Chapter

Fascioliasis: A Foodborne Disease of Veterinary and Zoonotic Importance

Tolulope Ebenezer Atalabi and Omotosho Taiye Lawal

Abstract

Fascioliasis is a food-borne neglected disease caused by digenetic trematodes in the genus *Fasciola.* There is a significant increase in the global prevalence of human fascioliasis with a strong correlation with a high infection rate among ruminant definitive hosts. *Fasciola* is a liver fluke with complex life cycle. Fascioliasis is endemic in every continent of the world with the exception of Antarctica. Discharge of the metabolites of liver flukes into the circulatory system of hosts has pathological consequences. Fascioliasis has been diagnosed by parasitological, immunological, and molecular means, and it is being reliably treated chemotherapeutically. The emerging drug-resistant strains of liver flukes have led to the need for vaccine development. Most vaccine candidates were first isolated as native proteins from adult worms. Several of the early antigens, including cathepsin L proteases, Glutathione S-transferase (GST), and fatty acid binding protein (FABP), significantly reduced worm burden, egg output, and liver pathology in cattle and sheep. Climate change, emerging drug resistance, and the development of new parasite strains through hybridization are the current challenges that could potentially alter the epidemiology of fascioliasis soon. Therefore, researchers need to produce promising vaccines that offer maximum protection to farm animals and humans.

Keywords: fascioliasis, veterinary, zoonosis, epidemiology, diagnosis, pathology, control, vaccines, Nigeria

1. Introduction

Fascioliasis is an ancient food-borne neglected zoonotic disease of medical importance caused by some species of macroscopic and leaf-like digenetic trematodes in the genus *Fasciola* [1]. The disease came into public health limelight in 1379 when Jehan De Brie, a French scientist, described the first ever known parasite, *Fasciola hepatica* [2]. In 1874, Professor James McConnell, a pathologist and resident physician in Calcutta, discovered *Clonorchis sinensis,* a Chinese human liver fluke, when he carried out autopsy on the corpse of a 20-year old carpenter [3]. Currently, suspected hybrid species of *F. hepatica* and *F. gigantica* are being investigated to ascertain their true taxonomic status [4].

Since every continent is infested with these trematodes, 180 million people are at risk, while an estimated 2.4 million people living in more than 70 countries of

the world are suffering from the scourge of fascioliasis [1, 5]. Meanwhile, it has been estimated that *F. hepatica* infects over 300 million cattle and 250 million sheep globally and in consonance with *F. gigantica* causes economic loss estimated at USD 3billion annually [6].

Recently, there has been a significant increase in the global prevalence of human fascioliasis [1, 7] with a strong correlation with a high infection rate among ruminant definitive hosts [8].

A broad range of cosmopolitan freshwater snails in the family Limnaeidae are responsible for the transmission of fascioliasis. For instance, *Austropeplea tomentosa, Hinkleyia caperata, Stagnicola corvus, Galba truncatula, Radix rubiginosa,* and *Pseudosuccinea columella*, which are endemic (but not limited) to Australia, North America, Europe, Africa, Asia, and South America, respectively, have been reported previously [9]. Fascioliasis due to *F. gigantica* is predominantly endemic in the lower altitudes of tropical and subtropical parts of the world. Consequently, more cases of the disease are reported in larger part of sub-Saharan Africa (SSA) where suitable snail intermediate hosts naturally inhabit [10–12].

2. Life cycle

Liver flukes have a complex life cycle with a wide range of mammalian definitive hosts [9]. Humans are accidental definitive host of *Fasciola* species [13]. The infective form of this parasite is the metacercariae which, upon infecting man, temporarily settle down in the peritoneal cavity for about 24 hours after burrowing through the wall of the small intestine. Various species of liver flukes have affinity for intrahepatic or extrahepatic biliary tree [14]. At hepatic stage, which is assumed to last for about 6–7 weeks in *F. hepatica* [15], metacercariae invade the parenchyma mechanically through the liver capsule and eventually find their way into the biliary duct; this is the biliary stage. They settle down there, attain maturity, and lay eggs after sexual reproduction [5, 15]. In humans, metacercariae attain maturity within 3–4 months [5].

