Archives of Razi Institute, Vol. 75, No. 1 (2020) 47-54

DOI: 10.22092/ARI.2018.122733.1227

Original Article

Genetic Affinity of *Echinococcus granulosus* protoscolex in Human and Sheep in East Azerbaijan, Iran

Zarrabi Ahrabi [1], S., Madani [1, 2] *, R., Shemshadi [1], B., Ranjbar Bahadori [3], Sh., Hashemzadeh Farhang [4], H.

1. Department of Microbiology, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Proteomics & Biochemistry section Biotechnology, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
3. Department of Parasitology, Faculty of Veterinary Medicine, Garmsar Branch, Islamic Azad University, Semnan, Iran
4. Department of Parasitology, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Received 14 August 2018; Accepted 13 September 2018
Corresponding Author: madanirasool@gmail.com

ABSTRACT

Echinococcosis caused by the larval form of *Echinococcus granulosus* (*E. granulosus*) is known as an important zoonotic disease in various parts of the world, including Iran. The genetic diversity of this parasite is very high, particularly in areas where the disease is endemic. It has been suggested in the literature from different parts of the world that diverse factors, such as parasite life cycle, transmission pathways, pathologic disease, immunization, and disease control can be affected by the genetic diversity of the parasite. Various studies indicated sheep strain G1 as the most common genotype throughout the world. This strain is commonly found in the liver and lung repeatedly causing echinococcosis in humans, sheep, and cattle. The present study was conducted to determine the genetic affinity between the protoscolex of *E. granulosus* in humans and sheep in East Azerbaijan province, Iran for the first time. A total of 120 hydatid cyst samples were collected, 60 of which were from people who referred to the hospitals of East Azerbaijan and 60 were from the sheep slaughtered in Tabriz slaughterhouse. Following DNA extraction, certain regions of the *cox1* gene were amplified and evaluated by the polymerase chain reaction. The replicated parts in all isolates had the same size of 450 bp. Electrophoresis was followed by selecting a total of 60 suitable samples, including 30 human samples and 30 sheep samples and sending them for genome sequencing. The overlap of the samples was investigated using the BLAST software. The results of BLAST, sequencing, and overlap demonstrated a genetic linkage of approximately 91.76% between the protoscolex of *E. granulosus* in human and sheep.

Keywords: DNA extraction, Genetic affinity, Hydatid cyst, PCR, Sequencing

Affinité Génétique *d'Echinococcus Granulosus* Protoscolex chez l'Homme et les Ovins dans l'Est Azerbaïdjan, Iran

Résumé: L'*échinococcose* causée par la forme larvaire *d'Echinococcus granulosus* (*E. granulosus*) est considérée comme une maladie zoonotique importante dans diverses parties du monde, y compris l'Iran. La diversité génétique de ce parasite est très élevée, en particulier dans les zones où la maladie est endémique. Plusieurs études provenant de différentes parties du monde ont suggéré que la diversité génétique du parasite avait des effets sur le cycle de vie du parasite, les voies de transmission, les maladies pathologiques, l'immunisation et le contrôle des maladies. Diverses études indiquent que la souche ovine G1 est le génotype le plus répandu dans le monde. Cette souche se retrouve couramment dans le foie et les poumons, provoquant à plusieurs reprises l'*échinococcose* chez l'homme, les ovins et les bovins. L'objectif de cette étude était de

déterminer l'affinité génétique pour la première fois entre le protoscolex d'*E. granulosus* chez l'homme et les ovins dans la province de l'Est Azerbaïdjan (Iran). Un total de 120 échantillons de kystes hydatiques ont été collectés, parmi lesquels 60 échantillons provenaient de personnes référées aux hôpitaux d'Azerbaïdjan de l'Est et les autres (n=60) provenaient d'ovins abattus à l'abattoir de Tabriz. Après l'extraction de l'ADN, certaines régions du gène cox1 ont été amplifiées et évaluées par la réaction en chaîne de la polymérase. Les parties répliquées dans tous les isolats avaient la même taille de 450 pb. L'électrophorèse a été suivie par la sélection d'un total de 60 échantillons, comprenant 30 échantillons humains et 30 échantillons d'ovins pour le séquençage du génome. La méthode BLAST a été utilisé pour étudier le chevauchement des échantillons. Les résultats du BLAST, du séquençage et du chevauchement ont démontré une similarité génétique d'environ 91.76% entre le protoscolex d'*E. granulosus* chez l'homme et les ovins.

