MICROBIOLOGICAL EVALUATION OF DRINKING WATER FROM CENTRALIZED AND SMALL COMMUNITY SUPPLY SYSTEMS IN KAUNAS REGION, LITHUANIA

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Summary. The aim of this study was to evaluate microbiological quality of drinking water from centralized and small community supply systems in Kaunas region, Lithuania. In total 1345 samples of drinking water were analyzed from both centralist and individual water supplies in 2004 – 2006. The results of our study showed that 94.4% of tested drinking water samples from centralized drinking water supply systems is of high microbiological quality and fulfill requirements of drinking water standard HN 24:2003.

Drinking water from dug wells is more often contaminated and does not fit drinking water standard requirements. We found that contamination of drinking water by coliforms, enterococci and *E. coli* exceeded microbiological requirements in 12.8 %, 23.4% and 16.7 % of tested samples respectively. Maximum number of *E.coli* and faecal enterococci, 4.22±0.85 and 3.14±0.90 cfu/100ml respectively, in drinking water from centralist water supply was detected in summer. Maximum number of coliforms- 1.12±0.02 cfu/100ml, in spring. The highest number of coliforms (5.04±2.66 cfu/100ml) in drinking water samples from individual water supplies was detected in summer. Whereas the highest contamination by *E.coli* and faecal enterococci was detected in autumn, 8.96±3.23 cfu/100ml and 8.02±2.56 cfu/100ml, respectively.

Key words: drinking water, microbiology, safety.

CENTRALIZUOTAI IR INDIVIDUALIAI TIEKIAMO GERIAMOJO VANDENS MIKROBIOLOGINIŲ RODIKLIŲ ĮVERTINIMAS KAUNO REGIONE (LIETUVA)

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Santrauka. Tyrimų tikslas – įvertinti geriamojo vandens mikrobiologinį užterštumą bei mikrobiologinių rodiklių atitikimą Lietuvos higienos normatyvinius HN 24:2003 „Geriamojo vandens saugos ir kokybės reikalavimai“ reikalavimus. Geriamojo vandens mėginiai buvo imami iš Kauno apskrities ir tiriami Nacionalinės veterinarijos laboratorijos Kauno skyriuje 2004–2006 metais. Iš viso ištirta 1345 geriamojo vandens mėginiai iš centralizuotos ir individualių vandens tiekimo sistemų.

Remiantis tyrimų rezultatais, Lietuvoje centralizuota tiekimo sistema vartotojus pasiekiantis vanduo daugeliu atveju (94,4 proc.) atitinka HN 24:2003 normatyvinius reikalavimus. Daugiausia higienos normos HN 24:2003 reikalavimų pažeidimų nustatyta mėginiuose iš individualių vandens tiekimo sistemų, ypač šachtinių šulinių vandenyje, – koliforminių bakterijų skaičius nustatytą normą viršijo 12,8 proc. visų tirtų mėginių, žarninių enterokokų – 23,4 proc. ir *E. coli* – 16,7 proc.

Atlikus statistinę duomenų analizę nustatyta, kad geriamojo vandens, gaunamo iš centralizuotos ir individualių tiekimo sistemų, bakterinis užterštumas didėja šiltuoju metų laiku, t. y. vasarą paimtuose mėginiuose nustatytas didžiausias koliforminių bakterijų, *E. coli* ir žarninių enterokokų skaičius.

Raktažodžiai: geriamasis vanduo, vandens tiekimas, mikrobiologiniai rodikliai.

Introduction. Water is essential to sustain life and a satisfactory supply of drinking water must be available to all consumers (WHO, 2006). The risk of contamination of drinking water supplies with microbial pathogens is minimized by modern approaches of water management, but continues to be of the major public health concern. Environmental issues such as increasing salinity and global warming are likely to affect the sustainability of our current drinking water supplies and increase the threat of waterborne disease outbreaks (Leder at al., 2002). New technologies, use of alternative water sources, such as rainwater tanks, water reuse and restrictions will undoubtedly be part of the solution to our diminishing water resources, but have the potential to introduce new health threats (Leder at al., 2002).

