplies in Finland, of which 61% distributed groundwater [3]. Estimates indicate that approximately 500 of these supplies serve fewer than 500 consumers in their distribution area and are, therefore, defined as small water supplies [4]. Most small water supplies are owned by cooperatives with operators who are often community members who work part-time, usually with no required professional training in the management of a water supply.

Small Finnish water supplies with fewer than 50 users (or with a water distribution of less than 10 m³ per day) are controlled by local health authorities according to the Decree of the Finnish Ministry of Social Affairs and Health [5] governing small water supplies. Supplies with more than 50 consumers are controlled on the basis of Finnish regulations governing the quality demands and surveillance of household water based on criteria stated in European Union drinking water directive 98/83/EC [6,7]. The microbiological and chemical quality of tap water is monitored in certain periods according to the number of consumers or the quantity of water distributed, but at least once every three years.

Many small European supplies have been found to have occasional contamination by surface water associated with increased coliform counts, indicating that they are at risk for fecal contamination [2,8]. However, usually the microbiological quality of groundwater supplies in Finland meets the quality criteria of the legislation, and most of the supplies distribute drinking water without disinfection treatment. Moreover, the chemical quality generally meets the criteria, but some chemical parameters, such as low pH and high iron and manganese concentrations, degrade the technical quality of the water in small private wells, as well as in some large water abstraction plants [9,10].

Occasional waterborne outbreaks have been reported annually in Finland. The National Institute for Health and Welfare has reported in 1998–2009 a total of 67 waterborne outbreaks, which caused illness for more than 27,000 people [11]. Most of these outbreaks originated from contaminated small groundwater supplies [12]. The most important microbial agents in the outbreaks have been noroviruses and *Campylobacter jejuni* [13]. No outbreaks associated with protozoan parasites *Giardia* or *Cryptosporidium* were reported in Finland prior to 2007 when sewage contaminated tap water in the

town of Nokia contained *Giardia* among other pathogens [14]. In a small number of the waterborne outbreaks, the causative agent remained unidentified [11].

The bedrock in Finland is dominated by Precambrian igneous rocks, the crust consisting mainly of plutonic and metamorphic rocks [15]. Although drilled wells utilizing deep groundwater flowing in the bedrock fractures are widely used in household water supply, the majority of municipal water supply relies on groundwater resources in shallow aquifers, *i.e.*, glaciofluvial and glacial deposits. Our aim was to study the microbiological quality, including the enteric pathogens (*Giardia* spp., *Cryptosporidium* spp., *Campylobacter* spp. and noroviruses), of small groundwater supplies and the association of microbiological quality with environmental risk factors that could increase the possibility of fecal contamination. In addition, we studied indicator bacteria and some chemical quality parameters of the small groundwater supplies. The supplies were selected on the basis of a recent history of coliform contamination or reported river or lake bank filtration, agricultural fields, household septic tanks or gravel mining pits close to the supply, potentially affecting groundwater quality in the studied shallow aquifers. The distance between the identified risk factors and the production wells ranged from less than 50 m to 200 m.

2. Results

2.1. Water Supplies

The characteristics of the 20 water supplies selected for the study are presented in Table 1. All studied water supplies were considered as small supplies since they served less than 500 inhabitants. The number of inhabitants in the area served by the water supplies varied from 38 to 450 (median 120 inhabitants). The average daily water intake was from 4 to 125 m³/d (median 30 m³/d) and the maximum water intake from 12 to 700 m³/d (median 138 m³/d). The majority of the studied wells derive groundwater from a shallow aquifer, the average thickness of a vadose zone being, in most cases, less than 5 m (Table 1). Only limited data on aquifer characteristics were available. These small groundwater supplies in Finland are prone to the surface water influence due to the insufficient depth of protective layers above the water table [2].

The water supplies were located around Finland and were regulated by the national Decree of the Finnish Ministry of Social Affairs and Health [5] when having fewer than 50 users or a water distribution of less than 10 m³ per day or by Finnish regulations based on European Union drinking water directive [6] when having more than 50 consumers or a water distribution of more than 10 m³ per day. Problems mentioned in the previous monitoring by the local public health protection authorities include the occasional detection of coliform bacteria (14 of the 19 water plants), low pH (11/19), and excess iron or manganese (7/19), or both. In six of the water supplies, the water was alkalized with either lye (NaOH), soda (Na₂CO₃), or limestone (CaCO₃), and in three supplies, excess iron or manganese was removed prior to distribution to consumers (Table 1). In three of the supplies, water was continuously disinfected with either UV light or sodium hypochlorite, in two supplies water was occasionally disinfected as reactive action to a contamination episode. In the majority of the water supplies (14/19), no disinfection procedure was performed. We have no information on the details of one supply.