Mots-clés: Extraction d'ADN, Affinité génétique, Kyste hydatique, PCR, Séquençage

INTRODUCTION

Hydatidosis is a parasitic disease caused by *Echinococcus granulosus* through the attachment of adult worm to the mucosal crypts of the small intestine of the definitive host, such as domestic dogs and some wild canids. Afterwards, larval stage or hydatid cyst forms in the intermediate hosts, including sheep, cattle, goat, horse, camel, and human. In addition, humans get accidentally infected by hydatid cysts through the consumption of contaminated vegetables and contact with dogs. This disease can be considered as the most remarkable zoonotic disease due to its increasing importance in medicine, veterinary medicine, and the economic aspects. The disease is of importance regarding the two dimensions of public health and economic damage, with a loss of 2.1 million infected animals in Iran (endemic) during 2002-2006 and an economic loss of about 76 billion Rials. Moreover, it will cost even more if indirect damages and costs due to human treatments are added (Tavakoli et al., 2008). In Iran, the adult worm has been isolated from dogs, jackals, and wolves. Cystic echinococcosis (CE) remains in the domestic cycle and survives in livestock farming areas where humans have free access to animal feed. Consequently, it is considered as a lasting zoonosis (Mitrea et al., 2014). Various cases of hydatid cyst have been reported in Iran. Sheep is one of the most common and important hosts of this parasite during its life cycle in Iran. Sheep can play an important role in disease transmission due to the presence of scolex in the liver cysts of this animal (Neva and Brown, 1994; John and Petri, 2006). The rate of sheep infection with larvae in different parts of Iran was 1%-7% (the maximum infection was reported for Fars province). According to the reports, the infection rate among cows was between 16%-58% with the maximum infection in Tabriz slaughterhouse and the minimum in buffaloes in Ahwaz indicating the severity of infection (John and Petri, 2006). In addition, the animal infection rate in the slaughterhouses of several regions of the country was reported to be 15%-74% (John and Petri, 2006). We constantly report human cases from different parts of Iran. Human infection has been reported in Isfahan, Fars, Khorasan, and Arak provinces, Iran. In East Azerbaijan, only 23 human cases of CE were reported during 2001-2006. However, in another study completed in Tabriz during 2011-2012, finding 206 cases showed an elevation in the incidence of disease among human (Bayat et al., 2014). Quite complete studies have been performed considering the extent of infection in the definitive and intermediate hosts. The highest infection (33.3%) was reported in the North and Northwest of Iran with the

