Research article
Volume 5:5, 2021

Journal of Microbial Pathogenesis

ISSN: Open Access

Human Lymph Node Samples in Urmia, Iran Using PCR with GRA6 Gene from 2020 to 2021

Mohammad Yousefzadeh* , Somayyeh Vakili Yekan

Department of Medical Parasitology, Nemati Clinical Laboratory, Urmia , Iran

Abstract

Background: Toxoplasmosis is one of the most common parasitic infections caused by a protozoan named Toxoplasma gondii. Humans and carnivores can become infected by eating tissue cysts in row or semi-cooked meat or oocysts from cat feces. The aim of this study was investigation of different genotypes of Toxoplasma gondii isolates from cat feces, lamb and human pathological samples to determine the dominant types in Urmia city by amplifying GRA6 gen using PCR method.

Materials and Methods: Meat samples were collected from butchery shops of Urmia city by cutting a small piece of diaphragm muscle. DNA extraction and PCR was done on these samples after being chopped. Stool samples from stray cats were collected from ruins and parks, and oocysts were concentrated by ethyl acetate formalin and were stained with acid fast technique. Microscopically positive samples were analyzed with PCR. The lymph node specimens were collected by reviewing the pathological records of Dr. Nemati Laboratory in Urmia city. The specimens that were suspected of toxoplasmosis, were also examined after hematoxylin eosin staining and microscopic confirmation, by PCR. Finally, 20 positive PCR products, including 5 stools, 5 lamb, 5 beef and 5 human lymph node samples were sent for sequencing.

Results: Of the 20 cat feces samples suspected of having Toxoplasma oocysts, 7 samples were confirmed by PCR and from the 100 selected Lymph Node specimens, 5 samples were confirmed microscopically and PCR .sixty percent of 60 meat samples were also infected. Also, thirty percent of 60 beef samples were positive with PCR. Finally, out of 100 samples of Goat milk, 8 samples were positive and we were able to determine with PCR. Analysis of DNA sequencing revealed that all 5 lymph node specimens were from ME49 (genotype II) strain. lamb specimens were shown to be from ME49 (2 samples), GT1 or genotype I (2 samples), and VEG or genotype III (one sample) genotypes and four of five oocysts from stools samples were ME49, VEG or genotype III (one sample) strain and all beef samples were related to ME49 (genotype II) strain.Also, 2 samples of goat milk are related to GT1 or genotype I strain and 3 other cases are related to ME49 (genotype II) strain. So most of our samples were of ME49 strain.

Conclusion: Genotyping is important because it can be used to identify the dominant genotype in the region and subsequently to take specific control measures as well as therapeutic measures against the risk of involved genotype. Most of the genotypes we obtained during this study, were of type II and this genotype appears to be predominant genotype in Urmia and regarding extremely high infection rate of meat samples, it seems that humans are mostly infected with Toxoplasma gondii by eating raw or undercooked meat.

Keywords: Genotyping • Toxoplasma gondii • GRA6 • Lymph Node

Introduction

Toxoplasma gondii was first seen in 1908 by Nicole and Mansou in the spleen and rodent liver of Ctenodactylus gondii, which had been kept for some time at the Pasteur Institute in Tunisia

Splendor, who worked on rabbits in Brazil, also described the organism. The reports were published three days apart without the researchers knowing about each other. The disease was later described in 1923 by Janko, who found Described pathological conditions including hydrocephalus and inflammation of the retina and choroid in one case of congenital toxoplasmosis [1]. But he did not isolate the organism. In 1939, Wolff and colleagues reported three cases of congenital infections, confirming Janko's observations and showing that the causative agent of the disease was transmissible to rabbits and mice.

***Address to Correspondence:** Mohammad Yousefzadeh, Department of Medical Parasitology, Nemati Clinical Laboratory, Urmia , Iran, Tel: 98914264284; E-mail: yousefzadeh_ir@yahoo.com

Received: November 02, 2021; **Accepted**: November 16, 2021; **Published**: November 23, 2021

Toxoplasmosis is one of the most common parasitic infections in humans and animals with a wide global distribution, caused by a protozoan called Toxoplasma gondii. This obligate intracellular parasite can infect humans as well as virtually all warm-blooded animals, including mammals and birds. According to estimates, approximately 1/3 of the world's population would be infectedby Toxoplasma gondii. Toxoplasma gondii infection represent the most prevalent parasitic zoonotic disease in world [2]. This parasite is present on all continents, and the rate of infection vary highly according to areas. However, climate change has led to an increase of Toxoplasma gondii infections in different regions of the world as a result of changing environmental conditions.

The evolution of this parasite takes place in two hosts. the intermediate hosts of this parasite is most warm-blooded vertebrates and The final host of this parasite is cats. In the life cycle of this parasite, there is an intestinal stage or isospore phase in the final host cell [3]. There is also another stage called the extraintestinal phase or toxoplasmic phase, which takes place in the tissues of all hosts (intermediate and final).

Human infection is acquired via ingesting food or water contaminated with sporulated oocysts or undercooked meat infected with latent cysts, Not through sterilized milk, by mother to fetus transmission, organ transplantation.