The number of eggs extruded by each adult worm per day varies from one definitive host to the other. Report has shown that as much as 25,000 eggs, 12,000 eggs, and 2,150 eggs could be extruded in sheep, cow, and black rats, respectively [16, 17]. Elsewhere, it has been reported that an individual liver fluke could extrude about 40,000 eggs per day [9]. These unembryonated eggs are transported in the bile medium to the small intestine, where they mix up with feces [18]. In ruminant definitive hosts, they are passed out in the pasture and undergo a period of embryonation under suitable ambient temperature and humidity.

Since freshwater body is crucial to the development of the larval stages of liver flukes [18], hatching takes place in response to external stimuli of light, temperature, and humidity [9, 19, 20]. The emerging free-swimming ciliated miracidia are genetically configured to locate a suitable Limnaeid snail intermediate host via thin films of water [21], in less than 24 hours through positive chemotactic and phototactic movements [9]. By means of their piercing stylets and proteolytic enzymes, they mechanically invade their snail hosts' body wall and tissues [20, 22] and develop into sporocysts. The sporocysts further metamorphose into mother rediae, which develop into the daughter rediae. The metamorphosis in the snail host culminates in the emergence of cercariae, which are capable of passively infecting suitable vertebrate hosts and humans who drink infested water [18, 23–25]. Relative humidity above 65%, annual rainfall >100 mm, and ambient temperature of between 25 and 30°C have been reported as the factors that are suitable for the growth and shedding of cercariae [26, 27].

Finally, the cercariae locate the wet leaves of herbaceous plants by negative geotactic movement, encyst, and metamorphose into metacercariae. When ingested by suitable ruminant definitive hosts during grazing, the cyst is digested by the hosts' enzyme and the metacercariae migrate to the duodenum where they re-encyst [18, 28]. **Figure 1** below shows a summary of the life cycle of liver flukes.

3. Epidemiology of fascioliasis

3.1 The distribution of fascioliasis

Fascioliasis is endemic in every continent of the world with the exception of Antarctica (**Figure 1**). The disease is being reported from Africa, Asia, the Caribbean, Europe, parts of Latin America, Middle East, and Oceania [29]. High transmission rate of human fascioliasis has been reported from the Andean highlands of Bolivia, Peru, the Nile valley, the Caspean sea basin, East Asia, and South East Asia [30].

In sub-Saharan Africa (SSA), fascioliasis has been reported in West Africa [12, 31], East Africa [32], and South African countries [33, 34]. However, it has also been reported in Egypt (outside SSA), North Africa [35, 36].

The distribution of fascioliasis in Nigeria covers every geo-political zone. There have been reports from North West [37, 38], North Central [39, 40], North East [41, 42], South West [43, 44], South South [45, 46] and South East [47, 48].