lowest infection (21.9%) observed in the West and Southwest of Iran (Alvarez Rojas et al., 2014). The infection was mostly found in the ruminants and livestock which were in direct contact with dogs (Acha and Szyfres, 2003). Cows do not play a role in disease transmission due to the absence of protoscolex in the bovine liver (Alvarez Rojas et al., 2014). In general, the infection rate among ruminants in the country is remarkable and 11.1% of sheep in East Azerbaijan were reported to be infected. It is noteworthy that the stray strain (G6) was isolated from a stray dog in East Azerbaijan. It seems that in the cycle of transmission and maintenance of the parasite between dogs and camels, some intermediate hosts play a secondary role (Spotin et al., 2017). Generally, in endemic areas, there is a relatively large genetic variation in *E. granulosus*, which is an intrinsic variety with many genetic strains adapted to evolve with different hosts (Fasihi Harandi et al., 2012). Genetic diversity among *Echinococcus spp.* is of particular importance because shows the difference between the types of infection in different hosts (Casulli et al., 2012). The relatively high genetic variation among the different genotypes leads to differences in the host characteristics, antigenicity, drug sensitivity, and life cycle. All the aforementioned factors are critical in the provision of vaccine, diagnosis, and treatment of the disease (Shamsi et al., 2016). Regarding the presence of different strains of hydatid cyst in Iran and due to the absence of cross-immunity between different strains, the protoscolex of the parasite should be evaluated for vaccine design. Genotypic differences between different species of echinococcus have resulted in the identification of ten genotypes (G1-G10) (Casulli et al., 2012). A common genotype that can cause disease in humans, cattle, and sheep is the genotype G1, which is classified as the sheep strain. Moreover, surgeries and biopsy samples have demonstrated that the disease could be caused by other genotypes (Shahnazi et al., 2011). Therefore, in terms of preventive and control measures, the exact determination of *E. granulosus* genotypes in endemic areas is necessary (McManus, 2002). Reports from the microscopic examination of Echinococcus protoscolex in Iran have pointed out the similarity of strains in sheep and humans. However, no comparative study has yet been conducted and no genetic link has been found in the East Azerbaijan region (Spotin et al., 2017). In domestic animals, such as cattle and sheep, compared to humans, little research has been carried out to improve the immunological diagnosis of hydatidosis. Detection of hydatidosis in hosts is majorly based on autopsy (Neva and Brown, 1994). Furthermore, the diversity in the gene expression profile of Echinococcus among humans and sheep in East Azerbaijan has not been investigated. A study conducted in 2016 on the genetic variation of the mitochondrial genome of hydatid cyst in Iran revealed no genetic difference between genotypes G1 and G3. Typically, cytochrome *oxidase 1* (*cox1*) is considered as a key marker in the structure of Echinococcus genes due to its repeated sequences in the genome (Casulli et al., 2012; Spotin et al., 2017). According to the literature, sheep is known as the source of disease in human. Therefore, the present study aimed to identify and determine the Echinococcus strains and investigate the extent of genetic relation between protoscolex in humans and sheep as the basis for further research on cross-immunity and vaccine production. Furthermore, the objective of this study was to compare the rate of convergence and the genetic difference between cystic protoscolex in humans and sheep. As a result, after coordination with the referral hospitals in East Azerbaijan, samples were taken from people with hydatid cyst. In addition, in the case of sheep, the samples were taken from Tabriz slaughterhouse.

MATERIAL AND METHODS

According to the main definitions, Echinococcosis, which is characterized by the emergence of cysts in most organs and the complications of cysts is common between humans and animals. A total of 120 hydatid cyst samples were collected, including 60 sheep

samples from Tabriz slaughterhouse and 60 samples from post-operative human cases in the referral hospitals of East Azerbaijan.

Protoscolex Isolation. To confirm the presence of protoscolex in the hydatid cysts, the cysts were subjected to microscopic examination.

A. Human Samples. For the isolation of protoscolex from human specimens, the hydatid cyst fluid was removed from the cyst after separating the cyst in the operating room. Afterwards, the fluid was diluted with sodium chloride and was centrifuged for 20 min at 1500 rpm. The wet samples were prepared from the sediments and following observing the protoscoleces, they were stored in distilled water in special tubes. Finally, the tubes were frozen in liquid nitrogen until the tests were carried out. It should be noted that all the steps were completed in sterile conditions (Oudni et al., 2004).

B: Sheep Samples. In order to isolate protoscolex from sheep samples, liver and infected lungs from the livestock were separated in the slaughterhouse and transferred to the laboratory. Next, hydatid cysts were sterilized by alcohol and heat and the fluid was discharged by syringe. Following centrifugation, the sediment containing protoscoleces was removed. Microscopic examination of the sample was followed by storing in distilled water in the tubes and was frozen in liquid nitrogen until the tests were carried out (Oudni et al., 2004) (Figure 1).

