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RESEARCH PAPER

Molecular Diagnosis of Fasciola hepatica in livestock using cox1 gene in Erbil Provence- Kurdistan Region/ Iraq

Muhammad J. Muhammad and Zuber I. Hassan
Department of Medical Laboratory Techniques, Shaqlawa Technical College, Erbil Polytechnic University, Erbil, Kurdistan Region-Iraq.

A B S T R A C T:

52 *Fasciola hepatica* worms (22 sheep, 3 goats and 27 cattle) from livers and bile ducts were collected. The prevalence rate of Fascioliasis was 7.28%, 3.37% and 10.19%, respectively. Among the three hosts regarding the sex, females of sheep and goats show a higher infection rate than males (4.3% and 2.25% versus 2.98% and 1.12%). Simultaneously, the prevalence rate was slightly higher in female cattle (5.28%) than male cattle (4.91%). Used the adult worms for DNA extraction followed by PCR amplification. The sequences of amplified products were identified under accession number (MT951586) of sheep were 99.51%, and (MT951587) of goat was 99.75% homologous to Iranian goat *Fasciola hepatica* (KF992219). Furthermore, the cattle *Fasciola hepatica* (MT951585) was 99.2% homologous with the reference of Iranian goat. Using phylogenetic tree analysis, isolated *F. hepatica* in sheep, goats, and cattle is positively related to various global *F. hepatica*.

KEY WORDS: *F.heatica, Cox1 gene, phylogenetic tree, Erbil Province* .
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1.INTRODUCTION :

Fasciola hepatica and *Fasciola gigantica* are two liver-flukes, and it is a zoonotic parasitic disease causing Fascioliasis (Zekarias and Bassa, 2019), which is distributed worldwide infecting sheep, goats, cattle, deer, and humans as final hosts (Moazeni et al., 2012, Sah et al., 2018). *F. hepatica* is a helminths parasite of animals belong to class Trematoda (Sah et al., 2018, Munita et al., 2019). It's localized in the liver's bile duct cause mortality in domestic ruminants and lead to significant economic losses (Magaji et al., 2014). Vast numbers of infective parasite platforms can cause a highly pathogenic sub-acute presentation in lambs, characterized by hepatic haemorrhage and lesions, resulting in immediate death (Nasr et al., 2016).

Chronic fasciolosis, showing reduced weight gain, anaemia, decreased fertility, reduced milk production and reduced feed conversion efficiency (Kantzoura et al., 2011, Munita et al., 2019). As is understood, the classification of the *Fasciola species* can't be performed by clinical, pathological, or immunological techniques. Serological findings do not differentiate between these species (Amor et al., 2011, Alajmi, 2019). Different molecular methods were employed in the analysis, taxonomy, and *fasciola* studies like complete genome sequencing and single nucleotide polymorphism (Moazeni et al., 2012, Raoof et al., 2020). The PCR amplification method improves many templates for initial priming that allow the primers to anneal the known conserved regions to amplify across unknown variable regions and better diagnose species (Eman et al., 2016, Mohammed et al., 2016, Hassan, 2018). Also, the intraspecific

* **Corresponding Author:**
Muhammad J. Muhammad
E-mail: Muhamad.jamal@epu.edu.iq
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genetic variations between liver flukes may show differences in virulence, host specificity and drug susceptibility or resistance.

Some genetic variation within *F. hepatica* and *F. gigantica* sequences of the mitochondrial DNA and ribosomal regions have been described previously from Iraq (Mohammed et al., 2016, Hamoo et al., 2019), Iran (Galavani et al., 2016, Baran et al., 2017), Turkey (Erensoy et al., 2009), Saudi Arabia (Shalaby and Gherbawy, 2013) and Egypt (Dar et al., 2012). Hence, this study aimed to investigate the genetic structure of the flukes using *cox1* sequences. *Compared Fasciola* populations from the three definitive host species to find host-specific genetic structures in Erbil Province, to assess their phylogenetic analysis.