Groundwater is the major source of the public water supply in Lithuania, therefore its protection is important both socially and economically. Data from recent years showed that nearly third of population used well- water in Lithuania (ref). The source of drinking water for 66 percent of population is centralist water supply (for 90–95% of townspeople and for 20–30% of country people). Animal waste from farms is the main pollution source of groundwater (Government news, 2006).

The new millennium represented a turning point, with the world population moving from rural to urban communities. In most developing cities (Mexico, Paris, Buenos Aires, Lima at al.) where groundwater is the major source of the public water supply, population growth precedes the development of an infrastructure capable of handling water and wastewater, which tends to lead to widespread contamination of the groundwater by domestic and industrial effluents (Mazari-Hiriart at al., 2005). Exposure to waterborne and foodborne pathogens can occur via drinking water (associated with fecal contamination), seafood (due to natural microbial hazards, toxins, or wastewater disposal) or fresh produce (irrigated or processed with contaminated water) (Rose at al., 2001). Nearly all studies as an indicator of faecal contamination of water used total coliforms, faecal coliforms or *E. coli*, measurments. Of these indicators *E. coli* are regarded as the most reliable measure of public health risks in drinking water (Wright at al., 2004).

Pathogenic bacteria like *Shigella* spp., *Leptospira* spp., *Salmonella paratyphi* B, *Vibrio, Staphylococcus aureus, Pseudomonas aeruginosa* and *Aeromonas* spp. can be isolated from drinking water samples (Ford, 1999; Rajendran at al., 2006; Levesque at al., 1994). A number of waterborne disease outbreaks of "unknown etiology" in the United States have been suspected to be caused by *Escherichia coli* and *Salmonella* serovar B (Goyal and Gerba, 1980). Epidemics of cholera have been reported from different parts of India. The outbreak was caused by *Vibrio cholerae* O1 isolated from municipal taps and wells (Sur at al., 2006). Diarrhea of unknown etiology was independently associated with drinking water from a household well contaminated with fecal enterococci and *Yersinia enterocolitica* (Eden, 1977; Borchardt at al, 2003). Outbreaks of waterborn *Campylobacter* infection are endemic in Northern Europe (Hänninen, 2003).

Waterborne pathogens, including a variety of viral, bacterial and protozoan agents, account for much of the estimated 4 billion cases and 2.5 million deaths from endemic diarrhoeal disease each year. Microbiologically contaminated water also contributes to the heavy burden of disease associated with cholera, typhoid, paratyphoid, hepatitis and gastroenteritis. Drinking water disinfection was shown to be an important public health protection measure. Low-income populations are particularly at risk of such diseases because of the lack of safe water and sanitation (Clasen at al., 2006; Akin at al., 1982).

Materials and methods. The samples of drinking water were tested at the National Veterinary Laboratory Kaunas branch. In total 1345 samples of drinking water from both centralist and individual water supplies were collected from Kaunas region and tested from August of 2004 till July of 2006 (Table 1). The samples of drinking water were tested by methods proposed by International Standardization Organization (ISO):

EN ISO 9308-1:2000 Water quality. Detection and enumeration of *Escherichia coli* and coliform bacteria. Part 1: Membrane filtration method.

LST EN ISO 7899-2:2000. Water quality. Detection and enumeration of intestinal enterococci. Part 2: Membrane filtration method.

LST EN ISO 6222:2001. Water quality. Enumeration of culturable micro-organisms. Colony count by inoculation in a nutrient agar culture medium.

