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Equine fasciolosis - a literature review

Diploma Thesis

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submitted by

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1. Introduction

Equine fasciolosis is an important re-emerging parasitic disease of horses, donkeys, and mules. The disease is caused by members of the trematode genus *Fasciola* which infect animals via ingestion of encysted metacercariae on plants. Infected animals show weight loss, lethargy, and to a less extent signs of hepatic failure. The disease is prevalent in areas with high rainfall and abundant intermediate hosts. Among lymnaeid host snails, *Galba truncatula* is the preferable host snail for *Fasciola hepatica*, the common liver fluke, which contributes to fluke infections in humans and herbivores animals in areas with moderate climate worldwide, while *Fasciola gigantica*, the tropical liver fluke, is restricted to sub-tropical areas (Hughes, 1985).

Infected donkeys are considered one of the important reservoir species that contribute to the maintenance of *Fasciola* infestation in the environment. Horses and mules may have mild to heavy infections and shed eggs of liver flukes onto the pasture. Diagnosis of *Fasciola* infection in equines continues to be difficult as several diagnostic techniques are applied with variable sensitivity and specificity. There is no single test to be considered a gold standard for the detection of infection in equids. Control of fasciolosis depends upon several inseparable steps including: livestock management, pasture management, and snail control. The aim of this work was to review the biology and pathophysiology of *Fasciola* infection with a focus on equids, to summarize the risk factors of equine fasciolosis, to describe and compare the available diagnostic techniques, and to review the current knowledge on the prevalence and pattern of spread of equine fasciolosis in different geographic areas.

2. Materials and Methods

The current work was a literature review based on a search of literature on equine fasciolosis using various search engines and websites. Google Scholar (https://scholar.google.com/scholar?hl=en&as_sdt=0%2C42&q=equine+fasciolosis+&btnG=) supplied multiple review and research articles. Swisscows (https://swisscows.com/) was another search engine that provided information which is not available on Google. Search Encrypt (https://www.searchencrypt.com/search/) and Bing search engines (https://www.bing.com/search?q=fasciolosis+in+equine&FORM=AWRE) support me with some online articles and some leaflets. PubMed Central (https://pubmed.ncbi.nlm.nih.gov/), Researchgate (https://www.researchgate.net/), Science direct (https://www.sciencedirect.com/) and CDC (https://www.cdc.gov/) websites gave me the ability to read more research articles, conference paper, and book chapters. Online Merck veterinary medicine also supported me with some information on the disease. Another website is vetlexicon (https://www.vetlexicon.com) provide some data about equine fasciolosis including pathogenic effect, life cycle and preventive and control measures.

The used keywords (used in various combinations) were: Equine, Fasciolosis, Fluke, Hepatic, Metacercaria, Human, Zoonosis, Horses, Donkeys, Mules, Flukicides

3. Results

3.1. Textbooks

Chapters from the following books were included:

- Chapter 4 from: Georgis' Parasitology for Veterinarians, 10th edition, editor: D.D. Bowman, 2014, pp 122-130, Elsevier Saunders, Philadelphia, USA
- Chapter 5 from Principles of Veterinary Parasitology, 1st edition, editors: D. Jacobs, M. Fox, L. Gibbons, C. Hermosilla, 2016, pp 138-141, pp published by John Wiley & Sons, Hoboken, NJ, USA
- Chapters 4 and 5 from: Fasciolosis: Causes, Challenges and Controls, 1st edition, editors: D.K. Singh, V. K. Singh, R. N. Singh, P. Kumar, 2021, Springer Nature, Singapore
- Chapter 1 from: Veterinary Parasitology, 4th edition, by M. A. Taylor, R. L. Coop, Richard L. Wall, 2016, Wiley-Blackwell, Oxford, UK
- Fasciolosis, 1st edition, editor J.P. Dalton 1999, CABI Publishing, Wallingford, UK
- Chapter 9: Diseases of the Liver, from: Veterinary Medicine - a Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats, 11th edition, editors: P. Constable, K. W. Hinchcliff, S. Done, W. Gruenberg, 2017, pp 641-645, Elsevier Saunders, Philadelphia, USA
- Chapter (27): Diseases associated with helminth parasites, from: Veterinary Medicine - a Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats, 10th edition, editors O. Radostits, C. Gay, K. Hinchcliff, P. Constable, 2009, pp 1576-1580, Elsevier Saunders, Philadelphia, USA

3.2. Review articles

Nine review articles were included:

- Review about the epidemiology and control of liver flukes in sheep and cattle (Howell and Williams, 2020)
- Review about the current challenges for fasciolicide treatment in ruminant livestock (Castro-Hermida et al., 2021)
- Review about the impact of human activities on fasciolosis transmission (Sabourin et al., 2018)
- A clinical commentary review on fasciolosis in horses as re-emerging disease (Williams and Hodgkinson, 2017)
- Review on the anthelmintic resistance and the control of human soil-transmitted helminths (Vercruysse et al., 2011)
- Review article on the recent advances in the diagnosis of *Fasciola hepatica* in cattle (Charlier et al., 2014)
- Resistance to fascioliasis (Haroun and Hillyer, 1986)

- Survival of *Fasciola hepatica* metacercariae in silage and stored forages on (John et al., 2019)
- Diseases of humans caused by trematodes in central Europe (Auer and Aspöck, 2014)

3.3. Original research articles

Research articles on equine fasciolosis mainly focused on the prevalence of fasciolosis among different equine species in different localities:

- Asia (Eslami et al., 2009; Hosseini et al., 2009)
- Africa (Getachew et al., 2010)
- South America (Mas-Coma et al., 2020; Mera et al., 2020).
- Europe (Howell et al., 2020; Quigley et al., 2017; Soykan and Oge, 2012; Umur and Acici, 2009).

The role of equine in epidemiology and transmission of *Fasciola* infection was discussed in two research articles (Mas-Coma et al., 2020; Mera et al., 2020).

3.4. Additional sources

- Leaflet about equine fasciolosis prepared by the university of Liverpool (http://www.animalwelfarefoundation.org.uk/wp-content/uploads/2017/12/Equine-liver-fluke-leaflet.pdf)
- PowerPoint presentation from SlideShare (https://www.slideshare.net/shafiiSheikh/fasciolosis-97464552).

4. *Fasciola* spp. and fasciolosis: General overview

4.1. Taxonomy

Liver flukes are digenean trematodes that appear as large leaf-like parasites with suckers on the anterior end. The adult flukes colonize the bile ducts of domestic or wild ruminants and produce large numbers of eggs in bile. The most important species of the family Fasciolidae are *F. hepatica* (common liver fluke), that mainly infects cattle, sheep, goats, horses, pigs and man, and *Fasciola gigantica* (tropical liver fluke) that infects cattle, buffaloes, horses, donkeys, sheep and camel (Bowman, 2014; Deplazes et al., 2016; Howell and Williams, 2020). Two other members of the Fasciolidae are *Fascioloides magna* (giant liver fluke); that infects white-tailed deer, caribou, red deer and fallow deer, and *Parafasciolopsis fasciolaemorpha* (moose liver fluke) which infects almost exclusively wild ruminants; elk, red deer, roe deer and bison (Deplazes et al., 2016). Recently, a new *Fasciola* species; *F. jacksoni,* was described from elephants and it was genetically characterized to be related to *Fascioloides*. It may induce significant pathogenic effects and could be responsible for fatal fascioliosis in elephants (Rajapakse et al., 2019).

