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Research Article

Molecular evaluation of *leptospira* species isolated from environmental surface waters in north of Iran

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ABSTRACT

Background: Leptospirosis is a zoonotic disease in humans and animals caused by spirochetes from the genus *leptospira*that is particularly prevalent in humid tropical and subtropical regions.

Objectives: The Purpose of this study is evaluated of various species of *leptospira*in surface waters of different regions in Mazandaranprovince.

Methods: This is a cross-sectional study. A total of 200 fresh water samples were collected from rice fields, water streams and rivers in rural areas of the Mazandaran province at different geographical areas in 19 counties.Multiplex PCR amplification was performed to distinguish between pathogenic and saprophytic *leptospira* species.

Results: Total of 200 samples analyzed 14(7%) were positive for leptospirainterrogans, 116(58%) were positive for *leptospirabiflexa* and 70(35%) samples were negative for *leptospira* spaces.

Conclusions: Identification of the level of water pollution with leptospira spp. can determine areas with higher risk of human contamination leptospirosis .In other hand preventive measures such as public education and medical prophylaxis in these areas could be feasible.

Key words: *leptospira spp.*, surface waters, Molecular evaluation

1- INTRODUCTION

Leptospirosis is a zoonotic disease in humans and animals caused by spirochetes from the genus *leptospira*that is particularly prevalent in humid tropical and subtropical regions(1-7).Annually, tens of millions of human cases occur worldwide, with case fatality rates ranging as high as 20%–25% in some areas(8). Humans infect to Leptospirosis through environmental surface waters contaminated by the urine of domestic and wild mammals chronically colonized with *leptospira*(9).Transmission occurs via skin abrasion or exposed mucous membranes. Flooding and seasonal rainfall are significant risk factors for exposure to water contaminated with leptospirosis(10). Human leptospirosis varies in severity from a mild influenza-like to a disease with a rapidly fatal course, renal failure, jaundice, and hemorrhages. The severity depends on the type of leptospira, the size of the infective dose, and host factors.

Pathogenic leptospires (L. interrogans) have been divided into 23 serogroups on the basis of serological cross-reactivity, with subdivision into 223 serovars(11).Human leptospirosis varies in severity from a mild influenza-like to a disease with a rapidly fatal course, renal failure, jaundice, and hemorrhages. The severity depends on the type of leptospira, the size of the infective dose, and host factors. Pathogenic leptospires (L. interrogans) have been divided into 23 serogroups on the basis of serological cross-reactivity, with subdivision into 223 serovars. TheFirstreport of leptospirosis in Mazandaran province was on 2006(12).Now leptospirosis is an endemic diseases in this region and this diseases has become a major cause of hospitalization of farmers , ranchers and other high risk people in Mazandaran province inrice cultivationSeasonsIdentification of the level of water pollution with leptospira spp. in different geographical regions can determine areas with higher risk of human contamination leptospirosis. Also preventive measures such as public education and preventive medicine in these areas could be possible.The Purpose of this study is evaluated of various species of *leptospira*in surface waters of different regions in Mazandaranprovince.

2- MATERIAL AND METHODS

This is a cross-sectional study.A total of 200fresh water samples (each one 100 ml) were collected from rice fields, water streams and riversin rural areas .Samplescast into sterile polypropylene tubes and transported to the laboratory immediately.

2-1- DNA extraction

The samples were centrifuged at 6,000 rpm for 30 min at room temperature. The pellet was suspended in 50 µL TE buffer [10 mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA], and the mixture was briefly mixed on a vortex mixer. The suspension was placed in a boiling water bath for 1 min, subjected to 3 freeze-thaw cycles alternating between -70℃ for 3 min and 100℃ for 2 min, and then centrifuged at 14000 rpm for 5 min. A 100 µL aliquot of the supernatant was transferred to a sterile tube and stored at -20℃ until PCR testing. The concentration and purity of DNA were determined by spectrophotometer .

2-2- PCR assay

The amplification of the LipL32, which encodes the outer membrane lipoprotein LipL32 and 16S ribosomal RNA genes, was performed using the primers previously described by (13). The forward primer of 16S rRNA was 5'-GGAACTGAGAC-ACGGTCCAT-3' and the reverse was 5'-GCCTCAGCGTCAGTTTTAGG-3'. The forward primer of LipL32 was 5'-AAGAATGTCGGCGATTATGC-3' and the reverse was 5'-CCAACAGATGCAACGAAAGA-3'. These two sets of primers allowed us to distinguish between pathogenic and saprophytic *leptospira*species. Multiplex PCR amplification was performed in a final reaction volume of 25 µL. All reactions contained 1X magnesium free PCR buffer, 2 mM MgCl2, 200 µM dNTPs, 10 µM of each primer, and 3 U Taq polymerase. PCR amplification was performed using the following conditions: one denaturation cycle at 95ºC for 5 min, 40 cycles of denaturation at 95ºC for 40 sec, annealing at 50ºC for 1 min and extension at 72ºC for 40 sec, and a final extension at 72ºC for 7 min.

