CHAPTER 18

EPIDEMIOLOGICAL AND MOLECULAR STUDIES OF ANIMAL FASCIOLIASIS IN IRAQ

Sara Omar Swar[1], Ahmed Basheer Mohammed[2], Wijdan Mohammed Salih Mero[2,4], Chira Abdullah Ramadhan[2], Mohammad Ismail Alberfkani[3] and Araz Ramadhan Issa[2]

[1]Food Technology Department, College of Agriculture Engineering Sciences, Salahuddin University, Erbil, Iraq
[2]Department of Biology, Faculty of Science, Zakho University, Zakho, Kurdistan Region, Iraq
[3]Department of Medical Laboratory Technology, College of Health and Medical Techniques, Duhok Polytechnic University, Duhok, Kurdistan Region, Iraq
[4]Scientific Research Center, Nawroz University, Duhok, Kurdistan Region, Iraq
*Corresponding author: sara.swar@yahoo.com

INTRODUCTION

Fascioliasis is considered as a neglected zoonotic disease of ruminants. This disease is caused by two species of Fasciola (F.) namely *Fasciola hepatica* and *F. gigantica*. They are distributed in more than 70 countries except Antarctica, in areas where sheep and cattle are reared (WHO 2016; CDC 2019). This disease was first recorded as early as 2000 BC. Fasciola spp. cause serious pathological effects to livestock (Abbas et al. 2019). The number of infected sheep exceeds 250 million and about 300 million cattle are infected globally with fascioliasis. The disease cause losses exceeding 3.2 billion $ yearly, due to decrease in milk and meat production and an increase in mortality rate in addition to liver condemnation and expenditure of anthelminthic (Hillyer and Apt 1997; Jaja et al. 2017).

Nowadays, attentions have been paid to fascioliasis due to its zoonotic feature, because it infects both humans and animals. Human cases are estimated to be around 2.6 million worldwide (Schweizer et al. 2005). Humans acquire fascioliasis incidentally through the ingestion of cercaria encysted on watercress or with drinking water. Human and animal infections have shown worldwide expansion which mainly related to climatic changes (Caravedo and Cabada 2020).

The geographic distribution of Fasciola spp. depends on the presence of the intermediate host, the snail, in an area which is responsible for spreading the disease and leading to the infection of new hosts (Mas-Coma et al., 2005). The intermediate host for *F. hepatica* is Lymnaea truncatula, that exists in cold and temperate zones, so it is prevalent in North America, Europe and Australia. While *F. gigantica* is common in sub-tropical and tropical zones of Asia and Africa (Mas-Coma et al. 1997).

Morphology

The both species of liver flukes; *F. hepatica* and *F. gigantica* (Fig. 1 and 2) can be differentiated morphologically by their shape, size, and the shape of cephalic cone (Keiser and Utzinger 2009). Adult *F. hepatica* is large, leaf-shaped measuring 3cm in length by 1.5 cm in width; the color is brown to pale grey. It possesses two unequal suckers; the smaller oral sucker which is powerful and located at the anterior end that extends into a conical projection, the cephalic cone, and the ventral sucker which is larger and located at the base of cephalic cone. The posterior end of the body is more rounded than anterior end. *F. gigantica* is the largest liver fluke which is longer and narrower than F. hepatica, measuring 7.5 cm in length and 1.2 cm. in width. It also possesses two unequal suckers; a shorter cephalic cone and the fluke is more oblong with a larger rounded posterior end (Jeandron et al. 2010; Abdisa and Jilo 2017; CDC 2019). Fasciola worm is hermaphroditic; it possesses two dendritic highly branched testes and one ovary that produce eggs excreted with animal feces. Even though both Fasciola spp. are separate, "hybrid forms" with intermediate morphological characters and genetic components of both species were recorded in some Asian and African countries, where both species were found (CDC 2019).

Classification of Fasciola Species

Fasciola belongs to Phylum Platyhelminthes, Class Rhabditophora, Order Plagiorchiida, Family Fasciolidae, Genus Fasciola and species *Fasciola hepatica*, Linnaeus 1758 and *Fasciola gigantica*, Cobblod 1855.

Life Cycle

Each adult fluke produces more than 20,000 eggs daily, which are deposited in the biliary passages of the host (Stein 2003). These eggs are oval, operculated, yellowish brown in color, large in size, the average length ranges from 130±150/63±90 µm for *F. hepatica* and 150±196/90±100 µm for *F. gigantica* (Marcilla et al. 2002; Stein 2003; Keiser and Utzinger 2009). The deposited eggs pass with the bile to the intestine then are excreted in animal feces. The life cycle (Fig. 3) begins when the eggs embryonate under favorable environmental conditions of temperature, oxygen tension, pH and humidity (at least a film of water).

The embryonation takes more than two weeks depending on the above-mentioned factors, then hatch into a free-swimming miracidium (Stein 2003). The miracidium penetrates the body of a suitable snail, the intermediate host, which is responsible

How to cite this chapter: Swar SO, Mohammed AB, Mero WMS, Ramadhan CA, Alberfkani MI and Issa AR, 2022. Epidemiological and molecular studies of animal fascioliasis in iraq. In: Abbas RZ, Khan A, Liu P and Saleemi MK (eds), Animal Health Perspectives, Unique Scientific Publishers, Faisalabad, Pakistan, Vol. I, pp: 137-146. https://doi.org/10.47278/book.ahp/2022.18

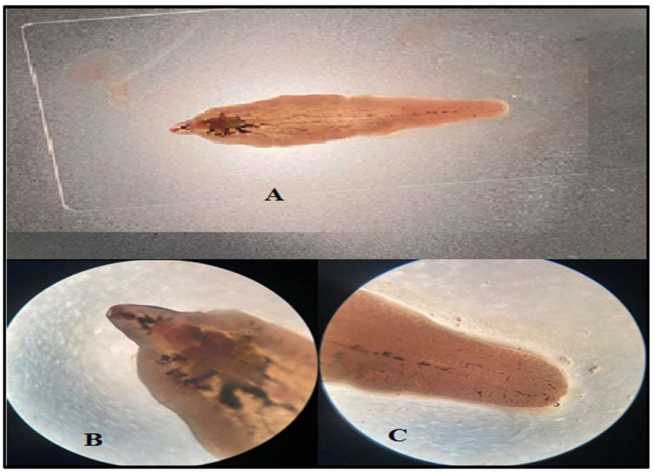

Fig. 1: *Fasciola hepatica* isolated from bile duct of sheep; A: Adult; B: Anterior end of Fasciola showing oral and ventral suckers, pharynx, genital pore, ovary and uterus; C: posterior end of the fluke; B and C under magnification power 20x.