4.2. Life cycle

Fasciola spp. have a complex life cycle which require intermediate hosts (freshwater snails) to complete their life cycle (Fig. 1). The life cycle starts with the presence of adult flukes in the bile duct of infected animals. Once the adult flukes reach sexual maturity, they commence oviposition of immature eggs in bile that are passaged with the intestinal content and excreted in faeces to be disseminated in the environment. Depending on humidity, oxygen concentration and ambient temperature, *Fasciola* eggs undergo embryonation and hatching occurs at temperature above 10 °C and the hatched miracidia begin to search for intermediate hosts (lymneid snails) depending upon chemotactic features of the snails as the mucus of snails contains short-chain fatty acids and macromolecular glycoconjugates that attract the miracidia and facilitate their penetration to snail body (Kalbe et al., 1997, 2000). Swimming miracidia have only 24 hrs to invade snail body, otherwise miracidia will die off. Infection of snail is a temperature dependant process that does not take place if temperature is below 5°C, being optimal at 15-20°C. Inside the snail tissues, clonal amplification occurs mainly in the digestive gland of the snail and the miracidium develops to a sporocyst followed by the redial stage with daughter rediae. The final stage in the snail is the cercaria. After several cycles of asexual multiplication, one miracidium entering a snail can give rise to 600 or more cercariae. The time elapsed between entrance of miracidium and release of cercariae is at least 7 weeks. Release of cercariae from infected snails occurs repeatedly over several weeks or months. Cercariae leave the snail through the birth pore and swim in water, attach to the leaves of plants. Shortly after their release from the snail, they become encysted and form a thick firm protective coat around their bodies. This protective coat is formed of keratinized proteins, mucopolysaccharides and mucoproteins, and secreted by a special gland (Constable et al., 2017; Deplazes et al., 2016; Jacobs et al., 2016).

Encysted metacercariae can resist adverse environmental conditions including cold weather and drought. Once plants with encysted metacercariae are ingested by the vertebrate host, encysted metacercariae undergo what is called “excystation” which is activated by the temperature of rumen and the presence of CO_2 and reducing agents. The encysted metacercariae start to produce intestinal proteases, cathepsins that digest the inner cyst wall and allow the emergence of metacercariae and convert into immature flukes which penetrate the intestinal wall of the infected animals and peritoneum (Deplazes et al., 2016). The penetrating flukes migrate toward liver; however, some flukes will lose their way and can infect other organs, e.g. lung or uterus, and become ectopic. Young flukes reach to liver after 4-6 days post excystation and they have a strong predilection for liver tissues, and once reaching the liver, they penetrate the liver capsule and migrate through the liver parenchyma for 6-7 weeks, forming haemorrhagic tunnels until they reach a bile duct. In the bile ducts, the immature flukes undergo maturation, reach to sexual maturity and begin to copulate and lay eggs that are passed with the faeces. The prepatent period of *Fasciola* spp. is usually 2-3 months depending upon the fluke burden. However, it has repeatedly been reported that flukes do not reach maturity in horses and eggs may not be passed with faeces, and if they do, prepatent period may be longer than in ruminants (Deplazes et al., 2016; Jacobs et al., 2016; John et al., 2019).

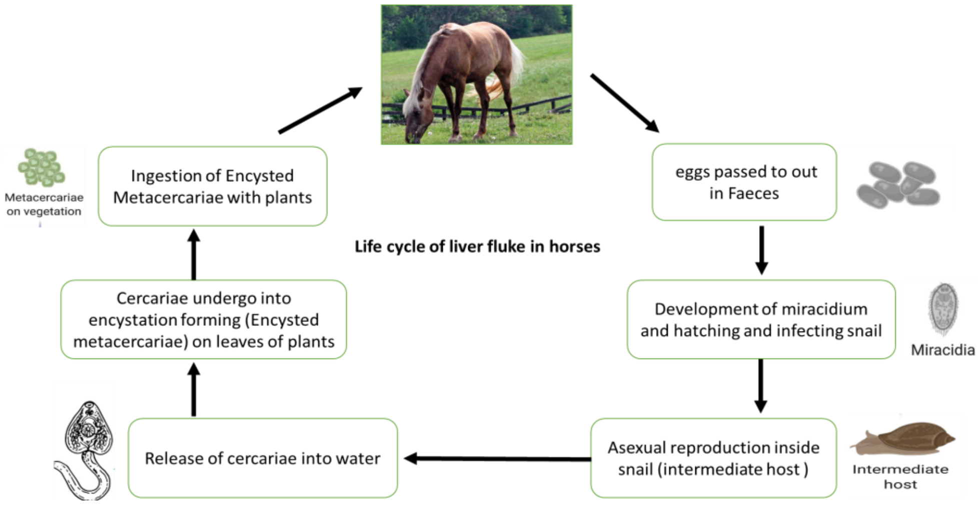

Figure 1: The life cycle of *Fasciola hepatica*. Copyright: R. Almatar.

4.3. Factors affecting the intramolluscan development of *Fasciola*

Development of *Fasciola* larval forms inside the host snail depends upon several factors that affect the quantity of cercariae during the shedding phase. These factors are either snails-related factors, parasite-related factors or environmental factors. Snails related factors include snail species, snail age and the exposure of snail to stress as temperature, starvation, and lower humidity (Boray, 1978; Gold, 1980). Regarding parasite related factors, the most important factor is the definitive host from which fasciola eggs originate. For instance, *Fasciola* egg from donkeys showed delayed embryonation, longer prepatent period in snails, lower cercarial production per snail, and lower metacercarial infectivity when compared with that from cattle and sheep (Mas-Coma et al., 2020).

Environment related factors have a crucial role in completion of Intramolluscan fasciola larval forms development. Climate in the region where the intermediate host lives has the greatest role in abundance of lymnaeid snails. The climatic conditions include temperature, relative humidity, oxygen concentration and high altitude. Other factors are pasture topography, vegetation growth, concentration of calcium salts in soil (Malone and Yilma, 1999; Mas-Coma et al., 2020).

4.4. Developmental stages of *Fasciola*

Pathogenic stages

In case of chronic fasciolosis, the pathogenic stage. is the adult fluke that appears brownish-grey in colour, laurel leaf-like with a distinct apical conus, measuring about 3.5 × 1 cm and localizing in the bile ducts. Adult flukes induce chronic inflammatory changes resulting in fibrosis and sometimes calcification of the bile ducts as fibrotic cholangitis (Bowman, 2014). In acute fasciolosis, juvenile migrating flukes are the pathogenic stages because they are responsible for induction of severe damage of liver by the action of their cathepsins enzymes that lyse liver tissue during migration, degrade proteins of liver into peptides and also destroy host antibodies. During migration, juvenile flukes result in liver necrosis and severe interstitial hepatitis (Deplazes et al., 2016).

Infective stage

The infective stage of *Fasciola* spp. is the encysted metacercaria attached to vegetation; and survival is dependent on moisture and ambient temperature. Encysted metacercariae have an oval or round shape and a thick tough coat that surrounds the immature fluke inside. Encysted metacercaria is approximately 250 µm in size with cyst wall consists of several layers that mainly composed of keratinised proteins, mucoproteins and mucopolysaccharides (Deplazes et al., 2016; Mas-Coma et al., 2018)

Diagnostic stage

Fasciola eggs are the diagnostic stage and appear yellowish brown (bile stained), oval, thin-walled with an operculum and their size is approximately 140 X 70 µm. They are excreted in faecal matter as fertile unembryonated eggs and embryonation requires special conditions as humidity, oxygen concentration, and temperature (Jacobs et al., 2016).