2. Materials and procedures

2.1- Parasite specimen:

The total livers of 656 slaughtered livestock (sheep (n=302), goats (n=89), and Cattle (n=265)) were carefully inspected visually (Hassan et al., 2017), and then the hepatic parenchyma was cut and squeezed directly towards the bile duct with both hands and the flukes getting out of the vents (Hamed et al., 2014). *Fasciola* specimens (n=52) were obtained from the bile duct or liver tissue of freshly slaughtered sheep, goats and cattle at regional slaughterhouses in Erbil province, Kurdistan Region-Iraq among July to November 2020. Flukes were morphologically classified as *Fasciola sp.* according to present keys and descriptions (Hamed et al., 2014, Piri et al., 2018, Hamoo et al., 2019). After collection, washed them thoroughly in Phosphate Buffer Saline (PBS), then fixed in 70 % ethanol.

2.2- Extraction of DNA and amplification of PCR

The posterior sections (without the uterus) of the samples were applied for DNA extraction, to avoid sampling sperm from another fluke (Tadayon et al., 2015). About 15 mg of adult *fasciola* worms were separated and crashed between two microscopic glass slides. The ethanol in each sample was allowed to evaporate for a few minutes and rinsed with PBS three times reference?. According to the manufacturer's directions, whole DNA was extracted utilizing the Wizard Genomic DNA Purification Kit (Promega). DNA was eluted in 100µL of elution buffer and stored at -20^{o}C till use. PCR was done according to the subsequent protocol: The fragment of *Cox1* was amplified applying the primers set Ita 8 (forward; 5`-ACGTTGGATCATAAGCGTGT-3` and Ita 9 (reverse; 5`-CCTCATCCAACATAACCTCT-3`) as explained by (Farjallah et al., 2013). Made PCR reactions (25 μl) with 3µl of DNA template, 12.5µl of (2X) Go-Tag master mix, 30 pmol (3µl) of each primers and 3.5µl nuclease-free water. Performed amplification result with the following conditions: Primary denaturation at 94^{0}C for 90 seconds was replaced by 33 cycles each at 94^{0}C for 90 seconds, annealing temperature 55^{0}C for 90 seconds, and extension at 72 for 120 seconds, the last step is the extension at 72^{0}C for 10 minutes.

2.3- Sequencing and Accession numbers:

The PCR product of all samples were loaded in 1.5% (w/v) agarose gels in tris-borate-EDTA (TBE) buffer staine with ethidium bromide. The electrophoresis is carried out for 45 min at 100 V and DNA was visualized under UV transilluminator, and the bands were visualized (figure 1) The amplified results were commercially sequenced in both directions (Macrogen Inc. South Korea). The DNA sequences undergo help analysis by applying bioinformatics tools and nucleotide alignment employing ClustalW (http://www.ebi.ac.uk/clustalw) for specific DNA sequence query (Thompson et al., 1997) and the identity matrix options of Bioedit (Hall, 1999), respectively. Saved the partial *Cox1* gene of the *F. hepatica* in the GenBank database below accession numbers MT951585- MT951587 through BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST). The phylogenic studies of *Cox*1 sequence data were carried by maximum likelihood using MEGA6 (Tamura et al., 2013).

2.4- Data analysis and presentation

MS Excel 2010 used for data entry and statistical analysis was performed using the t-test and Chi-square (X_2) test to discover the likelihood value (p-value) applying the graph pad prism 8.0.1.