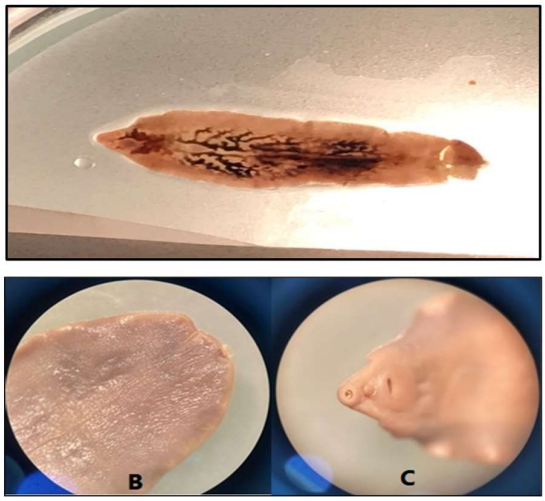

Fig. 2: *Fasciola gigantica* isolated from bile duct of Cattle; A: Adult; B: Posterior end of the fluke; C: Anterior end showing oral and ventral suckers, and genital pore, B and C under magnification power 20x

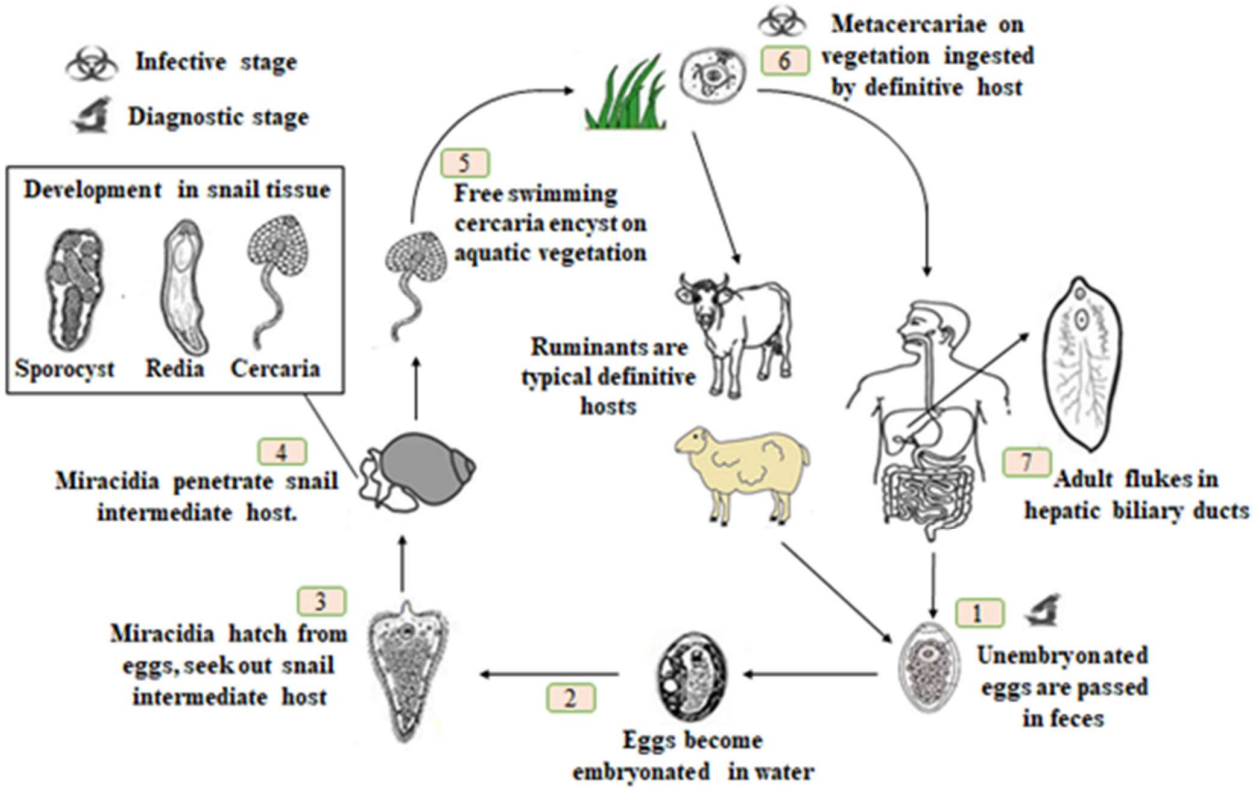

Fig. 3: The Lifecycle of Fasciola spp.

for the completion of the developmental stages and the transmission of the disease. The snails acting as intermediate hosts for Fasciola spp. inhabit a wide range of habitats such as rivers, lakes, springs, irrigation ducts, etc., (Thanh 2012). The snail Lymnaea truncatula acts as intermediate host for *F. hepatica* in Iraq and also, is prevalent in many temperate zones of America, Australia and Europe (Mas-coma et al. 1997, and 2009; Kock et al. 2003). While Lymnaea columella is very common in South Africa (Kock et al. 2003). In Egypt, the snail, L. cailliaudi acts as intermediate host for *F. gigantica*, while L. columella can transmit both *F. gigantica* and F. hepatica. In tropical and subtropical areas of Africa, *F. gigantica* is transmitted by Lymnae natalensis (Thanh 2012). In the intermediate host the miracidia develop into sporocysts, redia and cercaria.

The cercariae leave the snail and swim in water, for a short period of time, then encyst as metacercaria on aquatic vegetation (e.g., watercress) or other objects. Each snail infected with one miracidium can produces up to 4,000 free-swimming cercariae (Rinaldi et al. 2007). When the

metacercaria is taken by the susceptible definitive host, it will excyst by the effect of gastric and intestinal juices in the anterior part of the small intestine and penetrate its wall reaching peritoneal cavity, then to the liver.

The juvenile flukes migrate through the liver tissue and enter the biliary passages, where they become mature and produce eggs (CDC 2019). The incubation period ranges from 2-3 months depending on the number of the flukes and adult flukes may remain alive in the bile ducts of sheep for years, while in cattle most of them start shedding eggs within 5–6 months (Ballweber 2022).

Both Fasciola species can adapt to new animal hosts, thus they infect a wide range of domestic and wild animals and human can also be infected and regarded as incidental host. The commonest definitive hosts for Fasciola spp. are sheep, goats, cattle etc. Besides, other wild animals (deer, rabbits, llamas, rats, kangaroos, and beavers) can be infected with Fasciola spp. (Keiser et al. 2008; Keiser and Utzinger 2009).

Pathology and Symptoms of Fascioliasis

The pathological effects of the disease depend on the parasitic load. Therefore, the severity of the disease varies from asymptomatic to highly symptomatic (Pullan et al. 2008). These effects might be severe in mammalian hosts. This could be attributed to simultaneous infection by the parasite at different disease stages. Fascioliasis clinically can be divided into two phases:

Acute Phase

This phase of the disease starts when the immature Fasciola penetrate the liver tissue of the host (Pullan et al. 2008) causing tissue damage, hemorrhage, inflammation, and fibrosis of the liver. These effects are produced due to digestion of invaded liver tissue by the parasite. The burrows produced by flukes can be observed in the histological examination of liver sections.

The symptoms of the acute stage can be summarized as abdominal pain, gastrointestinal disorders and anemia (Pullan et al. 2008; Becker et al. 2011). Other symptoms of hepatic damage include jaundice, hepatosplenomegaly and ascites (CDC 2019). The infected definitive hosts of the livestock may die suddenly due to intense blood loss, liver dysfunction in addition to secondary bacterial infection with Clostridium novyi. Furthermore, in case of heavy infections, the parasite can penetrate the diaphragm tissue entering to the lung tissue causing pneumonia, infection and fibrosis of the plural membrane and producing lesions in the respiratory system (Pullan et al. 2008; Speich et al. 2010; Becker et al. 2011).

Chronic Phase

This phase of the disease begins when the parasite reaches the bile ducts and lay eggs. The main conspicuous symptoms of this phase include: dilatation and hyperplasia of biliary ducts, their calcification, thickness of gallbladder. In some cases of heavy infections, the bile ducts may be blocked causing severe complications such as, cholangiolitic, acute pancreatitis, cholecystitis and bacterial superinfections (Pullan et al. 2008; Becker et al. 2011).

Other pathological effects of the disease in livestock are intense anemia, since a single Fasciola fluke cane led to losing 0.2-0.5 ml of blood daily (Autissier 2008). Chronic fascioliasis of sheep and cattle may lead to bottle jaw disease (oedema under the jaw) in the infected animals (Duthaler 2012).