Table 1. Characteristics of the studied water supplies.

Water Supply	Aquifer Type	Average Thickness of Vadose Zone (m)	Well Type ¹	Well Depth (m)	Well Maintenance ²	Water Intake Constructed	Main Risk Factor	Water Treatment ³
1	Glaciofluvial esker, unconfined, sand and gravel	7	Driven well	8	3	1998	Bank filtration	1
2	Glaciofluvial esker, semi-confined, silty till and sand	<5	Dug well	NA	3	1987	Bank filtration	1
3	Ablation moraine, semi-confined, sandy till	<5	Spring well	2	3	1963	Bank filtration	0
4	Glaciofluvial esker, unconfined, till and gravel	<5	Dug well	6	2	1978	Bank filtration	0
5	Deep bedrock aquifer, confined	NA	Drilled well	NA	1	1979	Agriculture, sewage	0
6	Glaciofluvial esker, confined, clay and sand	NA	Spring well	3	2	1960	Agriculture	0
7	Ablation moraine, semi-confined, sandy till	<5	Driven + dug well	185	3	NA	Agriculture	2
8	Glaciofluvial sand formation, confined, clay and sand	NA	Dug well	4	2	1988	Agriculture	3
9	Moraine, confined, clay and silty till	NA	Dug well	10	1	1983	Agriculture	3
10	Littoral sand, semi-confined, clay and till	NA	Dug + spring wells	3	3	1940	Agriculture	0
11	Glaciofluvial esker, unconfined, sand and silt	3	Driven well	7	2	1988	Agriculture	1
12	Glaciofluvial ice-marginal formation, semi-confined, sand and silt	3	Driven well	10	3	1986	Sewage	3

Table 1. Cont.

Water Supply	Aquifer Type	Average Thickness of Vadose Zone (m)	Well Type ¹	Well Depth (m)	Well Maintenance ²	Water Intake Constructed	Main Risk Factor	Water Treatment ³
13	Glaciofluvial interlobate formation, semi-confined, sand, gravel and till	NA	Dug well	3	1–2	1962	Sewage	0
14	Littoral sand, semi-confined, till and sand	2	Dug well	4	1	1978	Sewage	NA
15	Moraine, semiconfined, till and sand	3	Spring wells	5–6	3	1962	Sewage	0
16	Littoral sand, semi-confined, till and sand	1	Dug wells	2–4	1	1948	Gravel mining	0
17	Deep bedrock aquifer and moraine, confined, till and gravel	NA	Dug well	NA	3	1993	Gravel mining	1
18	Ablation moraine, semi-confined, sandy till and gravel	6	Spring well	4 *	1	1992	Gravel mining	1
19	Littoral sand, semi-confined, till and sand	<5	Dug wells	NA	1–2	NA	Surface water runoff	0
20	Moraine, confined, till and sand	NA	Spring well	NA	1–2	NA	Flooding	0

Notes: ¹ Well type “Spring well” is a shallow dug well installed in close vicinity to a spring, the groundwater level reaching the ground surface; ² Well maintenance categories based on information on well structures and piping plus the surroundings of the production wells: 1 = poor, 2 = moderate, 3 = good; ³ Treatment after sampling point before water distribution; 0 = no treatment, 1 = alkalization, 2 = reverse osmosis, 3 = disinfection. NA = not available. * Well is located in a pit where gravel layers have been removed during previous gravel mining.

The well maintenance category presented in the Table 1 is based on information gathered from the personnel of the water supplies through the questionnaire. The well maintenance category is based on estimated technical state of well structures, piping, as well as general groundwater protection measures in the surroundings of the production wells. Instead of exact locations of the drinking water supplies, we report, herein, the results using the anonymous water supply number codes 1–20. All the results exceeding the drinking water quality standards have been announced to the corresponding water supply operators and health protection authorities to enable their corrective actions.

2.2. Microbiological Quality

The results of the microbiological analyses appear in Table 2. *Giardia* cysts were detected in autumn in samples from four small groundwater supplies (Table 2). All were later identified as *Giardia intestinalis*. No *Cryptosporidium* oocysts, *Campylobacter* spp. or noroviruses were detected in any of the samples.

Table 2. Microbiological quality parameters of 20 small water supplies in spring and autumn 2005.

