Anadolu Üniversitesi Bilim ve Teknoloji Dergisi C- Yaşam Bilimleri ve Biyoteknoloji
Anadolu University Journal of Science and Technology C- Life Sciences and Biotechnology

2017 - Volume: 6 Number: 1
Page: 31 - 37
DOI: 10.18036/aubtdc.269434
Received: 25 November 2016 Revised: 04 January 2017 Accepted: 10 February 2017

DETECTION OF *CRYPTOSPORIDIUM* OOCYSTS BY LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) IN SURFACE WATER FROM RIVER YEŞİLIRMAK AND STREAM TERSAKAN (SAMSUN-AMASYA)

Zeynep KOLÖREN [1,*], Burak K. DELİOĞLU [1], Beyhan TAŞ [1]

[1] Department of Biology, Faculty of Arts and Sciences, Ordu University, Cumhuriyet Campus, Ordu, Turkey

ABSTRACT

The water samples collected from six stations of Stream Tersakan which include Havza; Suluova-Celtek, Kanlıdere, Bogazkoy, Karasu, Tersakan-Last and eight stations of River Yesilirmak which include Amasya Bridge, Yassıcal, Durucasu, Tasova, Kumkoy, Carsamba entrance, Carsamba Bridge, Hacıomer in Amasya and Samsun Provinces were investigated for detection of *Cryptosporidium* oocysts by Loop Mediated Isothermal Amplification (LAMP).

The water samples were regularly collected in every month from the 14 stations in investigated area between 2010 December to 2011 November for LAMP tests.Ten of 14 investigated stations were found contaminated with *Cryptosporidium* oocyst by LAMP. The pollution with *Cryptosporidium* oocysts were determined in stations which are Tersakan–Havza; Tersakan-Suluova, Celtek; Tersakan-Kanlıdere; Tersakan-Bogazkoy; Tersakan-Karasu Tersakan-Last, Yesilirmak-Amasya Bridge, Yesilirmak-Yassıcal, Yesilirmak-Durucasu, Yesilirmak-Tasova. The high contamination rate of *Cryptosporidium* oocysts were found in Tersakan–Suluova, Celtek and Tersakan-Bogazkoy stations in the all investigated stations. However, River Yesilirmak which is last in Samsun more clean than Stream Tersakan which is last in Amasya in terms of the *Cryptosporidium* oocysts contamination. The reason in excess of the rate of *Cryptosporidium* contamination in Stream Tersakan are more animal husbandry in Havza and Suluova Boroughs and no processing domestic and agricultural waste water mixing to surface water.

Keywords: Amasya, *Cryptosporidium*, LAMP, Samsun, Surface waters

YEŞİLIRMAK NEHRİ VE TERSAKAN ÇAYI'NDAN (SAMSUN-AMASYA) ALINAN YÜZEYSEL SU ÖRNEKLERİNDE *CRYPTOSPORİDİUM* OOKİSTLERİNİN İLMİĞE DAYALI İZOTERMAL AMPLİFİKASYON (LAMP) YÖNTEMİYLE ARAŞTIRILMASI