Table 1. **Number of tested drinking water samples from different water supplies systems**

Water supply systems	Individual water supplies			Centralist water supply system
	Artezian boreholes	Groundwater boreholes	Wells	
Coliforms in 100 ml	31	80	40	1135
E. coli in 100 ml	32	85	49	1167
Faecal enterococci in 100 ml	31	87	48	1143
CFUN in 1ml 22^{0}C water	24	71	36	1074

Table 2. **Average of coliforms, *E. coli* and faecal enterococci in contaminated drinking water samples**

Water supply	Coliforms cfu/100 ml (X±mx)	*E. coli* cfu/100 ml (X±mx)	Faecal enterococci cfu/100 ml (X±mx)
Artesian boreholes	4.03±2.50	3.88±2.36	4.16±1.80
Groundwater boreholes	1.28±0.93	5.58±2.10	2.86±1.27
Shaft wells	3.73±1.87	4.82±1.99	5.58±2.39
Centralist water supply	0.63±0.15	2.52±0.32	2.10±0.32

Statistical data analysis was carried out using Microsoft Office Excel program The arithmetic mean value and standard deviation (X±mx) of variables, variance (σ) and coefficients of correlation (r) were calculated. The correlation was regarded to be statistically significant when $p \leq 0.01$.

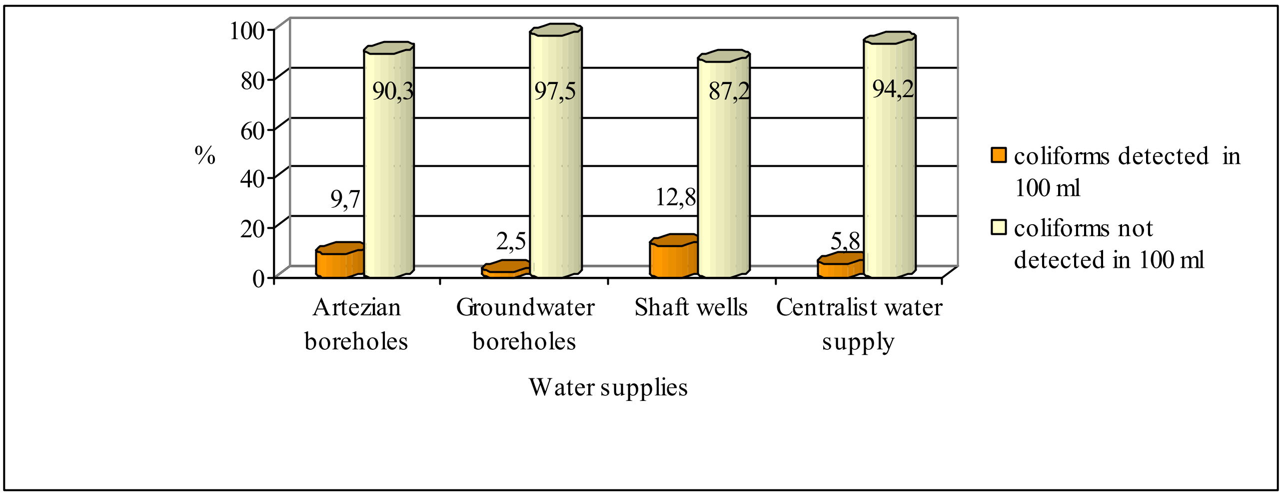

Figure 1. **Pollution by coliforms of drinking water from different water supplies**

