FABAD J. Pharm. Sci., 48, 2, 295-302, 2023
Doi: 10.55262/fabadeczacilik.1324184
RESEARCH ARTICLE

Parasite-Derived MicroRNAs: Potential Alternative Targets for Laboratory Diagnosis of Cystic Echinococcosis

Serra ÖRSTEN[*°], İpek BAYSAL[**], Salih MAÇİN[***]

Parasite-Derived MicroRNAs: Potential Alternative Targets for Laboratory Diagnosis of Cystic Echinococcosis

SUMMARY

Cystic Echinococcosis (CE) is a type of zoonotic infection that can be caused by a specific form of a parasite called Echinococcus granulosus sensu lato. Mainly, imaging techniques are utilized to diagnose CE. Serological tests are only used when imaging findings are atypical. Additionally, laboratory assays including, direct microscopy and PCR are used to confirm of diagnosis after treatment though obtained negative results with these tests cannot be ruled out the diagnosis. Specific miRNAs produced by the parasite could be used as markers to diagnose and monitor CE. This research investigates the diagnostic potential of parasite-derived miRNAs compared to the presence of protoscolex in animal-derived hydatid cyst samples. Accordingly, egr-let-7-5p, egr-miR71-5p, and egr-miR-9-5p were positive in 26, 25, and 11 out of 30 samples (86.6%, 83.3%, and (36.6%), respectively. There was no relationship between protoscolex presence and detection of either egr-let-7-5p or egr-miR-9-5p (p>0.05). On the other hand, egr-miR71-5p positivity was found to be statistically significant compared with protoscolex presence (p=0.04). As a result, egr-miR-71 is a promising potential target for the diagnosis of CE. Additional research is necessary to assess the diagnostic value of miRNAs in CE using a larger group of samples.

Key Words: *Echinococcus granulosus, cystic echinococcosis, diagnostics, microRNA*

Parazit Kaynaklı MikroRNA'lar: Kistik Ekinokokkoz'un Laboratuvar Tanısında Potansiyel Alternatif Hedefler

ÖZ

Kistik Ekinokokkoz (KE), Echinococcus granulosus sensu lato adlı parazitin larva formunun neden olduğu bir tür zoonotik enfeksiyondur. Çoğunlukla, KE tanısı için görüntüleme teknikleri kullanılmaktadır. Serolojik testler yalnızca görüntüleme bulguları atipik olduğunda tanıya yardımcı olmakta, tedavi sonrası tanının doğrulanmasında direkt mikroskopi ve PCR gibi laboratuvar tetkikleri de kullanılmaktadır. Ancak bu testlerle elde edilen negatif sonuçlar tanıyı dışlamamaktadır. Parazit tarafından üretilen spesifik mikroRNA'lar (miRNA), KE'yi teşhis etmek ve hastaları tedavi etkinliği açısından takip için belirteçler olarak kullanım potansiyeli taşımaktadır. Bu araştırmanın amacı, hayvan kaynaklı hidatik kist örneklerinde protoskoleks varlığına kıyasla parazit kaynaklı miRNA'ların teşhis potansiyelini araştırmaktır. Buna göre egr-let-7-5p, egr-miR71-5p ve egr-miR-9-5p, 30 örneğin sırasıyla 26, 25 ve 11'inde (%86,6, %83,3 ve %36,6) pozitif bulunmuştur. Protoskoleks varlığı ile egr-let-7-5p veya egr-miR-9-5p'nin saptanması arasında anlamlı bir ilişki (p>0.05) olmamasına karşın egr-miR71-5p pozitifliği ise protoskoleks varlığına göre istatistiksel olarak anlamlı bulunmuştur (p=0,04). Sonuç olarak, egr-miR-71, KE tanısı için umut verici bir potansiyel hedef olarak görülmektedir. İleri çalışmalarda, daha büyük bir örneklem kullanarak KE tanısında parazit kaynaklı miRNA'ların tanısal değerinin değerlendirilmesi gereklidir.