ÖZET

Amasya ve Samsun illerinde, Tersakan Çayı'nın geçtiği Havza, Suluova-Çeltek, Kanlıdere, Boğazköy, Karasu, Tersakan-son olarak adlandırılan altı istasyondan ve Yeşilırmak Nehri'nin geçtiği Amasya Köprüsü, Yassıçal, Durucasu, Taşova, Kumköy, Çarsamba girişi, Çarsamba Köprüsü, Hacıömer olarak adlandırılan sekiz istasyondan alınan su örneklerinde *Cryptosporidium* ookistleri İlmiğe Dayalı İzotermal Amplifikasyon (LAMP) yöntemi kullanılarak araştırılmıştır. 2010 Aralık-2011 Kasım döneminde 14 istasyondan her ay düzenli su örnekleri alınarak bu örneklere LAMP testi uygulanmıştır. On dört istasyonun 10'unda *Cryptosporidium* ookistlerine LAMP yöntemiyle rastlanılmıştır. *Cryptosporidium* ookistleriyle kontamine olmuş istasyonlar: Tersakan–Havza; Tersakan-Suluova, Çeltek; Tersakan-Kanlıdere; Tersakan-Boğazköy; Tersakan-Karasu; Tersakan-son; Yeşilırmak-Amasya Köprüsü; Yeşilırmak-Yassıçal; Yeşilırmak-Durucasu; Yeşilırmak-Taşova'dır. Araştırılan tüm istasyonların içinde Tersakan–Suluova, Celtek ve Tersakan-Boğazköy istasyonları *Cryptosporidium* ookistleri açısından en kirli istasyonlar olarak belirlenmiştir. Bununla beraber Amasya ve Samsun'dan geçen Yeşilırmak Nehri'nin Tersakan Çayı'na oranla daha temiz olduğu saptanmıştır. Tersakan Çayı'ndaki *Cryptosporidium* kontaminasyonun fazlalığı, hayvan besiciliğinin Havza and Suluova ilçelerinde yerleşim yerlerine çok yakın mesafelerde yapılmasına; evsel ve tarımsal atık suların hiçbir işleme tabi tutulmadan direk olarak Tersakan Çayı'na deşarj edilmesine bağlanmaktadır.

Anahtar Kelimeler: Amasya, *Cryptosporidium*, LAMP, Samsun, Yüzey suları

*Corresponding Author: zeynep.koloren@yahoo.com

1. INTRODUCTION

C. parvum causes cryptosporidiosis and it is a common protozoan parasite that can cause enteritis in endemic and epidemic proportions. *C. parvum* is found widely in nature since it has the potential for contamination by water and its oocysts are always infective and they are quite small (3.5-6.0 μm) and low sedimentation rate (0.5 m/s). Cryptosporidiosis infection can occur due to the release of the widely territory of animal manure by aerosol directly or contamination of water supplies indirectly. Large outbreaks are mainly caused by contacting with drinking water that gathered multiple sources or rested water. The routes of transmission of cryptosporidiosis include swimming pools, lakes, person-to-person transmission by directly or indirectly, raw milk ingestion, contact with infected animals, eating the contaminated fruits and vegetables and travel to endemic countries [1, 2].

Cryptosporidium oocysts are very resistant to chlorine and ozone applied to the drinking water. Drinking water supplies are contaminated with mixing the storage of fecal waste containing parasites. *Cryptosporidium* oocysts can survive in water at 4 °C up to one year. The little number of parasites for *cryptosporidiosis* infection (83-23 oocysts) is sufficient [3].

Cryptosporidium oocysts can remain live for months in a humid atmosphere. The wild and domestic animals can be host in nature. It is estimated that 0.6 to 4.3% of the world's population infected with *Cryptosporidium* [4].

Cryptosporidium oocysts of the surface waters are no enforcement to be tested regularly in many countries. However, surface waters used for recreation and drinking water [5].

The aim of this study was to investigate the occurence of *Cryptosporidium* spp. in the surface water samples of Stream Tersakan and River Yesilırmak in Samsun and Amasya Provinces at the Black Sea area by LAMP. The regular assessment of this parasite in surface water should be followed to better understanding of this pathogen threats.

2. MATERIALS AND METHODS

2.1. Sampling Area

The water samples were regularly collected in every month from the 14 stations in investigated area between 2010 December to 2011 November for LAMP tests**.**

Stream Tersakan which include Havza; Suluova-Celtek, Kanlıdere, Bogazkoy, Karasu, Tersakan-Last and River Yesilırmak which include Amasya Bridge, Yassıcal, Durucasu, Tasova, Kumkoy, Carsamba entrance, Carsamba Bridge, Hacıomer in Amasya and Samsun Provinces were selected for detection of *Cryptosporidium* oocysts as shown in Figure 1.