Acute toxoplasmosis is usually asymptomatic in healthy adults. However, people with AIDS, chemotherapy, immunocompromised individuals, and transplant recipients may be exposed to acute toxoplasmosis. Complications of acute toxoplasmosis may be It can include brain complications such as encephalitis, and ocular complications such as retinochoroiditis. Symptoms seen in the acute phase are similar to the symptoms of the flu with swollen lymph nodes, muscle cramps, etc.

Acute infection in pregnant women, acquired during or before gestation can lead to congenital toxoplasmosis. Acute infection in the immunocompetent host is followed by asymptomatic latent infection, during which the parasite encysts in various organs, brain parenchyma, muscles and retina. Latent infection can reactivate overtly in immunocompromised patients, with conversion of latent bradyzoites into rapidly replicating tachyzoites, causing death of the host. Infected infants through placental transmission may be born with any of these problems or brain abnormalities, although these complications are rare in infants [4]. Toxoplasma trophozoites, which cause acute toxoplasmosis, are commonly found in body fluids. Swollen lymph nodes are usually found in the neck or under the chin, followed by the armpits and groin. Swelling may occur at different times after the initial infection.

Toxoplasmosis, especially cerebral toxoplasmosis has become the most common opportunistic infection of the central nervous system during HIV infection in the world.

The highest rate of infection with this parasite is observed in hot and humid areas, so that proper humidity and heat can be one of the favorable factors for oocyst sporulation, while extreme heat and dryness, as well as extreme cold can be Prevent sporogony and their evolution. The infection is particularly important in women when they acquire the infection for the first time during their pregnancy, where an intrauterine transmission of the parasite may occur. Its severity vary depending on the time of infection in mothers.

Transmission rate increases with gestational age, whereas the severity of infection reduces by the time of infection. Food animals including pigs, sheep, goats, and reared birds, such as chickens and turkeys, can be infected by Toxoplasma and these animals can transmit the infection to humans through their meats. Birds are important intermediate hosts of Toxoplasma, since they serve as a source of infection for humans as well as an important reservoir for cats.Hosts that are easily infected with toxoplasmosis develop mild symptoms such as the Influenza, which sometimes appear within the first few weeks after exposure to the parasite. Except as noted, there are no visible symptoms at all. Does not cause signs in healthy human adults [5].

In most people with a complete immune system, the infection enters a latent stage during which only bradyzoites (in tissue cysts) are present. Tissue cysts can also occur in the alveoli of the lung (as an acute infection may mimic pneumocystis jiroveci infection. It can also involve tissue cysts in the heart, skeletal muscle, and central nervous system, including the brain. Cysts form in brain tissue upon infection with Toxoplasma gondii and remain stable in the host for as long as they are active Studies of serological studies estimate that 30-50% of the world's population is at risk for latent toxoplasmosis. However, the rate of infection varies from country to country. This type of toxoplasmosis is rare, but if a person is infected with this disease, it can cause skin lesions such as erythema, swelling and maculopapular lesions, urticaria. In infants, there may be macular lesions, ecchymoses occur . Diagnosis of cutaneous toxoplasmosis is based on the form of toxoplasma gondii tachyzoites in the epidermis. Symptoms in people with a healthy immune system are mild and transient, including mild fever and swollen lymph nodes. Centralized, seizures, mental disorders and brain calcification, pneumonia, myocarditis, etc. are known as opportunistic infections.

One of the most important advances in the last two decades in the field of molecular biology, especially in diagnostic applications is the polymerase chain reaction. Due to its high specificity, sensitivity, speed and ease, it has found a special place in molecular research and diagnosis. This reaction has been used to detect Toxoplasma DNA in body fluids, especially for the diagnosis of congenital, ocular and cerebral toxoplasmosis and diffuse toxoplasmosis.This method is an efficient method for detecting Toxoplasma. In order to allow the amplification of a piece of DNA in a short time. In this method, different genes can be used. Using these genesIt can be used individually or multidimensionally. To achieve high sensitivity, the multidimensional method is usually chosen, in other words, instead of using one gene, several genes are used. B1, GRA5, GRA6 GRA7, SAG1, SAG2, etc genes can be used. We used gra genes in this study. These genes were better due to their high diagnostic value and suitability for genotyping.

Genotyping is the process by which differences in a person's arrangement are determined by examining a person's DNA sequence using biological tests and comparing it to another personal sequence or a reference sequence. Genetic structure determines the alleles inherited from the parents. Traditionally, genetic structuring has been the use of DNA sequences to identify biological populations using molecular tools. This process usually does not involve identifying an individual's genes. Methods used to determine the genetic structure include identifying longitudinal polymorphisms in fragments of genomic DNA restriction enzymes (RFLP), detecting randomly

amplified genomic DNA polymorphisms (RAPD), and detecting Amplitude of amplified fragment length (AFLPD), polymerase chain reaction (PCR), DNA sequencing, allelespecific oligonucleotide probes (ASO), and hybridization to DNA microarrays or DNA beads.There are several genetic markers for the identification of Toxoplasma genotypes. Isolated Toxoplasma gondii genotypes such as surface antigens including SAG1 to SAG4, MAG1, BSR4 and SRS1 Secretory antigens including GRA1 to GRA4, GRA6 and Raptor proteins, one of which The most famous of these is ROP1. Dense granule antigens, called GRA proteins, which are predominantly expressed in tachyzoites are among the most important components for determining Toxoplasma isolates.