Water Supply	Risk Factor Category	Coliforms		<i>E. coli</i>		Intestinal Enterococci		HPC		<i>Aeromonas</i>		<i>Giardia</i>	
		CFUs/1000 mL		CFUs/1000 mL		CFUs/1000 mL		CFUs/mL		CFUs/1000 mL		cysts/100 L	
		Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
1	1	-	-	-	-	-	-	<1	<1	-	-	-	1
2	1	-	-	-	-	-	-	2	0	-	-	-	-
3	1	-	-	-	-	-	-	<1	<1	-	-	-	-
4	1	-	830	-	-	-	-	<1	18	-	9	-	-
5	2	-	-	-	-	-	-	3	14	-	12	-	1
6	2	-	10	-	-	-	-	<1	<1	1	-	-	-
7	2	-	-	-	-	-	-	1	<1	-	-	-	-
8	2	-	-	-	-	-	-	83	1	33	5	-	-
9	2	-	10	-	2	-	-	3	5	-	2	-	-
10	2	-	-	-	-	-	-	6	<1	-	-	-	-
11	2	-	-	-	-	-	-	<1	1	-	-	-	-
12	3	-	50	-	40	-	-	<1	8	-	-	-	-
13	3	-	-	-	-	-	-	3	0	15	3	-	1
14	3	-	-	-	-	-	4	296	10	-	-	-	2
15	3	-	70	-	-	-	5	30	25	5	-	-	-
16	4	-	-	-	-	-	-	267	1	-	-	-	-
17	4	-	-	-	-	-	-	14	8	-	-	-	-
18	4	-	-	-	-	-	2	200	214	-	-	-	-
19	5	-	-	-	-	-	-	4	8	-	-	-	-
20	5	-	150	-	-	-	-	<1	45	-	-	-	-

Notes: Recognized environmental risk factors: 1 = bank filtration, river or lake; 2 = agricultural load; 3 = wastewater treatment plant/household septic tanks <100 m; 4 = sand/gravel mining; 5 = multiple threats to contamination. HPC = heterotrophic plate count, - not detected/below the detection limit.

Low CFUs of coliform bacteria (10–150 CFUs/1000 mL) were found in five samples and a relatively high CFU (830 CFUs/1000 mL) in one sample. In the spring sampling, no coliforms were detected in any of the plants and the difference between the coliform counts at spring and autumn can be considered statistically significant ($p = 0.027$, Wilcoxon signed rank test). Low CFUs of *Escherichia coli* were found in two samples in autumn (2/1000 mL and 40/1000 mL), but in none of the samples in spring. Intestinal enterococci were found in three 1000-mL samples (2–5 CFUs/1000 mL), and no *Clostridium perfringens* was detected in any of the samples.

The CFUs of heterotrophic aerobic bacterial counts varied in the range of <1 to 300 CFUs/mL in spring and <1 to 200 CFUs/mL in autumn. No significant differences ($p = 0.795$, Wilcoxon signed rank test) in heterotrophic CFUs were detected between the spring and autumn samples.

Aeromonas spp. was detected in spring samples from four supplies and in autumn samples from five supplies; two of the positive wells were the same (Table 2). Further identification at the genospecies level showed that all *Aeromonas* species were mesophilic *A. salmonicida* [16].

2.3. Chemical Parameters

The chemical parameters appear in Table 3. Water temperature was significantly lower at the spring samples compared to the autumn samples ($p < 0.001$, Wilcoxon signed rank test). The pH of the wells studied varied from 5 to 7.6, the TOC varied from 0.5 to 11 mg/L, and no significant differences in the levels between the spring and autumn samples were detected (pH; $p = 0.657$ and TOC; $p = 0.159$, Wilcoxon signed rank test). Color, which is supposed to be about 5 mg Pt/mL, was high (20–40 mg Pt/mL) in two water supplies (14, 15) independent of the season (Table 3). In one of those supplies (14), turbidity was also significantly higher than in other samples. The iron concentrations were relatively high, ranging from 20 to 780 $\mu\text{g/L}$. The high concentrations were found mostly in the same wells both in spring and autumn. Manganese concentrations were clearly above the recommended limits in two wells (up to 180 $\mu\text{g/L}$), and the same wells had high concentrations in both seasons (Table 3). The concentration of nitrogen compounds was low in all samples: nitrate nitrogen varied from below 1 to 8.7 mg/L and nitrite nitrogen from below 0.01 to 0.02 mg/L.

2.4. Association of Recognized Risk Factors with Microbiological and Chemical Parameters

At one groundwater supply (15), where the septic tank of a household and a subterranean sand filter were located in close proximity (<100 and <500 m) to the well, TOC and color were high, and intestinal enterococci, coliform bacteria and *Aeromonas* spp. were detected. Intestinal enterococci and coliform bacteria were detected in autumn and *Aeromonas* spp. in spring. Conductivity was high in both seasons, and TOC, total phosphorus, color, and the nitrate nitrogen content were the highest of all the water supplies studied.

In one water supply (14), located in the middle of a small rural village and close to a common road, some of the study parameters showed increased values. A septic tank and a subsurface sand filter were located <100 m from the water supply. Intestinal enterococci and *Giardia* were detected in autumn, while TOC, color and turbidity values were high.