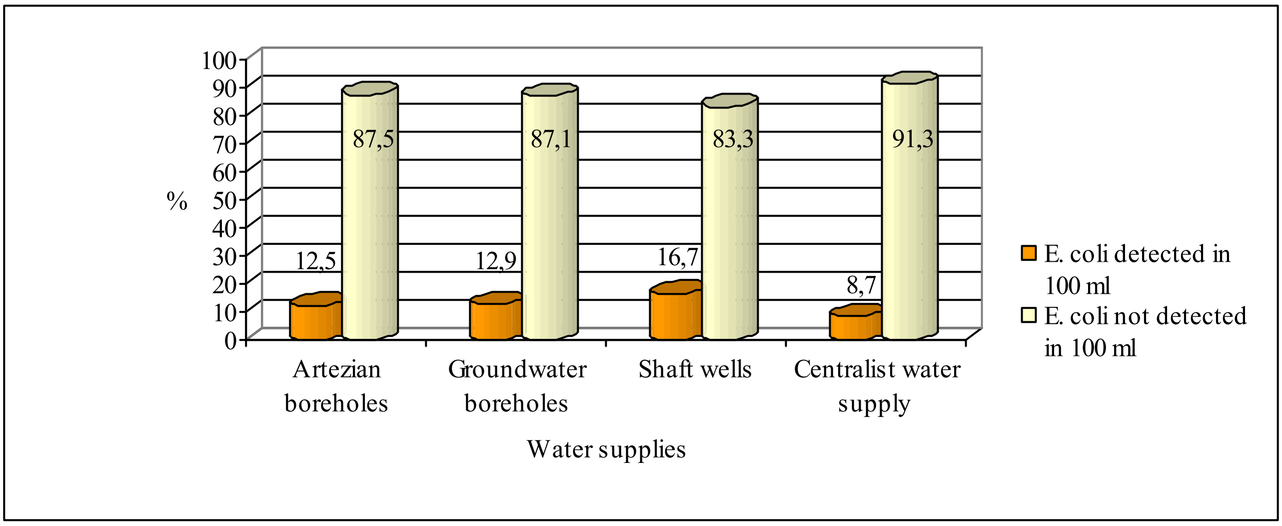

Figure 2. **Pollution of water from different water supplies by *E.coli***

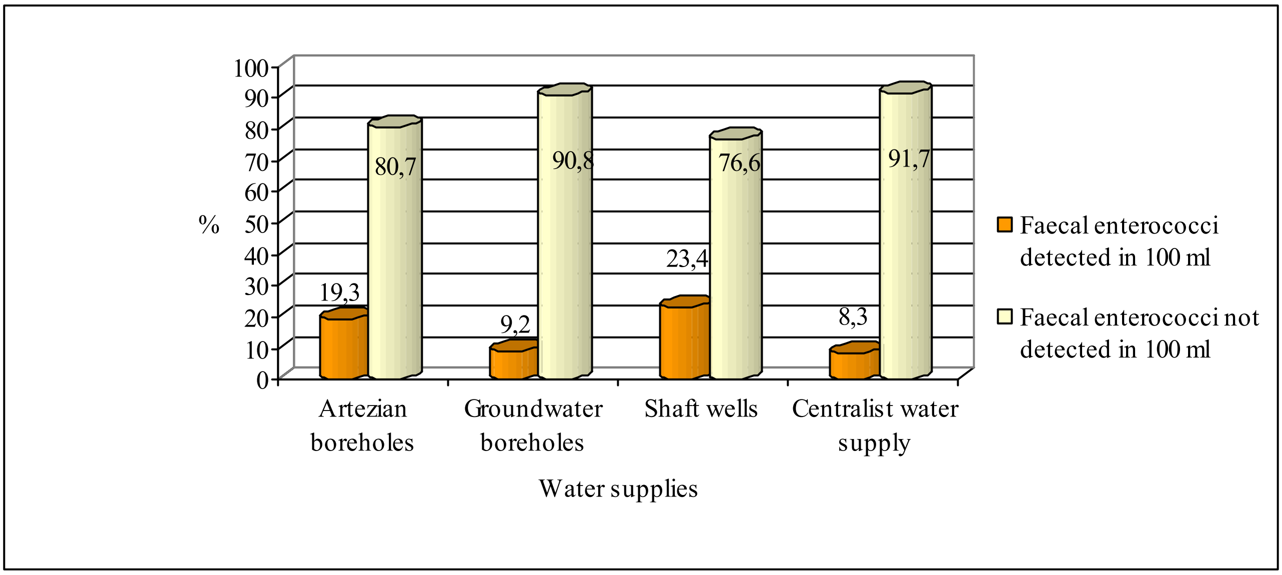

Figure 3. **Pollution of water from different water supplies by faecal enterococci**

Results. Coliforms were isolated from 12,8 %, 9,7 %, 5,8 % and 2,5 % of drinking water samples taken from wells, artesian boreholes, centralized water supply and ground water respectively.

E.coli were detected in 8.7 % of 1167 water samples from centralist water supply and in 16.7 % of 49 water samples from shaft wells. Of the 32 water samples from artezian boreholes tested 12.5 % contained these bacteria. *E.coli* were isolated from 12.9 % of 85 established water samples from groundwater boreholes, too (Figure 2).