For the treatment of toxoplasmosis, a combination of 25 to 100 mg daily of pyrimethamine and 6 grams of trisulfapyrimethamine daily for one month is prescribed. These compounds inhibit the synthesis of DNA and RNA in the parasite. Usually in combination with pyrimethamine folic acid. It is also prescribed to prevent bone marrow suppression. In people with immunodeficiency, spiramycin can be effective. Other drugs, such as clindamycin, are also used to treat toxoplasmosis. Also, a combination of pyrimethamine and sulfadiazine can be used to treat infections. Toxoplasma is used in the fetus. However, there are other regimens, including the use ofpyrimethamine in combination with clindamycin, avocado, clarithromycin, or azithromycin, or monotherapy with trimethoprim-sulfamethoxazole.

Methods

Study Area and Sample Collection

Collecting meat samples: To collect meat samples, he first went to the industrial slaughterhouse in Urmia. Due to the lack of cooperation of slaughterhouse staff, sampling from the slaughterhouse was stopped and to collect samples, he referred to the butchers in Urmia. Sampling of butchers Numerous and only one sample from each Mammals was taken from the diaphragm. After sampling, the samples were finally frozen to be ready for further experiments.

Collecting fecal samples: Stool samples were collected from the city of Urmia and from places, ruins, parks, etc. It was tried to collect samples from stray cats in the city of Urmia.

Collecting Lymph Node samples: To do this, we went to Dr. Nemati Laboratory and reviewed the reports of pathology samples that had been over the last two years.

Collecting milk samples: she went to farms and places where goats are raised and 50 cc of milk was collected from each goat and frozen for further experiments.

Collecting fecal samples: In a disposable glass, add 10 ml of 10% formalin to about 1 gram of feces and mix with the applicator to obtain a uniform suspension. Divide the gauze into 4 layers and place it in a glass funnel and place the funnel in the screw tube and pass the solution through the funnel so that 7 ml of it enters the screw tube. Add 3 ml of ethyl acetate and close the tube cap and shake it well to dissolve the ethyl acetate and formalin, then open the tube cap to remove the resulting gas.Centrifuge the tube for 1 minute at 2000 rpm. After centrifugation, 4 layers are formed. Which are from top to bottom: ethyl acetate and fat - fecal waste - formalin and the lowest layer of sediment.Using the applicator, separate the second layer from the inner wall of the pipe, then empty the top three layers by quickly overturning the pipe.

Fast acid staining method:

- First, we prepare a Smear of feces on the slide
- After the heat stabilization process, we cover the slide surface with carbol fuchsin paint.
- Heat the slide until the paint evaporates (turn the flame upside down and pass it through the slides repeatedly). When the paint evaporates from the slide, pass the flame under the slide again and then move it away. Do not allow the paint to boil or the area to dry. As the dye evaporates from the slide, we add more carbol fuchsin. This should take 5 minutes.
- Tilt the slide to remove excess paint and leave it to cool slightly and then wash it so that the slide does not break.
- Cover the surface of the slide with acid-alcohol and add the dye, which in this staining is 3% HCl in 95% ethanol (this alcohol is a very strong dye on alcohol) and let 15 Leave for 30 seconds. If the red color continues with the dye solution, pour alcohol on the acid slide again. When the red color does not come out, we do the next step.

Hematoxylin and Eosin staining of pathological specimens

Samples of paraffin tissue suspected of Toxoplasma were selected and cut by microtome and stained and examined by hematoxylin and eosin staining. This staining was done in Dr. Nemati laboratory.

Transparency stage

At this stage, the prepared slides are placed in 3 Xylene containers to be paraffinized. Then we placed the slides in each of the Xylene containers for 20 minutes so that the clarification process is done well.

Watering stage

This step is done with the help of alcohol in such a way that 2 containers containing absolute alcohol and 2 containers containing 96 degrees' alcohol were prepared and the slides were placed in containers related to absolute alcohol and 96 degrees' alcohol, respectively. It is better to insert and exit the slide samples in each container 10 times.

Staining stage

At this stage, the slide samples were first washed with water and then they were placed inhematoxylin dye, which is a specific dye for the nucleus, for 5 minutes, and then the rinses were washed again with water. Place to remove the color of the hematoxylin in the cytoplasm. Rinse again with water. In the next step, the slides are placed 3 times in lithium carbonate, which fixes the color of the core. It is washed again with water. In the last step, the slides are soaked in eosin paint for 3 minutes. Specific to the cytoplasm is placed.

Dehydration stage

This step is quite similar to the discharge step, except that in the dewatering step, the slides are placed first in low-grade alcohol and then in high-grade alcohol. Also at this stage, in addition to alcohol, the slides are placed in 3 Xylene containers.