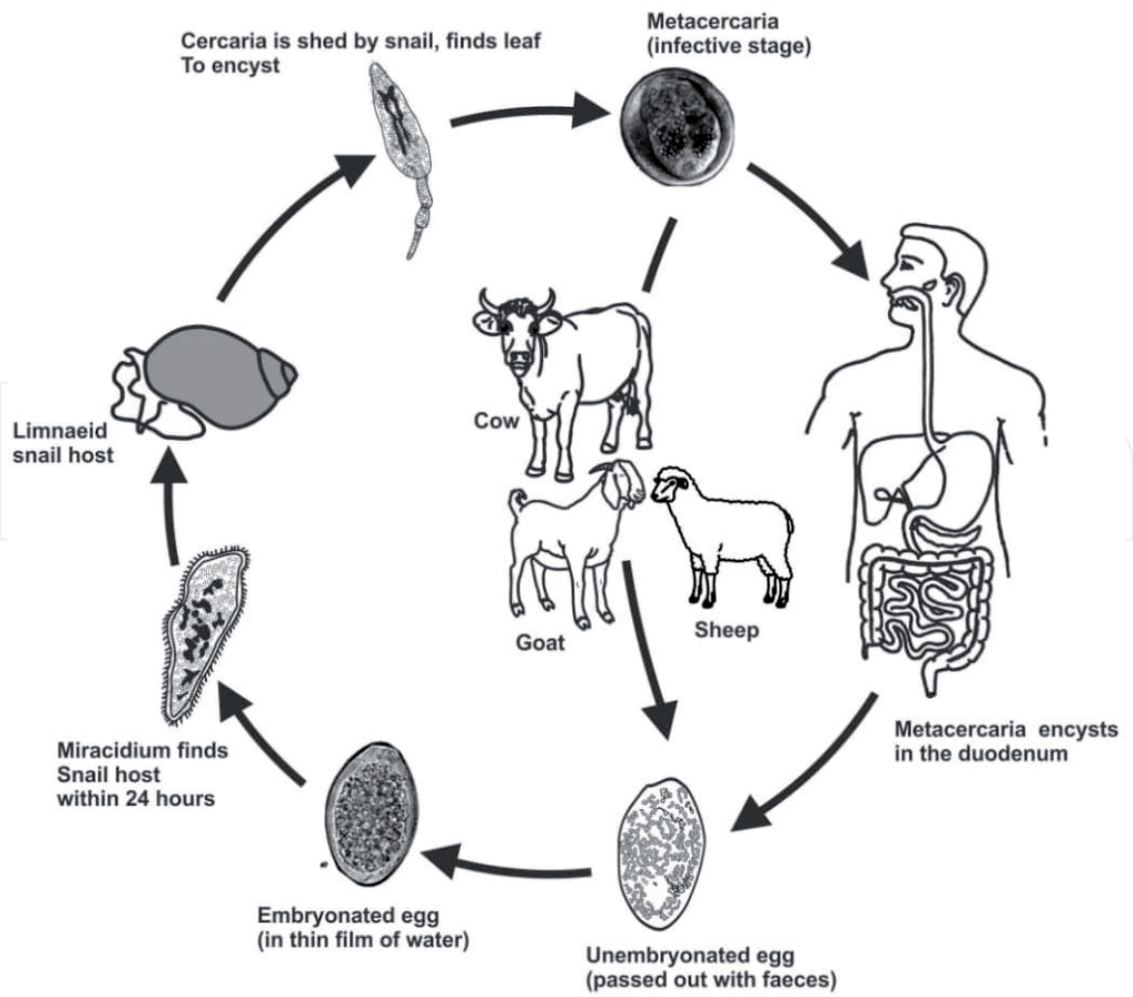

Figure 1.
Life cycle of liver flukes.

3.2 The ecology and transmission pattern of fascioliasis

In 1939, Eugene Pavlovsky, a Russian Academician propounded the theory of disease focality, which suggests that some disease-causing organisms (pathogens) naturally occur in specific ecosystems [49, 50]. This implies that the population of the pathogens is an integral part of that natural landscape [51]. Characteristically, pathogens are transmitted in such settings irrespective of the presence of humans. Consequently, humans become accidental definitive hosts when their ecological niche overlaps with suitable hosts from such landscapes and they become infected after establishing contact [52]. Researchers have found out that the epidemiology of fascioliasis has a strong link with the ecology of the settings where the disease is transmitted [30].

Pavlovsky's theory explains why fascioliasis is categorized as a focal infectious disease [29]. It could be transmitted independent of human presence. Drawing analogy from Pavlovsky's theory, there are three important elements that play key roles in the transmission pattern of fascioliasis. These are the snail intermediate hosts, ruminant vertebrate hosts, and humans who are the accidental hosts (see **Figure 2** below).