DNA Extraction. Following bringing the human and sheep samples to the laboratory, the DNA extraction process was performed according to the instructions of the manufacturer provided in the DNA extraction kit (Thermo Scientific #K0721, Lithuania). Afterwards, DNA concentration was evaluated using Nanodrop and the samples were stored at -70 °C.

Polymerase Chain Reaction. The *cox1* gene of the hydatid cyst was amplified using two standard forward and reverse primers (Table 1) (Bowels et al., 1992; Nikmanesh et al., 2014). Polymerase Chain Reaction (PCR) was performed in a final reaction volume of 50 µL containing 25 µL of 2X PCR Master Mix (Ampliqon, Denmark), 2 µL (0.5 µM) of each primer, and 100 ng of template DNA. After a short spin, the samples were placed in a thermal cycler and PCR was completed as the procedure demonstrated in Table 2. It should be noted that the PCR mixtures were prepared on ice (Bowles et al., 1992; Nikmanesh et al., 2014). The PCR amplifications were performed as follow: 94 °C for 5 min as an initial denaturation, 94 °C for 30 s, 56 °C for 45 s, 35 cycles of 72 °C for 35 s, and a final extension at 72 °C for 10 min (Spotin et al., 2017). Next, the PCR products (5 µL) were analyzed using 1% agarose gel electrophoresis.

Table 1. Oligonucleotides of cytochrome oxidase primer subunit 1 (*cox1*)

Gene	Primer	Gene length
COX1	F:5'-TTT TTT GGG CAT CCT GAG GTT TAT-3' R: 5'-TAA AGA AAG AAC ATA ATG AAA ATG-3'	450 bp

Table2. PCR procedure of sample

Step	Temperature (°C)	Time
Initial Denaturing	94	5 min
Denaturing	94	2 min
Annealing	50	45 sec
Extension	72	30-60 sec
Final Extension	72	5 min

Table 3. Average genetic similarity

	Query Cover	**Ident**
min	91.76	93.7

Sequencing. After electrophoresis, a number of qualitative samples, including 30 suitable sheep samples and 30 appropriate human specimens were selected and sent to the genome sequencing center for sequencing (Figures 4, 5). Following sequencing, the samples were crossed and evaluated one by one using the BLAST system (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

A total of 120 hydatid cyst samples were collected, including 60 sheep samples from Tabriz slaughterhouse and 60 samples from human cases after an operation in the referral hospitals of East Azerbaijan. Information

about patients and information about sheep were collected weekly from the hospitals and Tabriz slaughterhouse, respectively. The DNA of cystic protoscolex and mitochondrial DNA of *E. granulosus* were amplified by PCR and bands of 450 bp were obtained (Shamsi et al., 2016). Following the sequencing of human and sheep samples, the length of the fragment multiplied for the *cox1* gene was found to be around 450 bp for both of our sample models (Figure 2).

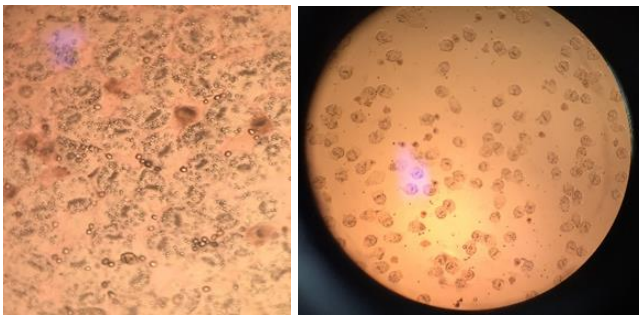

Figure 1. Microscopic examination of protoscolex.