Paraffin removal of pathology tissue samples

Cutting pathology specimens Cut these samples into 0.6 micron slices 10 to 15 times with a microtome and then pour them into the micro tube. Cut tissue samples that have already been placed in the micro tubes are removed and xylene is poured onto the samples inside the micro tubes. Alcohol 96 is then added and allowed to soak in the alcohol for a few minutes, then the samples are centrifuged and the supernatant is discarded. Finally, the lid of the micro tube is left open so that the excess water evaporates and only the tissue remains. Finally, the samples are prepared for DNA extraction.

DNA extraction

In this research from kia spin kit was used. This kit uses silica columns to purify DNA. Different samples are lysed with the help of different detergents and enzymes. Under high salinity conditions, DNA in cell lysis attaches to the silica membrane of the columns, and impurities leave the column and collect in the tube. The membrane is then washed with alcohol to remove proteins, salts and cellular additives. Finally, the column is washed with deionized water or low ionic strength buffer and pure DNA is collected in the new tube.

Select a primer

The reciprocating primers were blasted using the information available at http://www.ncbi.nlm.nih.gov and the required corrections were made. After making the necessary corrections, the reciprocating primers were selected as follows. Blast is visible on all areas to which the primer can be attached. The Primer-BLAST option, which is based on NCBI, allows simultaneous primer design and sequence blasting.

5´.....TTTCCGAGCAGGTGACCT.....3´

5´.....TCGCCGAAGAGTTGACATAG.....3´

PCR Perform

Pcr program			
First stage			
The name of the sub-step	Temperature	Number of cycles	Time
Initial heat	94°C	1 cycle	5 min
Second stage			
The name of the sub-step	Temperature	Number of cycles	Time
Denaturation	94°C	38 cycle	30 s
Annealing	57°C	38 cycle	1 min
Extention	72°C	38 cycle	2 min
Third level			
The name of the sub-step	Temperature	Number of cycles	Time
Extention	72°C	1 cycle	7 min

Add 13 microliters of master mix, add 7 microliters of deionized water, add 3 microliters of DNA extraction product, add 2 microliters of forward and reverse primers The above materials were first mixed in a 0.2 ml tube with a good sampler and then spin for 10 seconds in order to settle the material in the tube.

The microtubule was then placed inside the Thermal cycler. The Thermal cycler used in this study had a capacity of 25 micro tubes of 0.2 ml per reaction. In this study, the PCR reaction was performed according to the thermal schedule and schedule shown in the table.

Evaluation of quantity and quality of PCR

product in order to detect and confirm the reaction and the presence of PCR product, 5 microliters of the solution was placed in an order of 0.2 µl by electrophoresis with electrodes based on gel.

Detection using agarose gel:

At this stage, 1.5% agarose gel was used.

Preparation of 1.5% agarose gel:

- 0.3 g of agarose + 20 ml TBE buffer
- Heat for 45 seconds in the microwave at 180 ° C
- Cool to a temperature of 40 to 50 degrees
- Add Safe stain to 1 µl
- Pour into a mold containing a special comb
- Cool until the agarose gel is completely hard
- Remove the comb and mold from the agarose gel

10 µl of PCR product was electrophoresed by 1.5% agarose gel.

After making the gel, Ladder 100 bp was added to one of the wells.

Results

In this study, we collected 200 cat feces samples from the city, of which 20 were suspected to be toxoplasma oocysts. After PCR, we found that 7 of the samples were Toxoplasma oocysts.

Observation of Toxoplasma oocysts in fast acid staining

After staining the fast acid according to the mentioned protocol, Toxoplasma oocysts were observed under a light microscope.

The oocysts were measured using micrometer method and had a size between 12 and 18 microns. And they were well painted in color.

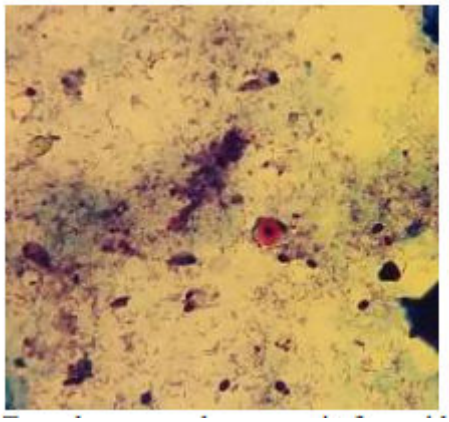

Figure1: Toxoplasma gondii oocysts in fast acid staining

Positive PCR results of fecal samples

All 7 samples were evaluated using PCR method and finally 5 cases had band sharp and 2 case had weak bands.

Results of meat samples:

Due to the fact that meat samples are first slaughtered in industrial slaughterhouses and then spread to the whole city, so sampling from the city was done randomly and out of 60 samples collected, about 60% of Toxoplasma infection was obtained Finally, the bands that were sharp were selected and sent for sequencing.

Results of beef samples: Of 60 beef samples, 30% were confirmed by pcr.

Results of Lymph Node samples: from the 100 selected pathologic specimens, 5 samples were confirmed microscopically and PCR

Results of Goat milk samples: Out of 100 milk samples collected, 10 were confirmed by PCR.