Feacal enterococci were isolated from 23.4 % of 48 and from 8.3 % of 87 water samples from shaft wells and from groundwater boreholes, respectively (Figure 3).

Evaluation of averages of microbial rates and there biases from different water supplies showed that the average of coliforms was 0.63±0.15 cfu/100ml, of *E. coli* was 2.25±0.32 cfu/100ml and the average of feacal enterococci was 2.10±0.32 cfu/100ml. (Table 2).

The average of colony forming units number in samples of artezian and shaft wells water was 630.8±360.1 cfu/ml and 528.5±164.6 cfu/ml. In samples of groundwater boreholes water it was 203.0±64.3 CU/ml (Figure 4).

The study showed that the season had influence either on number of faecal enterococci, either on number of *E.coli* and coliforms. The average of faecal enterococci number in winter was 0.57±0.25 cfu/100ml, of *E.coli* number – 0.56±0.31 cfu/100ml, coliforms were not found. The average of *E.coli* and faecal enterococci number in summer was 4.22±0.85 and 3.14±0.90 cfu/100ml, the average of coliforms number in spring was 1.12±0.02 cfu/100ml (Figure 5).

Microbial rates of water samples from individual water supplies (artezian boreholes, groundwater boreholes, shaft wells) were variated in period of one year. Maximum average of coliforms number (5.04±2.66 cfu/100ml) was detected in summer. Variation graph of faecal enterococci and *E.coli* number averages was in parallel with variation graph of coliforms in period of year. Maximum number of bacterium was fixed in autumn, the average of faecal enterococci number was 8.02±2.56, of *E. coli* - 8.96±3.23 cfu/100ml (Figure 6).

Evaluation of correlation coefficients between microbial rates showed that in water of artezian boreholes after increasing of coliforms number, the number of faecal enterococci increases too ($r = 0.89$; $p < 0.01$). Highly positive ($r = 0.82$; $p < 0.01$) correlations of groundwater boreholes were observed between *E.coli* and faecal enterococci number. Positive correlation ($r = 0.63$; $p < 0.01$) of shaft wells was estimated between *E.coli* and faecal enterococci number, too (Figure 6). Medium positive ($r = 0.58$; $p < 0.01$) correlation of centralist water supply was observed between *E.coli* and faecal enterococci number.

Discussion. The results of our study showed that microbial contamination occurs in both centralist and individuals water supplies and water does not always fulfill requirements of drinking water standard HN 24:2003 “Drinking water safety and quality requirements” in Lithuania.

Comparing water from boreholes and centralized water supply the quality of boreholes water is worst, contaminated withcoliforms in 12.8 % samples of drinking water, enterococci in 23.4% and *E. coli* in 16.7 % respectively. As observed by Strauss at al., in 2001, in Canada, 20% of hoseholds sampled, had indicator bacteria (total coliform or *Escherichia coli*) above the current Canadian and United States standarts for safe drinking water (Strauss at al., 2001). Over 4 million Canadians receive their drinking water from private water supplies, however numerous studies report that these supplies often exceed the minimal acceptable standarts for contamination and that testing of such water was minimal and done less frequently than recomended by the provincial government (Jones at al., 2006).

Current investigation showed that water of boreholes is less polluted than wells- water, coliforms were isolated from much less water samples (2.5 percent). Feacal enterococci were isolated from 8.3 percent water samples from boreholes, it is three times less than isolated from wells- water. As reported by Egwari and Aboaba, in Nigeria, water of boreholes was less contaminated by these microorganisms then wells- water. Poor town planning, dilapidated infrastructure and indiscriminate siting of wells and boreholes contributed to the low bacteriological quality of domestic water supplies (Egwari and Aboaba, 2002).

On comparison water samples taken from centralized water supply were less contaminated than samples taken from individual water supplies that is *E. coli* was isolated from 8.7 percent water samples, two times less than from water samples from wells. Contamination of drinking water by *E. coli* can occur from many different sources. But more often *E. coli* occurrence in water is associated with failures during water treatment or disinfection operations (WHO, 2006). Levasque at al., in 1994, in Canada, found that 22 percent samples of tap water were contaminated by at least one coliform or indicator bacterium and/or at least one pathogenic bacterium (Levesque at al, 1994).