Table 3. Chemical quality parameters of 20 small water supplies in spring and autumn 2005.

Water Supply	T (°C)		pH		EC (µS/cm)		TOC (mg/L)		Color mgPt/mL		Turbidity (FTU)		P (µg/L)		Fe (µg/L)		Mn (µg/L)	
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
1	7.1	10.2	5.6	6.2	92	93	1.7	2.0	5	5	0.50	1.10	7	11	440	780	25	32
2	2.2	8.4	6.1	6.5	48	53	5.7	1.0	5	<5	1.05	1.33	16	18	120	170	11	8.2
3	4.8	6.5	6.2	6.3	41	46	1.2	1.0	<5	<5	0.16	0.15	28	27	<20	<20	<5	<5
4	0.7	17.8	6.9	7.6	44	95	1.8	4.1	15	10	0.15	0.15	8	12	55	110	<5	56
5	7.5	7.3	7.5	6.9	328	337	1.1	1.2	<5	5	0.22	0.08	8	13	26	23	25	28
6	7.9	8.1	7.5	7.6	391	421	0.9	1.0	<5	<5	0.06	0.52	23	24	<20	<20	130	180
7	6.6	8.0	6.5	7.0	259 *	ns	1.2	1.3	<5	<5	0.06	0.04	9	11	<20	53	12	12
8	7.8	6.5	6.6	ns	178 *	ns	3.7	3.3	5	<5	0.69	0.35	11	12	150	83	79	46
9	6.8	5.7	6.5	ns	215 *	ns	1.3	1.2	<5	<5	0.80	0.70	14	9	71	126	<5	7
10	3.5	7.9	5.6	6.0	ns	ns	1.1	0.8	<5	<5	0.04	0.02	15	16	<20	<20	<5	<5
11	5.6	5.7	6.0	5.9	46	153	1.4	1.4	<5	<5	0.94	0.30	13	7	390	240	59	51
12	5.0	7.0	ns	6.5	148 *	150	0.8	0.9	<5	<5	0.52	0.92	5	11	38	220	<5	<5
13	5.2	8.0	7.0	6.9	214	196	1.0	1.0	5	<5	0.39	0.26	10	15	35	31	<5	<5
14	5.4	8.5	ns	5.4	12	202	5.3	5.2	20	20	3.7	3.46	20	21	440	120	<5	<5
15	4.3	8.5	6.1	6.0	231	479	11.0	10.8	40	40	0.73	0.79	182	108	180	150	100	110
16	1.5	9.7	5.5	5.9	ns	30	3.5	2.8	10	5	0.15	0.12	8	9	<20	<20	<5	<5
17	3.3	7.4	7.2	6.3	150	148	1.7	1.2	<5	<5	0.20	0.24	15	15	<20	<20	<5	<5
18	4.7	6.9	6.2	6.3	81	79	0.5	0.6	<5	<5	0.16	0.16	21	12	<20	<20	<5	<5
19	4.0	6.1	6.3	5.1	173	ns	1.8	1.5	<5	<5	0.18	0.16	16	15	31	29	<5	<5
20	3.5	7.1	5.1	5.0	ns	ns	1.8	1.8	<5	<5	0.12	0.15	12	13	<20	21	<5	<5

Notes: EC = Electrical Conductivity; TOC = Total Organic Carbon; ns = not defined; * = measured only in laboratory.

Giardia intestinalis was also detected in a water supply (1) in which no indicator bacteria were detected. Of the chemical parameters, only iron was high. The recognized risk factor in the supply was bank filtration, floodwater also had access to the well, and a wastewater treatment system was situated at a distance of 200–500 m.

The *Giardia*-positive well (5) also tested positive for *A. salmonicida* in autumn. Of the chemical parameters, conductivity was high (337 $\mu\text{s}/\text{cm}$). The identified potentially contaminating risk factor was agricultural load, but a wastewater treatment system was also located 200–500 m, and a wastewater drain 100–200 m, from the well.

Giardia intestinalis was also detected in a well (13), where *Aeromonas* spp. was detected in both spring and autumn samples, though no other indicators or pathogens were detected. The principal recognized threat to water safety was waste water treatment, and indeed the septic tank of a household was situated 50–100 m from the plant. Surface water also had access to the well.

Coliforms (830/1000 mL) were detected in an autumn sample from a well (4) located on a narrow isthmus between two lakes where bank filtration is substantial. The difference in the water temperature of the well between spring and autumn samplings was marked and suggests the close impact of large water bodies of the nearby lakes.