Anahtar Kelimeler: *Echinococcus granulosus, kistik ekinokokkoz, tanı, mikroRNA*

Received: 07.07.2023
Revised: 20.07.2023
Accepted: 21.07.2023

[*] ORCID: 0000-0002-9216-5413, Hacettepe University, Vocational School of Health Services, Ankara, Turkey
[**] ORCID: 0000-0002-9607-4199, Hacettepe University, Vocational School of Health Services, Ankara, Turkey
[***] ORCID: 0000-0002-1871-3629, Selçuk University, Faculty of Medicine, Medical Microbioloy, Konya, Turkey

[°] Corresponding Author; Serra Örsten, PhD
Phone: +90 312 305 14 33 Fax: +90 312 311 27 30

INTRODUCTION

Cystic Echinococcosis (CE) is a type of zoonotic infection that can be caused by a specific form of a parasite called *Echinococcus granulosus* sensu lato (McManus, 2001). *E.granulosus* s.l. is recognized as a group of concealed species, which comprises *Echinococcus granulosus sensu stricto* (*s.s.*) (G1 and G3 genotypes), *Echinococcus equinus* (G4 genotype), *Echinococcus ortleppi* (G5 genotype) and *Echinococcus canadensis* cluster (G6/7, G8, G10 genotypes) and *E.felidis* (Hüttner et al., 2008; Romig, Ebi, & Wassermann, 2015; Thompson, 2008; Vuitton et al., 2020). CE is a prevalent helminthic disease globally, especially where individuals are engaged in animal husbandry and have contact with roaming and, or herding canines (McManus, 2001). Transmission occurs with the fecal-oral route via eggs scattered around from definitive hosts' feces and ingested by intermediate hosts. The life cycle of parasites continues between canids, mainly dogs and livestock. Humans are accidentally involved in this cycle. Hence, early diagnosis and treatment in humans are affectless in parasite transmission but are essential for raising awareness and reducing the disease burden (Tamarozzi, Deplazes, & Casulli, 2020). The World Health Organization (WHO) has included CE in its roadmap for neglected diseases, aimed at preventing, controlling, eliminating, and eradicating them by 2030 (Organization., 2020).

The clinical manifestations of individuals with CE may range from having no noticeable symptoms to a severe illness. Most cases remain asymptomatic for years and may be diagnosed mainly by chance (Eckert, Gemmell, Meslin, Pawlowski, & World Health, 2001; Tamarozzi et al., 2016). The time of the beginning of infection is virtually impossible to determine due to the slow progression of hydatid cyst development and the absence of symptoms of acute infection (Tamarozzi et al., 2020). Although hydatid cysts may occur in any organ, 80% of the patients have a single cyst in the liver (4/5) or lungs (1/5) (Brunetti, Kern, & Vuitton, 2010; Brunetti et al., 2018).

The identification of CE primarily depends on imaging modalities like ultrasonography (US), computed tomography (CT), x-ray, and magnetic resonance (MR), and serological tests are mainly utilized when the imaging findings are not typical (Tamarozzi et al., 2016). To confirm the diagnosis, a protoscolex or hooklet examination is performed in the hydatid fluid obtained after treatment with light microscopy, but in the presence of a sterile cyst, the diagnosis cannot be confirmed microscopically (Schwarz et al., 2017). In addition, negative microscopy results cannot exclude the diagnosis of CE (Örsten S. , 2020; Schwarz et al., 2017). Today, molecular techniques are widely used to confirm the diagnosis of the obtained cyst material and distinguish between species and strains. However, PCR approaches cannot evaluate cyst viability and a negative PCR result cannot rule out the disease, creating confusion about the diagnosis (Schwarz et al., 2017).