Epidemiology of Fascioliasis

Fascioliasis has a global distribution in all continents where sheep and cattle are reared. Due to vast literature on the prevalence and distribution of the infection, in this section only the studies performed in Iraq and some of the neighboring countries during the last decades are reviewed.

The Epidemiology of Fascioliasis and its Relationship to some Factors in Iraq

Some studies have been performed in Iraq reporting the prevalence of fascioliasis in different parts of the country. Rasheed and Kadir (2008) in Kirkuk, reported rates of 2.63%, 0.50%, 0.43% and 4.00% in cattle, sheep goats and buffaloes, respectively. Furthermore, they observed seasonal variation in the rate of infection, since the prevalence was higher (5.0%) in cattle in Autumn, followed by Winter (2.08%). While the highest rates in sheep and goats was in winter, which were 0.88% and 0.68%, respectively. Because the larval stages of Fasciola spp. develop in freshwater snails and their presence depends on environmental conditions like humidity, rain fall, temperature etc. Furthermore, both definitive and intermediate hosts are involved with the external environment (Mas-Coma 2005).

In Erbil, Koyee et al. (2011) inspected slaughtered 53,868 sheep, 17632, goats and 14435, cattle and reported, infection rates of 4.11%, 3.62% and 3.44%, respectively, of fascioliasis. The highest prevalence in sheep was recorded in summer and autumn seasons which was 4.8% for each season, while in winter and summer lower rates (3.86% and 3.64% respectively) were reported. In goats, the highest prevalence (4.61%) was observed in autumn and the lowest (2.84%) in winter. In cattle, the highest prevalence was in spring (4.08%) and the lowest in winter (2.66%).

Al-Kassar (2012) reported the presence of Fasciola spp. stages at a rate of 16.04% in vegetables in Al-Nasiriyah, southern of Iraq. In Babylon, Abdalnabi (2012) performed serological survey for the prevalence of fascioliasis and reported rates of 14.30%, 35.00%, and 68.40% in cattle, sheep and goats, respectively. Hussain and Zghair (2017) reported a rate of 3.61% among 500 examined cows in Karbala, Iraq. Oleiwi et al. (2017) investigated the presence of fascioliasis in 220 blood samples collected from local breed sheep in Abu Gharib district, Baghdad governorate using ELISA test and recorded a seropositivity rate of 12.73% for *F. hepatica* among them.

Abass et al. (2018) investigated the prevalence of fascioliasis in slaughtered cattle, goats and sheep of Kirkuk from 2017 to 2018 and reported rates of 1.35%, 0.63% and 0.28%, respectively. With the highest prevalence (80%) in April and the lowest (6.0%) in November in cattle. On the other hand, the highest prevalence for sheep and goats was recorded in March which were 72.00% and 11.00%, respectively.

Gatie et al. (2018) investigated the prevalence of fascioliasis in cattle and buffalo in Thi-Qar and found 54.7% and 23.71% of them infected. In Koya city, Hassan (2018) investigated the prevalence of cattle and sheep on monthly basis and reported rates of 1.8% in cattle and 0.14% in sheep, with the highest rate (2.30%) among cattle in June and 0.98% among sheep in March while infection was not reported among goats.

In Kirkuk, Abass et al. (2019) investigated the rate of fascioliasis in slaughtered livestock and reported rates of 0.17%, 0.7%, 1.23% and 12.98% in sheep, goats, cattle and buffalo, respectively. Also, they further added that, the rates were higher in spring and winter and lower in summer and autumn.

Al-Mahmood and Al-Sabaawy (2019) in Mosul, reported a natural infection rate of 4% in slaughtered cattle. Al-Alo et al. (2019) investigated the prevalence of fascioliasis in sheep and cattle based on abattoir data of Al-Najaf, southern Iraq, and reported rates of 0.67% in sheep, and 0.66% in cattle. Furthermore, the highest rates in sheep and cattle were during April which were 0.69% and 0.80%, respectively and the lowest rate was 0.38% for both species in October.

Rasheed et al. (2019) investigated the prevalence of fascioliasis among slaughtered sheep in Tikrit and Balad cities, and reported rates of 17.54% and 8.79%, respectively. They further added that in Tikrit, the highest rate (30.58%) was in January and the lowest (10%) was in June, while in Balad city, they reported the highest rate (13.33%) in December and the lowest (3.86%) in June.

In Duhok governorate, Nerway et al. (2021) reported prevalence of 2.0% of fascioliasis among slaughtered livestock, with the highest prevalence of 3.27% in cattle, followed by sheep (1.76%) while the lowest (0.87%) was in goats. The local breed of livestock showed slightly higher rate as compared to imported breed (2.10% versus 1.88%). With regard to gender, the prevalence was higher (2.08%) in males than females (1.08%). The distribution of Fasciola species among livestock differs in sheep and cattle. *F. hepatica* was more common in sheep and goats; while most of the cattle were infected with *F. gigantica*.

Regarding the intensity of infection in livestock, heavy infections were reported in 38.46% of infected animals, with the highest rate (55.74%) being in sheep, followed by light infection which were reported in 35.90% of animals, with the highest rate (62.12%) in cattle, while 28.21% of the animals were infected with a moderate number of flukes with the highest rate (71.43%) being in goats. Most of the liver flukes were recovered from the bile ducts (67.69 %), followed by gall bladder (21.54%) and the least (10.77%) from both the bile ducts and gall bladder.

The Epidemiology of Fascioliasis in Countries Surrounding Iraq

A vast number of studies on fascioliasis, among countries neighboring Iraq were carried out in Iran. Only the studies performed during the period from 2005 to 2019 on sheep, goats and buffalos are listed in Table 1, which showed fluctuation in infection rates.

Regarding seasonal fluctuation of prevalence in Iran, cattle, sheep and goats showed the highest prevalence in spring and summer as reported by Ali et al. (2011), while, Khanjari et al. (2010) reported high prevalence among cattle in spring and among sheep in winter. On the other hand, some studies recorded higher prevalence in cattle, sheep and goats in winter, and low prevalence in summer (Kordshooli et al. 2017; Arbabi et al. 2018; Aminzare et al. 2018).

With respect to gender, some studies recorded significantly higher prevalence in females than males (Ali et al. 2011; Khanjari et al. 2014).

In Saudi Arabia few studies were carried out on the prevalence of fascioliasis in livestock. Sanad and Al-Megrin (2005) investigated the prevalence of fascioliasis in imported and local sheep in Riyadh city, and recorded a rate of 21.9%, with a higher rate among imported sheep as compared to local one, since only 4.96% of them were infected. Degheidy and Al-Malki (2012) investigated the prevalence of Fasciola infection and liver abscess among slaughtered imported cattle, in Al-Taif city and reported rates of 8.6% and 1.1% respectively, they further, added that fascioliasis caused 52.06% loss of liver and meat estimated to be around 75000 SR yearly. In another study in Al-Taif, Degheidy et al. (2013) recorded a prevalence of 3.1% in sheep.

In Jordan only the study performed by Maraqa et al. (2005) reported the prevalence of helminthic infection in imported and local sheep. *F. hepatica* was reported in 3.2% of sheep imported from Australia, while none of the local breed was found infected.