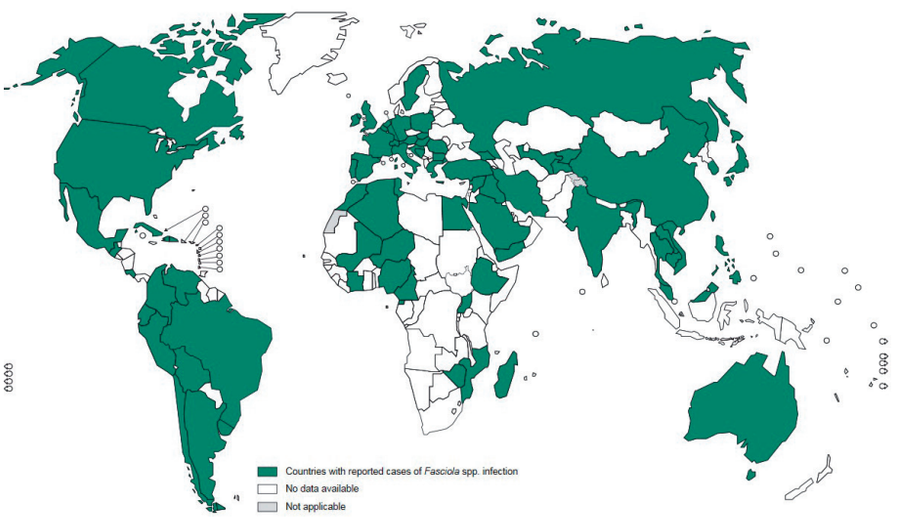

Figure 2.
Global distribution of fascioliasis. Source: Reprinted from [53].

The transmission patterns of fascioliasis vary in different epidemiological settings [30]. In the last 20 years, some researchers proposed that the patterns of the disease can be classified as fascioliasis due to influx of immigrants, human endemic/non-human (animal) endemic areas, native or isolated cases, and the three human degrees of endemicity. The fourth classification is further grouped as hypoendemic [prevalence rate < 1% while Arithmetic Mean Intensity of Infection (AMII) < 50 eggs/gram of feces], mesoendemic [prevalence rate of 1–10% while AMII 50–300 eggs/gram of feces], and hyper-endemic [prevalence rate > 10% while AMII >300 eggs/gram of feces]. In mesoendemic settings, school-age children (SAC) [5–15 years] may have higher prevalence rates while in hyper-endemic settings, SAC usually record higher prevalence rates [54, 55].

3.3 The effects of climate change on fascioliasis transmission

The role of climate in the ecology of disease-causing organisms cannot be overemphasized. In fact, climate is regarded as a basic concept of ecology. Climate triggers environmental changes [56], which in turn affect the ecosystems where parasites are transmitted, reproduced, and complete their life cycles. Because life

cycles, transmission rates, and pattern *vis a vis* the biology of their intermediate hosts are weather-sensitive, climate change has the capacity to significantly increase the prevalence and intensity of infection with liver flukes [57]. Besides, it could widen the geographical distribution [58] as well as determine the survival and transmission of the infective stage (metacercariae) [56, 59].

3.4 The risk factors of fascioliasis

Factors that predispose humans and animals to infectious diseases are referred to as risk factors. At different times and locations, many researchers have carried out spatial regression analysis of environmental variables to determine the risk factors of fascioliasis in humans and domestic ruminants. Consequently, significant associations have been described between fascioliasis and streams, wetlands, pastures [60], raising more than five sheep, dog ownership, familiarity with aquatic plants, drinking alfalfa juice, dizzy spells, history of jaundice, peripheral eosinophilia, presence of *Ascaris lumbricoides* eggs in feces [61], seasonal precipitation, temperature, elevation, and several land covers [62].

Meanwhile, gender, age, epidemiological settings (rural, urban, or rural-urban), feeding habit, familial, and social factors have been reported as the major risk factors of fascioliasis among humans [55].

3.4.1 Gender

In areas that are hyper-endemic for human fascioliasis (e.g., Egypt and Bolivia), females have reportedly recorded higher prevalence and intensity rates [55, 63, 64].

3.4.2 Age

All age groups have been found to be at risk of infection with fascioliasis but school-age children (5–15 years) have the highest prevalence and intensity [30, 64].