A type of small non-coding RNAs called microRNAs (miRNAs) are naturally plentiful and have been conserved throughout evolution. These have been discovered in a wide array of organisms, ranging from viruses to more complex higher eukaryotes. Additionally, they are accepted as primary regulators of gene expressions (Ameres & Zamore, 2013; Faruq & Vecchione, 2015). miRNAs are stable and are found in tissues and several body fluids (Mitchell et al., 2008). Therefore, they have great potential as diagnostic and prognostic biomarkers with high specificity and sensitivity (Faruq & Vecchione, 2015). During various developmental phases of its life cycle, *E. granulosus* expresses numerous miRNAs (Cucher et al., 2011). This study aims to evaluate whether parasite-derived miRNAs known to be abundantly expressed in the metacestode form of the parasite, have the potential for laboratory diagnostic of CE compared with protoscolex presence.

MATERIALS and METHODS

Sample Selection, Direct Examination, and Molecular Characterization

Hydatid fluids were obtained from different abattoirs in the Central Anatolia region of Turkey. The ethics committee approval is not necessary since the used samples were obtained from the slaughterhouse. Collected cyst fluids were examined for protoscolex or hooks under a light microscope. For this purpose, fluid was centrifuged at 3000 xg for 3 minutes to obtain a residue. In case of hooks or protoscoleces in microscopy were detected, the cyst was accepted as fertile. A commercial DNA Purification Kit was used for DNA extraction from the hydatid cysts. (GeneAll Biotechnology, Korea) following the instructions provided by the manufacturer. PCR amplification of a segment of the mitochondrial gene cytochrome c oxidase subunit 1 (mtCO1) was carried out using a protocol that had been published previously (Nakao, Sako, Yokoyama, Fukunaga, & Ito, 2000). Amplicons were evaluated via electrophoresis and the products were considered positive when a band size of ~875 bp was observed. Subsequently, sequencing was performed to identify all positive amplicons. The chromatograms were analyzed via FinchTV 1.4.0 (Geospiza Inc., Seattle, Washington, USA). The Basic Local Alignment Search Tool (BLAST) database (http://www.ncbi.nlm.nih.gov/BLAST/) was utilized to confirm the species. Thirty isolates from 28 cattle and two sheep confirmed to be E.granulosus were included in the study.

RNA Isolation, cDNA Synthesis, and miRNA Detection

RNA extraction from the hydatid cysts was performed using RNA Extracol (EURx) according to the manufacturer's recommendations. To measure the concentration and assessment of the purity of RNA, a FLUOstar Omega Microplate Reader (manufactured by BMG LABTECH) was used in conjunction with an LVis plate. The cDNA synthesis was performed using a commercial kit per the manufacturer's guidelines (cDNA Synthesis Kit with RNase Inh. (High Capacity) by A.B.T.™, Turkey).

To determine *E. granulosus*-specific miRNAs in the hydatid cyst material, miR-71a-5p, miR-9-5p, and let-7-5p were chosen for assay. MiRNA primers were used in conjunction with SYBR Green Master Mix (manufactured by A.B.T.™, Turkey) for conducting real-time polymerase chain reaction (RT-PCR) with ViiA™ 7 RT PCR System (Thermo Fisher Scientific) (Örsten et al., 2022) (Table 1). Data were collected with the previously published protocol (Örsten et al., 2022). As a negative control, nuclease-free water was used.

Table 1. Primer Sequences

miRNA	Primer Sequence
egr-let-7- 5p	TGAGGTAGTGTTTCGAATGTCT
egr-miR-9- 5p	TCTTTGGTTATCTAGCTGTGTGT
egr-miR-71a-5p	TGAAAGACGATGGTAGTGAGA

Statistical Analysis

The data collected were analyzed using the Chi-Square test with SPSS 23 (SPSS Inc., Chicago, IL, USA) program. A significance level was accepted as $p \leq 0.05$.

RESULTS AND DISCUSSION

The study investigated 30 hydatid cysts obtained from sheep and cattle, with 60% (18/30) of the cysts found in the liver and the remaining 40% (12/30) in the lungs. Upon microscopic examination, 60% (18/30) of the cysts were classified as fertile, while the remaining cysts were categorized as sterile. Most fertile cysts (13/18, 72.2%) were located in the liver. The BLAST algorithm confirmed that all samples were *E. granulosus* s.s. as per the PCR results.