In one water supply (12), coliforms (50/1000 mL) and *E. coli* (40/1000 mL) were detected in autumn, though UV light disinfection was in use after the point of sampling. A septic tank as well as an oil tank, gravel pit, and a cultivated field were situated <50 m from the well.

Some of the water quality parameters associated with the recognized environmental risk factor categories. Heterotrophic CFUs were significantly higher in the supplies at gravel mining category compared to the bank filtration category supplies ($p = 0.020$, Kruskal-Wallis test). Electrical conductivity and manganese concentrations were more elevated at supplies close to agricultural fields than supplies with other risk factors ($p = 0.005$ and 0.009 , respectively, Kruskal-Wallis test). Elevated water color and turbidity associated with supplies in close proximity of septic tanks ($p = 0.022$ – 0.023 , Kruskal-Wallis test). The iron concentration was lower at the supplies associated with gravel mining than supplies in other risk factor categories ($p = 0.005$, Kruskal-Wallis test).

2.5. Associations within Microbiological and Chemical Parameter Results at Spring and Autumn

Coliform bacteria, *E. coli*, intestinal enterococci and *Giardia* findings were occasional and originated solely from the autumn samples (Table 2). Physico-chemical parameters pH, TOC, color, turbidity, and the concentrations of phosphorus, iron, and manganese were more stable and showed significant relation between the spring and autumn samples in the correlation analysis (Spearman correlation coefficient, $r_s > 0.719$, $p = 0.001$).

As regards associations between the microbiological parameters, intestinal enterococci counts associated with heterotrophic CFUs ($r_s = 0.319$, $p = 0.045$) when all samples were considered ($n = 40$) in the Spearman correlation analysis. The associations between the chemical parameters were strong: TOC associated with color ($r_s = 0.693$, $p < 0.001$) and iron concentration ($r_s = 0.418$, $p = 0.007$) and the iron concentration was also associated with color ($r_s = 0.413$, $p = 0.008$) and turbidity of the water ($r_s = 0.727$, $p < 0.001$). Manganese concentration was associated with the electrical conductivity of the water ($r_s = 0.468$, $p = 0.008$).

Overall, the associations between microbiological and chemical parameters were rare. When analyzing the autumn samples ($n = 20$), there was a weak association between the water color and the detection of *Giardia* ($r_s = 0.463$, $p = 0.040$) and when all samples were considered, water temperature associated with the *Giardia* counts ($r_s = 0.377$, $p = 0.016$). Furthermore, *Aeromonas* counts were associated with the water pH ($r_s = 0.420$, $p = 0.011$) and electrical conductivity ($r_s = 0.438$, $p = 0.014$).

3. Discussion

The definition of a small drinking water supply varies in Europe and in the United States. In the USA, a small supply is one that supplies drinking water to fewer than 10,000 people. In the EU the DWD (Drinking Water Directive 98/83/EC) [7] does not require reporting from the supplies which either provide water to fewer than 50 users or which distribute less than 10 m³/d. The EU research program WEKNOW collected data from European small and very small water supplies and defined a small supply as one which provides water to fewer than 5000, but more than 50 people, and distributes 10–1000 m³/d [8]. The small water supplies in our study had 38 to 450 users and were thus not totally within any of the EU definitions mentioned above. Of the 20 Finnish small groundwater supplies studied, 4 contained *Giardia intestinalis* in autumn samples. The detected *Giardia* cyst counts were low (1–2 cysts/100 L). Due to the lack of exact recovery rates of the method used, we cannot rule out the possibility of false negative results in the *Giardia* and *Cryptosporidium* analysis. However, the detection of *Giardia* in these samples was proved and can be considered as a reliable result. No other studied enteric pathogens, *Campylobacter* spp. or noroviruses were detected. In addition, intestinal enterococci, coliforms and *E. coli* were detected in 8 of the 20 wells. All these results indicate that these water supplies are at increased risk for fecal contamination, thus extending the results of the studies of Corkal *et al.* [17] from Canada and of Rutter *et al.* [18] as well as those of Richardson *et al.* [1] from England and Wales. In our study, a trend emerged in which indicator organisms were detected more often in autumn samples than in spring samples, suggesting the impact of weather. Spring 2005 was dry, and occasional rain was common in autumn.

Giardiasis, as an endemic disease in Finland, has most likely been underestimated. Hörman *et al.* [19] estimated on the basis of their meta-analysis study that there could be as many as 4664 unregistered symptomatic *Giardia* cases per 100,000 general population compared to 5.38 registered symptomatic cases per 100,000 general population (ratio 1:867). In our previous studies, we identified *Giardia* cysts in Finnish surface water samples [20] as well as in municipal wastewater samples [21] and in tap water contaminated with sewage [14]. Our present study is the first Finnish study to identify *Giardia* cysts in a small drinking water supply. No data were available on potential asymptomatic carriers or symptomatic *Giardia* cases among the water users. If contaminated water caused the human illness, the number of patients was most likely so low that they remained undetected. The impact of small water supplies as source of *Giardia* infections in humans requires further study. *Giardia intestinalis* is a zoonotic pathogen, thus the contamination source of the supplies can be of either human or animal origin.