2-3-Electrophoresis

The amplified products were electrophoresed on sybr green-stained. 2% agarose gels and observed using UV light. The LipL32 and 16S rRNA primers produced 279 and 430 bp fragments, respectively, as estimated using a 100-bp ladder.

2-4-Statistically analysis

The data wereanalyzed by SPSS (ver. 16) and descriptive statistics were used.

RESULTS

Samples were collected from rice fields, water streams and rivers in rural areas of different geographical areas in 19 counties in Mazandaran province. Total of 200 samples analyzed 14(7%) were positive for *leptospirainterrogans*, 116(58%) were positive for *leptospirabiflexa* and 70(35%) samples were negative for *leptospira* spaces.Figure 1 illustrates Agarose gel electrophoresis of positive strains.

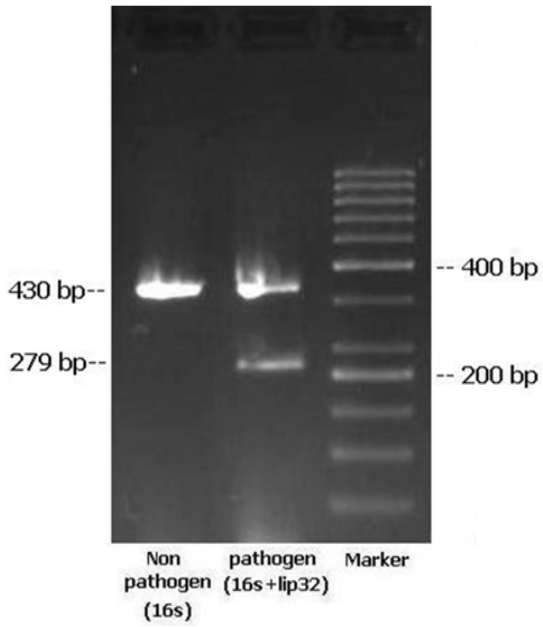

Figure 1: The result of electrophoresis of the PCR product

DISCUSSIONS

Leptospirosis has a very wide range of natural rodent, and non-rodent reservoir hosts. The animals act as carriers of the leptospira and excrete large number of leptospires in their urine, thus responsible for the contamination of large and environmental surface waters as well as soil flooding and drainage congestion may be risk factors for contamination of water bodies with infected animal urine. Water logged areas may force rodent population to abandon their burrows and contaminate the stagnant water by their urine.Infection is acquired from contact through skin, mucosa/ conjunctiva with water or soil contaminated with the urine of rodents, carrier or diseased animals in the environment. Ingestion of contaminated water may also cause infection. There is no documentation of human to human transmission. In our study 7% of surface water sample was contaminated with pathogenic leptospirosis. Issazadeh et al determine the distribution of aerobic spirochetes Leptospira in Surface waters in Guilin province(14).They found that 4% of rice field water contaminated with leptospirosis interrogans which their finding is close to our results. In 2006 Gonaza et al determine the presence of pathogen-related Leptospira in environmental water samples from rural and urban sites in the Peruvian Amazon was (11 [52%] of 21) for L. interrogans and (5 [24%] of 21) for L. santarosai. Gonaza et al mentioned that in the Amazon region of Peru, leptospirosis is an important cause of morbidity and mortality (8). Reis et al estimated the prevalence of Leptospira infection and identify risk factors for infection in the urban slum setting. Theyfound the main source of infectious to leptospirosis is water contamination in urban regions with low health level(15).InTansuphasiri et al study, out of 100 water samples analyzed, 23 samples were positive for pathogenic *Leptospira*.In Thailand, leptospirosis is found to be sporadic in many regions of the country similar to Mazandaran and Guilanregion inIran. In recent years outbreak of a re-emerging leptospirosis occurred and expanded to provinces in the northernIran and the outbreak corresponded with the rainy season and most infections occurred in agricultural workers, primarily rice producers(13, 16-19).PCR- based detection and identification of *leptospira*, which avoid the problems such as isolation of fastidious organisms from contaminated sources, can be useful for assessing and monitoring risk of human to *Leptospira*-contaminated water.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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