4.5. Epidemiology

Geographic distribution

The most common and most widely distributed *Fasciola* species is *F. hepatica.* It has a cosmopolitan distribution in temperate areas, while *F. gigantica* is restricted to African tropical countries, the Middle East, eastern Europe and southern and eastern Asia (Knubben-Schweizer et al., 2021)

Liver flukes are present in Africa, Asia and Europe and some countries in South and Central America. A high prevalence of infection with *F. hepatica* was reported due to the abundance of *Galba truncatula* snails that permit the transmission of flukes all year round at high altitudes in Bolivia, Peru, Chile and Argentina (Mas-Coma et al., 2009).

In the Afro-Mediterranean lowlands, like the Nile Delta area in Egypt and the Iran, the abundance of several lymnaeid snails (*Galba fossaria* and *Radix* spp.) facilitate the distribution of both *F. hepatica* and *F. gigantica* in a seasonal pattern (Mas-Coma et al., 2005).

In South-east Asian lowland countries like Vietnam, the presence of *Radix* spp. give rise to fasciolosis epidemics that are related mainly to *F.* gigantica (Mas-Coma et al., 2005).

In Central and Western Europe (Germany, Austria, Poland, Switzerland, France, Spain and the UK) the abundance of *G. truncatula* constitute the main factor in the distribution and colonization of *F. hepatica* in this continent beside the presence of sheep, cattle and goats which are considered the main reservoirs of *F. hepatica* and are responsible for shedding of large numbers of *Fasciola* eggs, leading to permanent contamination of grazing pasture (Knubben-Schweizer and Torgerson, 2015; Mas-Coma et al., 2009)

In Austria, *F. hepatica* is prevalent in cattle populations in the western Austrian provinces, Vorarlberg and Tyrol, which are considered the main endemic regions. Also, *F. hepatica* is reported in Salzburg, Carinthia, and Lower Austria. Many factors affect the distribution of fasciolosis in Austria, such as the prevailing husbandry conditions. For instance, in western Austria, the animal husbandry system is usually small farms while eastern Austria is characterized by flat land with larger agricultural holdings. Also, the rainfall rate is much higher in western areas and the wet, humid conditions are more favourable for the abundance and reproduction of snails and the different developmental stage fluke to survive. The presence of *G. truncatula* which is the most common intermediate host for *F. hepatica* is reported throughout Europe including Austria (Husch et al., 2020).

Lymnaeid snails are mainly aquatic in nature, and they are restricted to non-acidic, wet areas with slowly moving water and also in areas with small streams, springs, blocked drainage or spillages. Frequent irrigation of lands with water from water streams or water ways may be act as a source of infection with metacercariae (Radostits et al., 2007). Lymnaeid snails are mainly found in areas subjected to flooding and desiccation and they are more likely to be found in intermittently wet areas rather than permanently wet areas. Distribution of intermediate host snails is not uniform and may vary between areas. Snails can travel large distances by drifting water during floods and drifting in water (Ollerenshaw, 1971). Equines; mules and donkeys play a role in distribution of lymaneid snails over large areas as the snails were found in mud poached in hooves of animals and so equines were cosidered to be responsible for introduction of intermediate host snail into new areas (Mas-Coma et al., 2001).

Seasonal distribution

Seasonal distribution of *Fasciola* is affected by rainfall, environmental temperature, solar radiation, and global warming which are common conditions that promote the reproduction and distribution of *Galba truncatula* (Jaja et al., 2017).

Temperatures above 10˚C are necessary for the breeding of the snail host and for the development of different *Fasciola* stages inside snails and so this development will not take place during winter season in many countries especially those with adverse winter season (Constable et al., 2017).

Infection of snails with miracidia mainly takes place in two seasons. The summer infection of snail occurs mainly in late spring and early summer and so cercarial shedding from infected snails occur 1-2 month later resulting in contamination of pasture with encysted metacercariae and the infection of susceptible hosts will occur either in late summer or early autumn. Infection of snails with miracidium in early summer is called one year cycle and the release of cercariae and conversion into encysted metacercariae occurs mainly in late summer until the end of grazing season that considered a period of high risk for definitive host to get infection (Constable et al., 2017).

The winter infection of snails is described but the development of *Fasciola* stages inside snails will cease during the adverse climatic condition in winter, but it resumes again in the following year when temperature rises, this initiates a two-year cycle. The importance of this cycle depends upon the rate of snail mortality during the winter season that differ from area to area depending upon the climatic changes. In central Europe, the availability of metacercariae is generally higher in late summer and early autumn so it considered a period of high risk of infection while early summer is a period of low risk. Adverse winter conditions result in mass mortality of cercaria while in case of mild climatic condition, metacercariae may overwinter and infect susceptible hosts in spring. The presence of wetter lands may allow accumulation of metacercariae and so could be a source of infection (Deplazes et al., 2016; Radostits et al., 2007).

The most important hosts

Fasciola spp. have a complicated life cycle that includes definitive host and intermediate host. Regarding definitive hosts, fasciolosis is a disease of multiple species and was proven to infect them as definitive hosts either by natural or experimental infection. Young, first-season grazing animals are usually the highly susceptible to *Fasciola* infections including sheep, cattle, goats and buffaloes. Sheep develop the acute form of disease in case of heavy infection while cattle and buffaloes are likely to develop chronic fasciolosis. Sheep have significant epidemiological role in fasciolosis as they can carry the parasite in bile ducts for several years and shed huge amounts of *Fasciola* eggs into the pasture. Other ruminants including camels, antelopes, alpaca and African wild ungulates; hartebeest and wildebeest and European wild ungulates; red deer, roe deer and fallow deer are susceptible to infections with *Fasciola* (Alba et al., 2021; Haridy et al., 1999; Howell and Williams, 2020; Radostits et al., 2007; Sattmann et al., 2014).

Horses, donkeys, and mules are less susceptible to infection than ruminants. Horses have some resistance and can overcome the migrating flukes. However, few numbers can reach to liver and bile duct and they are able to develop the chronic form of fasciolosis. *Fasciola* infection is considered uncommon disease in horses based on failure to induce the infection of horses experimentally however, there was a report that discussed the infection of horses with *Fasciola* during mixed grazing with cattle (Alves et al., 1988; Williams and Hodgkinson, 2017). Donkeys and ponies are more susceptible than horses and they may develop acute or subacute form of fasciolosis (Haridy et al., 2002; Mera et al., 2020; Owen, 1977). A report from Scotland discussed a case of severe cholangiohepatitis in a pony which showed on necropsy an overwhelming infestation with flukes in biliary tract (Raftery et al., 2017).

Pigs have a mild susceptibility as they resist the penetration of the liver parenchyma by migrating flukes. However, it was reported that pigs supported the whole life cycle of *F. hepatica* and participated in its transmission; therefore, pigs are considered a definitive host for *F. hepatica* ,,(Constable et al., 2017; Mas-Coma et al., 2021; Mezo et al., 2013)

Lagomorphs including rabbits were used as an experimental model for fasciolosis. Other species including hare, nutria, kangaroos, and other herbivores animals could be a carrier for the parasite (Deplazes et al., 2016).

Rats and mice showed susceptibility to experimental infection and developed liver pathology; necrotic haemorrhagic tracts and granulomatous reaction in liver and the parasite reached bile duct and induced hyperplasia accompanied by enlargement of the bile duct walls. Rats develop a partial resistance to *F. hepatica* reinfections and they could be used as model for immunopathological research studies of chronic fasciolosis while mice could be a useful model to study host-parasite interactions (De Paula et al., 2010; Doy et al., 1978; Valero et al., 2017).