Only three of the water supplies distributed disinfected tap water, revealing that in most cases there were no preventive barriers between the aquifer and consumers' taps. The *Giardia* positive wells (Supplies 1, 5, 13, and 14) seem to be unprepared for the microbiological risk as there was no advanced water treatment or disinfection in place after the sampling point. The *E. coli* positive wells

were better prepared presumably due to the earlier noncompliance with the microbiological water quality standards as the supplies 9 and 12 had disinfection in use. Fecal contamination of drinking water increases the risk for enteric illness among users even if no such documented data are available from our study region. Small supplies have been shown to be frequently prone to fecal contamination in Finland [2,11] and elsewhere. Richardson *et al.* [1] analyzed the microbial quality of 11,233 private drinking water supplies within England and found that *E. coli* was detected in at least one sample from 32.4% of the water supplies. In accordance with the increased risk for fecal contamination of small water supplies, analysis of the distribution of waterborne outbreaks in England and Wales showed that small supplies were associated with 36% of all drinking water outbreaks even though they serve only 0.5% of the entire population [22]. The impact of small supplies on the disease burden of their users remains unknown. The life-long consumption of drinking water from a contaminated source could, on the other hand, also lead to acquired immunity [23,24].

In our study, all *Giardia*-positive wells were located near wastewater treatment activities; either a wastewater treatment plant for municipal human sewage was located at a distance of 200–500 m or the septic tank of a household was located within 50–100 m. One of the *Giardia*-positive wells (14) also had increased levels of multiple indicator parameters (intestinal enterococci, TOC, electrical conductivity, and turbidity), indicating surface water access to the well. However, neither *E. coli* nor *C. perfringens*, suggested as suitable indicators for *Giardia* and *Cryptosporidium* [25,26], were detected in the 100-mL samples. This well was known to be located in the middle of a rural village and close to a road.

One *Giardia*-positive well (1) had no increased levels of indicator bacteria or chemical indicators of surface water contamination even though a wastewater treatment system was located at a distance of 200–500 m and surface water was known to have access to the well. In two of the *Giardia*-positive wells, *A. salmonicida* was also detected, but not coliform bacteria. In another study, protozoa and total coliform levels were clearly correlated [27]. High turbidity (>1.0 FTU), which indicated surface water access to the groundwater source, was observed in three autumn samples, two of which were *Giardia*-positive.

The wells studied were known to be located where contamination sources such as roads, sewage treatment/treatment plants, and habitations were known to be rather close to the aquifer. Contamination by surface water after snow thawing or rainfalls was possible in most of the wells. Most of the water supplies were opened decades ago, when habitation and human activities around the wells were most likely much less than today.

Ten of the wells were located close to a river or lake, a common location for small aquifers in Finland because this kind of location will guarantee a consistent supply of water. The location may allow bank filtration of river or lake water into the well during the dry season, when the groundwater table is low. After a heavy rain, floodwater may also contaminate the well. Studies of past Finnish waterborne outbreaks have shown that some outbreaks were associated with groundwater wells located close to a river or lake [11]. All of the four plants with *Giardia* findings were located less than 50 m from a lake, river or ditch.

A. salmonicida was found in four samples in spring and in five samples in autumn. *A. salmonicida* is a common bacterium in natural waters as well as in well water, and has been previously isolated in Finnish groundwater wells [28,29] and elsewhere [30]. Its isolation in groundwater well may indicate surface water contamination. *A. salmonicida* was not connected to fecal contamination in a study by Hirotani *et al.* [31]. The pathogenicity of *Aeromonas* spp. as an enteric pathogen is not confirmed, and

mesophilic *A. salmonicida* in particular has been regarded as an environmental organism with low pathogenic potential for humans. In addition to indicating surface water contamination, its presence could be associated with increased heterotrophic plate counts [32].

In 14 of the water supplies examined, coliform bacteria had been detected in previous samplings, and *E. coli* had occasionally been detected in five of the wells. These wells placed consumers at increased risk for acquiring waterborne illness, especially because only four of them had undergone disinfection treatment with either UV light or hypochlorite solution before distribution. Two of these wells tested positive for *E. coli*, indicating fecal contamination. *E. coli* was detected in the 1000-mL sample, but not in the 100-mL, suggesting that the use of volumes larger than 100 mL would be more accurate in monitoring fecal contamination. Larger volumes have proved useful in tracking contamination sources associated with waterborne epidemics [33]. Both *E. coli*-positive wells were located in either a pit or flat ground. The other was located near (under 50 m) the septic tank of a household, and both were near a cultivated field and ditch.