3.Results and Discussion

The infection rate of fasciolosis in sheep, goats and cattle were 7.28%, 3.37% and 10.19% respectively as shown in Table 1. The result agreed with (Kordshooli et al., 2017) in Iran, who

detected that the prevalence rate of *F. hepatica* were 5.22% of sheep, 2.15% of goat, and 11.15% of cattle. *Fasciola species* are usually found in herbivores in various parts of Iraq (Hamoo et al., 2019, Raoof et al., 2020, Abdulwahed and Al-Amery, 2019). The observation could be a return to husbandry practices and climatic variation including rain and warmth which had significant effects on the remainder of both intermediate host and the larval stages (miracidium and cercariae) of the parasite. Other factors; soil effect according to the epidemiological aspects of fasciolosis, distribute intermediate host in the environment (Kordshooli et al., 2017). In this study, The prevalence was higher in cattle, followed by sheep and goats, which may be due to free-ranged pasture grazing. But sheep and goats held either tethered or free-ranged, and the prevalence was distinctly higher in free-ranged than tethered. It may be due to exposing more further contact to the hazard factors such as the presence of metacercaria (Oryan et al., 2011, Yadav et al., 2015). The results were interpreted according to the infection rate among each host's males and females individually from the total number of all investigated livestock. Discovered the highest rate of infection in female sheep and goats 13(4.3%), 2(2.25%) as distinguished to male sheep and goats 9(2.98%), 1(1.12%), respectively. The results agreed with (Isah, 2019), who showed that female sheep and goats reported higher prevalence (44.6% and 42.6%) than males (30.7% and 30.4%) respectively; this variation could be since females stay longer in flock/herd for reproduction and breeding. Simultaneously, the prevalence rate was slightly higher in female cattle (5.28%) versus male cattle (4.91%) as revealed in table 2. Statistically, there was no significant variation (P= 0.8143) between all studied hosts. These agreed with (Yadav, 2015), who revealed that the female cattle 42 (82.3%) were more acceptable to infected in compared to the males 9 (17.7%). But the results disagreed with (Isah, 2019), who showed that the male cattle were more infected (55.3%) than female's cattle (41.3%). The potential reason may be that, sexes are travelling together for food and water and that thus the risk of infection is high for both sexes. (Magaji et al., 2014).

Table 1: prevalence of *F.hepatica* among slaughtered sheep, goat and cattle in new Erbil Slaughtered house

Hosts	Examined number	Number of infected Fasciolosis	Percentage (%)
Sheep	302	22	7.28
Goats	89	3	3.37
Cattle	265	27	10.19
Total	656	52	7.93

Table 2: The Prevalence of Fasciolosis among all slaughtered animals of both sexes

Hosts	No of slaughtered male animals	No of infected male animals	Prevalence (%)	No of slaughtered female animals	No of infected female animals	Prevalence (%)
Sheep	189	9	2.98	113	13	4.30
Goats	51	1	1.12	38	2	2.25
Cattle	187	13	4.91	78	14	5.28
Total	427	23	3.51	229	29	4.42

Statistically significant ($P < 0.05$)

In the current study, adult individuals of *Fasciola species* were found from the liver of sheep, goats, and bile ducts of infected cattle. It is subjected to confirmation with the taxonomic key (Yakhchali et al., 2015). Previously, PCR technology and DNA sequencing technologies have been facilitated to identify Trematoda species' analysis (Abdulwahed and Al-Amery, 2019), especially the partial mt genes like *Cox1* (Moazeni et al., 2012, Meshgi et al., 2017, Buathong et al., 2019). In the present study, multiple alignments compared with Trematoda references (KF992219) available in the NCBI database (Shafiei et al., 2014). The results of *Cox1* sequence analysis showed that the species of *F. hepatica* under the accession no. MT951586 in sheep was 99.51% homologous to Iranian goat *Fasciola hepatica* (KF992219) due to nucleotide substitution (T-G) at positions 254 and (A-G) at position 320. On the other hand, MT951587 of goat was 99.75% homologous to KF992219; due to nucleotide substitution (A-G) at position 320. Furthermore, the cattle *F. hepatica* (MT951585) was 99.2% homologous with the reference of Iranian goat, as shown in figure 2. The *Cox1* gene can be applied to properly identify *Fasciola species* and become a valuable tool for the specific diagnosis of the liver fluke causal factor of Fascioliasis in livestock (Simsek et al., 2011). The sequence of *Cox1* gene with only nucleotide polymorphism could be due to hybrids in our isolates, as mentioned by (Bozorgomid et al., 2020), the advantage of low substitution rate genes making it easier to interpret mixed infections (Amor et al., 2011). Figure 3 shows that the phylogenetic analysis of the *Cox1* gene sequence and the worms' species were identical to gene sequences from *F. hepatica* accession no. MK447946, LC273112, LC485089, KT893716, AB207170, AB553810 and KF992219 recovered from Iran, Egypt, Algeria, Iran and Japan, respectively. Phylogenetic analyses help understand population differentiation, species formation and ecological adaptation (Lotfy et al., 2008). Still, it was difficult to identify exactly genotype utilizing a host, suggesting that host associations are not suitable for *Fasciola* genotype classification (Tadayon et al., 2015).