3.4.3 Epidemiological settings (rural, urban, or rural-urban)

People from low-middle income countries are more likely to suffer from fascioliasis. However, inhabitants from developed countries could be infected when they feed on imported infested plants that elude quarantine measures [30]. During field trips, urban inhabitants could be at a high risk of infection due to fascioliasis [55].

3.4.4 Human feeding habits

Source of food and water consumed is an important epidemiological factor of human fascioliasis. Uncontrolled markets of vegetables (like carrot, cucumber, cabbage, onions, tomatoes, spinach, etc.) coupled with drinking infested water or beverages/juice made from local plants could predispose humans to infection since liver flukes have affinity for all plants. Reports have also shown that consumption of raw liver plays a vital role in infection transmission [55, 65].

3.5 The prevalence and intensity of fascioliasis in Nigeria

Information on the prevalence of fascioliasis in Nigeria is more available compared to the intensity of infection: researchers seem to report the former more than the latter. *Fasciola gigantica* has a higher geographical coverage than *F. hepatica* and other helminthes of veterinary importance [38].

In a recent cross-sectional study conducted in North Central Nigeria where 686 fecal samples were collected from cattle in 11 villages, 110 were found to test positive for the eggs of *F. gigantica*, implying a prevalence of 16% (95% CI: 13–19%) [31]. However, a decade long study (2005–2014) of the prevalence of bovine fascioliasis in the States in that geopolitical zone shows a prevalence of 32.34% (95% CI: 30.28–34.46%) [40].

Meanwhile, a study carried out in South-South Nigeria revealed a fascioliasis prevalence (due to *F. gigantica* and *F. hepatica*) of 44.8 and 36% in cattle and goats, respectively. Intensity of infection showed that for every cattle infected, 8–10 liver flukes were recovered. Conversely, 4–5 flukes were recovered from the liver of every goat infected [45]. A similar study carried out elsewhere in the same zone reported a low prevalence of 5.34% from a total number of 712 randomly sampled cattle [46].

Nevertheless, in the North-East, a fascioliasis (without distinction) prevalence of 28.2% was reported where 262 gall bladders of White Fulani cattle were examined [42]. In a recent longitudinal study carried out in another part of North-East, Nigeria, where 7640 samples of feces and gall bladders were collected from slaughtered cattle, sheep, and goats in seven local government areas, 3092 were positive for the eggs and adults of *F. gigantica* and *F. hepatica*, giving a prevalence of 40.5% [41].

Moreover, a cross-sectional study on bovine fascioliasis in southwestern Nigeria where 905 samples of feces were screened for the eggs and adult of both species shows the predominance of *F. gigantica* (84.38%) over *F. hepatica* (1.56%) with an overall prevalence of 7.07% [44]. Conversely, the report of a longitudinal study conducted between 1994 and 2004 in another part of the zone revealed a prevalence of 2.31% after a total of 1,640,095 cattle were screened [66].

Furthermore, a cross-sectional survey carried out in North-West Nigeria reported a prevalence of 27.68% after fecal and bile samples were examined from 224 cattle [37]. Another cross-sectional survey of slaughtered cattle, sheep, and goats carried out in similar zone reported a prevalence of 29.6% for *F. hepatica* [67].

Finally, a longitudinal study carried out in South-East 8 years ago reported a prevalence of 17.2% after fecal and liver samples from 367 slaughtered sheep were examined for the presence of *F. gigantica* [47]. Interestingly, in a more recent study carried out 2 years ago in the same geo-political zone, a prevalence of 16.4% was reported for *F. hepatica* after the liver of 128 slaughtered cattle were examined [48].

The pattern of the prevalence rate of fascioliasis in Nigeria has proven the focal nature of the disease irrespective of the class of ruminant animals examined.

4. Pathology of fascioliasis

Oriental forms of liver flukes cause cholangiocarcinoma, a type of liver cancer that is peculiar to areas where *Opisthorchis felineus, O. viverrini*, and *Clonorchis sinensis* are endemic [68]. Bile ducts and gall bladders become enlarged when liver flukes establish themselves in these locations in large numbers. Discharge of the metabolites of liver flukes into the circulatory system of hosts has reportedly led to anemia, increased level of serum enzyme concentration, and dysfunction of the thyroid and adrenal glands [69].