Intestinal enterococci were found in three water supplies, and all of these wells were located within 100 m of the septic tank of a household. They were found in samples of either 500 mL or 1000 mL, but not 100 mL. These results again suggest that using larger volumes of water may more often facilitate the detection of indicators of contamination than do the 100-mL samples used in the EU Directive [7].

Our study supports previous findings showing that the pH of Finnish groundwater is typically low (<6.5) [9]. In approximately half of the groundwater wells monitored, pH was lower than recommended (pH 6.7). Low pH may cause the corrosion of iron water pipes. In the project questionnaire, 11 of 19 water supplies reported low pH as their problem. In six of the water supplies, the water was alkalized prior to distribution to consumers.

Another common problem in Finnish groundwater is its high content of iron and manganese [9]. Iron and manganese in excess decrease the usability of water as drinking water or for household use. In our survey, 20% of the water supplies contained excess iron (up to 780 µg/L, with a recommended maximum of 200 µg/L), and 25% of them contained excess manganese (up to 180 µg/L, with a recommended maximum of 50 µg/L). In the questionnaire, seven water supplies reported the problem. In only three of them, the iron and manganese were removed before distribution to consumers.

4. Experimental Section

4.1. Selection and Characterization of the Water Supplies

Water samples were collected from 20 small groundwater supplies around Finland in April and September–October 2005. The sampled supplies were selected based on a questionnaire answered for 248 small groundwater supplies owned mostly by cooperatives and operated by part-time working persons in the preceding year. Data on the water supply characteristics, microbiological quality and factors considered as potential fecal contamination threats of each water supply were obtained from the questionnaire and analyzed. The sites were selected according to these evaluations and the location of a water supply. The main selection criteria were the presence of an existing potential fecal contamination source in the neighborhood and the occasional detection of coliforms in the water quality compliance monitoring of the local health authorities.

4.2. Sampling and Analysis

Water samples were taken at a water supply from a tap or from a tap and well (if the tap was located after a collection tank), or only from a well with a submersible pump in the absence of a tap at the water plant. One sample was taken from the overflow of a well. The samples were taken prior to any potential treatments. For the microbiological analyses, all the equipment was disinfected before the sampling. The filters were removed from the taps, which were sterilized by flaming. The submersible pump was disinfected by submerging it in 10 mg/L hypochlorite solution for a minimum of 30 min. Each sample was taken with a new hose. Sterile containers were rinsed twice with sample water before sampling. For the *Giardia* and *Cryptosporidium* analyses, 100 L of water were filtered through an Envirochek® HV filter capsule (PALL Life Sciences, Port Washington, NY, USA). Other samples were taken with disinfected plastic containers and with a sterile glass bottle. Temperature, pH, and electrical conductivity were measured at the sampling site, and the evaluation of sensory qualities, such as odor, color, and turbidity, was performed repeatedly in a laboratory.

The water samples were refrigerated and transported to laboratories in Helsinki and Kuopio within 24 h and stored at refrigerated temperature prior to examination.

4.3. Detection of Enteric Pathogens

For the detection of *Giardia* spp. and *Cryptosporidium* spp., Envirochek® -concentrated samples were further treated according to the USEPA Method 1623 [34] as described by Rimhanen-Finne *et al.* [35]. In brief, for further concentration, the sample was first filtrated through a polycarbonate filter, which was then spooled with 10 mL of PBS-Tween20. The cysts and oocysts in the suspension were captured by using the immunomagnetic separation technique (Dynabeads® GC-Combo, Dynal Biotech ASA, Oslo, Norway). The final concentrate of 100 µL was divided into two portions, one of which was stored frozen at -20 °C for further molecular PCR and sequencing analyses. The other portion was immunostained using an Aqua-Glo G/C Direct Comprehensive Kit (Waterborne Inc., New Orleans, LA, USA). (Oo) cysts were counted under an epifluorescence microscope by using positive controls, *i.e.*, the cysts of *Giardia intestinalis* (H3 isolate, Waterborne Inc. New Orleans, LA, USA) and the oocysts of *Cryptosporidium parvum* (Iowa isolate, Waterborne Inc. New Orleans, USA) as described by Rimhanen-Finne *et al.* [35]. The positive control cysts and oocysts were stored at 4 °C. The numbers of purified cysts and oocysts was enumerated from five stock solution aliquots in hemocytometer resulting in a mean concentration of 1.1×10^6 cysts mL⁻¹ and 11.8×10^6 oocysts mL⁻¹ [35]. DNA from frozen water concentrate that tested positive for *Giardia* in microscopy was isolated through five rounds of the frozen-thaw procedure following DNA isolation [35]. *Giardia*-specific PCR was performed with glutamate dehydrogenase gene-targeted PCR using the primers GDH1 and GDH4 [36].