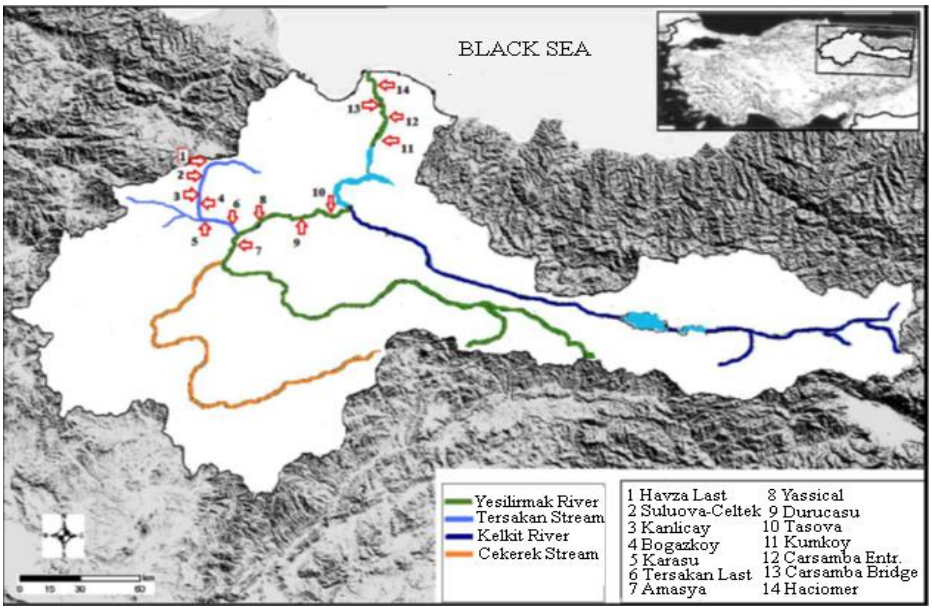

Figure 1. The map of the investigated sampling sites

2.2. Aluminum Sulfate Flocculation and Sucrose Gradient Centrifugation

Five litters of water samples from investigated area were collected and they were flocculated with treatment in Aluminum Sulfate [$Al_2(SO_4)_3$] and concentrated by sucrose gradient centrifugation for water pellets as described by Karanis and Kimura [6] and Kourenti et al. [7] and Karanis et al. [5] Koloren et al. [8]

2.3. Genomic DNA Extraction

Genomic DNA was isolated by the QIAamp DNA Mini Kit (Qiagen) as described by the manufacturer, following the addition of 10 freeze-thaw cycles according to Plutzer et al. [9]. All samples were frozen by immersion in liquid nitrogen for 1 min and thawed in a water bath with boiling water, until the frozen samples were completely dissolved. DNA was eluted in 50 µL TE buffer in a clean tube and kept at -20°C until Polymerase Chain Reaction (PCR) .

2.4. LAMP Assay

The S-adenosyl-L-methionine synthetase (SAM) LAMP method was performed on DNAs from water samples as previously described by Karanis et al. [10], Bakheit et al. [11], and Koloren et al. [8].
C. parvum, *C. hominis* and *C. meleagridis* is amplified with the SAM- LAMP method as described before Bakheit et al. [11], When we use this assay we can determine that one of these species is found in the sampling samples.

Briefly, all LAMP reactions include 40 mM Tris-HCl, 20 mM KCl, 16 mM MgSO4, 20 mM (NH4)2SO4, 0.2% Tween 20, 1.6M betaine and 2.8 mM each deoxynucleoside triphosphate, 8 U Bst DNA polymerase, 1.3 ml primer mixture (40 pmol each of the FIP and BIP primers, 20 pmol each of the LF and LB primers and 5 pmol each of the F3 and B3 primers), 2 ml DNA and 8.2 ml distilled water. The samples were incubated at 63 ºC for 60 minutes and consequently heated at 85 ºC for 5 minutes to terminate the reaction. Positive (*C. parvum*) and negative controls (distilled water) were included for

each test. The LAMP products were analyzed by agarose gel electrophoresis and visualized under UV light after ethidium bromide staining.

3. RESULTS

In this study, a total of 168 surface water samples from Tersakan and Yesilirmak Rivers in Amasya and Samsun Provinces were analysed by LAMP. Sixty nine surface water samples (41.1%) were found positive for the occurrence of *Cryptosporidium* oocysts in the surface water samples collected in the period between December, 2010 and November, 2011. Table 1 presents the results from the occurrence of *Cryptosporidium* oocysts in investigated area by LAMP assay.