5. Diagnosis of fascioliasis

The parasitological means of examining fecal samples for the presence of liver flukes' eggs is the use of microscope [68]. Eggs become visible after 8–10 weeks post

infection. This, however, varies from one host species to another. The limitation of this method is that the sensitivity of the Fecal Egg Count (FEC) may be undermined by factors like the age of the host, quantity of water in each fecal sample, and how representative the number of aliquots is per fecal sample examined [70]. Furthermore, a report has shown that in definitive hosts suffering from the acute phase of the disease, adverse effects of fascioliasis become evident much earlier before the pre-patent period [71]. Consequently, at necropsy, quantitative fecal examination and finding the hepatic fluke load will grossly downplay the severity of the disease [69, 72].

Quite a number of relatively cheap antibody detection indirect enzyme-linked immunosorbent assays (ELISA) with high sensitivity and specificity have been developed. Most of these techniques are based on excretory-secretory products and cathepsin L proteases [70, 73].

Increase in parasite-specific IgG (which becomes detectable after 4 weeks post infection) is peculiar to infection with fascioliasis [74]. The limitation of this technique is that after many months of successful treatment, antibodies could remain in serum, giving a false impression that the infection status is positive [70].

Excellent specificity and sensitivity has been reported for a serodiagnostic technique developed in 2011 for human fascioliasis. SeroFluke, as it is called, is a lateral flow test which has fared better compared with ELISA test (MM3-SERO) [70, 75].

Nonetheless, report has shown the superiority of antigen detection to that of antibody in the diagnosis of human fascioliasis. Coproantigens (antigens in fecal samples) are preferred to antigenemia (the presence of antigens in blood) because in the latter, circulating antigens disappear soon in the serum of patients. Besides, most of them appear in form of immune complex which are not freely detectable [76].

Less than a decade ago, a nested-PCR was developed to boost the sensitivity and specificity of current diagnostic techniques with the view that the fascioliasis could be detected in the feces of sheep 2 weeks post infection. This method entails the amplification of a 423 bp fragment of the Cytochrome C Oxidase 1 gene [70, 77]. Interestingly, similar result was achieved a year later in lesser time by amplifying a 292 bp fragment of ITS2 gene [70, 78]. Because molecular diagnosis using PCR is not readily available everywhere and as well undermined by irreproducibility of published methods, loop-mediated isothermal amplification (LAMP) has been introduced as an alternative. LAMP has proven to be more specific and sensitive by detecting fascioliasis 1 week post infection in sheep within a much shorter time—about 2½ times faster than PCR [70, 79].

6. Control of fascioliasis in Nigeria

6.1 Chemotherapeutic control

Chemotherapeutic approach has been in practice in fascioliasis control for 20 years. Based on its effectiveness, it has been predicted that the *status quo* shall be maintained in the future [80, 81]. Epidemiological and meteorological data-based treatment with drugs of choice is important for the control of fascioliasis [82]. Such drug categories include: "halogenated phenol (niclofolan, bithionol, hexachlorophene, and nitroxynil); salicylanides (rafoxanide, oxyclozanide, and closantel), benzimidazoles (triclabendazole and albendazole); sulphonamides (clorsulon), and phenoxyalkanes (diamphenethides)" [82, 83]. These drugs differ in their effectiveness against adults and immature liver flukes. However, Triclabendazole (TCZ) has been the preferred drug for treating fascioliasis since 1983 as a result of its high efficacy against the adult and all larval stages of liver flukes [82, 84–86].

The single dose of 10 mg/kg body weight is effective against the adult in the bile ducts and on immature flukes migrating through the liver [80, 85]. However, TCZ resistance has been reported in animals [87, 88] and in humans [89]. Reports have shown that drug resistance could frustrate fascioliasis control programmes [18]. Besides, TCZ is not commercially available because it is solely distributed by Novartis Pharma. Inc. (Basel, Switzerland). Consequently, it is not recommended for mass administration of medicines (MAM) [80, 90]. The unavailability of the drug, specifically to treat fascioliasis, has been reported to result in outbreaks of the disease more than a decade ago [91].