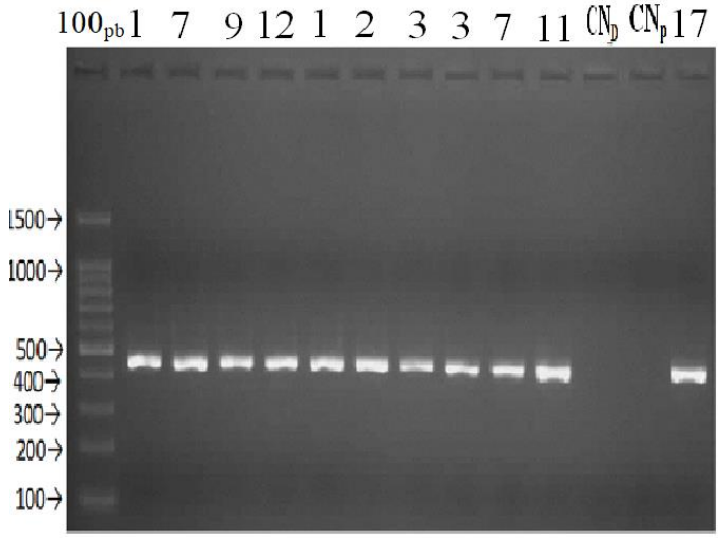

Figure 1: Amplified Cox1 region in agarose gel electrophoresis. Lanes (1,7, 9 and 12) denote sheep fluke samples, (1 to 3) denote goat fluke samples, (3,7,11 and 17) denote cattle fluke samples amplified as a single band of 425 bp; CN_D denotes DNA extraction negative control; CN_P denote PCR negative control, and 100_{bp} denote 100bp ladder molecular weight marker.

