Ann Agric Environ Med 2008, 15, 199–203

ENTEROVIRUSES IN WATER ENVIRONMENT – A POTENTIAL THREAT TO PUBLIC HEALTH

Barbara Rajtar, Magdalena Majek, Łukasz Polański, Małgorzata Polz-Dacewicz

Department of Virology, Medical University of Lublin, Lublin, Poland

Rajtar B, Majek M, Polański Ł, Polz-Dacewicz M: Enteroviruses in water environment – a potential threat to public health. *Ann Agric Environ Med* 2008, **15**, 199–203.

Abstract: Enteroviruses belong to the Picornaviridae family and are the smallest, non-enveloped viruses known to infect both humans and animals. The spread of enteroviral infections is mainly by the faecal-oral and oral-oral route, but also through direct contact with secretions from ophthalmic and dermal lesions. Water, food and soil contaminated by infected feaces are an exogenous infection source which creates many opportunities for the transfer of the infection, and cause an epidemic outbreak in a short period of time. Enteroviruses are being isolated from all types of water: ground, sea, sewage and fresh water environments but also – and what is the most important from the epidemiological point of view – drinking water. They are resilient organisms, able to withstand high concentrations of sodium chloride (NaCl) and large changes in temperature. These abilities allow the viruses to flourish in a water environment, their natural reservoir. The number of infections in temperate climate regions peak in summer months and early autumn. Detection of enteroviruses in the water environment is performed by virus isolation in cell cultures and the use of molecular techniques. Many researches conducted in different countries with the use of methods mentioned above, reveal widespread environmental contamination by enteroviruses.

Address for correspondence: Dr. Barbara Rajtar, Department of Virology, Medical University of Lublin, W. Chodźki 1, 20-930 Lublin, Poland. E-mail: b.rajtar@am.lublin.pl

Key words: enteroviruses, epidemiology, water, laboratory techniques, public health.

INTRODUCTION

Enteric viruses – important causative agents of human diseases – transit easily to water environments due to varied human activity. They are usually present in insufficiently treated drinking water, ground water, rivers and seas. Impurities from human households are a main source of water contamination. Enteroviruses may cause a wide variety of pathological symptoms and enteroviral infections that affect especially young children. Enteroviral epidemics are predominantly waterborne; therefore water contamination poses an absolute threat to human health [28].

CHARACTERISTIC OF THE SPECIES

Human enteroviruses belong to the Picornaviridae family (pico = SMALL – RNA viruses), Enterovirus genus [5, 12, 13]. Traditional division organizes this taxonomic group into the subgenera polioviruses, coxackieviruses (group A, B), echoviruses and a group of enteroviruses marked according to their serotype number (66–71 and newly identified 73–75, 77, 78) [5, 20]. The group of polioviruses includes 3 different serotypes; type 1 and 3 are recognized as epidemic while type 2 as endemic. Type 1 is the cause of a particularly severe form of paralytic Polio infection [22]. Coxackieviruses are divided into 2 groups A and B. Group A includes 24 serological types, whereas B group comprises 6 serotypes [4, 20]. In 2003, the International Committee on Taxonomy of Viruses created a new taxonomy classification. Enteroviruses henceforth were divided into 5 groups of species based on their molecular properties (Tab. 1) [20].

Enteroviruses are icosahedral, non-enveloped and small – only 27–30 nm in diameter, particles. The genome is made of single stranded, positive polarity RNA molecule composed of 7.5 thousand base pairs. Replication of enteroviruses takes place in vertebrate's cell cytoplasm [20, 31].

Received: 31 August 2007
Accepted: 8 March 2008

Enteroviruses are highly tolerant, not only to residual chlorine from sewage treatment, but also salinity and temperature fluctuations. These properties significantly facilitate survival in the water environment, which is a main reservoir for these pathogens [13, 23]. These viruses can be easily eliminated in water temperature above 50°C, but they remain infectious in refrigerators and freezers [12, 31].

Enteroviruses are one of the most common causes of human infections [20]. The spread of infections is mainly by the faecal-oral and oral-oral route, but also through direct contact with secretions from ophthalmic and dermal lesions. Infection is transmitted by contact with water, food and ground contaminated with infected feaces [23]. It poses a threat of transmission of infection and even an epidemic outbreak [12, 31]. The average incubation period for enteroviral contagion is 3–10 days [4, 31]. Replication in intestinal lumen initiates when the pathogen has successfully penetrated via the oral route. If, however, replication conditions are restricted, the course of infection becomes asymptomatic. The virus, after breaking the gastrointestinal tract barrier, is transmitted via the blood stream to every organ of the body. Enteroviruses reveal tropism towards organs like the heart, skin, and in particular the central nervous system [4, 12, 31]. It has been proved that infected people excrete large quantities of the virus, amounting from 105–1011 parts per gram of stool, for a period of even 16 weeks [12, 13].