Evaluation of averages of microbial rates and there biases from different water supplies showed that water from centralist water supply was contaminated by minimal number of pathogens. The quality of water from individual water supplies was worse. However, after comparison of values of microbial rates odds, the odds of averages were not significantly different ($P>0.05$).

Examination of drinking water microbial rates showed that most water quality disturbances were in individual water supplies, however average of pathogenic bacterium number wasn‘t much different than number of pathogens in centralist water supply. Even so in some cases in water samples from individual water supplies it was observed extremely high number of pathogens, for example in three water samples the number of *E. coli* 18 times exceed settled average. It may cause the risk of waterborne contamination for consumers, because with lesser quantity of water the infective dose could enter into organism.

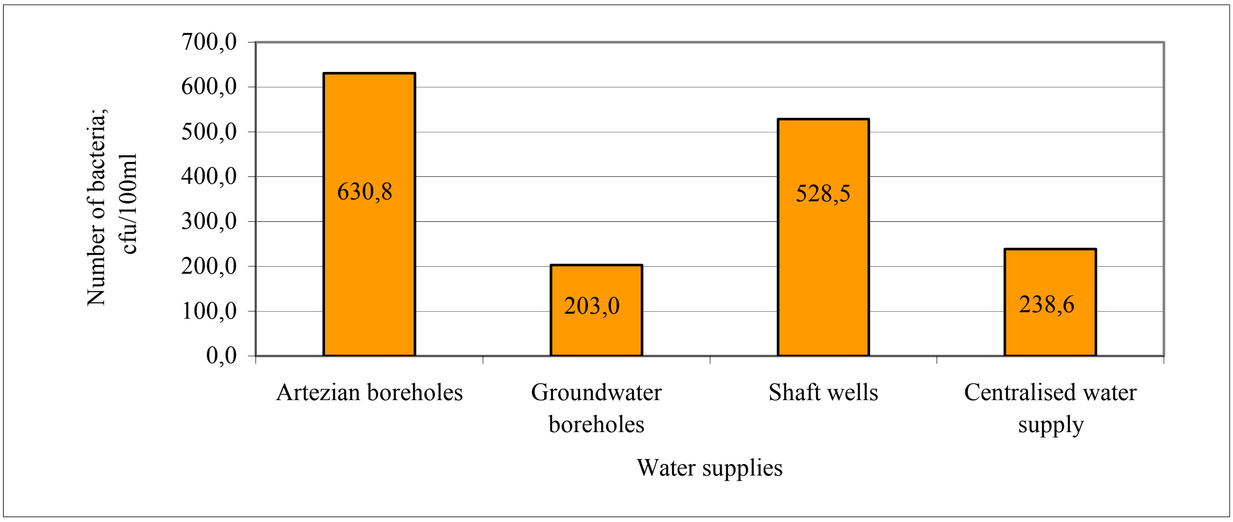

Figure 4. **Pollution of water by heterotrrophic microorganisms**

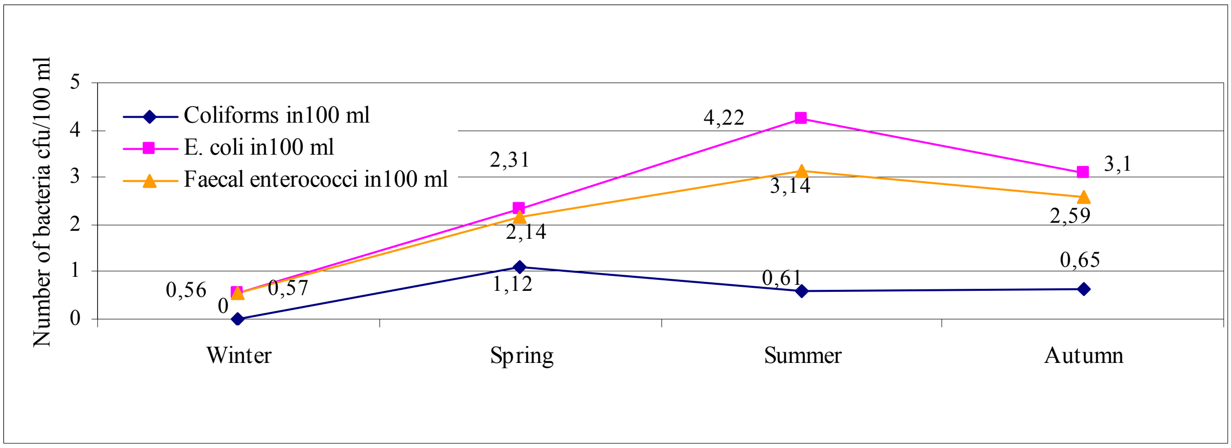

Figure 5. **The influence of the season on the microbiological quality of drinking water from centralist water supply**