RT PCR was used to assess the existence of parasite-specific miRNAs, namely egr-let-7-5p, egr-miR71-5p, and egr-miR-9-5p. Accordingly, egr-let-7-5p and egr-miR71-5p were positive in 26 and 25 out of 30 samples (86.6% and 83.3%), respectively. On the other hand, egr-miR-9-5p (11/30, 36.6%) was found to be positive in 11 out of 30 samples (36.6%). The positivity rates of egr-let-7-5p, egr-miR71-5p, and

egr-miR-9-5p in liver cysts were 89.9%, 83.3%, and 38.9%, respectively. In addition, the positivity rates of egr-let-7-5p, egr-miR71-5p, and egr-miR-9-5p in lung cysts were 83.3%, 91.7%, and 33.3%, respectively. On a sample basis, specific parameters such as cyst location, results of protoscolex examination, and miRNA detection status are given in Table 2.

Table 2. The parameters evaluated in this study

Isolate number	Location	Protoscolex	Let-7	miR-71	miR-9
1	Lung	-	+	+	-
2	Lung	-	+	+	-
3	Lung	-	+	+	-
4	Lung	-	-	+	+
5	Lung	-	-	+	-
6	Lung	+	+	-	+
7	Lung	+	+	+	+
8	Lung	+	+	+	-
9	Lung	-	+	+	+
10	Lung	-	+	+	-
11	Lung	+	+	+	-
12	Lung	+	+	-	-
13	Liver	+	+	+	+
14	Liver	+	+	+	+
15	Liver	+	+	+	-
16	Liver	-	-	+	+
17	Liver	-	+	+	-
18	Liver	+	+	+	-
19	Liver	+	+	-	-
20	Liver	-	+	+	-
21	Liver	-	+	+	-
22	Liver	+	-	+	-
23	Liver	+	+	+	+
24	Liver	+	+	+	-
25	Liver	+	+	+	+
26	Liver	+	+	-	-
27	Liver	-	+	+	-
28	Liver	+	+	-	+
29	Liver	+	+	+	-
30	Liver	+	+	+	+

(+): Positive, (-): Negative

There was no relationship between protoscolex presence and detection of either egr-let-7-5p or egr-miR-9-5p ($p>0.05$). On the other hand, egr-miR71-5p positivity was found statistically significant compared to protoscolex presence in molecularly confirmed samples ($p=0.04$) (Figure 1).