Humans can also serve as definitive hosts and fasciolosis is categorized as a neglected tropical foodborne disease of humans. The estimated current cases of human fasciolosis are about 17 million in more than 70 countries worldwide (Howell and Williams, 2020; Mas-Coma et al., 2009; WHO, 2013). The main source of infection in humans is ingestion of fresh wild plants, e.g. watercress, besides drinking contaminated water (Mas-Coma et al., 2018). The disease is more prevalent in developing countries in Latin America, African countries, and some Asian countries, while in developed countries, disease is less prevalent. For instance, there were few reports about human fasciolosis in Austria - between 1980 and 2013, 41 human cases had been registered and most of cases were reported in the provinces Vorarlberg, Tyrol, Salzburg, and Upper Austria (Auer and Aspöck, 2014).

With respect to the intermediate host, lymnaeid snails are intermediate hosts for all *Fasciola spp*. At least 20 species of lymnaeid snails are reported to serve as a potential intermediate host in the life cycle of *Fasciola spp*. (Correa et al., 2010; Torgerson and Claxton, 1999). For *F. hepatica*, *G. truncatula* is the preferred snail host in most parts of the world and parts of Africa and some Latin American countries and it showed a high rate of infection and high cercarial production during experimental infection (Boray, 1978).

In Europe, *G. truncatula* inhabits both mountainous and lowland areas and they live in water or on wet grounds. In North America, *Galba cubensis* and *Galba bulimoides* are the main host species for both *F. magna* and *F. hepatica*. They can be found in shallow margins of ditches, streams, and in damp parts of pastures. *Galba truncatula* snail prefers clean, standing or slow moving water with optimal oxygen concentration and pH value ranges between 5 and 9. For *F. gigantica, Lymnaea natalensis* and *Lymnaea rufescens* are the main host snails in Africa and in the Indian subcontinent, respectively (Deplazes et al., 2016; Howell and Williams, 2020).

4.6. Fasciolosis

General harmful effects of *Fasciola* infections

Fasciolosis is a parasitic disease that affects health and productivity of different animal species and in some areas of the world also human health. The harmful effect induced by liver flukes depends upon the number of encysted metacercariae ingested with herbage, the number of immature flukes that succeed to penetrate liver parenchyma and the number of mature flukes lodged in the bile duct of infected animals beside the capability of immune system to attack and overcome the invading parasite. In animal that ingested a high number of encysted metacercariae over short time, an acute form of fasciolosis can develop, while in hosts that repeatedly ingested low numbers of encysted metacercariae over a longer time period a chronic form of disease develops which is the most predominant (Constable et al., 2017). If migratory flukes miss their ways to liver and invade other organs, they can induce pneumonia, fibrous pleuritis and also infect the foetus in the uterus of an infected dam (Behm and Sangster, 1999).

Clinical and pathological consequences of *Fasciola* infections

In general, clinical and pathological changes induced by Fasciola depend upon several factors including; infective dose, strain of *Fasciola*, host species and age, body condition of the final host and the healthy status. Fasciolosis can present in three clinical forms; acute, subacute and chronic forms depending upon the number of ingested metacercariae over time. Acute form is rare in cattle and more common in sheep and goats. In case of heavy infections, the infected animal dies suddenly and discharges bloody froth from nostril and anus. If the disease is observed clinically, the affected animal shows dullness, lethargy, lack of appetite, and sometimes pale mucous membrane. On necropsy, the liver of affected animal is congested and enlarged and shows dark red multiple perforations with sub-capsular haemorrhage. Acute *Fasciola* infection induces interstitial hepatitis that is manifested by the increase in demarcation of hepatic lobules. Some flukes may migrate from liver back to peritoneum and cause small round and sharp-edged outlet opening on the liver surface. Peritoneal cavity may contain bloody stained fibrinous clots and immature flukes. Histopathological examination of liver shows infiltration of leucocytes in liver parenchyma besides the presence of haemorrhagic tracts that may contain immature flukes (Constable et al., 2017; Deplazes et al., 2016; Torgerson and Claxton, 1999).

Subacute fasciolosis is an intermediate form of disease between acute and chronic forms. The affected animals suffer from weight loss, pallor mucous membrane with submandibular oedema "bottle jaw" in some cases. In necropsy, the affected liver has many migratory tracts and early stage of fibrosis may be seen microscopically (Torgerson and Claxton, 1999).

Chronic fasciolosis is the common form of infection in cattle and sheep. The clinical affection appears several weeks post-infection. It occurs mainly due to repeated exposure to low dose infection over long period. The infected animals show anaemia and jaundice and suffer from chronic weight loss, submandibular oedema, loss of production either loss of wool in sheep or decrease of milk production in cattle. On necropsy, liver of infected animals appears marbled in colour due to presence of haemorrhagic tunnels and becomes hardened and shows chronic long-lasting hepatitis while bile ducts are fibrosed, thickened, calcified and protruded on the surface of liver. Some flukes may remain in liver parenchyma and become encapsulated by tissue reaction. Histopathological examination reveals chronic hepatitis and hyperplastic cholangitis with calcification in bile ducts. Adult flukes localize in the lumen of bile ducts (Behm and Sangster, 1999; Deplazes et al., 2016; Radostits et al., 2007; Torgerson and Claxton, 1999).

4.7. Control of fasciolosis

Treatment against *Fasciola* spp. in ruminants

The use of flukicides (Table 2) in treatment of fasciolosis is the corner stone method employed to control the diseases among livestock. Flukicides are not equally effective in elimination of fasciolosis as some of them are effective against both mature and immature flukes while the others are effective only against adult flukes. Several types of anthelmintic drugs are used for treatment of fasciolosis (Constable et al., 2017; Shokier et al., 2013; Torgerson and Claxton, 1999). The commonly used flukicides are mentioned in Table 2 and they are used for prophylaxis and strategic treatment of fasciolosis in farm animals. Among all of the used anthelmintic drugs, Triclabendazole is the most effective one against mature and immature flukes which make it the drug of choice in treatment regimens (Fairweather, 2011).

However, there are many challenging factors facing the use of flukicide in control of fasciolosis, including few effective substances against early stages of *Fasciola*, anthelmintic resistance and the withdrawal period of anthelmintics used for treatment of livestock. So, selection of flukicides should be carried out depending upon the physiological status of the animals, stage of infection and withdrawal period (Castro-Hermida et al., 2021). Long term use flukicidal drugs should be avoided to prevent anthelmintic resistance. The administration of flukicidal drugs should be either rotated or used in combination (James et al., 2009; Vercruysse et al., 2011).

Table 1: Common flukicides used in ruminants.