Enteroviral infections are characterized by high heterogeneity of clinical manifestations [5]. Poliovirus can be the cause of infections of either subclinical or severe course [31]. The most common and serious diseases are aseptic meningitis and poliomyelitis (infantile paralysis) [12]. Neuropathic changes evoked by poliovirus are associated with the direct, destructive effect on anterior horn cells. The spread of poliovirus infections has been considerably limited since the fifties through common preventive vaccination. Immunization was conducted with use of 2 vaccines: oral attenuated (Sabin) and inactivated (Salk). Salk is a trivalent inactivated polio vaccine (IPV) given by injection and stimulates serum IgM, IgG, and IgA, but not secretory IgA, immunity being induced by antibody transuding into the oropharynx.

Sabin trivalent oral live attenuated polio vaccine (OPV) is composed of 3 live attenuated strains of polioviruses – 1, 2, and 3 – grown in cell culture. Sabin vaccine is not only cheaper than Salk but also can be administered orally, and therefore stimulates production of local secretary IgA in addition to serum antibody production [16].

Coxackieviruses, depending on the group they belong to, vary in induced disease symptoms. Group A demonstrates strong organotropism toward muscle tissue, while group B is more likely to evoke brain tissue and parenchymal organ necrosis. Coxackie infections predominatly afflict the upper respiratory and gastrointestinal tracts, but also can produce more serious conditions such as insulin dependent diabetes and heart diseases [12, 14]. Other clinical syndromes caused by these pathogens are: meningitis, aseptic encephalitis, paralysis (A group – flaccid, B group – spastic), haemorrhagic conjunctivitis, herpangina, epidemic pleurodynia – Bornholm disease, enanthema and cutaneous eruptions [31].

Symptoms of echoviral infections are usually very similar to those produced by Polio or Coxackie viruses. Echoviruses are more likely to cause the common cold, but can also inflict serious conditions such as aseptic meningitis [5, 12, 22].

Serotypes of Enterovirus subgenera numbered from 68–71 are the least explored; however their contribution to causing bronchiolitis, conjunctivitis, meningitis and paralysis similar to poliomyelitis is broadly postulated [12].

Immunoglobulin production is one of the protective mechanisms generated by the diseased organism in order to neutralize and eliminate the virus. IgM antibodies can be present in blood serum after only 1week of infection and remain there for approximately 6 months, whereas IgG are detectable for more than 2 years. Nonetheless, the majority of enteroviral infections stimulate the development of acquired immunity for life [31].

PREVALENCE OF ENTEROVIRUSES IN AQUATIC ENVIRONMENTS

Enteroviral infections are the second most common viral infections, next to the Rhinoviruses which cause the common cold. It is estimated that in the United States alone 30–50 million new enteroviral infections occur a year, of which only 5–15 million are symptomatic. Severe CNS infections requiring hospitalization account for 30–50 thousand cases [13].

Enteroviruses are very resilient to the environmental conditions of the gastrointestinal tract – virions remain stable at pH 3–5 for 1–3 hours, are not susceptible to proteolytic enzymes nor to bile salts. Stability of the virus in external environmental conditions depends on the temperature, humidity and UV radiation. In order to inactivate 90% of salt-water dwelling polioviruses (PV), 671 days at a temperature of 4°C are required; at the temperature of 25°C that period is reduced to 25 days. In comparison, exposition to daylight for 24 hours has led to inactivation of 99.9% of PV [12, 18, 33]. The risk of viral infection via contaminated liquids is 10–10,000 times greater than through drinking water containing a similar numbers of bacteria of faecal origin [8, 12, 13, 15]. Due to the above-mentioned factors, the greatest numbers of enteroviral infections occur during the summer and early autumn months in the temperate climate regions; meanwhile, the tropical and subtropical areas maintain a constant level of infections throughout the year [12].

Due to the stability of the virion in the environment, multiple studies have been conducted in order to demonstrate the presence of enteroviruses in surface waters worldwide. A study conducted by Lodder *et al.* demonstrated that local sewage which is being discarded into the river Maas in

Table 1. Classification of human enteroviruses [15].