Thermophilic campylobacters were identified in 4000-mL samples using the ISO 17995 method [37] with Bolton enrichment (LabM, Lancashire, UK) and modified Charcoal Cefoperazone Deoxycholate Agar (Oxoid, Cambridge, UK) incubated in a microaerobic atmosphere.

Noroviruses were analyzed from a 1000-mL water sample that was concentrated by filtering it through a positively charged nylon membrane [38]. Norovirus detection was carried out using the RT-PCR method, and the result was confirmed with microplate hybridization [39].

4.4. Analyses of Indicator Bacteria

CFUs of *Escherichia coli* and coliform bacteria were analyzed from both 100- and 1000-mL samples according to the SFS 3016 standard [40] using m-Endo LES (Merck KGaA, Darmstadt, Germany) agar plates and membrane filtration. The heterotrophic plate count was determined according to the ISO 6222 standard [41] on tryptone-yeast agar (Oxoid) incubated at $(22 \pm 1)^\circ\text{C}$ for three days. The CFUs of intestinal enterococci were analyzed from 100-mL, 500-mL and 1000-mL samples according to the ISO 7899-2 standard [42] with membrane filtration and a Slanetz-Bartley medium (Oxoid).

Clostridium perfringens CFUs were counted in 100-mL and 1000-mL samples with the membrane filtration method on Tryptose-Sulphite-Cycloserine agar (Difco) using the ISO/CD 6461-2 method.

The CFUs of *Aeromonas* spp. were counted in 100-mL and 1000-mL samples by using the membrane filtration technique on ADA (Ampicillin-Dextrin Agar) plates with ampicillin as a selective substance [43]. After 24 h of incubation at 30°C , typical yellow colonies were subcultivated on blood agar for further identification. A total of 28 colonies from five *Aeromonas*-positive water supplies from autumn 2005 were further identified as *Aeromonas* spp. with API20 NE (bioMérieux, Marcy l'Etoile, France). For genospecies identification, the DNA of the colonies was isolated, and a fragment of the 16S rRNA gene was amplified with PCR according to the method described by Borrell *et al.* [16]. PCR products were digested with *AluI* and *MboI*. The pattern of fragments on agarose gels was compared with the results of Borrell *et al.* [16], and genospecies identification was performed on the basis of an RFLP pattern.

4.5. Chemical Analyses

Temperature, pH, and conductivity were measured on site using a YSI 556 MPS multiple parameter instrument or a pH/Cond 340i WTW-meter. Color was measured according to the ISO 7887-4 standard [44], and turbidity according to the ISO 7027 standard [45]. Iron and manganese concentrations were measured according to the SFS 5502 standard [46]. Nitrite nitrogen and nitrate nitrogen was measured using ISO 13395 standard method [47].

4.6. Statistical Analyses

Statistical analyses were performed using SPSS Statistics 22. For the results below the detection limit, half of the detection limit was used as a numerical value. The normality of microbiological and chemical parameter results from spring and autumn samples was tested using Shapiro-Wilk test and by visual evaluation of frequency distributions. Non-parametric methods were used, because normal distributions of the variables could not be obtained. Wilcoxon signed rank test was used to determine if there were statistically significant differences between results obtained from spring and autumn samples. The variation of the water quality results in the recognized environmental risk factor categories were tested with non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA) test. Spearman's correlation coefficients were used to display the relationships between the water quality parameters. Differences and correlations were considered statistically significant when $p < 0.05$.

5. Conclusions

We detected *Giardia intestinalis* for the first time in the Finnish groundwater supplies. *E. coli* and coliform bacteria, as well as intestinal enterococci were detected in some wells, which together with *Aeromonas* findings indicate surface water access and possible contamination from the surroundings to the wells. These findings suggest an increased health risk associated with small drinking water supplies, even though among public they are usually considered safe. In addition, high iron and manganese concentrations, and low pH, which have also been detected previously in Finnish groundwater, degraded the quality of drinking water.

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Author Contributions

Ilkka T. Miettinen, Marja-Liisa Hänninen, Matti Valve, Anna-Liisa Kivimäki and Kirsti Lahti conceived and designed the experiments; Tarja Pitkänen, Tiina Juselius and Eija Isomäki performed the experiments; Tarja Pitkänen, Tiina Juselius and Anna-Liisa Kivimäki analyzed the data; Matti Valve produced the graphics; Tarja Pitkänen, Tiina Juselius, Anna-Liisa Kivimäki and Marja-Liisa Hänninen wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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