Drug	Dose (mg/kg), route of administration	Fluke stage targeted	Reference
Triclabendazole	10-12 mg/kg per os	Immature and mature flukes	(Shokier et al., 2013; Torgerson and Claxton, 1999
Albendazole	Cattle: 10 mg/kg per os Sheep: 7.5 mg/kg per os	Mature flukes	Shokier et al., 2013; Torgerson and Claxton, 1999
Oxyclozanide	Cattle: 10 mg/kg per os Sheep:15 mg/kg per os	Immature and mature flukes	Shokier et al., 2013; Torgerson and Claxton, 1999
Closantel	10 mg/ kg parenteral (subcutaneous)	Mature flukes	Constable et al., 2017
Rafoxanide	Cattle and sheep: 7.5 mg/kg per os	Mature flukes	Shokier et al., 2013
Clorsulon	Cattle and sheep: 2 mg /kg parenteral (subcutaneous)	Mature flukes	Torgerson and Claxton, 1999
Netobimin	Sheep: 20 mg/kg per os	Mature flukes	Constable et al., 2017
Nitroxynil	Cattle and sheep: 10 mg/kg parenteral (subcutaneous)	Immature and mature flukes	Torgerson and Claxton, 1999

Vaccination

Vaccination against *Fasciola* was first developed in 1980s by using radiation-attenuated metacercariae, a mixture of secreted parasite proteins and crude somatic parasite extracts. In the 2000s, several antigens supposed to induce protective immune response against *Fasciola* in sheep and cattle were described. These antigens include fatty acid-binding proteins (FABP), the cysteine peptidase cathepsin L, and leucine aminopeptidase (Haroun and Hillyer, 1986; Kumar et al., 2012; McManus and Dalton, 2006; Spithill et al., 1997).

The use of FABP antigen vaccine resulted in reduction of fluke size and egg production and minimized the pathological effect caused by the flukes. The recombinant cathepsin L antigen induced up to 79 % protection against infection in cattle and sheep; the use of vaccine contains combined cathepsin L1 and L2 antigens affected the egg production by liver flukes and also eggs viability. Aminopeptidase antigen application resulted in fluke burden reduction of up to 89 % (Constable et al., 2017; Dalton et al., 2003; Singh et al., 2021a).

Snail control

Snail control plays an important role in the reduction of pasture contamination. Diminishing of the intermediate host in pasture could be performed through different ways. Use of molluscicidal agents such as copper sulphate or sodium pentachlorophenate or n-trityl morpholine could be applied before the breeding season or when snails are plentiful. Chemical molluscicidal agents are, however, generally prohibited due to their toxic effect on the macrofauna in freshwater environment (Duke and Moore, 1976; El-Gindy et al., 1991; Rawi et al., 1994). A newly developed plant-based biomolluscicide (*Citrullus colocynthis, Bauhinia variegata and Mimusops elengi*) showed efficacy against *Fasciola* snail vectors and also *Fasciola* cercaria without effects on normal fauna of fresh water (Chawech et al., 2017; Singh et al., 2012). Biological methods could be carried out by breeding of snail predators; aquatic birds, and fish that prey on snails as this strategy is of low cost, technically simple and easy to apply (Singh et al., 2021b). Another method is the physical control by hand picking of snails and slugs, burning and burying them at a depth of 70–80 cm in area away from the grazing pasture. Draining of boggy lands, irrigation canals and water troughs is helpful in reduction of snails in their habitats (Constable et al., 2017; Sabourin et al., 2018; Singh et al., 2021b).

Herd management

Susceptible animals should have a limited access to snail habitats by isolation of herds from natural water sources (i.e. snail habitats) and fencing of water bonds besides providing water tanks to herds. Moving of animals to uncontaminated pasture and avoid green forage during spring and early summer could be helpful in reduction of infections. It is recommended to allow animals to graze in safe and uncontaminated pasture and also to set-up of a surveillance system to monitor infection in herds on regular basis. An alternative method, using of deworming plants*; Agrimonia eupatoria, Urtica dioica, Calendula officinalis*, could be applied in combination with rotation of animals into safe, uncontaminated pasture beside increasing genetic resistance and tolerance to fasciolosis in livestock population (Sabourin et al., 2018).

5. Literature review on equine fasciolosis

Disease in equines is mainly subclinical and the affected animals can show lethargy, poor performance, inappetence, rough coat, diarrhoea, jaundice and weight loss. Clinical pathology may reveal an increase in liver enzymes. The disease is mainly acute in donkeys and ponies, and subacute to chronic in horses and mules (Mera et al., 2020; Raftery et al., 2017).

The equine Immune system can overcome the invading immature flukes at an early stage and so few immature flukes can reach the liver and induce either subclinical or chronic disease. The migratory immature flukes burrow through the liver parenchyma resulting in formation of migratory tracks. Also, severe cholangiohepatitis was reported as a consequence of fluke infection (Nansen et al., 1975; Radostits et al., 2007; Raftery et al., 2017). Once the flukes settle in the bile duct, they start to feed, inducing anemia, inflammation of the bile duct (cholangitis) and physical obstruction of the bile duct and consequently bile retention in bile ducts and liver, and thus elevation of bile salts and bilirubin in blood plasma (Lalor et al., 2021).

Gross and histopathological examination in donkey suffering from fasciolosis revealed that hypertrophy of the bile ducts and fibrosis of the large portal area of the liver (Getachew et al., 2010).

5.1. Prevalence of equine fasciolosis

The prevalence of equine fasciolosis in Europe and especially in Austria needs further investigation to get more accurate data about the actual prevalence as many of studies were performed on horses slaughtered in abattoirs while the actual status of disease in field is unknown duet lack of field studies.

For instance, data from an abattoir study in UK in 2017 showed that 8.7% and 3.9% of slaughtered horses from UK and Ireland were positive for *F. hepatica* respectively in the ES-ELISA. By liver inspections, only 2.2% of the UK horses and none of the Irish horses were positive (Howell et al., 2020).

In a survey carried out in Iran in 2009 on fasciolosis among livestock including cattle, sheep, buffaloes and horses, a total of 79 horse faecal samples were examined for presence of *Fasciola* eggs by using flotation concentration techniques. The flotation was performed by using a mixture of saturated Zinc and Sodium chloride solution. About 50 % of the horses were positive for *Fasciola* infection. The author assumed that beside wild boars, horses could be a reservoir for fasciolosis and plays a minor role in transmission of *Fasciola* to other animals and also to human (Eslami et al., 2009).

A cross -sectional study in Ethiopia in 2015 was performed to investigate the epidemiology and pathogenicity of *Fasciola* infections in donkeys. The study work included coproscopical (faecal) and retrospective post-mortem examination. A total of 803 faecal samples were subjected for sedimentation-centrifugation technique and the sediment was examined microscopically. It was found that 44.4 % of examined faecal samples were positive. In post-mortem examination, 112 donkeys were necropsied, and 41.9% of them had parasites in the bile ducts. Bile ducts of the infected animals showed hyperplasia and thickening of bile ducts while livers were fibrosed. The author showed that donkeys were susceptible to *Fasciola* infestation and may play a role in the epidemiology of fasciolosis in both livestock and humans (Getachew et al., 2010).

A study from Ireland in a period between 2013 and 2014 investigated the prevalence of liver fluke infection in Irish horses and assessed the use of ELISA in diagnosis of equine fasciolosis. Faecal samples and blood of 200 horses were collected before slaughter and livers of the same animals were inspected in abattoir. In total, 9.5% (19/200) of the examined horses were positive for *F. hepatica*. From the 19 positive horses; 12 were positive for *Fasciola* based on liver inspection, four were positive on inspection and faecal egg count, two were positive by faecal egg count and one had a juvenile fluke in histological examination. The cathepsin L1 ELISA with sera of examined horses showed a high specificity but a low sensitivity in contrast to the high specificity and sensitivity of the test in detection of *Fasciola* infections in cattle (Quigley et al., 2017).

In Argentina a study was performed in 2020 to investigate the prevalence of fasciolosis in mules and the role of experimentally infected snails (*Galba truncatula*) in shedding of cercariae. A total of 81 respectively 127 faecal samples were collected from mules from two different localities in Argentina, and faecal egg counts were performed. Coproscopical analysis showed that 39.5% respectively 24.4% mules were had *Fasciola* eggs in their faeces. Also, one snail experimentally infected with one miracidium of *F. hepatica* could shed from 3 to 237 cercarial stage after 70-90 days post infection (Mera et al., 2020).