Table 1. The results from the occurrence of *Cryptosporidium* oocysts in positive samples collected from Yesilirmak River and Tersakan Stream.

Investigated area and number code	**Number of analyzed samples**	**Number of positive samples by LAMP**
Amasya		
Tersakan River		
Suluova-Celtek (2)	12	**11**
Kanlıcay (3)	12	5
Bogazkoy (4)	12	**12**
Karasu (5)	12	5
Tersakan-last (6)	12	6
Subtotal	60	39
% positive		(65%)
Yesilirmak River		
Amasya bridge (7)	12	5
Yassical (8)	12	6
Durucasu (9)	12	7
Tasova (10)	12	6
Subtotal	48	24
% positive		(50%)
Samsun		
Tersakan River		
Havza last (1)	12	6
Subtotal	12	6
% positive		(50%)
Yesilirmak River	12	-
Kumkoy (11)	12	-
Carsamba entrance (12)	12	-
Carsamba bridge (13)	12	-
Haciomer (14)		
Subtotal	48	0
% positive		0%
Total positive (%)	168	69 (41.1%)

Of the 60 surface water samples collected at River Tersakan (code number 2 to 6) and 48 surface water samples collected at River Yesilirmak (code number 7 to 10) in Amasya, thirty nine (65%) and twenty four (50%) water samples were found contaminated by *Cryptosporidium* oocysts. When comparing the two rivers with the occurrence of *Cryptosporidium* oocysts, River Tersakan especially (Suluova-Celtek and Bogazkoy sites) more contaminated with oocysts than River Yesilirmak. While six positive surface water samples (50%) for the presence of *Cryptosporidium* were found in River Tersakan of Samsun, there was not any contamination with *Cryptosporidium* oocysts at River Yesilirmak in Samsun. Figure 2 represents surface water samples positive with *Cryptosporidium* oocysts by LAMP.

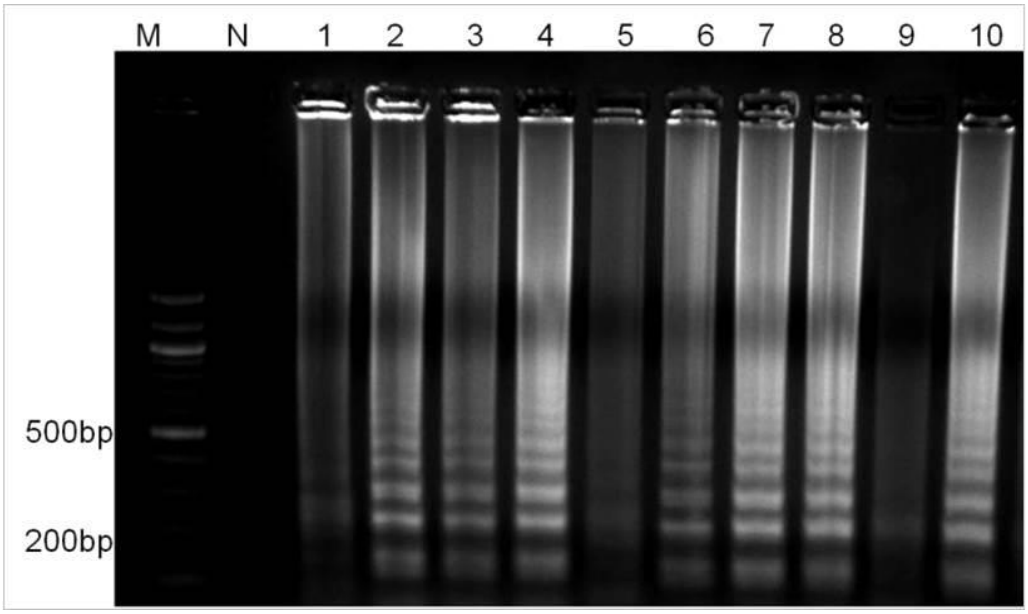

Figure 2. LAMP assay for *Cryptosporidium* spp. with SAM gene in positive samples collected from Yesilirmak River and Tersakan Stream. Lane (M): 100 bp ladder; lane (N): negative control (distilled water); lanes 1-9: river waters; lanes 10: positive control (*C. parvum* Iowa).