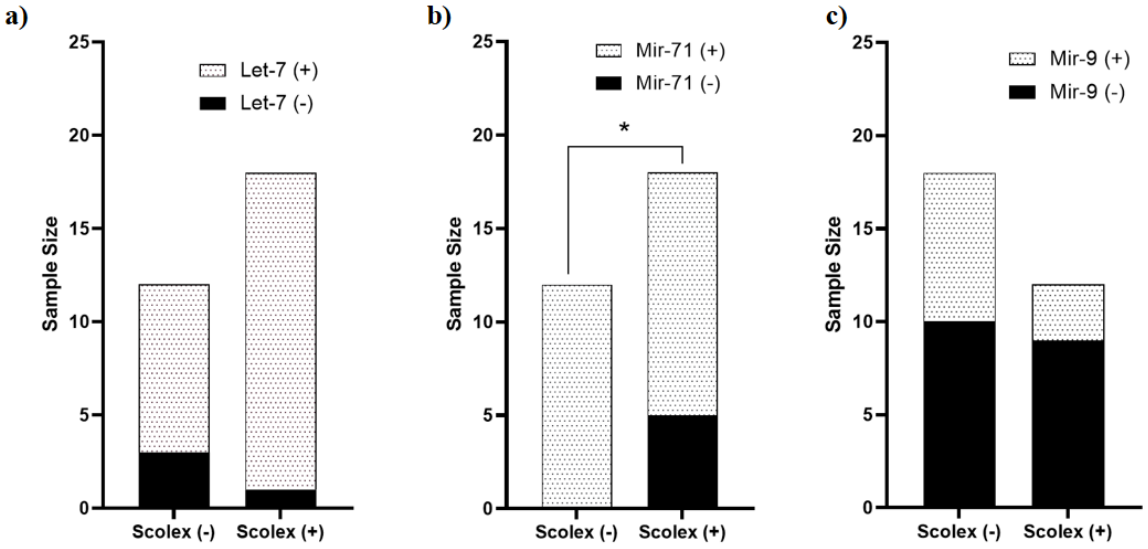

Figure 1. Presence of scolex and miRNA detection rates

Currently, imaging techniques serve as the primary diagnostic tool for CE in human patients, while laboratory methods are employed solely for confirmation purposes albeit their potential inadequacy. For instance, serological tests used for confirmation in humans are not standardized (World Health Organization, 2020). Characteristic protoscolex examination in the hydatid cyst fluid is performed with light microscopy after the treatment to confirm the diagnosis. However, it cannot be confirmed microbiologically in sterile cysts (Brunetti et al., 2010). Molecular techniques, such as PCR, are regarded as specific and sensitive methods that can be used in the species identification of parasites and confirmation of CE diagnosis (Grimm et al., 2021). However, it is known that PCR negativity targeting the DNA of *E.granulosus* does not rule out the diagnosis (Schwarz et al., 2017). Consequently, there is a need for alternative targets that can be used in the diagnosis of CE. In this study, the diagnostic potential of miRNAs was evaluated by comparison with protoscolex examination in hydatid cyst material derived from intermediate hosts, including cattle and sheep. For this purpose, specific parasite miRNAs, namely egr-let-7, egr-miR71a, and egr-miR-9, were chosen. The outcomes showed that 86.6% of all samples were positive for egr-let-7, while egr-miR71a and egr-miR-9 were detected in 83.3% and 36.6% of the samples, respectively.

Several studies have consistently found that cysts derived from sheep display notable fertility and viability, while cysts from cattle tend to exhibit a significant level of sterility (Fikire, Tolosa, Nigussie, Macías, & Kebede, 2012; Kebede, Mitiku, & Tilahun, 2009). According to studies from Turkey, cyst fertility rates have changed between 5.42-93.3% in hydatid cysts derived from cattle (Macin et al., 2021; Yildiz & Gurcan, 2003; Yildiz & Tunçer, 2005). Consistent with the literature, in this study fertility rate of hydatid cysts from cattle was found to be 57.1% (16/28). In addition, according to the organ location of the hydatid cysts, protoscolex positivity was found to be 72.2% in the liver and 41.6% in the lung.

Investigation of *E. granulosus*-specific antigens in the hydatid fluid and showing the parasite's DNA (eg PCR) are considered more advanced techniques that can be used in the diagnosis, especially in sterile cysts (Eckert et al., 2001). A recent study showed that 25 out of 39 samples accepted sterile by light microscopy were PCR positive. This result reveals the neces-

sity of examining the negative samples by light microscopy with molecular methods as well (Örsten S. , 2020). The study revealed that protoscolex-negative samples exhibited a positivity of 75% for egr-let-7-5p and 100% for egr-miR71a-5p, respectively. However, only the egr-miR71a-5p positivity showed statistical significance (p=0.04). Surprisingly, the egr-miR-9-5p positivity rate was observed as 25% and 44.4% in protoscolex-negative and protoscolex-positive samples, respectively. Prior research found that specific miRNAs derived from the parasite, namely let-7, miR71a, and miR-9, were significantly more abundant in the serum of patients with CE compared to the healthy subjects (with fold changes of >200, 107.90, and 126.56, respectively), with a statistical significance level of $p<0.05$. Furthermore, based on its expression levels in active and inactive CE patients, egr-miR-9-5p was identified as the most effective discriminatory miRNA (Örsten et al., 2022). In this study, it was surprising to find that egr-miR-9-5p exhibited the lowest miRNA positivity rate in the cyst sample. This might be due to its abundant presence as the highest circulating miRNA in the host serum. It is important to interpret the empirical findings presented in this study while acknowledging certain limitations. One such limitation is the restriction of the hydatid materials used to those of animal origin. Therefore, conducting extensive research involving hydatid material derived from humans is imperative to ascertain the diagnostic capabilities of the findings.