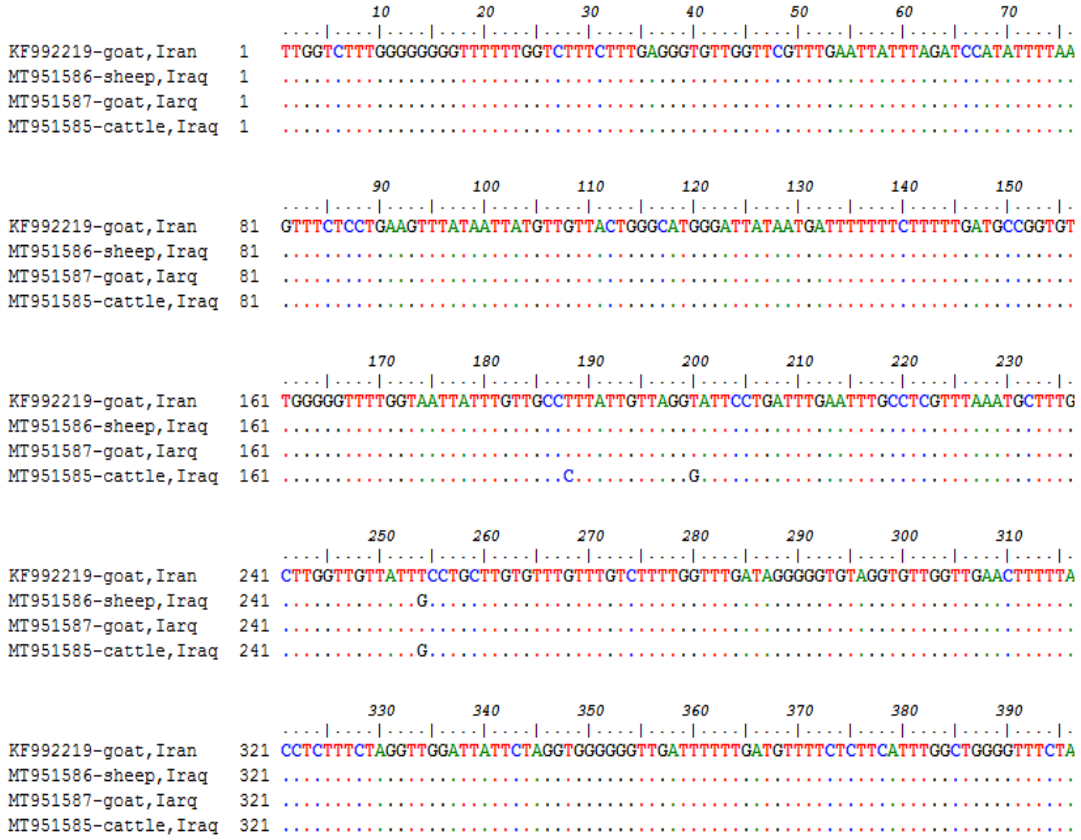

Figure 2: multiple Alignment of detected *Fasciola hepatica* with the (KF992219) (Shafiei et al., 2014). (Shafiei et al., 2014) Dots represent sequence similarities

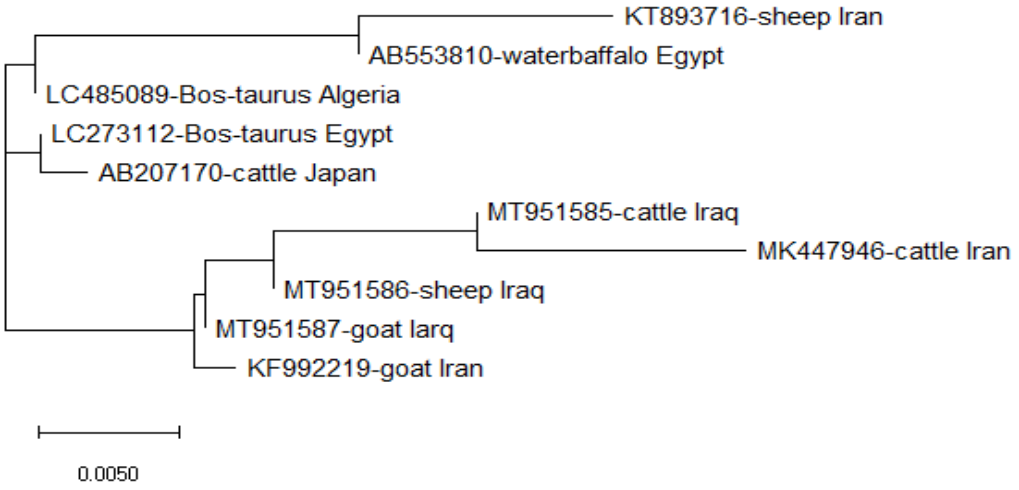

Figure 3: The nucleotide sequences of the partial *Cox1* and the adjacent procedure are a phylogenetic tree of *F. hepatica*. Molecular analysis reveals that among Iraqi *F. Hepatica* people three separate genotypes.