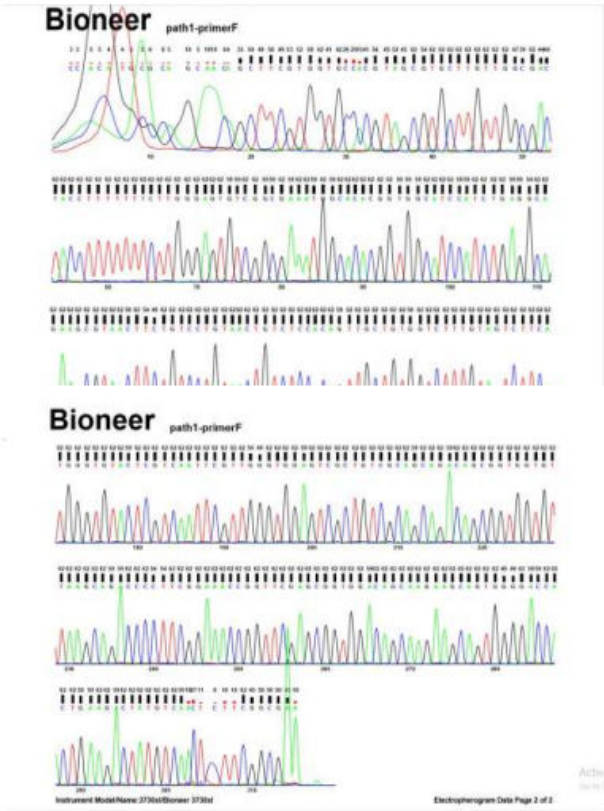

Figure 2: Toxoplasma gondii positive samples on gel electrophoresis

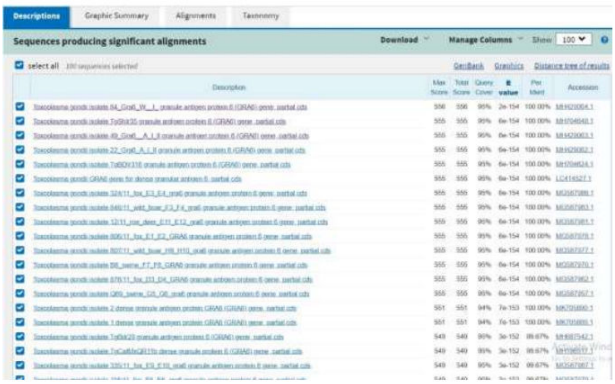

Figure3: Figure 2: Toxoplasma gondii positive samples on gel electrophoresis.

Observation of tissue cysts in pathology slides

Samples suspected of Toxoplasma were cut using a microtome after collection and then stained with hematoxylin-eosin.

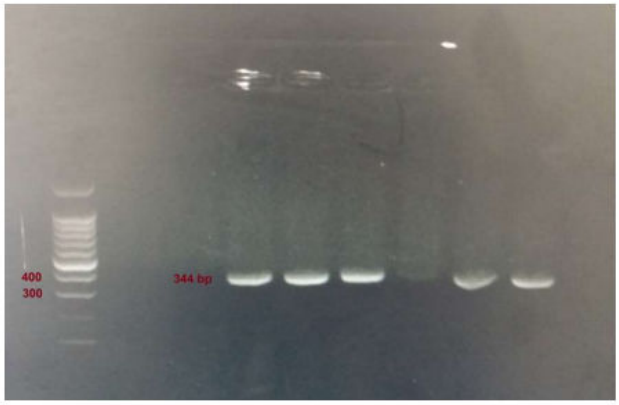

Figure 4: Toxoplasma gondii tissue cyst in lymph node specimen.

Sequencing results

From each of the tested samples, 5 that had a better band in PCR were selected and sent for sequencing.

Sample	The amount of sample collecte d	Positive result confirme d by PCR	Percenta ge of pollution	ME49 (genotyp e II)	VEG (genotyp e III)	GT1 (genotyp e I)
fecal samples	200	7	3.50%	4	1	0
lamb samples	60	36	60%	2	1	2
beef samples	60	18	30%	5	0	0
Lymph Node samples	100	10	10%	5	0	0
Goat milk samples	100	8	8%	3	0	2

Figure 5: Diagram of sequenced samples.

As shown in the images above, the sequencing process is good and there is no interaction and overlap between the nucleotides.

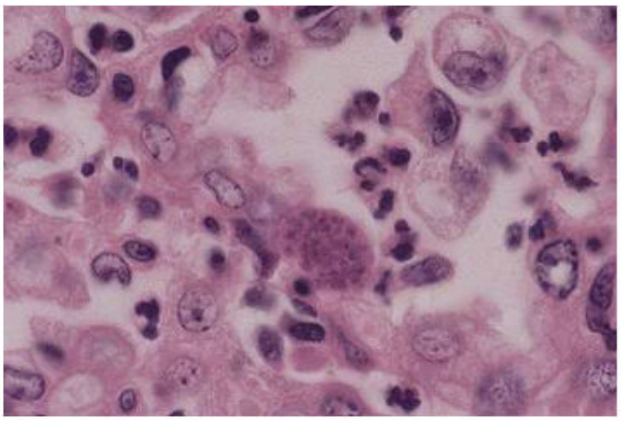

Figure 6: An image of a sequence of nucleotides in mega software.