4. DISCUSSION

The simple oocyst morphology and host specificity properties are used for the support of molecular taxonomy in the diagnosis of *Cryptosporidium* species. The definitive diagnosis of *Cryptosporidium* species, but can be performed using molecular methods. In recent years, advances in molecular parasitology provides more reliable results in a diagnosis of protozoa.

LAMP technique which is one of the most preferred molecular methods is not required complex and professional tools. The reaction mixture of LAMP can be evaluated in an easy way with the help of the turbidity or fluorescent dye. However, PCR and other molecular techniques can be applied only if the well-equipped laboratories [12].

Though less than 10 years since the LAMP invention of the first time, LAMP technology was regarded as an original method in over 250 publication [13].

LAMP reaction has high specificity because six primer is used to recognize the eight region of the target DNA. LAMP assay can be evaluated as a suitable method for rapidly spreading infection diseases which is important early diagnosis and use under field conditions.

PCR method is inhibited with DNA polymerase inhibitors in water samples [14] while, LAMP assay does not affect any inhibitors in water samples [15].

The results of our study revealed *Cryptosporidium* DNA from 65% of samples at River Tersakan and 50% of samples at River Yesilirmak. This results were smiliar to previously findings of *Cryptosporidium* spp. in environmental water samples from the Lower Rhine area in Germany at the ratio of 43.6% by LAMP [16] and *Cryptosporidium* spp. from rivers in the north of Iran with 50 % by LAMP [17] and *Cryptosporidium* spp. in sea and tap water samples from Sinop and Ordu Provinces, Black Sea, Turkey at the ratio of 65.5% by LAMP [18]**.**

In our study, the prevalence of the parasite in Tersakan Stream was more greater than Yesilirmak River flowing in Amasya and Samsun by LAMP. The reasons of excess the rate of *Cryptosporidium* oocysts in Tersakan Stream were animal husbandry made widely in Havza and Suluova and slaughterhouse waste water without being subject to any processing is mixing with surface waters. When Yesilirmak River in Amasya and Yesilirmak River in Samsun compared to each other, the reasons of excess the rate of *Cryptosporidium* oocysts in Yesilirmak River in Amasya were Tersakan Stream flows through Yesilirmak River in Amasya and the waste water come from settlements in Tasova Borough is being discharged into the river directly.

The contamination of Yesilirmak River, Tersakan Streams in Amasya mainly stems from livestock. The livestock activities in Yesilirmak River Basin occupies an important place. A very large part of the animal waste generated from especially intensive farming activities in Suluova is given to directly Tersakan Stream and in this case constitutes a very heavy pollution in the flow decreasing stream. In addition, the mix of agricultural pollution to rivers even more simplified due to the batter of the land.

5. CONCLUSION

Yesilirmak River's water in Samsun and Amasya Provinces and their boroughs has been used drinking water, daily needs and agricultural irrigation. The lack of waste water treatment plant in this area should be considered because Yesilirmak River Basin is to permanently contaminated with sewage. *Cryptosporidium* infection in the people who live in this area may probably occur because they benefit from this river as drinking water and daily needs. Firstly, it is necessary to regularly check the water resources in the region for not to occurrence of infections linked to water pollution. In addition, blocking the arrival of wastewater to basin or wastewater after the treatment draining to the basin is quite important to protect the health of people living in this region.

REFERENCES

[1] Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium*: transmission, detection and identification. Int J Parasitol 2000; 30:1305-1322.

[2] Chen X, Keithly JS, Paya CV, Russo L, Nicholos F. Kriptosporidiyoz, The New Eng J Med 2002; 346(22): 1723-1731.

[3] Smith HV, Cacciò SM, Cook N, Nichols RAB, Tait A. *Cryptosporidium* and *Giardia* as foodborne zoonoses. Vet Parasitol 2007; 149: 29–40.