A field survey and complete experimental study were performed in Bolivia in 2020 to assess the epidemiological role of donkeys in the transmission of fasciolosis. It was found that donkeys are susceptible to infection and supported the whole life cycle of *Fasciola;* however, *Fasciola* did not appear to be fully adapted to donkeys compared to cattle and sheep. The infection in donkey had a long prepatent period and *Fasciola* eggs from donkeys showed delayed embryonation (Mas-Coma et al., 2020).

Donkeys were considered as a secondary reservoir of *Fasciola* and they contributed to contamination of pasture as cattle and sheep did. The role of donkeys as reservoirs for *F. hepatica* was investigated depending upon the ability of parasite to develop and mature, being able to produce huge number of eggs, the excreted eggs must be able to embryonate and hatch and the miracidium is capable of infecting the intermediate host. Also, larval stages must be able develop inside the snail and produce cercariae which shed in sufficient numbers and metamorphose into encysted metacercariae. Finally, the encysted metacercariae are able to infect a susceptible host and develop into mature adult flukes inside the bile duct. With respect to donkeys, it was found that they were able to develop the role of potential reservoir of fasciolosis as they produce huge number of eggs, fluke egg from donkeys were able to embryonate and hatch, and the hatched miracidia were able to infect intermediate host, develop into cercaria that shed and metamorphose into encysted metacercariae. The infectivity of encysted metacercariae of donkey's fluke isolates showed lowest infectivity rate in wester rates, and its transmission capacity is less efficient than that of sheep and cattle but it still has the potential to infect (Angles et al., 2022; Mas-Coma et al., 2020; Mas-Coma et al., 1997).

5.2. Risk factors

Animal risk factors

Fasciolosis is a disease in multiple species. Cattle, sheep, buffaloes, horses, donkeys, mules, camelids, pigs, wild ruminants and also humans are susceptible to infection. Subclinically infected cattle and sheep are considered the main reservoir and they shed large numbers of *Fasciola* eggs into the environment especially sheep that can harbour the parasite in their bile ducts for several years with high egg output. Also, the untreated animals continue to contaminate pasture with eggs for long period (Mas-Coma et al., 2014; Mera et al., 2020; Radostits et al., 2007).

Some works found no correlation between prevalence rate of fasciolosis among equine and breed classification, animal age, season, sex, animal body condition and the ante-mortem assessment (Quigley et al., 2017) and most of equine fasciolosis may be under-recognized as a cause of hepatic disease (Howell et al., 2020). Other authors declared that horses and donkeys should be considered within the preventive and control measures as the prevalence rate of disease among donkeys and horses was variable (Haridy et al., 2002; Mas-Coma et al., 1997).

Environment risk factors

Wet or damp environment is considered a preferable habit for snail breeding. In general, snails prefer non-acidic swampy area like slowly moving water, irrigation canals, ditches, lands with small streams or blocked drainage. Frequently, irrigated lands are considered more hazardous and highly suitable for transmission (Constable et al., 2017; Sabourin et al., 2018; Takeuchi-Storm et al., 2017), , .

Fasciola spp. infect a wide variety of intermediate hosts of the group of lymnaeid snails that reproduce by self-fertilization and are able to colonize new habitats. These snails so far have overcome all attempts of eradication (Sabourin et al., 2018).

Management risk factors

Agricultural practices may alter the risk of infection. For instance, application of lime leads to reduction of soil acidity, allowing snail colonization and creating favourable conditions for proliferation of the snail species, and consequently increase the prevalence of *Fasciola* infections. The use of inadequately treated wastewater from animal farms in irrigation or use of untreated animal faecal matter as fertilizer are often associated with a high prevalence of disease (Constable et al., 2017; Sabourin et al., 2018).

In animal husbandry, shared pasture between cattle, sheep and equids is considered a hazard for horses as it increases infection risk. Sheep and cattle act as chronic reservoirs with higher fluke egg output (Mas-Coma et al., 2020; Mas-Coma et al., 1997; Mera et al., 2020). Pasture with suitable habitat to sustain the snails could be a source of *Fasciola* infection to equids (Howell et al., 2020; Nelis et al., 2010).

5.3. Diagnosis

Diagnosis of *Fasciola* infection is a difficult task as it depends on clinical symptoms and signs beside laboratory diagnosis. Diagnosis based on clinical signs is usually not suitable as an infection may go unnoticed as affected animals frequently do not show clinical signs until 80% of liver become damaged. Then affected animals show poor performance, inappetence, fatigue, diarrhea, and jaundice (Divers and Barton, 2018; Quigley et al., 2017).

Laboratory diagnosis of fasciolosis depends upon parasitological examination, haematological and hepatic parameters, immunodiagnostics and molecular tools (Table 1). The most important tool in fasciolosis diagnosis is the detection of fluke eggs in faecal samples of patently infected animals. Detection of eggs is performed by sedimentation techniques. The large, oval, yellow, operculated eggs are frequently shed by ruminants in the chronic phase of infection (Taylor et al., 2016). However, equids lack a gall bladder that concentrates eggs before passage in the intestines, which is assumed to negatively affect the sensitivity of copro-microscopical methods in the detection of fasciolosis. Extended prepatent period or lack of egg excretion have also been described in equids infected with *F. hepatica*. Faecal egg count is a poor indicator of infection when fluke burden is low or when immature flukes are migrating. Microscopical examination is laborious and time-consuming, and unsuitable for large-scale or herd-level testing (Munita et al., 2019; Quigley et al., 2017; Singh et al., 2021a).

Blood parameters and liver enzymes is useful tool to assess liver affections including hepatic fasciolosis. Liver enzymes, including glutamate dehydrogenase, gamma-glutamyltrasferase and aspartate aminotransferase, are used as an indication for liver damage. Also, hypoalbuminemia and hyperglobulinemia are indicative parameters for fluke infection. In horses, elevation of gamma-glutamyltrasferase occurs three months post infection, remains at peak for one month and then decreases thereafter. The lack of constant elevation of liver enzymes post infection indicates that they are useful in diagnosing liver fluke infection beside the lack of specificity as these biomarkers are elevated in all cases related to liver damage and so the possibility of misdiagnosis is very high. Another issue is that hepatic biomarkers are not seen elevated in blood until 50 % of hepatic tissue is damaged (Divers and Barton, 2018; Quigley et al., 2017; Singh et al., 2021a).

Serodiagnosis or immunodiagnostics are used extensively for rapid diagnosis of fasciolosis in animals. It depends upon the detection of antibodies against *Fasciola* antigens, both from immature and mature flukes, including excretory-secretory antigen ELISA and Cathepsin L antigen ELISA. Antibody detection ELISAs are considered reliable tests, easy to perform, timesaving and have the capacity to detect early infections with immature flukes with high specificity and sensitivity. The main drawbacks of the serodiagnostic approach are the inability to discriminate between previous and current infection and cross-reactions with other trematodes. Coproantigen ELISA allows detection of *Fasciola* antigen in faecal samples. This test is able to detect the intensity of fluke infection in the liver (Adedokun et al., 2008; Munita et al., 2019).

Molecular tools - PCR-based method and next-generation sequencing - have been applied for genetic characterization, identification, and differentiation of *Fasciola* species by using faecal samples from animals (Mejia et al., 2013; Roeber et al., 2012), but they are not implemented for routine field testing.