Groups of enteroviruses	Species of enteroviruses	Main pathological syndromes
HEV-A	Coxackievirus A2–8, 10, 12, 14, 16	herpangina, meningitis, hand, foot and mouth disease, Gianotti-Crosti-like eruption, acute bronchitis
	Enterovirus 71, 76, 89, 90, 91	hand, foot and mouth disease, poliomyelitis
HEV-B	Coxackievirus A9	herpangina, meningitis, hand, foot and mouth disease
	Coxackievirus B1-6	myocarditis, pleurodynia, meningitis
	Echovirus 1–7, 9, 11–21, 24–27, 29–33	meningitis, pleurodynia, exanthema, eruptive pseudoangiomatosis, vesicular stomatitis with exanthema
	Enterovirus 69, 73–75, 77–78, 79–88, 100–101	pharyngitis
HEV-C	Coxackievirus A1, 11, 13, 17–22, 24	common cold, acute epidemic haemorrhagic coniunctivitis, carditis, meningitis
	Poliovirus 1–3	acute paralytic poliomyelitis: vaccine-associated, acute poliomyelitis: unspecified, acute non-paralytic poliomyelitis
HEV-D	Enterovirus 68, 70	acute bronchitis, encephalitis, meningitis, acute haemorrhagic coniunctivitis

Holland has very high concentrations of enterovirus particles [24]. A different study of the waters of the Gulf of Mexico revealed the presence of Enteroviruses in 14% of water samples and in 72% of samples of the sea bed silt. Water wells in the USA were also examined. In wells located in 35 US states, over 30% of samples proved positive for Enteroviruses [1].

The contamination of both fresh and salt water environments with viral particles was reflected in a study of mussels tested for presence of enteroviral RNA. The study conducted in Holland returned 14 positive results out of 64 [25]. Not only mussels but any food that comes in contact with contaminated water and is improperly prepared can be a source of an epidemic outbreak.

Third world countries and areas undergoing recurrent flooding are at greatest risk of developing enteroviral epidemics. Poor sanitary conditions and overcrowding are also contributing factors. Studies conducted in South African countries and areas of Indonesia demonstrated that over 90% of children aged 5 have systemic antibodies proving contact with at least one enterovirus [11].

The largest European outbreak of enterovirus-related infections occurred in Minsk, Belarus, in 2003. The source of infection was established to be water contaminated mostly with ECHO 30, 6 and Coxackie B5 viruses. The number of hospital referred patients reached 1,300 [2].

The World Health Organization (WHO), in order to decrease the frequency of enteroviral infections, recommends basic hygiene techniques as the most important factor in the termination of the epidemic chain. Simple hand washing after contact with potentially contagious material practically eliminates the risk of oral transmission of enteroviruses. The use of chloride and iodine containing disinfectants effectively neutralizes viruses [32].

A separate article should be written on the epidemiology of Polioviruses, but the fact that these viruses belong to the genus Enterovirus, justifies a short note on this topic in this paper.

Poliovirus type 3 is the most common strain in the world. It is endemic to Nigeria, with 2 small foci in northern India, southern Afghanistan and Pakistan. Type 1 of PV is endemic to northern Nigeria where it coexists with PV type 3. Since February 2006, the list of countries with endemic presence of wild poliovirus strains comprises 4 countries: Afghanistan, with 9 confirmed cases of polio in 2005, India – 66 cases, Nigeria – 799 and Pakistan – with 28 cases. It is a disturbing fact that infection is occurring in countries so far free of wild PV strains. Since July 2005, new infections were identified in Angola, Bangladesh, Chad, Ethiopia, Indonesia, Nepal, Somalia and Yemen. The greatest epidemiological threat worldwide comes from Nigeria. It has been confirmed, that 94% of global PV infections have been caused by a virus originating from that area. In Poland, the last isolation of wild PV took place in 1982 and 1984 [9, 10].