4.Conclusion:

According to this study, the direct inspection method is still the best approach for estimating the prevalence of liver fluids in animals. More steps are recommended to formulate appropriate control strategies to decrease diseases and financial loss due to the condemnation of infected livers in Erbil Province. The current investigation also showed that *Fasciola* isolates from domesticated animals were *F. hepatica* by using molecular methods. Classification of F. hepatica based on *cox1* genes sequencing method is an accurate, simple, quick, and available tool that can differentiate *F. hepatica* and *F. gigantica* species.

5.References:

ABDULWAHED, T. K. & AL-AMERY, A. M. 2019. Morphological and molecular study of Fasciola spp. in sheep in Alkut city. *International Journal of Biosciences (IJB),* 14**,** 121-130.

ALAJMI, R. A. 2019. Molecular characterization of Fasciola flukes using mitochondrial 28S rRNA gene in Naimi Saudi sheep. *Saudi J Biol Sci,* 26**,** 112-117.

AMOR, N., HALAJIAN, A., FARJALLAH, S., MERELLA, P., SAID, K. & BEN SLIMANE, B. 2011. Molecular characterization of Fasciola spp. from the endemic area of northern Iran based on nuclear ribosomal DNA sequences. *Exp Parasitol,* 128**,** 196-204.

BARAN, A. I., SARAY, H. C. & KATIRAEE, F. 2017. Molecular determination of Fasciola spp. Isolates from domestic ruminants fecal samples in the northwest of Iran. *Iranian Journal of Parasitology,* 12**,** 243.

BOZORGOMID, A., ROUHANI, S., HARANDI, M. F., ICHIKAWA-SEKI, M. & RAEGHI, S. 2020. Genetic diversity and distribution of Fasciola hepatica haplotypes in Iran: Molecular and phylogenetic studies. *Veterinary Parasitology: Regional Studies and Reports,* 19**,** 100359.

BUATHONG, S., WEBSTER, B. L., LEELAYOOVA, S., MUNGTHIN, M., RUANG-AREERATE, T., NAAGLOR, T., TAAMASRI, P., SUWANNAHITATORN, P., JANEKITKARN, S. & TAN-ARIYA, P. 2019. MOLECULAR CHARACTERIZATION OF FISH-BORNE TREMATODE METACERCARIAE INFECTING FRESHWATER FISH IN RICE FIELD OF CENTRAL THAILAND. *Southeast Asian Journal of Tropical Medicine and Public Health,* 50**,** 25-35.

DAR, Y., AMER, S., MERCIER, A., COURTIOUX, B. & DREYFUSS, G. 2012. Molecular identification of Fasciola spp.(digenea: Fasciolidae) in Egypt. *Parasite: journal de la Société Française de Parasitologie,* 19**,** 177.

EMAN, K., SHERIF, M. & REDA, S. 2016. Molecular characterization of Fasciola hepatica infecting cattle from Egypt based on mitochondrial and nuclear ribosomal DNA sequences. *J. Parasitol,* 11**,** 61-66.

ERENSOY, A., KUK, S. & OZDEN, M. 2009. Genetic identification of Fasciola hepatica by ITS-2 sequence of nuclear ribosomal DNA in Turkey. *Parasitology research,* 105**,** 407.

FARJALLAH, S., BEN SLIMANE, B., PIRAS, C. M., AMOR, N., GARIPPA, G. & MERELLA, P. 2013. Molecular characterization of Fasciola hepatica from Sardinia based on sequence analysis of genomic and mitochondrial gene markers. *Exp Parasitol,* 135**,** 471-8.

GALAVANI, H., GHOLIZADEH, S. & TAPPEH, K. H. 2016. Genetic characterization of Fasciola isolates from West Azerbaijan province Iran based on ITS1 and ITS2 sequence of ribosomal DNA. *Iranian journal of parasitology,* 11**,** 52.

HALL, T. A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series, 1999. [London]: Information Retrieval Ltd., c1979-c2000., 95-98.

HAMED, N., AYADI, A. & HAMMAMI, H. 2014. Epidemiological studies on fasciolosis in northern Tunisia. *Revue Méd. Vét,* 165**,** 49-56.