Despite the overlap in electrophoretic findings, the numbers obtained from BLAST software and sequencing indicated the overlap and genetic relationship between humans and sheep as 91.76% (Table 3). Electrophoresis was followed by selecting and sending a number of high-quality samples to the genome sequencing center for sequencing. These samples did not have false bands and were shown to be exactly 450 bp and included 30 sheep samples and 30 human specimens. The overlaps of forward and reverse sequences of the samples were cross-sectioned one by one utilizing the BLAST system. As could be seen in the qualitative results demonstrated in Figure 6, the larger the red line drawn at the end of the table, the greater the overlap will be. Our findings obtained by electrophoresis were similar to the previous results concerning protoscolex. Furthermore, the comparison between humans and sheep showed no difference in the amount of electrophoretic band, which suggests no variety in the qualitative formation of the electrophoresis band (figures 2, 3).

DISCUSSION

Hydatid cyst is a common international disease of humans and animals (Pissuwan et al., 2007). The World Health Organization (WHO) listed *E. granulosus* under the group of neglected tropical diseases (Fasihi Harandi et al., 2012). This agent has a high prevalence attributed to the numerous intermediate hosts in Iran. It is considered as one of the important zoonotic diseases whose proper and timely diagnosis is a necessity (Shamsi et al., 2016). In Ilam, using human mitochondrial *cox1* genes from human and sheep isolates, genotypes G1, G2, and G3 belonging to the *E. granulosus* were identified (Shamsi et al., 2016).

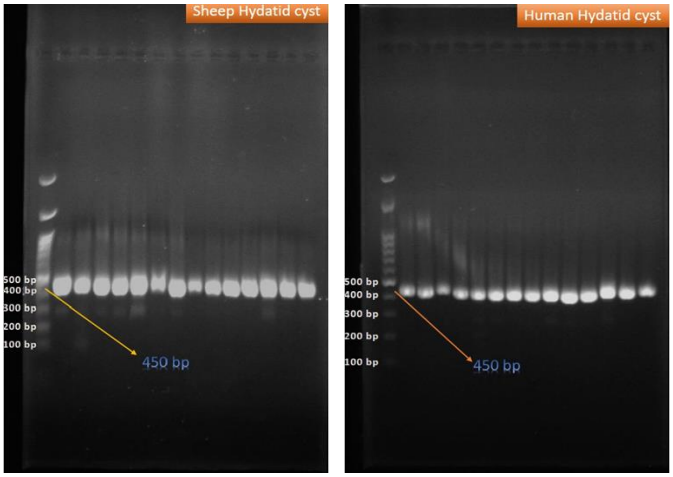

Figure 2. PCR outcomes of the *cox1* fragment from the human and sheep samples

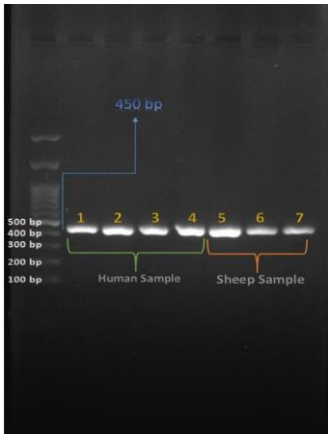

Figure 3. Comparison of the PCR results of *cox1* fragment between human and sheep samples

Investigation of *cox1* gene in 70 stray dogs in Lorestan province showed that 75% of the samples had genotype G1, 10% had genotype G2, and 15% had genotype G3 proving the presence of G1, G2, and G3 in that area (Parsa et al., 2012). Isolation of *E. granulosus* strains in Turkey was accomplished by the enzymatic digestion of the *cox1* gene, which in most cases was the sheep strain G1 (Utuk et al., 2008). Sequencing of the *cox1* gene has been carried out in various studies, whose results show that the sheep strain G1 is predominantly isolated from sheep, cattle, goats, and humans. Isolation of buffalo strain G3, in addition to the sheep and cattle strains, from two cases of human infection was reported for the first time by Pezeshki et al. in 2013.