The most effective way of dealing with polio transmission is a worldwide programme of vaccinations. According to WHO reports and predictions from the late XX century, PV was to be entirely eliminated by the year 2005. This goal so far has not yet been achieved – lack of funds and international agreement issues are the most important factors in the above failure. WHO estimates that in the year 2004 only 80% of children worldwide were administered with 3 doses of OPV3 (oral polio vaccine 3) with large regional differences – in Europe almost 94% of children were vaccinated, while in countries where polio is still endemic the percentage of immunized children varied from 39% in Nigeria to 70% in India. However, a noteworthy success is the elimination of wild polio virus from the western hemisphere, countries of the Western Pacific and Europe, as well as a significant decrease in cases of acute flaccid paralysis – AFP – the most severe complication of poliomyelitis [9]. Areas of low percentage of oral vaccination and populations' immunity to PV, poor sanitation, great concentrations of humans, warm and humid climate, create a new threat. Widespread research among patients with

AFP, conducted in the years 1998–2002, has shown that all isolated polioviruses were derivatives of the attenuated strain [17, 30]. The so-called circulating Vaccine-Derived Polio Viruses – cVDPVs are an increasing problem. They originate from attenuated strains used in vaccines and occurs due to loss of main mutations that cause inactivation of the virion. The majority of cVDPVs are recombinants of the Sabin strain and Human Enterovirus C-HEV C. cVDPVs are hazardous because they can cause fully symptomatic poliomyelitis. The largest numbers of the paralytic form of polio infection related with cVDPVs were noted in 2005 on the island of Madura, Indonesia. 46 cases were reported during that outbreak [9, 10].

METHODS OF DETECTION OF ENTEROVIRUSES IN WATER ENVIRONMENT

Enteroviruses are isolated from different kinds of aquatic environments such as seas, rivers, streams, drinking water, ground water, and sewage. The process of virological reservoir control comprises of the following steps: water sample concentration and purification, then virus isolation on cell cultures or detection with the use of molecular techniques.

The amount of enteroviral particles in natural environment is so minimal that before each detection procedure large quantities of water must be condensed [11, 24]. For this purpose various filters and filtering techniques are used. Due to the small proportions of viruses, filtration based on absorption-elution rather than mechanical methods is employed. These techniques use special filters with an adequate electrical charge. After percolation, the virus particles are adsorbed on filters, which are then rinsed in order to remove viruses from the filter, and the process of identification, based on cultural and molecular methods, begins [19, 27].

PCR (Polymerase Chain Reaction) method and cell cultures are used to isolate and identify viruses in condensed and purified water. The procedure of enteroviruses insulation is based on a wide variety of cell culture systems, such as Buffalo green monkey kidney cell culture (BGM), rhabdomyosarcoma cell culture (RD), human colonic carcinoma cell line (CaCo2), human hepatoma cell line (PLC/PRF/5) [7, 11, 23, 26]. When selected lines are infected, the presence of cytopathogenic effect (CPE) – that is, damaged and dead cells, is assessed [12]. Ehlers *et al.* state that in spite of recent developments in the technology of molecular detection that made it possible to perform quick and more sensitive non-culture-based diagnostic procedures such as RT-PCR with nucleic acid hybridization, the golden standard in diagnosis of enteroviral infections still remains virus isolation on cell lines [6, 11].

Molecular methods utilized for detection of enteroviruses, both in clinical and environmental trials, became popular in the nineties. PCR technique are based on the disclosure of highly conservative and homologous for the Enterovirus genus parts of viral genome. Amplified virus genetic material can be extracted either directly from water samples or infected cell lines [7]. Various methods are employed in nucleic acids detection. One of them depends on nucleic acids extraction with guanidine izothiocyanate, immobilization in a silicone column, and washing out with nonorganic solvents [24]. Another method uses columns filled with fibreglass which, with assistance of chaotropic salts, bind specific nucleic acids [7].

Sample analysis based on PCR methods definitely preponderates over researches using cell cultures only. PCR technique has proved to be especially quick, specific and sensitive in the diagnosis of enteroviral infections [29]. By using different primers, this method allows detection of defined groups of viruses or even specific types and serotypes [19]. PCR procedure, due to its high sensitivity, is a reliable mode of localizing enteroviruses in samples despite small quantities, even if the virus is not inducing CPE [23]. This low limit of detection of the method poses a threat of noncontiguous particles participating in the process of amplification, which can easily falsify results [7]. The traditional method of PCR hinders the estimation of the exact amount of viruses in a tested sample. For that reason, few variants of PCR technique were developed: “nested PCR”, “multiplex PCR” and “real-time PCR”. These procedures, of enhanced sensitivity and specificity, enable the performance of quantative determination of viral genetic material.

In “nested PCR”, 2 pairs of primers are used: external, which is complementary to ends of quested sequence, and internal which are attached closer to the centre of the amplified part of DNA. After the first reaction, the next is immediately performed in the course of the chain reaction, with usage of products of the previous reaction, which increases sensitivity and specificity of the method [11, 19].