Yildirim et al. (2007) recorded prevalence of *Fasciola hepatica* in 65.2% cattle of Turkey using ELISA test, with significantly higher rate among females than males (70.7% versus 47.8%). Significantly different rates were observed in different age categories while the association among animal was found nonsignificant. In the black sea area, Acici et al. (2017) investigated the rate of F. hepatic in sheep in three areas using ELISA, and reported rates of 32.40%, 25.40% and 34.90% in Samsun, Sinop and Tokat, respectively. Furthermore, Celik and Celik (2018) performed another study on fascioliasis and recorded rates of 7.5% and 14.14% for *F. hepatica* among sheep and goats, respectively. With regard to Syria and Kuwait, fascioliasis was not investigated in these countries.

The Genome of Fasciola spp.

Fasciola gigantica complete nuclear genome is 1.04 Gb, having a total of 20858 genes (Pandey et al. 2020). While, F. hepatic possess the largest parasitic genome accounting of 1.3Gb (Cwiklinski et al. 2015). It possesses 10 pairs of chromosomes, 32% of this genome is composed from repetitive DNA. This genome contains 12 gene-coding proteins, two rRNA genes and 22 transfer RNA genes, with the absence of mitochondrial genes that encode ATP synthase membrane subunit 8 (atp 8 gene) (Huang et al. 2013; Liu et al. 2014). The targeted genes in each genome must be long enough to show reasonable variable differential and similar features allowing differentiation between the genes of the targeted species (Huang et al. 2004). Because some nucleotides remain constant and others are divers, this will lead to the occurrence of small numbers of mutations in the active constraint regions of the genome. Coding regions possess more functional constraints as compared with non-coding regions; therefore, their information is used in phylogenetic studies (Shaw et al. 2005). Phylogenetic and genetic studies of Fasciola spp. have been performed using r DNA and mitochondrial DNA (mt DNA) markers (Le et al. 2000; Huang et al. 2004).

DNA Markers

The DNA markers are modern tools used for the differentiation between Fasciola species, since morphological differences do not give the precise identification of the species due to the overlapping of the morphological features between both Fasciola species in addition to time consuming (Rokni 2008; Hasanpour et al. 2020). Therefore, recent molecular techniques were used for distinguishing both

species of flukes, as it is necessary to identify them accurately because intermediate forms of both species have been raised creating difficulties for taxonomical identification (Walker et al. 2006). For this reason, molecular techniques are considered as the golden method for discrimination between both species.

So far, the used markers include first Internal Transcribed Spacers (ITS-1) of 5.8S and second Internal Transcribed Spacer (ITS2) of 28S ribosomal ribonucleic acid (rRNA) (Marcilla et al. 2002; Le et al. 2008) and the mitochondrial DNA markers (Walker et al. 2006).

Internal Transcribed Spacers (ITS)

The rDNA markers are valuable markers, because they have a number of variable regions between two conservative regions making them a perfect molecular marker in molecular studies (Hills and Dixon 1991). These markers are non-coding regions situated between18S, 5.8S and 28S ribosomal RNA genes as illustrated in Fig. (4). The ITS1 and ITS2 are used in phylogenetic studies for differentiation between Fasciola spp. isolated from various hosts and different locations. This will help in identifying the intermediate forms of Fasciola and in the precise identification of the known Fasciola spp. (Itagaki and Tsutsumi 1998; Itagaki et al. 2005; Prasad et al. 2008).

Mitochondrial DNA Markers

These markers are used in phylogenetic studies of Fasciola spp., they included the cox1, and the Mitochondrial Nicotinamide Dinucleotide Dehydrogenase Subunit-1 (nd1). Both these markers have been used in many molecular studies for determining the relatedness among Fasciola spp. (Chaichanasak et al. 2012). Such studies have been carried out in Bulgaria, China, Turkmenistan, Turkey and Russia using one or both of these markers (nd1 and cox1) for determining the lineages of F. hepatica, and they reported 13 haplotypes using nd1 and 10 using cox1 (Semyenova et al. 2005).

Molecular Studies on Fascioliasis

Molecular Studies of Fascioliasis in Iraq

In Iraq some molecular investigations were performed using different DNA markers for identifying Fasciola spp. Mohammed et al. (2016) used ITS-1 and ITS-2 ribosomal DNA markers to identify *F. hepatica* isolated from cattle in Duhok governorate. Abdulwahed and Al-Amery (2019) used ITS2 and RFLP to identify *F. hepatica* and *F. gigantica* isolated from infected sheep in Al-Kut city, central region of Iraq. In addition, they also, reported a new intermediate form of Fasciola. In Baghdad, Hassone and Salah (2019) performed a phylogenetic study of *F. hepatica* isolated from cattle using cox1 gene and recorded 12 *F. hepatica* closely related forms which were similar to sequences from Spain.

Hamoo et al. (2019) used ITS-1 rDNA as a marker for identifying Fasciola spp. isolated from cattle liver in Kirkuk. They reported *F. gigantica* which showed 100% similarity with globally isolates sequences. The same researchers conducted another study in Aqrah (Hamoo et al. 2020) using the 18S rRNA gene for the identification of *F. gigantica* removed from the liver of infected cattle.

Table 1: The prevalence of Fasciola spp. among livestock in Iran

Hosts	Province in Iran	Prevalence (%)	References
Sheep	Fars	38.30	Sayari et al. 2008
Goats	Fars	5.00	
Cattle	East Azerbaijan	32.10	Eslami et al. 2009
Sheep	East Azerbaijan	32.00	
Calves	East Azerbaijan	0.00	
Buffaloes	East Azerbaijan	17.00	
Horses	East Azerbaijan	50.00	
Cattle	Khuzestan	49.50	Ahmadi and Meshkehkar 2010
Sheep	Khuzestan	28.70	
Goats	Khuzestan	35.90	
Cattle	Isfahan	2.40	Ali et al. 2011
Sheep	Isfahan	6.90	
Goats	Isfahan	4.10	
Cattle	Ilam	53.00	Abdi et al. 2013
Sheep	Ilam	36.50	
Goats	Ilam	10.50	
Cattle	Lorestan	7.60	Ezatpour et al. 2014
Sheep	Lorestan	7.10	Ezatpour et al. 2015
Goats	Lorestan	3.90	
Cattle	Fars	1.65	Mohamadzadeh et al. 2016
Sheep	Fars	0.33	
Goats	Fars	0.24	
Cattle	Jahrom	11.15	
Sheep	Jahrom	5.22	Kordshooli et al. 2017
Goats	Jahrom	2.15	
Cattle	Arak	0.76	
Sheep	Arak	0.75	Arbabi et al. 2018
Goats	Arak	0.42	
Cattle	Guilan	9.00	Khademvatan et al. 2019
Sheep	Guilan	4.20	
Goats	Guilan	3.10	

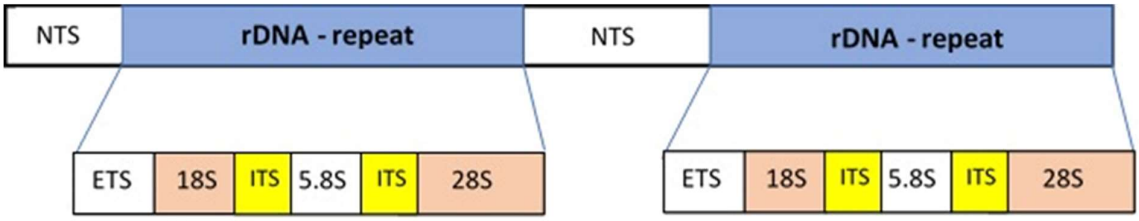

Fig. 4: Diagram of ITS-1 and ITS-2 markers.

Raoof et al. (2020) used the mitochondrial 28S rRNA gene in a molecular study and phylogenetic analysis for Fasciola species isolated from sheep and goats of Sulaymaniyah governorate. They obtained two sequences of *F. gigantica* that showed high similarity with sequences of Indian strains, while four *F. hepatica* sequences were analogous to sequences from Iran and Saudi Arabia.