First, we sequenced the results with mega alignment software and reviewed the results.The results of mega software showed that all sequences except (in a few nucleotides) corresponded to each other.

Figure 7: Comparison of the sequences obtained in this study with other sequences in the World Gene Bank

As can be seen in the pictures, the sequences obtained in this study show 100% correspondence with the sequences of many sequences registered in the World Gene Bank.Analysis of DNA sequencing revealed that all 5 lymph node specimens were from ME49 (genotype II) strain. lamb specimens were shown to be from ME49 (2 samples), GT1 or genotype I (2 samples), and VEG or genotype III (one sample) genotypes and four of five oocysts from stools samples were ME49, VEG or genotype III (one sample) strain and all beef samples were related to ME49 (genotype II) strain. Also, 2 samples of goat milk are related to GT1 or genotype I strain and 3 other cases are related to ME49 (genotype II) strain. So most of our samples were of ME49 strain.

Standard genes for VEG, GT1 and ME49 strains registered with the World Gene Bank

#Toxoplasma gondii GRA6 GT1 JX044183

ATGGCACACGGTGGCATCCATCTGAGGCAGAAGCGTAACTTC
TGTCCTGTAACTGTCTCCACAGTTGCTGTGGTCTTTGTAGTCTTC
ATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGCAGC
AGACAGCGGTGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCG
AGCGGTGGACAGCAAGAAGCAGTGGGGACCACTGAAGACTATG
TCAACTCTTCGGCGATGGGCGGTGGCCAAGGCGACTCGTTAGC
TGAAGATGATACAACCTCCGAAGCGGCGGAGGGCGACGTTGAC
CCTTTTCCCGTGCTGGCGAATGAGGGGAAGTCGGAGGCGCGTG
GCCCGTCGCTCGAGGAAAGAATCGAAGAACAGGGCACAAGACG
ACGTTACTCCTCTGTTCAAGAACCACAAGCGAAGGTGCCTAGCA
AACGAACACAGAAACGCCACAGACTCATTGGTGCTGTGGTGTTG
GCAGTATCTGTGGCAATGCTTACCGCTTTCTTTCTTCGAAGGACT
GGACGACGCTCTCCCCAAGAACCATCTGGGGATGGTGGTGGAA
ATGATGCAGGCAATAATGCTGGGAACGGTGGGAATGAAGGCAGA

GGTTACGGAGGCAGAGGTGAAGGAGGAGCCGAGGATGACAGG
CGCCCGTTGCACCCGGAACGTGTGAATGTGTTTGATTATTAA

#Toxoplasma gondii GRA6 ME49 AF239285

ATGGCACACGGTGGCATCTATCTGAGGCAGAAGCGTAACTTC
TGTCCTTTAACTGTCTCCACAGTTGCTGTGGTCTTTGTAGTCTTC
ATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGCAGC
AGACAGCGGTGGTGTTAGGCAGACCCCTTCGGAAACCGGTTCG
AGCGGTGGACAGCAAGAAGCAGTGGGGACCACTGAAGACTATG
TCAACTCTTCGGCGATGGGCGGTGGCCAAGGCGACTCGTTAGC
TGAAGATGATACAACCTCCGATGCGGCGGAGGGCGACGTTGAC
CCTTTTCCCGCGCTGGCGAATGAGGGGAAGTCGGAGGCGCGTG
GCCCGTCGCTCGAGGAAAGAATCGAAGAACAGGGCACAAGACG
ACGTTACTCCTCTGTTCAAGAACCACAAGCGAAGGTGCCTAGCA
AACGAACACAGAAACGCCACAGACTCATTGGTGCTGTGGTGTTG
GCAGTATCTGTGGCAATGCTTACCGCTTTCTTTCTTCGAAGGACT
GGACGACGCTCTCCCCAAGAACCATCTGGGGGTGGTGGTGGAA
ATGATGCAGGCAATAATGCTGGGAACGGTGGGAATGAAGGCAG
A
GGTGAAGGAGGCGAGGATGACAGGCGCCCGTTGCACCCGGGA
AGTGTGAATGAGTTTGATTTT AA

#Toxoplasma gondii GRA6 VEG JX044209

ATGGCACACGGTGGCATCCATCTGAGGCAGAAGCGTAACTTC
TGTCCTTTAACTGTCTCCACAGTTGCTGTGGTCTTTGTAGTTTTC
ATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGCAGC
AGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGA
GCGGTGGACAGCAAGAAGCAGTGGGGACCACTGAAGACTATGT
CAACTCTTCGGCGATGGGCGGTGGCCAAGGCGACTCGTTAGCT
GAAGATGATACAACCTCCGATGCGGCGGAGGGCGACGTTGACC
CTTTTCCCGTGCTGGCGAATGAGGGGAAGTCGGAGGCGCGTGG
CCCGTCGCTCGAGGAAAGAATCGAAGAACAGGGCACAAGACGA
CGTTACTCCTCTGTTCAAGAACCACAAGCGAAGGTGCCTAGCAA
ACGAACACAGAAACGCCACAGACTCATTGGTGCTGTGGTGTTGG
CAGTATCTGTGGCAATGCTTACCGCTTTCTTTCTTCGAAGGACTG
GACGACGCTCTCCCCCAGAACCATCTGGGGATGGTGGTGGAAA
TGATGCAGGCAATAATGCTGGGAACCGTGGGAATGAAGGCAGA
G
GTTACGGAGGCAGAGGTGAAGGAGGAGGCGAGGATGACAGGC
GCGCGTTGCACCCGGAACGTGTGAATGTGTTTGATTATTAA