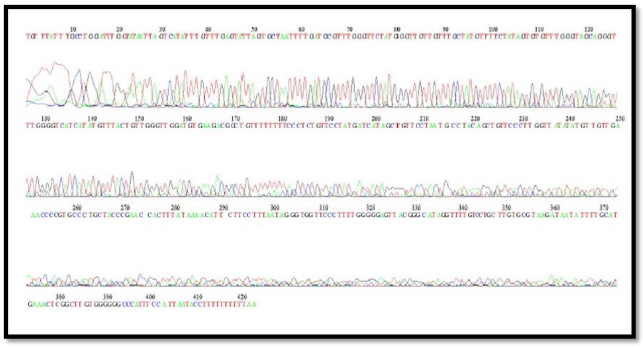

Figure 4. Sequencing result for *cox1* gene derived from a human sample

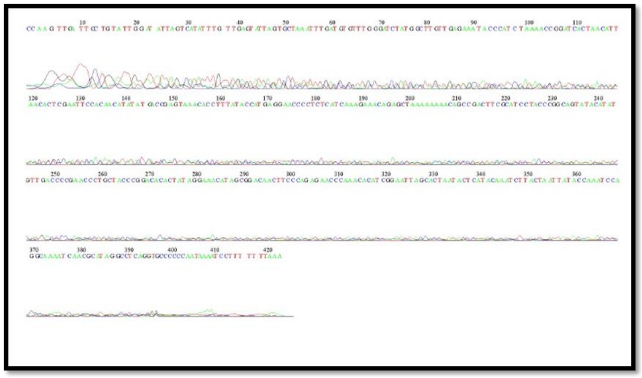

Figure 5. Sequencing result for *cox1* gene derived from sheep sample

Until now, several molecular investigations have been performed in distinct countries, including Iran to identify different genotypes of this parasite. These studies were conducted mainly using the PCR technique. Moreover, to complete these similar results, the sequences of mitochondrial genes were used to determine the genotype, most of which indicated genotype G1 (McManus, 2002). Sheep strain G1 was isolated from humans, cattle, sheep, and a small number of camels in Isfahan. Isolation of camel strain G6 from human, cow, and camel suggests that this strain is the leading cause of infection in humans (Shahnazi et al., 2011). According to a study completed in Ilam, the geographical distribution of *E. granulosus* strains in different regions can vary. However, it has become clear that in areas where sheep are kept together with other domestic animals, humans are also at the risk of infection with sheep strain G1 due to contact with the final host, especially dog (Shamsi et al., 2016). As mentioned above, *cox1* is typically known as an important marker in the structure of Echinococcus genes due to repetitive sequences in the genome and most researches on the genotype of Echinococcus addressed this gene. In order to determine the genetic diversity of *E. granulosus*, a variety of sequences of the *cox1* gene were used in different hosts present in different geographical areas of Iran (Casulli et al., 2012; Spotin et al., 2017).

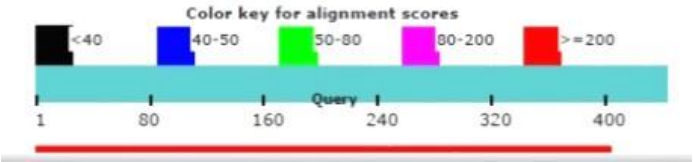

Figure 6. Graphical overview of overlap

Numerous *E. granulosus* strains have been reported in human and animal isolates using different molecular methods. Specified patterns of restriction fragment length polymorphism were reported to be identical between the isolates of horses and cows after digestion with Msp1 and Alu1 enzymes, as well as other distinct patterns of enzymes, namely Rsa1, CFO, and ITSI (Bowles et al., 1992). Considering the molecular and morphological characteristics of the isolates derived from humans and animals, the two life cycles of dog-sheep and dog-camel are considered as the active life cycles of the parasite (McManus, 2002). The genetic