Mohammed et al. (2021) carried out another molecular study and phylogenetic analysis of Fasciola spp. isolated from cattle, sheep and goats in Duhok using ITS1 and ITS2 as DNA marker. They sequenced 13 products from the study; 7 amplicons were recognized as *F. hepatica* and 5 as *F. gigantica* except one sequence of *F. gigantica* was clustered as monophyletic, these sequences were deposited in GenBank.

Molecular Studies on Fascioliasis in Countries Surrounding Iraq

Simsek et al. (2011) used PCR-RFLP method for targeting mitochondrial cox1 with the help of the restriction enzymes AluI and RsaI for identification of Fasciola spp. isolated from domestic livestock in Turkey. Both fluke species were reported.

In Turkey also, Canakoglu et al. (2019) used molecular tools for identifying Fasciola spp. isolated from cattle and sheep using PCR-RFLP on β-tubulin isotype 3 gene and the enzymes MboII, HphI and HindII. Polymorphism in β-tubulin isotype, 3 genes were reported among *F. hepatica* isolated from sheep and cattle of the two studied parts of Turkey.

Shalaby and Gherbawy (2013) performed a molecular study of Fasciola species isolated from imported sheep in Saudi Arabia, using Random Amplified Polymorphic DNA (RAPD) PCR in amplifying ITS1. Both species of Fasciola were recorded in their study in addition to a hybrid form with different genetic constituents of both species.

Amor et al. (2011) used morphological and molecular techniques for the identification of Fasciola species isolated from buffaloes and goats in Iran. They used the sequences of both ITS-1 and ITS-2 rDNA. The performed tree of these sequences showing 48.1% identity to the haplotypes that most frequently identified for F. hepatic and 38.45% identity to *F. gigantica*. But these sequences differ from each other in the position of some nucleotides of the ITS region. Also, a new intermediate form with 13.45% similarity was reported which showed overlapping of nucleotides in all positions between the two Fasciola species.

Also, Shahbazi et al. (2011) used ITS1 and RAPD-PCR techniques for molecular identification of Fasciola removed from sheep and cattle. They reported intra-specific variation within both species. Shafiei et al. (2014) investigated the morphological and molecular analysis of *F. hepatica* and *F. gigantica* using the ITS1, ITS2, and mitochondrial genes (nd1 and cox) in the southwest of Iran. They isolated *F. hepatica* from sheep and goats, while both species (*F. hepatica* or *F. gigantica*) were isolated from cattle.

In west region of Iran, Kobra et al. (2018) used morphological characters and molecular tools like PCR-RLFP, to identify both Fasciola spp. and their intermediate forms isolated from sheep, goats and cattle. Another study in west of Iran, Shokouhi et al. (2019) carried out a genotyping investigation on Fasciola spp. isolated from sheep and cattle targeting ITS1 by PCR and PCR-RFLP techniques with Rsa1 restriction enzyme and they reported *F. hepatica* in their study.

Javanmarda et al. (2020) identified Fasciola spp. taken from sheep, goats, cattle and humans using multiplex PCR and RLFP-PCR for targeting the genes phosphoenolpyruvate carboxykinase (pepck) and DNA polymerase delta (pold). They found that all isolates were *F. hepatica* without the detection of any hybrid form. But the sequences isolated from cattle were with higher diversity in the three genes than those of sheep and goats. In addition, phylogenetic analyses showed a close relation between *F. hepatica* isolates of human and sheep.

Diagnosis of Fascioliasis in Livestock

The control and management of fascioliasis depend on the early identification and treatment of the disease in infected hosts. Different diagnostic methods have been used for the diagnosis of the disease in livestock such as coprological, morphometric, serological, imaging techniques and molecular techniques (Thanh 2012).

Coprological and Morphometrical Methods

In coprological diagnosis, the stool samples are examined using microscopy which is effective for detecting Fasciola eggs in the stool samples and in bile and duodenal fluid samples. Stool samples can be examined using sedimentation and Kato-Katz method (Hanpithakpong et al. 2008; Bhamidipati et al. 2009). The copromicroscopic techniques are cheap, and can easily be performed. Using this method, the intensity of infection can be estimated that help in determining the effect of anthelminthic used in treatment of livestock (Cesar et al. 2011).

Fasciola eggs are quite big and characteristic, as they are oval, operculated and unembryonated. So morphological characters can be helpful in diagnosis, except during migration of immature flukes in the liver tissue and in such cases, it will be difficult to detect the eggs in stool samples (Hillyer 1999; Marcilla et al. 2002). The copromicroscopy can be good for diagnosing chronic infections, since mature flukes only can lay eggs, but in the cases of acute and ectopic infections this method is useless (WHO 2006).

The morphometric methods are used in many countries for the differentiation of adult Fasciola flukes depending on measurements of body length and other morphological

characters which are differential such as, body length, width, cone length and width, size of suckers, and other measurable characters (Periago et al. 2006; Ashrafi et al. 2006). For precise diagnosis, it is useful to combine morphometric method with Computer Image Analysis System (CIAS). This technique has a wide application in many countries where fascioliasis is prevalent (Periago et al. 2008; Itagaki et al. 2009; Thanh 2012). Morphological features are not used for identification of intermediate forms of Fasciola species, because these forms are genetically different and such diagnostic method do not give accurate results (Knowlton 1993). Furthermore, morphological identification requires a lot of experience, so using molecular techniques can give a more confirmative identification of a species (Hebert et al. 2003).

Immunodiagnosis Methods

The most widely used immunological methods include Enzyme-Linked Immunosorbent Assays (ELISA), which is indirect serological test used for detecting antibodies produced by the parasite (Rokni et al. 2002; Espinoza et al. 2005; WHO 2006). ELISA helps to diagnose even infections with low parasite load in addition to ectopic infections (Mas-Coma et al. 2005; WHO 2006). Although, immunodiagnostic techniques are highly sensitive, but they have some difficulties in recommending the precise treatment, since the circulating antibodies remain for long time after treatment. Furthermore, cross reacting antibodies with other trematodes may develop (Boulard et al. 1995; Hillyer et al.1999).

Molecular Methods

Molecular Methods are the most confirmative means for diagnosis as compared with other methods. In this method polymerase chain reaction (PCR) are used and they gave an accurate diagnosis of both parasite species and even strains (Mas-Coma et al. 2005; Thanh 2012).

For precise identification of species and the measurement of their boundaries in addition to studying their phylogenetic relationships, many molecular methods are used including mitochondrial and nuclear DNA markers, fingerprinting, hybridization methods, sequencing of selected genes, DNA barcoding and microsatellites (Maddison 1997; Baker and Bradley 2006; Thanh 2012). All these methods can be used for differentiation of Fasciola species eggs and from eggs of other trematodes and even can be used in identifying the parasite in infected snail host (Ai et al. 2011).

Treatment of Animal Fascioliasis

For treatment of animal fascioliasis, numerous anthelminthic drugs have been used. Some of these drugs are effective in treatment for adult parasite stages (Spithill 1999). The drug Triclabendazole is effective for mature and immature stages of *F. hepatica* and *F. gigantica*. Therefore, it is used in the treatment of acute and chronic stages of fascioliasis (Waruiru et al. 1994). This drug is given as one dose of 10 mg/ Kg body weight for treatment of adult flukes inhabiting the biliary passages and the migratory juvenile flukes within liver tissue (Mahmoud 2008). Three doses per year are recommended for treatment starting from winter season for treating chronic infection. The second dose is recommended in spring to minimize the pasture contamination by parasite eggs and the third dose in autumn, at the end of dry season (Pfukenyi and Mukaratirwa 2004).