An example of the genes obtained in this study

>path1-primerF

CCCACATGCGCAGCAACAGCTTCGTGGTGCCACGTAGCGTG
CTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATG
GCACACGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCC
TGTAACTGTCTCCACAGTTGCTGTGGTCTTTGTAGTCTTCATGGG
TGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGCAGCAGACA
GCGGTGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGG
TGGACAGCAAGAAGCAGTGGGGACCACTGAAGACTATGTCAACT
CTTCGGCGAA

>stool1-primerF

GAAAACTGCGCAGCAACAGCTTCGTGGTGCCACGTAGCGTG
CTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATG
GCACACGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCC
TGTAACTGTCTCCACAGTTGCTGTGGTCTTTGTAGTCTTCATGGG
TGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGCAGCAGACA
GCGGTGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGG
TGGACAGCAAGAAGCAGTGGGGACCACTGAAGACTATGTCAACT
CCTTCGGCGAA

>tiss1-primerF

GCATCAGGAGACACTGAGCTTCGAATTGGGAGTTTTAATTTTG
ATAGAATTCCAGTCTATTTTTCTTGGGAGTGTCGGCGAAATGGCA
CACGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTGT
AACTGTCTCCACAGTTGCTGTGGTCTTTGTAGTCTTCATGGGTGT
ACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGCAGCAGACAGC
GGTGGTGTTAAGCAGACCCCATGTGTGTTTTTTAAATATGGGATA
CACTTTGTGTCCACATGTCTCAAACTCCTTCAAGCCAACTTCCTA
TGTCAACTCCTTCGGCGAA

Discussion

Toxoplasmosis is one of the most common parasitic infections common to humans and animals with a wide global distribution, caused by a protozoan called Toxoplasma gondii. This protozoan has the power to infect most warm-blooded vertebrates. Based on serological studies, it is estimated that one third of the adult population in most countries of the world is infected with this parasite. The evolution of this parasite takes place in two hosts. The final host of this parasite is cats and felines and most warm-blooded vertebrates are hosts. They are the mediators of this parasite. In the life cycle of this parasite, there is an intestinal phase or isospore phase that takes place in the epithelial cells of the intestinal wall of the final hosts. There is also another stage called the extraintestinal phase or the toxoplasmic phase, which takes place in the tissues of all hosts (intermediate and final). Proper humidity and heat can be desirable factors for oocyst sporulation, while extreme heat and dryness as well as extreme cold can prevent sporulation and their development. The infection is often asymptomatic. Symptoms in immunocompromised individuals may include fever, lymphadenopathy, muscle aches, and headache. Affected infants may experience visual impairment and mental retardation. Congenital infections may be generalized and localized, but can also affect the retina and brain. Toxoplasma's focal chorioretinitis can also lead to visual impairment. Toxoplasma can also cause classic symptoms such as hydrocephalus, seizures and intracranial calcification. Brain Lymphadenitis in infants is also the most common symptom of toxoplasma in humans. On the other hand, encephalitis is one of the most important and severe symptoms of toxoplasmosis in diarrheal patients infected with AIDS. Other symptoms may include headache, sleep disturbance, hemiparesis, reflex changes, and seizures that can sometimes lead to death Humans and carnivores can become infected by consuming raw or undercooked meat and cat feces containing tissue cysts, which transmit at least 30% of human toxoplasmosis cases. Toxoplasma can enter the host body orally in the form of tissue cysts or oocysts can be described in 4 forms of acquired toxoplasmosis, congenital, ocular and disease in immunocompromised individuals. The pathogenic mechanism of this parasite in acute toxoplasmosis is related to the effect This protozoan is directly on the cells in which it multiplies. The standard method for diagnosing Toxoplasma is serological methods, especially ELISA. In this study, Toxoplasma gondii GRA6 gene, which has a 344 bp fragment, was used. The GRA6 gene is also used to observe and diagnose toxoplasmosis and its role in determining the antigenic potency of the parasite. The GRA6 coding region shows a higher rate of polymorphism than other genes encoding Toxoplasma. The ability

of GRA6 gene to differentiate into three types 1, 2 and 3 is remarkable. Based on the characteristics of GRA6 nucleotide polymorphisms, three types of Toxoplasma 1, 2, and 3 can be well differentiated by PCR reaction and sequencing. For example, when digesting SAG2 gene, two digestive enzymes should be used, while using GRA6 gene, if Toxoplasma tachyzoites are high or well reproduced in Toxoplasma culture medium, only using one enzyme and simple PCR method the desired results can be obtained. The use of PCR method in addition to high sensitivity has many features.Recent studies have shown that the three main isolates of Toxoplasma gondii usually dominate in different geographical areas. Type II and III types are prevalent on all continents, as well as in Europe, Africa and North America. In addition, types I and II are the most common types in North America, and types I and III are also the most important types in Africa. The importance of genotyping is important because it can be used to identify the dominant genotype in the region and subsequently other measures such as control and special prevention of that genotype as well as treatment measures to the risk of the genotype.