[4] Redlinger T, Corella-Barud V, Graham J, Galindo A, Avitia R, Cardenas V. Hyperendemic *Cryptosporidium* and *Giardia* in housholds lacking municipal sewer and water on the United States-Mexico Border. Am J Med Trop Hyg 2002; 66 (6): 794-798.

[5] Karanis P, Sotiriadou I, Kartashev V, Kourenti C, Tsvetkova N, Stojanova K. Occurrence of *Giardia* and *Cryptosporidium* in water supplies of Russia and Bulgaria. Environ Res 2006; 102: 260–71.

[6] Karanis P, Kimura A. Evaluation of three flocculation methods for the purification of *Cryptosporidium parvum* oocysts from water samples. Lett Appl Microbiol 2002; 34: 444–449.

[7] Kourenti C, Heckeroth A, Tenter A, Karanis P. Development and application of different methods for the detection of *Toxoplasma gondii* in water. Appl Environ microb 2003; 69: 102–106.

[8] Koloren Z, Karanis P, Sotiriadou I, Investigations and comparative detection of *Cryptosporidium* species by microscopy, nested PCR, and LAMP in water supplies of Ordu, Middle Black Sea. Ann Trop Med Parasitol 2011; 105 (8): 607–615.

[9] Plutzer J, Karanis P, Domokos K, Torokne A, Marialigeti K. Detection and characterisation of *Giardia* and *Cryptosporidium* in Hungarian raw, surface and sewage water samples by IFT, PCR and sequence analysis of the SSUrRNA and GDH genes. Int J Hyg Environ Health 2008; 211: 524–533.

[10] Karanis P, Thekisoe O, Kiouptsi K, Ongerth J, Igarashi I, Inoue N. Development and preliminary evaluation of loop-mediated isothermal amplification (LAMP) for sensitive detection of *Cryptosporidium* oocysts in fecal and water samples. Appl Environ microb 2007; 73: 5660–5662.

[11] Bakheit MA, Palomino L, Thekisoe OMM, Mate PA, Ongerth J, Karanis P. Sensitive and specific detection of *Cryptosporidium* species in PCR-negative samples by loop-mediated isothermal DNA amplification and confirmation of generated LAMP products by sequencing. Vet Parasitol 2008; 158: 11–22.

[12] Notomi T, Okayama H, Yonekawa T, Watanabe K, Amino N, Hase T. Loop- mediated isothermal amplification of DNA. Nucleic Acids Res 2000; 28: 63.

[13] Karanis P, Ongerth JE. LAMP - an emerging technology fort he detection of water and food borne protozoan parazites. Trends Parasitol 2009; 25:498-499.

[14] Sotiriadou I, Kartashev V, Kourenti C, Tsvetkova N, Stojanova K. Investigations on *Giardia* and *Cryptosporidium* in drinking water supplies of Rostov region (Southern Russia) and Sofi a (Bulgaria). Environ Res 2006; 102:475-81.

[15] Alhassan A, Thekisoe OM, Yokoyama N, Inoue N, Motloang MY, Mbati PA, et al. Development of loop-mediated isothermal amplification (LAMP) method for diagnosis of equine piroplasmosis. Vet Parasitol 2007; 143: 155-60.

[16] Gallas-Lindemann C, Sotiriadou I, Plutzer J, Noack MJ, Mahmoudi MR, Karanis P. *Giardia* and *Cryptosporidium* spp. dissemination during wastewater treatment and comparative detection via immunofluorescence assay (IFA), nested polymerase chain reaction (nested PCR) and loop mediated isothermal amplification (LAMP). Acta Trop 2016; 158: 43–51.

[17] Mahmoudi MR, Kazemi B, Mohammadiha A, Mirzaei A, Karanis P. Detection of Cryptosporidium and Giardia (oo)cysts by IFA, PCR and LAMP in surface water from Rasht, Iran. Trans R So Trop Med Hyg 2013; 107(8):511-7.

[18] Koloren Z, Kaya D. Avsar C. Detection of *Cryptosporidium* Species in the Sea and Tap Water Samples of Black Sea, Turkey. J Parasitol 2013; 99(3):554-557.