similarity of *E. granulosus* between humans and sheep in the East Azerbaijan region was not studied so far and only general research was performed on the genetic recognition of species in the country. On the other hand, a recent investigation reported an increase in the incidence of this disease in the East Azerbaijan region (Bayat et al., 2014). Whit this background in mind, the necessity of this research is proved. Investigation of the genetic convergence between human and sheep might lead to the recognition of the root cause of this disease in humans. In addition, it can be useful in vaccine preparation and preventive protocol design as recent researches suggest it as a serious problem in public health in the region (Boufana et al., 2015; Spotin et al., 2017). To our knowledge, most of the work carried out until now was on the identification and determination of genotypes and common strains of *E. granulosus*. Moreover, the relationship between strain and pathogenicity in humans and host animals was assessed. All the specimens for determining the genotype have been authenticated by the National Gene Bank. According to the available comprehensive studies, the genotype G1 (i.e., common bovine strain) was confirmed as a common strain in human infection.

Genetic variation and similarity, as well as the presence of different serotypes in one species of parasite, may only result in changes in the expression of a gene or a sequence of amino acid. The results obtained by electrophoresis in the present study revealed the level of genetic similarity and overlap between sheep and human as 91.76%. Due to the absence of genetic similarity in about 8% of the cases, it can be concluded that there may be a difference in any of the sequences resulting in a lack of cross-immunity against the parasite. Genotypic and strain variation in any of the infected cases, including humans and sheep, is still possible as the result of individual variations. Although a similarity of 91.76% is quantitatively significant in evaluating genetic similarity, the presence of different serotypes, each of which has the ability to cause disease in humans and sheep, cannot qualitatively be a reliable number. Therefore, according to these interpretations, the provision and production of a vaccine, even in the native and regional conditions, does not seem to be a successful experience in preventing disease. Due to the high ability of the parasite, it seems that the disease can spread among different regions. According to the reports, G6 genotype or camel strain has also been reported in Tabriz. All the previous findings concerning the different strains of *E. granulosus* in Iran suggest a very high strain variation in the country. Furthermore, the most infecting strain in humans was found to be sheep strain. Therefore, the current research intended to prove the level of genetic similarity. In addition, we investigated whether this similarity is of importance in the development of immunity between two human and sheep species. Further investigation is needed in adults to show a 91.76% qualitative score. Moreover, the analysis of genetic and molecular research is not sufficient for judging the cross-immunogenicity and antigenicity features.

Consequently, an effective program can be taken regarding the issues of prevention, control, and preparation of recombinant vaccines suitable for cystic Echinococcosis in humans and animals. In order to combat and control the parasite cycle in humans, ruminants, and carnivores in the region, useful measures should be taken.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contribution

Study concept and design: salar zarrabi ahrabi- rasoul madani- bahar shemshadi
Acquisition of data: salar zarrabi ahrabi-rasoul madani
Analysis and interpretation of data: salar zarrabi ahrabi

Drafting of the manuscript: salar zarrabi ahrabi
Critical revision of the manuscript for important intellectual content: shahrokh ranjbar bahadori
Statistical analysis: salar zarrabi ahrabi- rasoul madani
Administrative, technical, and material support: hosein hashemzadeh farhangh

References

Acha, P.N., Szyfres, B., 2003. Zoonoses and communicable diseases common to man and animals, Pan American Health Org, New York, pp. 184-199.

Alvarez Rojas, C.A., Romig, T., Lightowlers, M.W., 2014. Echinococcus granulosus sensu lato genotypes infecting humans--review of current knowledge. Int J Parasitol 44, 9-18.

Bayat, A., Shirazi, S., Zarabi, S., Nejhad-Partovi, A., 2014. The epidemiologic survey of operated patients with haydatid cyst in Emam Reza medical and research and training hospital in Tabriz city during 2011-2012. 6th International Congress of Laboratory and Clinic, Tehran, Iran, pp. 287-294.

Boufana, B., Lett, W.S., Lahmar, S., Buishi, I., Bodell, A.J., Varcasia, A., *et al.*, 2015. Echinococcus equinus and Echinococcus granulosus sensu stricto from the United Kingdom: genetic diversity and haplotypic variation. Int J Parasitol 45, 161-166.