HAMOO, R., AL-RUBAYE, F. & MUSTAFA, N. 2019. Molecular characterization and phylogenetic analysis of fasciola gigantica in iraqi sheep using ITS1. *Adv. Anim. Vet. Sci,* 7**,** 256-260.

HASSAN, Z. I. 2018. Molecular Characterization of Cutaneous Leishmaniasis Isolated from Human in Erbil Province-Kurdistan Region/Iraq. *Zanco Journal of Pure and Applied Sciences,* 30**,** 76-85.

HASSAN, Z. I., MEERKHAN, A. A., BOUFANA, B., HAMA, A. A., AHMED, B. D., MERO, W. M. S., ORSTEN, S., INTERISANO, M., POZIO, E. & CASULLI, A. 2017. Two haplotype clusters of Echinococcus granulosus sensu stricto in northern Iraq (Kurdistan region) support the hypothesis of a parasite cradle in the Middle East. *Acta Tropica,* 172**,** 201-207.

ISAH, U. M. 2019. Studies on the prevalence of fascioliasis among ruminant animals in northern Bauchi state, north-eastern Nigeria. *Parasite Epidemiol Control,* 5**,** e00090.

KANTZOURA, V., KOUAM, M. K., DEMIRIS, N., FEIDAS, H. & THEODOROPOULOS, G. 2011. Risk factors and geospatial modelling for the presence of Fasciola hepatica infection in sheep and goat farms in the Greek temperate Mediterranean environment. *Parasitology,* 138**,** 926-38.

KORDSHOOLI, M. S., SOLHJOO, K., ARMAND, B., DOWLATKHAH, H. & JAHROMI, M. E. 2017. A reducing trend of fasciolosis in slaughtered animals based on abattoir data in South of Iran. *Vet World,* 10**,** 418-423.

LOTFY, W. M., PERERA, V. B. V. P., LOKER, E. S., BRANT, S. V., RAJAPAKSE, R. P. V. J., LAURSEN, J. R., DEMIASZKIEWICZ, A., DEJONG, R. J. & LE, T. H. 2008. Evolutionary Origins, Diversification, and Biogeography of Liver Flukes (Digenea, Fasciolidae). *The American Journal of Tropical Medicine and Hygiene,* 79**,** 248-255.

MAGAJI, A. A., IBRAHIM, K., SALIHU, M. D., SAULAWA, M. A., MOHAMMED, A. A. & MUSAWA, A. I. 2014. Prevalence of Fascioliasis in Cattle Slaughtered in Sokoto Metropolitan Abattoir, Sokoto, Nigeria. *Advances in Epidemiology,* 2014**,** 1-5.

MESHGI, B., JAHANI, Z. & AMININIA, N. 2017. Fasciola gigantica of Ruminants: The phylogenetic analysis based on COX1 sequences. *Iranian Journal of Veterinary Medicine,* 11**,** 21-29.

MOAZENI, M., SHARIFIYAZDI, H. & IZADPANAH, A. 2012. Characterization of Fasciola hepatica

genotypes from cattle and sheep in Iran using cytochrome C oxidase gene (CO1). *Parasitology research,* 110**,** 2379-2384.

MOHAMMED, A. B., MUSTAFA, A., ISSA, A. R. & MERO, W. M. 2016. Molecular Characterization of Fasciola Spp. Isolated From the Gallbladder of Infected Cattle in Duhok Province, Kurdistan Region Iraq. *Science Journal of University of Zakho,* 4**,** 37-42.

MUNITA, M. P., REA, R., MARTINEZ-IBEAS, A. M., BYRNE, N., MCGRATH, G., MUNITA-CORBALAN, L. E., SEKIYA, M., MULCAHY, G. & SAYERS, R. G. 2019. Liver fluke in Irish sheep: prevalence and associations with management practices and co-infection with rumen fluke. *Parasit Vectors,* 12**,** 525.