“Multiplex PCR” allows simultaneous usage of a few pairs of primers in the reaction compound for identification of different virus types. Reaction products must have varied lengths in order to be able to differentiate them. “Multiplex PCR” is considered to be a very useful diagnostic technique due to the fact that only small quantities of material are required, and the result can be obtained in only few hours [3, 12].

Quantative DNA assay is performed by means of “real-time PCR”. This system enables synchronized monitoring of the reaction during the time it runs. This is performed due to the presence of the additional element in reaction compound. This element is marked with fluorochromium and a quencher probe complementary to the quested sequence. By extending the number of PCR products detected, the fluorescence rate increases and is presented diagrammatically on the computer. Gregory *et al.* [13] proved the effectiveness of the procedure in the control of enteroviral water contamination.

Compared to cell cultures, PCR technique considerably increases the effectiveness of determining enteroviral inherence in water samples, but it still does not give direct

information about the contagiosity of pathogens. Methods of integrated cell cultures and PCR have been devised, based on the hypothesis that after viruses are internalized into cell lines, only the contagious particles will multiply. After an adequate incubation period, viral genetic material is sampled from cell cultures and tested with PCR to estimate the presence of enteroviruses. This particular method is also recommended to detect pathogens that proliferate on cell lines without triggering CPE effect [12, 19, 21].

CONCLUSION

Virological controls of reservoirs and water intakes are not a routine procedure in water purity testing. Enteroviruses are one of the most common causes of infections in human population. Wider and easier access to methods allowing isolation and identification of these microorganisms in water could be helpful in reducing the risk of waterborne epidemic outbreak. Research performed in many countries with the use of cell culture techniques and molecular methods directed at detection of enteroviruses in water environment, report widespread environmental contamination with these pathogens.