Table 2: Comparison between different techniques used in diagnosis of equine fasciolosis.

Test	**Detection after infection**	**Ability to discriminate between current and previous infection**	**Value of the test in detection**	**References**
Microscopic egg detection	After 8 weeks post infection	Able to detect current infection	Lower sensitivity (30-70%)	(Alvarez Rojas et al., 2014; Mas-Coma et al., 2020)
Antibody in serum	After 2 weeks post infection	Unable to discriminate between previous and current infection	ES ELISA has (71 % sensitivity and 97% specificity) CI-1 ELSIA has lower sensitivity (45%) & high specificity (95.6%)	(Alvarez Rojas et al., 2014; Howell et al., 2020; Quigley et al., 2017),
Antigen detection in faeces	After 4 weeks post infection	able to discriminate between previous and current infection	Lower sensitivity (30%) & higher specificity (99%)	(Palmer et al., 2014)

5.4. Treatment and control

There is no single effective strategy to reduce fasciolosis transmission. All strategies for control and reduction of fasciolosis should be inseparable steps including the use of sensitive and specific tests to diagnose *Fasciola* infection in both definitive and intermediate hosts, subsequent treatment of livestock which are the main reservoirs, prevention of infection during grazing, and reduction of snail population in grazing pasture (Castro-Hermida et al., 2021). Application of integrated parasite control allows countries like Peru to reduce the prevalence of fasciolosis among dairy cattle from 63% to 14 % within two years (Raunelli and Gonzalez, 2009; Sabourin et al., 2018).

Treatment of equine fasciolosis

Currently, no drug is licensed to be used for treatment of equine fasciolosis in European countries. Trials were conducted to control and treat equine fasciolosis with flukicidal drugs registered for ruminants. Most of flukicidal drugs used for treatment of *Fasciola*-infected animals are not effective against all developmental stages of *Fasciola*. The drug of choice in treatment of fasciolosis is triclabendazole as it is effective against both adult and immature flukes beside *Fasciola* eggs. Oral triclabendazole was used for control and treatment of fasciolosis in horses and donkeys at a dose rate 12 mg/kg bodyweight. It showed a good efficacy with 100% cure rate (Castro-Hermida et al., 2021; Rubilar et al., 1988; Trawford and Tremlett, 1996). Closantel oral medication was used at dose rate of 10-20 mg/kg of bodyweight orally with efficacy in elimination of flukes older than 4 weeks and delayed oviposition in adult flukes (Alcaino, 1985; Constable et al., 2017). Nitroxynil was administered to infected foals at a dose of 7 mg /kg of bodyweight subcutaneously but it had a low efficacy in elimination of fasciolosis as it resulted in only 50 % cure rate (Rubilar et al., 1988). Table 3 summarizes the anthelmintic drugs used in treatment and control of fasciolosis in equids.

Table 3: The most common flukicides used to treat fasciolosis in equids.

Drug	**Dose (mg/kg) route of administration**	**Regimen of treatment**	**Fluke stage targeted**	**Reference**
Triclabendazole	15 mg/kg per os	Single dose	From 2 weeks old	(Knottenbelt and Malalana, 2015)
Closantel	10 mg/kg per os	Two doses two months in between	From 6-8 weeks old	(Alcaino and Aguilar, 1985)
Nitroxynil	7 mg /kg parenteral (subcutaneous)	Single dose	From 6-8 weeks old	(Rubilar et al., 1988)

6. Discussion

Fasciolosis is an economically important parasitic disease of livestock with high prevalence rate among the domestic ruminants and lower prevalence among equines. In Europe, the infection rate among domestic ruminants is highly variable especially in Alpine upland farms but this does not reflect do not reflect the prevalence rates in horses. The change in climatic condition and the increase in animal's movement led to expansion of fasciolosis among large areas (Howell and Williams, 2020; Knubben-Schweizer and Torgerson, 2015).

In Austria, fasciolosis is prevalent among cattle especially in Vorarlberg and Tyrol while the prevalence of equine fasciolosis is questionable and it needs more investigation. Several factors could facilitate equine infections with *Fasciola spp*. including the presence of susceptible snails; *G. truncatula* as well as humid conditions in western Austria, which are favourable for breeding and reproduction of snails, and enhance the development of *Fasciola* stages in the intermediate host (Husch et al., 2020). Equines are less susceptible to *Fasciola* infection than ruminants and the prevalence of fasciolosis among different equine species is variable. The most common *Fasciola* species known to infect equine is *F. hepatica* (Getachew et al., 2010; Mas-Coma et al., 2020; Quigley et al., 2017).

In addition, *Fascioloides magna* was detected in liver of mare in US, it was present as adult fluke of F. magna in cyst that contains dark semiliquid substance, and so it seems to induce severe granulomatous inflammation (McClanahan et al., 2005). Therefore, prevalence of *F. hepatica* in equines may reflect the susceptibility to infection among different species of family Equidae. Fasciolosis is more prevalent in donkeys than in mules and horses whereas the prevalence rate was 41.9-44.4% in donkeys (Getachew et al., 2010). This high prevalence rate could be attributed to the co-grazing with highly susceptible animals such sheep and cattle and the application of pasture rotation strategy in nematodes control program and so the exposure rate for *Fasciola* infective stage is high. In horses, the prevalence rate range from 2.2- 9.5% that was attributed to the ability of horses to resist the migratory flukes, to overcome the infection and so few number of immature flukes can reach the bile duct and establish infection (Howell et al., 2020; Quigley et al., 2017). Experimental infection of horses with encysted metacercariae was evaluated. Results showed that horses exhibit a pronounced resistance to the establishment of a liver fluke infection even at a high dose of infective stages and the infection only was established in 10 % of the inoculated horses. Most of migratory flukes either eliminated or immobilized in the peritoneal cavity and it did not reach to liver (Alves et al., 1988; Nansen et al., 1975).

Severity of disease in equines depends upon their degree of susceptibility and the number of flukes that reach to bile duct. Ponies and donkeys develop acute disease which may ended by death of the infected animal. Horses and to less extent donkeys develop subacute to chronic infection, and the infected animals may be asymptomatic, and act as reservoirs that shed eggs in their faeces for a long time period, resulting in contamination of pasture however their role is lower than that of ruminants (Getachew et al., 2010; Mas-Coma et al., 1997; Raftery et al., 2017). *Fasciola* developmental stage from equine origin especially donkeys seem to be different in egg embryonation, intramolluscan cycle and also some characteristics of metacercarae, but it still has a potential for induction of infection in susceptible hosts even it is lower when compared with that from ruminant origin (Mas-Coma et al., 2020). Co-grazing of equine with ruminants should be taken in attention as a potential risk for exacerbating fasciolosis among equines. The use of pasture rotation strategy may be appropriate for nematode control, but could increase the risk for liver fluke infections, especially during the rainfall seasons. Besides the presence of *Fasciola*-free ruminants, the co-grazing strategy could be effective in dry pastures during dry season that does not support the breeding or the presence of the intermediate host. The approach to co-grazing of horses with ruminants on fluke-risk pasture has to remain aware of the potential for fluke infection, and the application of a test and treat strategy should be performed promptly if signs of fasciolosis are seen ((Alves et al., 1988; Howell et al., 2020).