In the present study, the prevalence of Toxoplasma in sheep is significant and shows a prevalence of approximately 60% in the city of Urmia, which is a risk for all members of the community, especially pregnant and immunocompromised. Also, the prevalence of beef and goat milk is 30%and 8% respectively. Use of grilled meat or meat Undercooked and raw can increase the transmission of Toxoplasma in this case. In another study conducted in 2009 by Tavassoli et al., The abundance of Toxoplasma was examined using the B1 gene, which was one of 45 sheep Toxoplasma infection was detected, which could be due to the increased transmission of Toxoplasma in recent years. Also, the use of GRA6 gene is better than B1 gene in differentiating genotypes and is highly sensitive. Also, in another study that Asgari et al. In 2011 sampled from various organs of sheep and goats slaughtered in Shiraz slaughterhouse and analyzed the results using PCR method, they concluded that the prevalence of gonorrhoeae in the gut was: 37%and in goats is 22.7%.

Conclusion

Our results indicate a significant increase in Toxoplasma gondii in recent years. In this study, the prevalence of Toxoplasma gondii in cats in Urmia is low and about 3.5%, which could be due to the fact that cats during their lifetime Oocysts are excreted only once, in a short period of time. For example, in a study conducted by Tavassoli on cats in 2009, Toxoplasma infection was examined among 7 cats, of which no parasites were received. This may be because oocysts are excreted by the cat only for a short time Also, from the Lymph Node samples that were adopted, we were able to obtain only 5 positive samples, which may be due to the priority of serological and molecular tests in the diagnosis of Toxoplasma over pathological methods. Some conditions such as geographical and cultural conditions and ecology, etc. of the region can cause the spread of certain types in a particular region. The superiority and high prevalence of type II can be due to having more adaptability and high reproductive power for competition and persistence compared to other genotypes and also due to the high ability of the parasite to form other tissue cysts and other tissue cysts and other races. Type II is also the leading type in opportunistic infections in immunocompromised individuals and congenital toxoplasmosis in Europe. In our results, the most genotype was related to genotype. In other words, out of 13 samples that were sent for sequencing, 9 were related to genotype II, 2 were related to genotype I and 2 others were related to genotypeIII. In 2007, Zia Ali et al., By molecular analysis of 105 sheep by RFLP-PCR method, were able to isolate four strains of Toxoplasma gondii, so that two of them were type II strains and the other two were type III strains. GRA6 marker can clearly distinguish between 3 main genotypes of Toxoplasma gondii as well as some atypical genotypes. In 2012, Habibi et al. Examined 18 aborted sheep embryos in Qazvin province by RFLP-PCR and showed that 66% of the aborted sheep embryo samples were infected with type I infection and became infected with type I tuberculosis. It is one of the causes of abortion in sheep. Seed China et al. In 2013 were able to separate Toxoplasma gondii DNA from 54% of aborted embryonic brain by RFLP-PCR in Khorasan Razavi province. All isolates were based on GRA6 locus, depending on type I. Based on the studies that have been done, it seems that humans in the city of Urmia are mostly infected with Toxoplasma parasite through raw meat. It also seems that genotype II has more adaptability than other genotypes in the city of Urmia and that is why the genotype is dominant in this region.

References

1. Weiss LM, Dubey JP. Toxoplasmosis: A history of clinical observations. Int J Parasitol. 39 (2009):895-901
2. Wolf, Abner, Cowen David, and Paige Beryl. "Human toxoplasmosis: occurrence in infants as an encephalomyelitis verification by transmission to animals." Science 89 (1939): 226-227.
3. Ferguson, David JP. "Toxoplasma gondii: 1908-2008, homage to Nicolle, Manceaux and Splendore." Mem Inst Oswaldo Cruz 104 (2009): 133-148.
4. Moncada, Pablo A., and Jose G. Montoya. "Toxoplasmosis in the fetus and newborn: an update on prevalence, diagnosis and treatment." Expert Rev Anti Infect Ther 10 (2012): 815-828.
5. Tenter, Astrid M., R Anja. Heckeroth, and. Weiss Louis M. "Toxoplasma gondii: from animals to humans." Int J Parasitol 30 (2000): 1217-1258.

How to cite this article: Yousefzadeh M, Yekan Somayyeh Vakili. "Molecular Detection and Genotyping of Toxoplasma gondii Strains from Beef, Lamb,Goat milk, Cat Feces, and Human Lymph Node Samples in Urmia, Iran Using PCR with GRA6 Gene from 2020 to 2021." *J Microb Pathog*5 (2021) : jmp-21-39547