REFERENCES

1. Abbaszadegan M, Stewart P, LeChevallier M: A strategy for detection of viruses in groundwater by PCR. *Appl Environ Microbiol* 1999, **65**, 444-449.
2. Amvrosieva TV, Paklonskaya NV, Biazruchka AA, Kazinetz ON, Bohush ZF, Fisenko EG: Enteroviral infection outbreak in the Republic of Belarus: principal characteristics and phylogenetic analysis of etiological agents. *Cent Eur J Public Health* 2006, **14**, 67-73.
3. Bednarska A, Podlasin R, Karczewski G, Łoch T, Horban A, Radkowski M: Ocena użyteczności metody multiplex PCR w diagnostyce zmian w ośrodkowym układzie nerwowym pacjentów zakażonych HIV i chorych na AIDS. *Przegl Epidemiol* 2005, **59**, 873-879.
4. Bennett NJ: Enteroviral infections. Available from: http://www.emedicine.com/med/topic688.htm.
5. Bernit E, de Lamballerie X, Zandotti C, Berger P, Veit V, Schleinitz N, de Micco P, Harle JR, Charrel RN: Prospective investigation of a large outbreak of meningitis due to echovirus 30 during summer 2000 in Marseilles, France. *Medicine (Baltimore)* 2004, **83**, 245-253.
6. Binduga-Gajewska I, Gut W: Diagnostyka zakażeń enterowirusami niepoliomyelitycznymi – aktualne kierunki i problemy. *Post Mikrobiol* 2002, **41**, 367-381.
7. Binduga-Gajewska I, Gut W, Wielkopolska A, Jarząbek Z: Zastosowanie metody RT-PCR i nested-PCR do wykrywania zakażeń enterowirusami. *Med Dośw Mikrobiol* 1999, **51**, 375-381.
8. Bosch, A: Human enteric viruses in the water environment. *Int Microbiol* 1998, **1**, 191-196.
9. Centers for Disease Control and Prevention (CDC): Progress toward interruption of wild poliovirus transmission-worldwide, January 2005–March 2006. *MMWR Morb Mortal Wkly Rep* 2006, **55**, 458-462.
10. Centers for Disease Control and Prevention (CDC): Update on vaccine-derived polioviruses. *MMWR Morb Mortal Wkly Rep* 2006, **55**, 1093-1097.
11. Ehlers MM, Grabow WO, Pavlov DN: Detection of enteroviruses in untreated and treated drinking water supplies in South Africa. *Water Res* 2005, **39**, 2253-2258.
12. Fong TT, Lipp EK: Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. *Microbiol Mol Biol Rev* 2005, **69**, 357-371.
13. Gregory JB, Litaker RW, Noble RT: Rapid one-step quantitative reverse transcriptase PCR assay with competitive internal positive control for detection of enteroviruses in environmental samples. *Appl Environ Microbiol* 2006, **72**, 3960-3967.
14. Griffin DW, Donaldson KA, Paul JH, Rose JB: Pathogenic human viruses in coastal waters. *Clin Microbiol Rev* 2003, **16**, 129-143.
15. Haas CN, Rose JB, Gerba CP, Regli S: Risk assessment of viruses in drinking water. *Risk Anal* 1993, **13**, 545-552.
16. Howard RS: Poliomyelitis and the postpolio syndrome. *BMJ* 2005, **330**, 1314-1318.
17. Jarząbek Z: Rola I badania Krajowego Ośrodka ds. diagnoostyki zakażeń poliowirusami akredytowanego przez Światową Organizację Zdrowia w programie eradykacji polomyelitis. *Przegl Epidemiol* 2002, **56**, 531-540.
18. Johnson DC, Enriquez CE, Pepper IL, Davis TL, Gerba CP, Rose JB: Survival of Giardia, Cryptosporidium, poliovirus and *Salmonella* in marine waters. *Water Sci Technol* 1997, **35**, 261-268.
19. Katayama H, Shimasaki A, Ohgaki S: Development of a virus concentration method and its application to detection of enterovirus and norwalk virus from coastal seawater. *Appl Environ Microbiol* 2002, **68**, 1033-1039.
20. Khetsuriani N, Lamonte-Fowlkes A, Oberst S, Pallansch MA; Centers for Disease Control and Prevention: Enterovirus surveillance – United States, 1970-2005. *MMWR Surveill Summ* 2006, **55**, 1-20.
21. Kim HJ, Shin YO, Kim SH: Detection of enteroviruses and mammalian reoviruses in Korean environmental waters. *Microbiol Immunol* 2006, **50**, 781-786.
22. Kmetzsch CI, Balkie EM, Monteiro A, Costa EV, dos Santos GP, da Silva EE: Echovirus 13 aseptic meningitis, Brazil. *Emerg Infect Dis* 2006, **12**, 1289-1290.
23. Lee HK, Jeong YS: Comparison of total culturable virus assay and multiplex integrated cell culture-PCR for reliability of waterborne virus detection. *Appl Environ Microbiol* 2004, **70**, 3632-3636.
24. Lodder WJ, de Roda Husman AM: Presence of noroviruses and other enteric viruses in sewage and surface waters in The Netherlands. *Appl Environ Microbiol* 2005, **71**, 1453-1461.
25. Lodder-Verschoor F, de Roda Husman AM, van den Berg HH, Stein A, van Pelt-Heerschap HM, van der Poel WH: Year-round screening of noncommercial and commercial oysters for the presence of human pathogenic viruses. *J Food Pro* 2005, **68**, 1853-1859.
26. Moce-Llivina L, Lucena F, Jofre J: Double-layer plaque assay for quantification of enteroviruses. *Appl Environ Microbiol* 2004, **70**, 2801-2805.
27. Moce-Llivina L, Lucena F, Jofre J: Enteroviruses and bacteriophages in bathing waters. *Appl Environ Microbiol* 2005, **71**, 6838-6844.
28. Nwachuku N, Gerba CP: Health risks of enteric viral infections in children. *Rev Environ Contam Toxicol* 2006, **186**, 1-56.
29. Pusch D, Ihle S, Lebuhn M, Graeber I, Lopez-Pila JM: Quantitative detection of enteroviruses in activated sludge by cell culture and real-time RT-PCR using paramagnetic capturing. *J Water Health* 2005, **3**, 313-324.
30. Rosińska M, Stefanoff P, Jarząbek Z: Eradykacja poliomyelitis w Polsce – ocena realizacji programu. *Przegl Epidemiol* 2004, **58**, 185-196.
31. Sina S: Enteroviruses. Available from: http://www.emedicine.com/med/topic681.htm.
32. WHO Fact sheet: Enteroviruses – non polio. Available from: http://www.who.int/ediacentre/factsheets/fs174/en/
33. Wetz JJ, Lipp EK, Griffin DW, Lukasik J, Wait D, Sobsey MD, Scott TM, Rose JB: Presence, infectivity, and stability of enteric viruses in seawater: relationship to marine water quality in the Florida Keys. *Mar Poll Bull* 2004, **48**, 698-704.