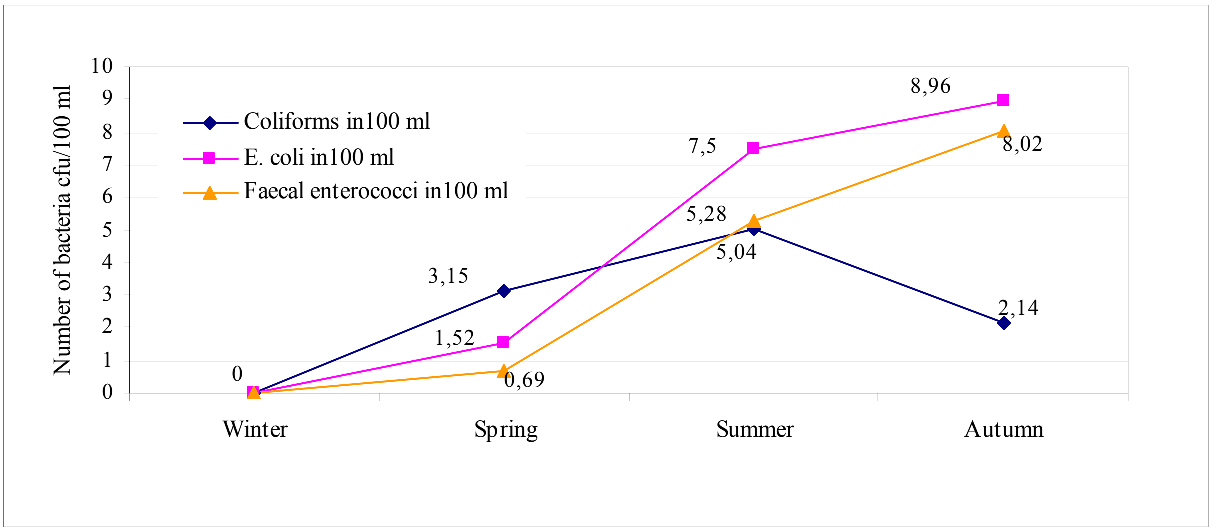

Figure 6. **The influence of the season on the microbiological quality of drinking water from individual water supplies**

Inpurity of water samples were valuated by colony forming units or evidence of heterotrophic micro-organisms. Maximum number of colony forming units was established in samples of artezian and shaft wells water, minimum – in boreholes water. Colony forming unit is important on valuation of desinfection efficiency, water supply condition, it is sensitive on breakdown of water- supply or after outside water passed into drinking water. Low colony forming unit indicate better quality of water, however European union directives doesn‘t refer norm of this unit. During evaluation of this unit it is important to value variation of this unit in certain period, and to take into account water sampling place, time and season (Sartory, 2004). At the time of current investigation it was lack of information about each consumer of water sample, place and condition of water supply and variation of this unit in certain period, therefore we inability to do steady conclusions on drinking water and water supply quality.