Prevention and Control of Fascioliasis

It is necessary to control the snail hosts which harbor the larval stages of the parasite and contaminate the pasture preventing disease spreading among definitive hosts (Pfukenyi and Mukaratirwa 2004). In this step, molluscicides and pesticides are used (Mzembe and Chaudhry 1981). Molluscicides are used for reducing the snail populations in rivers, springs dams or for the amphibious snails living on the surface of plants (Spithill 1999). The other step is to keep away the animal herds from sites that contain heavy growth of aquatic plants used for animal feeding that might be contaminated with parasite infective stages.

Conclusions

This review indicates that fascioliasis is endemic in Iraq, since both *F. hepatica* and *F. gigantica* in addition to their intermediate forms were reported in the livestock besides the presence of the snail hosts required for the completion of the parasite life cycle. Thus, the application of preventive and control measures are necessary,

REFERENCES

Abass KS et al., 2018. Prevalence of Liver Fluke Infections in Slaughtered Animals in Kirkuk Province, Iraq. Journal of Animal Sciences and Livestock Production 2: 01-05.

Abass KS et al., 2019. Study of bovine and ovine pulmonary and hepatic abscessation at Kirkuk abattoir. Plant Archives 19: 1640-1644.

Abdalnabi RA, 2012. Epidemiological study on *Fasciola hepatica* in children and animals at Babylon city. Al-Mustansiriyah Journal of Sciences 23: 19-26.

Abdi J et al., 2013. New features of fascioliasis in human and animal infections in Ilam province, Western Iran. Gastroenterology and Hepatology from Bed to Bench 6: 152-155.

Abdisa T and Jilo K, 2017. Review on ovine fasciolosis in Ethiopian. Journal of Veterinary Science and Research 2: 1-10.

Abdulwahed TK and Al-Amery AM, 2019. Morphological and molecular study of Fasciola spp. in sheep in Alkut city. International Journal of Bioscience 14: 121-130.

Acici M et al., 2017. Serologic detection of antibodies against *Fasciola hepatica* in sheep in the middle Black Sea region of Turkey. Journal of Microbiology, Immunology and Infection 50: 377-381.

Ahmadi NA and Meshkehkar M, 2010. Prevalence and long-term trend of liver fluke infections in sheep, goats and cattle slaughtered in Khuzestan, southwestern Iran. Journal of Paramedical Sciences 1: 26-31.

Ai L et al., 2011. Genetic characterization species differentiation and detection of Fasciola spp. by molecular approaches. Parasites and Vectors 4: 101-106.

Al-Alo KZ et al., 2019. Retrospective survey of liver flukes in sheep and cattle based on Abattoir Data in Al-Najaf Province, Iraq. Acta Scientific Veterinary Sciences 1: 2-8.

Ali TS et al., 2011. Prevalence of liver flukes' infections in slaughtered animals in Kashan, Isfahan province, central Iran. Institute of Integrative Omics and Applied Biotechnology Journal 2: 14-18.

Al-Kassar NR, 2012. Prevalence of different parasitic stages in commercial Vegetables in Al-Nasiriyah city, Iraq. Journal of Education for Pure Science 2: 172-186.

Al-Mahmood SS and Al-Sabaawy HB, 2019. Fasciolosis: grading the histopathological lesions in naturally infected bovine liver in Mosul city. Iraqi Journal of Veterinary Sciences 33: 379-387.

Aminzare M et al., 2018. Prevalence of liver flukes' infections and hydatidosis in slaughtered sheep and goats in Nishapour, Khorasan Razavi, Iran. Veterinary world 11: 146- 150.

Amor N et al., 2011. Molecular characterization of Fasciola spp. from the endemic area of northern Iran based on nuclear ribosomal DNA sequences. Experimental Parasitology 128: 196-204.

Arbabi M et al., 2018. Epidemiology and economic loss of fasciolosis and dicrocoeliosis in Arak, Iran. Veterinary World 11: 1648-1655.

Ashrafi K et al., 2006. Phenotypic analysis of adults of Fasciola hepatica, Fasciola gigantica and intermediate forms from the endemic region of Gilan, Iran. Parasitology International 55: 249-260.

Autissier C, 2008. Ethical review in biomedical research. Medicine Sciences 24: 437-442.

Baker RJ and Bradley RD, 2006. Speciation in mammals and the genetic species concept. Journal of Mammalogy 87: 643-662.

Ballweber LR, 2022. *Fasciola hepatica* in ruminants. https://www.merckvetmanual.com

Becker SL et al., 2011. Comparison of the Flotac-400 dual technique and the formalin-ether concentration technique for diagnosis of human intestinal protozoon infection. Journal of Clinical Microbiology 49: 2183-2190.

Bhamidipati RK et al., 2009. Simultaneous determination of OZ277, a synthetic 1, 2, 4- trioxolane antimalarial, and its polar metabolites in rat plasma using hydrophilic interaction chromatography. Journal of Chromatography B. 877: 2989-2995.

Boulard C et al., 1995. Evaluation of nitroxinil and closantel activity using ELISA and egg counts against *Fasciola hepatica* in experimentally and naturally infected cattle. Veterinary Research 26: 249-255.

Canakoglu SS et al., 2019. Preliminary evidence for the occurrence of β-tubulin isotype 3 polymorphisms in *Fasciola hepatica* isolates in cattle and sheep in Turkey. Helminthologia 56: 87-92

Caravedo MA and Cabada MM, 2020. Human fascioliasis: current epidemiological status and strategies for diagnosis, treatment and control. Research and Reports in Tropical Medicine 11: 149-158.

CDC-Centers for Disease Control, 2019. **Error! Hyperlink reference not valid.**. www.cdc.gov.

Celik ÖY and CELiK BA, 2018. Investigation of the Prevalence of *Fasciola hepatica* in Small Ruminants in the Siirt Region, Turkey. Iranian Journal of Parasitology 13: 627-631.

Cesar IC et al., 2011. Liquid chromatography–tandem mass spectrometry for the simultaneous quantitation of artemether and lumefantrine in human plasma: Application for a pharmacokinetic study. Journal of Pharmaceutical and Biomedical Analysis 54: 114-120.

Chaichanasak P et al., 2012. Identification of Fasciola flukes in Thailand based on their spermatogenesis and nuclear DNA, and their intraspecific relationships based on mitochondrial DNA. Parasitology International 61: 545-549.

Cobbold TS, 1855. Description of a new trematode worm (*Fasciola gigantica*). The Edinburgh New Philosophical Journal, Exhibiting a View of the Progressive Discoveries and Improvements in the Sciences and the Arts. New Series, II, 262–267.

Cwiklinski K et al., 2015. The *Fasciola hepatica* genome: gene duplication and polymorphism reveal adaptation to the host environment and the capacity for rapid evolution. Genome Biology Journal 16: 71-76.

Degheidy NS and Al-Malki JS, 2012. Epidemiological studies of fasciolosis in human and animals at Taif, Saudi Arabia. World Applied Science Journal 19: 1099-1104.

Degheidy NS et al., 2013. The incidence of Fascioliasis and some types of bacteria incurred causing lack meat and livers quality of slaughtered animals at Al-Taif, KSA. Assiut Veterinary Medical Journal 59: 136-141.

Duthaler UP, 2012. Artemisinin for the treatment of fascioliasis: progress in preclinical and diagnostic research. Doctoral dissertation, University of Basel.