CONCLUSION

In conclusion, the diagnostic potential of certain miRNAs was evaluated using hydatid isolates originating from cattle and sheep. This study has indicated that parasite-derived miRNAs can be used as an alternative target molecule in the laboratory diagnosis of CE. Especially, egr-miR71a-5p has shown promising diagnostic potential compared to the other miRNAs. Additional research is necessary to assess the diagnostic value of miRNAs in CE using a larger group of samples.

ACKNOWLEDGMENTS

We are grateful to veterinarians for their help collecting samples in slaughterhouses. The author declares that no funds, grants, or other support were received during the preparation of this manuscript.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest

AUTHOR CONTRIBUTION STATEMENT

S.Ö: Developing hypothesis, experimenting, preparing the study text, literature research. İ.B: Statistics, analysis, and interpretation of the data, reviewing the text. S.M: Sample collection, literature research, reviewing the text.

REFERENCES

Ameres, S. L., & Zamore, P. D. (2013). Diversifying microRNA sequence and function. *Nat Rev Mol Cell Biol, 14*(8), 475-488. doi:10.1038/nrm3611

Brunetti, E., Kern, P., & Vuitton, D. A. (2010). Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop, 114*(1), 1-16. doi:10.1016/j.actatropica.2009.11.001

Brunetti, E., Tamarozzi, F., Macpherson, C., Filice, C., Piontek, M. S., Kabaalioglu, A., . . . Dietrich, C. F. (2018). Ultrasound and Cystic Echinococcosis. *Ultrasound Int Open, 4*(3), E70-e78. doi:10.1055/a-0650-3807

Cucher, M., Prada, L., Mourglia-Ettlin, G., Dematteis, S., Camicia, F., Asurmendi, S., & Rosenzvit, M. (2011). Identification of Echinococcus granulosus microRNAs and their expression in different life cycle stages and parasite genotypes. *Int J Parasitol, 41*(3-4), 439-448. doi:10.1016/j.ijpara.2010.11.010

Eckert, J., Gemmell, M. A., Meslin, F. o.-X., Pawlowski, Z. S., & World Health, O. (2001). WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern / edited by J. Eckert ... [et al.]. In: Paris, France: World Organisation for Animal Health.

Faruq, O., & Vecchione, A. (2015). microRNA: Diagnostic Perspective. *Front Med (Lausanne), 2*, 51. doi:10.3389/fmed.2015.00051

Fikire, Z., Tolosa, T., Nigussie, Z. M., Macías, Á., & Kebede, N. (2012). *Prevalence and characterization of hydatidosis in animals slaughtered at Addis Ababa abattoir, Ethiopia*.

Grimm, J., Krickl, J., Beck, A., Nell, J., Bergmann, M., Tappe, D., . . . Brehm, K. (2021). Establishing and evaluation of a polymerase chain reaction for the detection of Echinococcus multilocularis in human tissue. *PLoS Negl Trop Dis, 15*(2), e0009155. doi:10.1371/journal.pntd.0009155

Hüttner, M., Nakao, M., Wassermann, T., Siefert, L., Boomker, J. D., Dinkel, A., . . . Ito, A. (2008). Genetic characterization and phylogenetic position of Echinococcus felidis (Cestoda: Taeniidae) from the African lion. *Int J Parasitol, 38*(7), 861-868. doi:10.1016/j.ijpara.2007.10.013

Kebede, N., Mitiku, A., & Tilahun, G. (2009). Hydatidosis of slaughtered animals in Bahir Dar Abattoir, Northwestern Ethiopia. *Trop Anim Health Prod, 41*(1), 43-50. doi:10.1007/s11250-008-9152-3

Macin, S., Orsten, S., Samadzade, R., Colak, B., Cebeci, H., & Fındık, D. (2021). Human and animal cystic echinococcosis in Konya, Turkey: molecular identification and the first report of E. equinus from human host in Turkey. *Parasitol Res, 120*(2), 563-568. doi:10.1007/s00436-021-07050-w

McManus, R. C. A. T. a. D. P. (2001). Aetiology: parasites and life-cycles. In G. M. Eckert J, Meslin FX, Pawlowski ZS. (Ed.), *In: WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern.* Paris OIE: WHO.

Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., Pogosova-Agadjanyan, E. L., ... Tewari, M. (2008). Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A, 105*(30), 10513-10518. doi:10.1073/pnas.0804549105

Nakao, M., Sako, Y., Yokoyama, N., Fukunaga, M., & Ito, A. (2000). Mitochondrial genetic code in cestodes. *Mol Biochem Parasitol, 111*(2), 415-424. doi:10.1016/s0166-6851(00)00334-0

Örsten, S., Baysal, İ., Yabanoglu-Ciftci, S., Ciftci, T., Ünal, E., Akıncı, D., . . . Akhan, O. (2022). Can parasite-derived microRNAs differentiate active and inactive cystic echinococcosis patients? *Parasitol Res, 121*(1), 191-196. doi:10.1007/s00436-021-07382-7

Örsten S. , Ü. E. (2020). Radyolojik Olarak Aktif Hidatik Kisti Olan Olgulardan Perkütan Aspire Edilen Kist Sıvılarında Protoskoleks Varlığı ile PCR Sonuçları Arasındaki Korelasyonun Araştırılması. *Genel Tıp Dergisi, 30*(4), 271-275.

Romig, T., Ebi, D., & Wassermann, M. (2015). Taxonomy and molecular epidemiology of Echinococcus granulosus sensu lato. *Vet Parasitol, 213*(3-4), 76-84. doi:10.1016/j.vetpar.2015.07.035

Schwarz, N. G., Loderstaedt, U., Hahn, A., Hinz, R., Zautner, A. E., Eibach, D., . . . Frickmann, H. (2017). Microbiological laboratory diagnostics of neglected zoonotic diseases (NZDs). *Acta Trop, 165*, 40-65. doi:10.1016/j.actatropica.2015.09.003

Tamarozzi, F., Covini, I., Mariconti, M., Narra, R., Tinelli, C., De Silvestri, A., . . . Brunetti, E. (2016). Comparison of the Diagnostic Accuracy of Three Rapid Tests for the Serodiagnosis of Hepatic Cystic Echinococcosis in Humans. *PLoS Negl Trop Dis, 10*(2), e0004444. doi:10.1371/journal.pntd.0004444

Tamarozzi, F., Deplazes, P., & Casulli, A. (2020). Reinventing the Wheel of Echinococcus granulosus sensu lato Transmission to Humans. *Trends Parasitol, 36*(5), 427-434. doi:10.1016/j.pt.2020.02.004

Thompson, R. C. (2008). The taxonomy, phylogeny and transmission of Echinococcus. *Exp Parasitol, 119*(4), 439-446. doi:10.1016/j.exppara.2008.04.016

Vuitton, D. A., McManus, D. P., Rogan, M. T., Romig, T., Gottstein, B., Naidich, A., . . . Menezes da Silva, A. (2020). International consensus on terminology to be used in the field of echinococcoses. *Parasite, 27*, 41. doi:10.1051/parasite/2020024

World Health Organization (2020). *Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030*. Retrieved from

Yildiz, K., & Gurcan, S. (2003). Prevalence of hydatidosis and fertility of hydatid cysts in sheep in Kirikkale, Turkey. *Acta Vet Hung, 51*(2), 181-187. doi:10.1556/AVet.51.2003.2.6

Yildiz, K., & Tunçer, C. (2005). [Prevalence of hydatid cysts in cattle in the province of Kirikkale.]. *Turkiye Parazitol Derg, 29*(4), 247-250.