NASR, S. M., BAZH, E. K. A. & FADLY, R. S. 2016. Molecular Characterization of Fasciola hepatica Infecting Cattle from Egypt Based on Mitochondrial and Nuclear Ribosomal DNA Sequences. *Research Journal of Parasitology,* 11**,** 61-66.

ORYAN, A., MANSOURIAN, M., MOAZENI, M., NIKAHVAL, B. & BARBAND, S. 2011. Liver distomatosis in cattle, sheep and goats of Northeastern Iran. *Glob Vet,* 6**,** 241-246.

PIRI, K., SAIDIJAM, M., MAGHSOOD, A., MATINI, M. & FALLAH, M. 2018. Prevalence of Animal Fasciolosis and Specification of Fasciola spp. Isolated from Sheep, Goats and Cattle by Molecular Method: Hamadan Province, West of Iran. *Iran J Parasitol,* 13**,** 524-531.

RAOOF, H. S., MARIF, H. F., RAHMAN, H. S., OMAR, M., SHEIKH, B. & SAN AHMED, A. M. 2020. Molecular characterization and phylogenetic analysis of Fasciola species in sheep and goats in Sulaymaniyah Province, Northern Iraq.

SAH, R., KHADKA, S., LAKHEY, P. J., PRADHAN, S., SHAH, N. P., SINGH, Y. P. & MAS-COMA, S. 2018. Human case of Fasciola gigantica-like infection, review of human fascioliasis reports in Nepal, and epidemiological analysis within the South Central Asia. *Acta Parasitol,* 63**,** 435-443.

SHAFIEI, R., SARKARI, B., SADJJADI, S. M., MOWLAVI, G. R. & MOSHFE, A. 2014. Molecular and Morphological Characterization of Fasciola spp. Isolated from Different Host Species in a Newly Emerging Focus of Human Fascioliasis in Iran. *Vet Med Int,* 2014**,** 405740.

SHALABY, I. & GHERBAWY, Y. 2013. Molecular Characterization of Fasciola sp. isolated from imported sheep in Taif region (Saudi Arabia). *Tropical biomedicine,* 30**,** 1-12.

SIMSEK, S., UTUK, A. & BALKAYA, I. 2011. Molecular differentiation of Turkey cattle isolates of Fasciola hepatica and Fasciola gigantica. *Helminthologia,* 48**,** 3-7.

TADAYON, S., SHARIFIYAZDI, H., MOAZENI, M. & DIVAR, M. R. 2015. Molecular differentiation of Fasciola species and characterization of genetic diversity of F. gigantica using NADH dehydrogenase I (ND1) gene in the endemic areas of Iran. *Iranian journal of parasitology,* 10**,** 9.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. & KUMAR, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution,* 30**,** 2725-2729.

THOMPSON, J. D., GIBSON, T. J., PLEWNIAK, F., JEANMOUGIN, F. & HIGGINS, D. G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic acids research,* 25**,** 4876-4882.

YADAV, S., AHADUZZAMAN, M., SARKER, S., SAYEED, M. & HOQUE, M. 2015. Epidemiological survey of fascioliasis in cattle, buffalo and goat in Mahottari and Dhanusha, Nepal. *J. Adv. Parasitol,* 2**,** 51-56.

YADAV, S. K. 2015. Epidemiological Survey of Fascioliasis in Cattle, Buffalo and Goat in Mahottari and Dhanusha, Nepal. *The Journal of Advances in Parasitology,* 2**,** 52-56.

YAKHCHALI, M., MALEKZADEH-VIAYEH, R., IMANI-BARAN, A. & MARDANI, K. 2015. Morphological and molecular discrimination of Fasciola species isolated from domestic ruminants of Urmia city, Iran. *Iranian journal of parasitology,* 10**,** 46.

ZEKARIAS, T. & BASSA, T. 2019. Prevalence of Ovine Fasciolosis in Damot Sore Woreda, Wolayta Zone, Ethiopia.