Eslami A et al., 2009. Animal Fasciolosis in North of Iran. Iranian Journal of Public Health 38: 132-135.

Espinoza JR et al., 2005. Fas2-ELISA in the detection of human infection by Fasciola hepatica. Journal of Helminthology 79: 235-240.

Ezatpour B et al., 2014. A slaughterhouse study on prevalence of some helminths of cattle in Lorestan province, west Iran. Asian Pacific Journal of Tropical Disease 4: 416-420.

Ezatpour B et al., 2015. Prevalence of liver fluke infections in slaughtered animals in Lorestan, Iran. Journal of Parasitic Diseases 39: 725-729.

Gatie JA et al., 2018. Prevalence of Gastrointestinal Parasites in Cattle and Buffaloes in Thi-Qar Province, South of Iraq. Basrah Journal of Veterinary Research 17: 847-856.

Hamoo RN et al., 2019. Molecular characterization and phylogenetic analysis of Fasciola gigantica in Iraqi sheep using ITS1. Advance Animal Veterinary Science 7: 256-260.

Hamoo RN et al., 2020. Genotyping study of Fasciola gigantica isolated from cattle in Aqrah city, Iraq. Iraqi Journal of Veterinary Sciences 34: 123-127

Hanpithakpong W et al., 2008. A liquid chromatographic tandem mass spectrometric method for determination of artesunate and its metabolite dihydroartemisinic in human plasma. Journal of Chromatography B 876: 61-68.

Hassan NO, 2018. Prevalence of some infections in liver and lung of slaughtered ruminants in Koya abattoir Erbil, Iraq. Tikrit Journal of Pure Science 20: 1-6.

Hasanpour H et al., 2020. Molecular Characterization of Fasciola spp. from Some Parts of Iran. Iranian Journal of Public Health 49: 157.

Hassone WS and Salah MHK, 2019. Phylogenetics of *Fasciola hepatica* in cattle in Iraq. Online Journal of Veterinary Research 23: 776-781.

Hebert PD et al., 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B Biological Sciences 270: 313-321.

Hills DM and Dixon MT, 1991. Ribosomal DNA molecular evolution and phylogenetic inference. The Quarterly Review of Biology 66: 411-453.

Hillyer GV and Apt W, 1997. Food-borne trematode infections in the Americas. Parasitology Today 3: 87-88.

Hillyer GV, 1999. Immunodiagnosis of human and animal. Dalton JP. Fasciolosis. Oxon, UK: CABI publishing. 435-449.

Huang WY et al., 2004. Characterization of Fasciola species from Mainland China by ITS-2 ribosomal DNA sequence. Veterinary Parasitology 120: 75-83.

Huang Y et al., 2013. The carcinogenic liver fluke, Clonorchis sinensis: new assembly, reannotation and analysis of the genome and characterization of tissue transcriptomes. PloS One 8: e54732.

Hussain KL and Zghair ZR, 2017. Prevalence of fasciolosis in ruminant in Karbala city. Journal of Entomology and Zoology Studies 5: 364-369.

Itagaki S et al., 2009. In vitro and in vivo antioxidant properties of ferulic acid: a comparative study with other natural oxidation inhibitors. Food Chemistry 114: 466-471.

Itagaki T and Tsutsumi KI, 1998. Triploid form of Fasciola in Japan: genetic relationships between *Fasciola hepatica* and Fasciola gigantica determined by ITS-2 sequence of nuclear rDNA. International Journal for Parasitology 28: 777-781.

Itagaki T et al., 2005. Molecular characterization of parthenogenic Fasciola spp. in Korea on the basis of DNA sequences of ribosomal ITS1 and mitochondrial NDI gene. Journal of Veterinary Medical Science 67: 1115-1118.

Jaja IF et al., 2017. Financial loss estimation of bovine fasciolosis in slaughtered cattle in South Africa. Parasite Epidemiology and Control 2: 27-34.

Javanmarda E et al., 2020. Multigene typing and phylogenetic analysis of Fasciola from endemic foci in Iran. Infection, Genetics and Evolution 80: 104-202.

Jeandron A et al., 2010. Accuracy of the Kato-Katz, adhesive tape and FLOTAC techniques for helminth diagnosis among children in Kyrgyzstan. Acta Tropica 116: 185-192.

Keiser J and Utzinger J, 2009. Food-borne trematodiases. Clinical Microbiology Reviews 22: 466-483.

Keiser J et al., 2008. Efficacy and safety of artemether against a natural *Fasciola hepatica* infection in sheep. Parasitology Research 103: 517-522.

Khademvatan S et al., 2019. Prevalence of fasciolosis in livestock and humans: A systematic review and meta-analysis in Iran. Comparative Immunology, Microbiology and Infectious Diseases 65: 116-123.

Khanjari A et al., 2010. A retrospective survey of fasciolosis and dicrocoeliosis in slaughtered animals in Meisan abattoir, Tehran, Iran (2005-2008). In Veterinary Research Forum 1: 174-178.

Khanjari A et al., 2014. Prevalence of fasciolosis and dicrocoeliosis in slaughtered sheep and goats in Amol Abattoir, Mazandaran, northern Iran. Asian Pacific Journal of Tropical Disease 4: 120-124.

Knowlton N, 1993. Sibling species in the sea. Annual Review of Ecology and Systematics 24: 189-216.

Kobra PIRI et al., 2018. Prevalence of animal fasciolosis and specification of Fasciola spp. Isolated from sheep, goats and cattle by molecular method: Hamadan province, west of Iran. Iranian Journal of Parasitology 13: 524-527.

Kock KN et al., 2003. Distribution and habitats of the snail Lymnaea truncatula, intermediate host of the liver fluke Fasciola hepatica, in South Africa. Journal of the South African Veterinary Association 74: 117–122.

Kordshooli MS et al., 2017. A reducing trend of fasciolosis in slaughtered animals based on abattoir data in South of Iran. Veterinary World 10: 418-423.

Koyee QM et al., 2011. Prevalence of Some Parasitic Helminthes Among Slaughtered Ruminants (Sheep, Goats and Cattles) in Hawler Slaughter House During 2010. In Hawler, Kurdistan Region, Iraq 4th International Scientific Conference Salahaddin University, Erbil-Iraq. 10: 1-3.

Le T et al., 2000. Mitochondrial genomes of human helminths and their use as markers in population genetics and phylogeny. Acta Tropica. 77(3):243-256.

Le T et al., 2008. Human fascioliasis and the presence of hybrid/introgressed forms of *Fasciola hepatica and Fasciola gigantica* in Vietnam. International Journal for Parasitology 38:725–73

Liu GH et al., 2014. Complete mitochondrial genomes of the 'intermediate form of Fasciola and *Fasciola gigantica*, and their comparison with F. hepatica. Parasites and Vectors Journal 7: Article# 1

Linnaeus C. 1758. Systema Naturæ per Regna Tria Naturæ, Secundum Classes, Ordines, Genera, Species, cum Haracteribus, Differentiis, Synonymis, Locis. Tomus I. Holmiæ: Impensis Direct. Laurentii Salvii.50.

Maddison WP, 1997. Gene trees in species trees. Systematic Biology 46: 523-536.

Mahmoud MS, 2008. Immune response in Fasciola gigantica experimentally infected rabbits treated with either carnosine or Mirazid. Research Journal of Parasitology 3: 40-49.

Maraqa A et al., 2005. An abattoir survey of liver and lung helminthic infections in local and imported sheep in Jordan. Turkish Journal of Veterinary and Animal Sciences 29: 1-2.