Bowles, J., Blair, D., McManus, D.P., 1992. Genetic variants within the genus Echinococcus identified by mitochondrial DNA sequencing. Mol Biochem Parasitol 54, 165-173.

Casulli, A., Interisano, M., Sreter, T., Chitimia, L., Kirkova, Z., La Rosa, G., *et al.*, 2012. Genetic variability of Echinococcus granulosus sensu stricto in Europe inferred by mitochondrial DNA sequences. Infect Genet Evol 12, 377-383.

Fasihi Harandi, M., Budke, C.M., Rostami, S., 2012. The monetary burden of cystic echinococcosis in Iran. PLoS Negl Trop Dis 6, e1915.

John, D.T., Petri, W.A., 2006. Markell and Voge's medical parasitology-e-book, Elsevier Health Sciences, Philadelphia, USA, pp. 287-296.

McManus, D., 2002. The molecular epidemiology of Echinococcus granulosus and cystic hydatid disease. Trans R Soc Trop Med Hyg 96, S151-S157.

Mitrea, I.L., Ionita, M., Costin, II, Predoi, G., Avram, E., Rinaldi, L., *et al.*, 2014. Occurrence and genetic characterization of Echinococcus granulosus in naturally infected adult sheep and cattle in Romania. Vet Parasitol 206, 159-166.

Neva, F.A., Brown, H.W., 1994. Basic clinical parasitology, Appleton & Lange, New York, USA, pp, 217-224.

Nikmanesh, B., Mirhendi, H., Ghalavand, Z., Alebouyeh, M., Sharbatkhori, M., Kia, E., *et al.*, 2014. Genotyping of Echinococcus granulosus isolates from human clinical samples based on sequencing of mitochondrial genes in Iran, Tehran. Iran J Parasitol 9, 20-27.

Oudni, M., M'Rad, S., Mekki, M., Belguith, M., Cabaret, J., Pratlong, F., *et al.*, 2004. Genetic relationships between sheep, cattle and human Echinococcus infection in Tunisia. Vet Parasitol 121, 95-103.

Parsa, F., Fasihi Harandi, M., Rostami, S., Sharbatkhori, M., 2012. Genotyping Echinococcus granulosus from dogs from Western Iran. Exp Parasitol 132, 308-312.

Pezeshki, A., Akhlaghi, L., Sharbatkhori, M., Razmjou, E., Oormazdi, H., Mohebali, M., *et al.*, 2013. Genotyping of Echinococcus granulosus from domestic animals and humans from Ardabil Province, northwest Iran. J Helminthol 87, 387-391.

Pissuwan, D., Valenzuela, S.M., Miller, C.M., Cortie, M.B., 2007. A golden bullet? Selective targeting of Toxoplasma gondii tachyzoites using antibody-functionalized gold nanorods. Nano Lett 7, 3808-3812.

Shahnazi, M., Hejazi, H., Salehi, M., Andalib, A.R., 2011. Molecular characterization of human and animal Echinococcus granulosus isolates in Isfahan, Iran. Acta Trop 117, 47-50.

Shamsi, M., Dalimi, A., Khosravi, A., Ghafarifar, F., 2016. The phylogenetic similarity of hydatid cyst isolated from humans and sheep in Ilam Province southwest of Iran. Comp Clin Pathol 25, 1221-1226.

Spotin, A., Mahami-Oskouei, M., Harandi, M.F., Baratchian, M., Bordbar, A., Ahmadpour, E., *et al.*, 2017. Genetic variability of Echinococcus granulosus complex in various geographical populations of Iran inferred by mitochondrial DNA sequences. Acta Trop 165, 10-16.

Tavakoli, H., Bahonar, A., Joneydi, N., 2008. Epidemiology of hydatidosis in Iran during 2002-2006. Iran J Parasitol 42, 71-76.

Utuk, A.E., Simsek, S., Koroglu, E., McManus, D.P., 2008. Molecular genetic characterization of different isolates of Echinococcus granulosus in east and southeast regions of Turkey. Acta Trop 107, 192-194.