The study showed that there was a season variation of microbiological quality of water from centralist and individual water supplies. The season had influence either on number of faecal enterococci, either on number of *E.coli* and coliforms. Analysis of *E.coli* variation graph showed that the odds of averages between winter and spring ($P<0.001$), winter and summer ($P<0.001$) and winter and autumn ($P<0.005$) differ significantly. Variation graph of faecal enterococci was in parallel with *E.coli* variation graph in period of year, except the odds of averages reliability between winter and summer ($P<0.01$). Reliability of averages odds of coliforms number were established between winter and spring ($P<0.001$), winter and summer ($P<0.025$) and winter and autumn ($P<0.005$). The odds of averages between spring and summer ($P<0.05$) were reliable too. Analysis of water pollution results in period of one year showed that best quality of drinking water was in winter. In spring, summer and autumn quality of water was worst.

Maximum average of coliforms number during the year was fixed in summer. Graphics of faecal enterococci and *E.coli* variated in parallel with coliforms. Maximum number of microorganisms was fixed in autumn.

Analysis of variation graph of coliforms number averages in season showed that established odds of averages were not reliable. Analysis of variation graph of faecal enterococci revealed that the odds of averages between winter and summer ($P<0.025$), winter and autumn ($P<0.01$) and spring and autumn ($P<0.05$) were statistical reliable. Reliability of averages odds of *E.coli* number averages were established between winter and summer ($P<0.05$), winter and autumn ($P<0.05$) and spring and autumn ($P<0.01$). In the issue we can maintain that worst quality of water was in summer and autumn. The best quality of water from individual water supplies same as water from centralist water supply was in winter. As reported Nogueira at al., in 2003, in Brasil, the season had influence on number of faecal coliforms and total coliforms. They were established that in warm-weather period was highest percentage of contaminated samples; and in cold– weather period it was lower (Nogueira at al., 2003). Many of microorganisms are not cold resistant and surface outgoes doesn‘t permeate freezing ground (Cordy, 2001, LeChevallier, 2003).

Highly significant positive correlations were observed between *E.coli* and faecal enterococci number. Relation between these two microorganisms was detected because determination of indikator *E.coli* showes the presens of other pathogens in drinking water (Wright at al., 2004).

On purpose to keep good quality of centralist water supply water it is important to handle objects of water extraction and provision, to control condition of water quality and equipment, carry on a programm supervision of water. As far as possilble, water sources must be protected from contamination by human and animal waste, whitch can contain a variety of bacterial, viral and protozoan pathogens (WHO, 2006). An importance of water treatment (chlorination, sedimentation, filtration ozonation) was determined in 1985 (Payment at al., 1985). To accurately assess risks from waterborne disease, it is necessary to understand pathogen distribution and survival strategies within water distribution systems and to apply methodologies that can detect not only the presence, but also the viability and infectivity of the pathogen (Ford, 1999).

Recommendations are made for government actions that would increase the efficiency of efforts to ensure water quality; protect watersheds; strengthen waterborne disease surveillance; and protect the health of vulnerable populations (Gostin at al., 2000). Improved dissemination of information on water testing and various media pertaining to private water supplies in this population is needed (Jones at al., 2006).

Conclusions:

1. The results of our study showed that 94.4 % of drinking water from centralist drinking water supplies in Lithuania fulfill requirements of drinking water standard HN 24:2003 and is safe to drink.

2. Drinking water from dug wells is more often contaminated and does not fit always drinking water standard requirements. We found that contamination of drinking water by coliforms, enterococci and *E. coli* exceeded microbiological requirements in 12.8 %, 23.4% and 16.7 % of tested samples respectively.

3. Maximum number of *E.coli* and faecal enterococci, 4.22 ± 0.85 and 3.14 ± 0.90 cfu/100ml respectively, in drinking water from centralist water supply was fixed in summer. Maximum number of coliforms- 1.12 ± 0.02 cfu/100ml, in spring.

4. The highest number of coliforms (5.04 ± 2.66 cfu/100ml) in drinking water samples from individual water supplies was detected in summer. Whereas the highest contamination by *E.coli* and faecal enterococci was detected in autumn. 8.96 ± 3.23 cfu/100ml and 8.02 ± 2.56 cfu/100ml, respectively.

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