Also, *Fasciola* -infected equine are considered long-term shedders of eggs and may be responsible for the introduction of fasciolosis into free areas when conditions are appropriate Surveillance for equine fasciolosis faces many obstacles including; lack of gall bladder in horses that may account for a reduction in egg shedding in relation to cattle. *Fasciola* is not associated with significant changes in blood parameters in the infected horses as the lack of elevation of liver biomarkers such as GGT and GLDH enzymes limit its use of this biomarker in diagnosis of fasciolosis (Quigley et al., 2017). Damage of equine liver is often cumulative and so liver disease indicative biomarkers could not be seen in blood until damage exceeds 50% of equine liver and signs of liver insufficiency will be not observed until 80% of liver is damaged (Divers and Barton, 2018). Besides, the assessment of impact of fluke on liver performance and animal welfare is a difficult task as infection mainly is subclinical and therefore is gone undiagnosed and subsequently untreated. Further research is required to evaluate the haematological, biochemical and immunological response of the naive horse to fluke infection (Howell et al., 2020).

Surveillance of equine fasciolosis should be applied in areas with high cattle and sheep fasciolosis. ELISAs are the rapid, highly sensitive and specific serodiagnostic tests for serosurveillance as they are reliable, easy to perform tests and able to detect the early infection status, beside the ability of coproantigen ELISA to determine the intensity of infection and fluke burden. However, ELISAs are unable to discriminate between recent and previous infection, and incapable of differentiating between *Fasciola* infection and other cross-reacting trematodes (Howell et al., 2020).

The combination of more than one test in diagnosis of fasciolosis will increase the accuracy in detection and evade the false positive and false-negative results. For instance, the use of ELISA and molecular techniques could be beneficial in diagnosis and tracing back of infection besides characterization and genetic identification of *Fasciola* species. Accurate diagnosis is crucial for estimation of prevalence and distribution of fasciolosis among livestock including equine and for establishment of successful control program beside evaluation of alteration in distribution of disease following treatment regimens (Alvarez Rojas et al., 2014). Among control measures, deworming approach could be useful in reduction of the incidence rate of fasciolosis in susceptible livestock including equine. The application of flukicides in equine has some restrictions, as there is no licensed drug for equine fasciolosis and most of available flukicides are not effective against different *Fasciola* stages. Triclabendazole (TCZ) is the drug of choice in treatment of equine fasciolosis but the inappropriate use leads to emergence of TCZ-resistant *Fasciola* (Castro-Hermida et al., 2021). Therefore, there is a need for using anthelmintics cautiously, which is of paramount importance to minimize the spread of resistance and also, the repeated use of the same flukicidal drug over extended period should be minimized to avoid selection of resistance gens beside the use of combined formulations or flukicidal drug with different mechanism of action is fully recommended.

7. Summary

Equine fasciolosis is an important re-emerging parasitic disease of different equine species. The disease is caused by *F. hepatica* that induces subclinical infection and may go undiagnosed. Donkeys are the highly susceptible equine species while horses display some resistance. The snail *Galba truncatula* is the most important intermediate host for *F. hepatica* in Europe and it is responsible for the persistence of infection among livestock including equines. The disease is prevalent among livestock in Europe including western Austrian provinces due to the suitable climatic conditions that are favourable for breeding of intermediate host. During the life cycle, *Fasciola* developmental stages of equine origin showed some variation when compared with that of ruminant origin. The adult flukes colonize the bile duct and induce fibrotic obstructive cholangitis and so the affected animal may show dullness, lethargy, lack of appetite, and jaundice. Diagnosis of *Fasciola* infection in equine is difficult because the disease is mainly subclinical. Serodiagnostic techniques including ELISA is beneficial in detection of subclinically infected animals and ELISAs are reliable tests, easy to perform, timesaving and have the capacity to detect early infections with immature flukes with high specificity and sensitivity however, it is unable to discriminate between previous and current infection. Coproantigen ELISA test has the ability to detect *Fasciola* antigen in faecal matter and it has the capability of determining the fluke burden in the liver. Molecular tools and NGS are promising techniques in characterization and identification of *Fasciola* species but they are not included in routine diagnostics. Control of fasciolosis depends upon several inseparable steps including; livestock management, pasture management, and snail control. There is no licensed drug approved for treatment of equine fasciolosis in European Union. The extra-label use of triclabendazole (TCZ) was conducted to treat and control equine fasciolosis at dose rate 12 mg/kg bodyweight. The use of TCZ showed high cure rate of up to 100% but the uncontrolled use of TCZ in ruminants resulted in emergence of TCZ-resistant *Fasciola*. Several attempts were conducted to develop *Fasciola* vaccine but there is no commercial vaccine available until now.

8. Zusammenfassung

Die equine Fasziolose ist eine wichtige, wiederauftretende parasitäre Erkrankung bei verschiedenen Pferdeartigen. Die Krankheit wird durch *F. hepatica* verursacht, die eine subklinische Infektion verursacht und diagnostisch übersehen werden kann. Esel sind besonders anfällig, während Pferde eine gewisse Resistenz haben. Die Schneckenart *Galba truncatula* ist der wichtigste Zwischenwirt für *F. hepatica* in Europa und verantwortlich für die Persistenz der Infektion bei Nutztieren, einschließlich Pferden. Die Krankheit ist aufgrund der geeigneten klimatischen Bedingungen, die für die Vermehrung von Zwischenwirten günstig sind, bei Nutztieren in Europa, einschließlich der westösterreichischen Bundesländer, weit verbreitet. Während des Lebenszyklus‘ zeigen die Entwicklungsstadien von *Fasciola* bei Pferden im Vergleich zu denen von Wiederkäuern einige Unterschiede. Die erwachsenen Egel besiedeln den Gallengang und induzieren eine fibrotische obstruktive Cholangitis, sodass das betroffene Tier Mattigkeit, Lethargie, Appetitlosigkeit und Gelbsucht zeigen kann. Die Diagnose einer *Fasciola*-Infektion bei Pferden ist eine schwierige Aufgabe, da die Krankheit hauptsächlich subklinisch verläuft. Serodiagnostische Techniken, einschließlich ELISA, sind beim Nachweis subklinisch infizierter Tiere von Vorteil. ELISAs sind zuverlässige Tests, einfach durchzuführen, zeitsparend und in der Lage, frühe Infektionen mit unreifen Egeln mit hoher Spezifität und Empfindlichkeit zu erkennen, sie können jedoch nicht zwischen früheren und aktuellen Infektionen unterscheiden. Der Coproantigen-ELISA-Test kann *Fasciola*-Antigen in Kot nachweisen und die Egelbürde in der Leber bestimmen. Molekulare Werkzeuge und NGS sind vielversprechende Techniken zur Charakterisierung und Identifizierung von *Fasciola*-Spezies, sie werden jedoch nicht in die Routinediagnostik aufgenommen. Die Kontrolle der Fasziolose hängt von mehreren untrennbaren Schritten ab, darunter: Nutztierhaltung, Weidewirtschaft und Schneckenbekämpfung. In der Europäischen Union gibt es kein zugelassenes Medikament, das zur Behandlung der Fasziolose beim Pferd zugelassen ist. Triclabendazol (TCZ) wurde zur Behandlung und Bekämpfung von Fasziolose bei Pferden in einer Dosis von 12 mg/kg Körpergewicht verabreicht. Die Verwendung von TCZ zeigte eine hohe Heilungsrate von bis zu 100 %, aber die unkontrollierte Verwendung von TCZ führte zum Auftreten von TCZ-resistenten *Fasciola*. Es wurden bereits mehrere Versuche unternommen, einen *Fasciola*-Ipfstoff zu entwickeln, aber bis jetzt ist kein kommerzieller Impfstoff verfügbar.

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10. Tables and Figures