Marcilla A et al., 2002. A PCR-RFLP assay for the distinction between *Fasciola hepatica* and *Fasciola gigantica*. Molecular and Cellular Probes 16: 327-333.

Mas-Coma S and Bargues MD, 1997. Human liver flukes: a review. Research and Reviews in Parasitology 57: 145-218.

Mas-Coma S, 2005. Epidemiology of fascioliasis in human endemic areas. Journal of Helminthology 79: 207–216.

Mas-Coma S et al., 2005. Fascioliasis and other plant-borne trematode zoonoses. International Journal for Parasitology 35: 1255-1278.

Mas-Coma S et al., 2009. Fasciola, lymnaeids and human fascioliasis with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Advances in Parasitology 69: 141-146.

Mohamadzadeh T et al., 2016. A study on prevalence of some helminthic infections of the liver and lungs among ruminants in abattoir of Fars province, Iran. Archives of Razi Institute 71: 245-251.

Mohammed AB et al., 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.

Mohammed AB et al., 2021. Molecular Identification of Fasciola spp. Isolated from Domestic Animals Based on DNA Sequencing of the Nuclear Ribosomal ITS1 -ITS2 Markers, Kurdistan Region, Iraq. Pakistan Veterinary Journal (in press).

Mzembe SAT and Chaudhry MA, 1981. The epidemiology of fascioliasis in Malawi part II. Epidemiology in the definitive host. Tropical Animal Health and Production 13: 27-33.

Nerway CA et al., 2021. Prevalence of Fasciola spp. Among Slaughtered Livestock in Zakho City, Duhok Governorate-Iraq. Academic Journal of Nawroz University 10: 199-206.

Oleiwi KI et al., 2017. Detection of *Fasciola hepatica* in Abu-Ghraib district, Iraq. Journal of Entomology and Zoology Studies 5: 1068-1072

Pandey T et al., 2020. Draft genome of the liver fluke *Fasciola gigantica*. Journal of the American Chemical Society Omega 5: 11084-11091.

Periago MV et al., 2006. Phenotypic comparison of allopatric populations of *Fasciola hepatica* and Fasciola gigantica from European and African bovines using a computer image analysis system (CIAS). Parasitology Research 99: 368-378.

Periago MV et al., 2008. First phenotypic description of *Fasciola hepatica*/*Fasciola gigantica* intermediate forms from the human endemic area of the Nile Delta, Egypt. Infection. Genetics and Evolution Journal 8: 51-58.

Pfukenyi DM and Mukaratirwa S, 2004. A retrospective study of the prevalence and seasonal variation of Fasciola gigantica in cattle slaughtered at the major abattoirs of Zimbabwe between 1990 and 1999. Onderstepoort Journal of Veterinary Research 71: 181-187.

Prasad PK et al., 2008. Molecular identification of the Indian liver fluke, Fasciola (Trematoda: Fasciolidae) based on the ribosomal internal transcribed spacer regions. Parasitology Research 103: 1247–1255.

Pullan RL et al., 2008. Human helminth co-infection analysis of spatial patterns and risk factors in a Brazilian community. PLOS Neglected Tropical Diseases 2: e352.

Raoof HS et al., 2020. Molecular characterization and phylogenetic analysis of Fasciola species in sheep and goats in Sulaymaniyah Province, Northern Iraq. Journal of Zankoy Sulaimani 22: 297-305.

Rasheed MM et al., 2019. Prevalence of Liver Flukes in Some Areas of Salahalddin Province. Tikrit Journal for Agricultural Sciences 19: 29-34.

Rasheed SA and Kadir MA, 2008. Prevalence of some parasitic helminths among slaughtered ruminants in Kirkuk slaughter house, Kirkuk, Iraq. Iraqi Journal of Veterinary Sciences 22: 81-85.

Rinaldi L et al., 2007. Crenosoma vulpis in dog: first case report in Italy and use of the FLOTAC technique for copromicroscopic diagnosis. Parasitology Research 101: 1681-1684.

Rokni MB, 2008. The present status of human helminthic diseases in Iran. Annals of Tropical Medicine and Parasitology 102: 283-295.

Rokni MB et al., 2002. Diagnosis of human fasciolosis in the Gilan province of Northern Iran: application of cathepsin L-ELISA. Diagnostic Microbiology and Infectious Disease 44: 175-179.

Sanad M and Al-Megrin W, 2005. Fascioliasis among local and imported sheep in KSA: Parasitological and serological diagnosis. Journal Egyptian Society of Parasitology 35: 1121-1134.

Sayari M et al., 2008. A comparative study of prevalence of hydatidosis and fasciolosis (liver) rote in small ruminant of Ahwaz. Scientific Research Iranian Veterinary Journal 3: 89-95.

Schweizer G et al., 2005. Estimating the financial losses due to bovine fasciolosis in Switzerland. Veterinary Record 157: 188-193.

Semyenova SK et al., 2005. Polymorphism of internal transcribed spacer 2 (ITS-2) sequences and genetic relationships between *Fasciola hepatica* and *F. gigantica*. Acta Parasitologia 50: 240- 243.

Shafiei R et al., 2014. Molecular and morphological characterization of Fasciola spp. isolated from different host species in a newly emerging focus of human fascioliasis in Iran. Veterinary Medicine International 2014: 1-14.

Shahbazi A et al., 2011. Identification and genetic variation of Fasciola species from Tabriz, North-Western. Iran. Iranian Journal of Parasitology 6: 52-59.

Shalaby I and Gherbawy Y, 2013. Molecular Characterization of Fasciola sp. isolated from imported sheep in Taif region (Saudi Arabia). Tropical Biomedicine 30:1-12.

Shaw J et al., 2005. The tortoise and the hare 2: Relative utility of noncoding chloroplast DNA sequence for phylogenetic analysis. American Journal of Botany 92: 142-166.

Shokouhi S et al., 2019. Genotyping of *Fasciola hepatica* isolated from domestic animals in the west of Iran. Infectious Disorders-Drug Targets 19: 409-413.

Simsek A et al., 2011. Molecular differentiation of Turkey cattle isolates of *Fasciola hepatica* and *Fasciola gigantica*. Helminthologia Journal 48: 3-7.

Speich B et al., 2010. Comparative cost assessment of the Kato-Katz and FLOTAC techniques for soil-transmitted helminth diagnosis in epidemiological surveys. Parasites and Vectors 3: 7-134.

Spithill TW, 1999. *Fasciola gigantica*: epidemiology, control, immunology and molecular biology. Fasciolosis Journal 465-525.

Stein M, 2003. Animal-Health-Online "Leberegel" Survey [cited 2011 15.05.2011].

Thanh NT, 2012. Zoonotic fasciolosis in Vietnam: Molecular identification and geographical distribution (Doctoral dissertation, Tesis de Doctorado, Ghent University, Bélgica).

Walker SM et al., 2006. Stage-specific differences in fecundity over the life-cycle of two characterized isolates of the liver fluke Fasciola hepatica. Journal of Parasitology 133: 209-216

Waruiru TM et al., 1994. The efficacy of triclabendazole and oxyclozanide against *Fasciola gigantica* in naturally infected dairy cattle in Kenya. Bulletin of Animal Health and Production in Africa. 42:205–209.

World Health Organization. (2006). Report of the WHO Informal Meeting on use of triclabendazole in fascioliasis control. World Health Organization, Headquarters Geneva.17: 18.

WHO. 2016. https://www.who.int/foodborne_trematode_infections/fascioliasis/en/.

Yildirim A et al., 2007. Prevalence and risk factors associated with *Fasciola hepatica* in cattle from Kayseri province, Turkey. Revue de Medicine Veterinaire 158:613-617.