Periodic antihelmintic use at 12–13 week intervals is effective against both mature and immature flukes. By this strategic control measure, intensity of infection with liver flukes significantly reduces over time. In the tropics where incidence of fascioliasis occurs all year round, annual treatment of up to four times is recommended [82, 92].

In Nigeria, some parts have reported seasonal trends in fascioliasis while some Southern parts of the country have reported an all-year-round occurrence [93]. A recent 46- year meta-analysis of the prevalence and distribution of helminthes of veterinary and zoonotic importance in Nigeria has identified failure of control programmes in the area of strategic deworming, snail host control, and adequate sanitation as the reason for the highest pooled prevalence in southwestern Nigeria [38]. Some authorities recommend that cattle be dewormed regularly [94], while others recommend treatment upon onset of clinical fascioliasis. Meanwhile, two or three annual treatments have been proposed: at the start of the rainy season, mid rainy season, and at the start of the dry season [95]. Currently, the anthelminthic drugs in use in Nigeria include: albendazole, nitroxynil, clorsulon and levamisole. However, a report has shown that the drug of choice against fascioliasis (triclabendazole) is not available for use in Nigeria [82].

6.2 Vaccination against fascioliasis

The emerging drug-resistant strains of liver flukes have led to the need for vaccine development. Despite the immense effort of researchers in this regard, no commercial vaccine is available yet [96]. Cysteine proteases produced by every stage in the life cycle of the liver flukes are common virulence mediators [97], which mediate biological functions like excystment, tissue invasion, and immune evasion [98].

Adult fluke cathepsin L and newly excysted juvenile (NEJ) cathepsin B are the prominent proteolytic enzymes of their respective excretory secretory (ES) materials [97]. Cathepsin L5 and cathepsin B synthesized by *F. hepatica* as well as cathepsin L1 synthesized by *F. gigantica* are promising targets for vaccines against fascioliasis [99–101].

Recombinant protein expression is critical to the assessment of cysteine protein vaccine potential [97]. The yeast expression system has been a useful tool for the functional expression of cathepsin L1 and L2 [102], cathepsin L5, and cathepsin B [101].

A larger part of these vaccine candidates was first isolated as native proteins from adult worm ES products. Several of these early antigens, including cathepsin L proteases, glutathione S-transferase (GST), and fatty acid binding protein (FABP) significantly reduced worm burden, egg output, and liver pathology in cattle and sheep [96, 103].

7. Conclusion

Fascioliasis has been established as an important foodborne disease of veterinary and zoonotic importance. Climate change, emerging drug resistance, and

the development of new parasite strains through hybridization are the current challenges that could potentially alter the epidemiology of the disease in the nearest future [70]. To this end, researchers need to step up their effort to produce promising vaccines that offer maximum protection to farm animals and humans and as well contribute immensely to global elimination of the disease by reducing its prevalence and intensity. Government of countries in the tropics and subtropics should endeavor to provide more funds for researchers.

Acknowledgements

The authors sincerely appreciate the effort of Mr. Osuntuyi Mabayoje Pius who did the art work on the Life Cycle of liver flukes. We extend our gratitude to the World Health Organization for granting us the permission to use the map of the world showing the global distribution of fascioliasis under the request ID 312094.

Conflicts of interest

The authors do not have any conflicts of interest to declare.

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References

[1] World Health Organization. Foodborne trematode infections: Fascioliasis [Internet]. 2019a. Available from: https://www.who.int/foodborne_trematode_infections/fascioliasis/en/ [Accessed: 04 November 2019]

[2] Hugh-Jones ME, Hubbert WT, Hagstad HV. Chronology of Events in Zoonoses: Recognition, Control, and Prevention. Iowa: Iowa State University Press; 1995. p. 11

[3] Wikivisually. Ascaridida [Internet]. 2019. Available from: https://wikivisually.com/wiki/Ascaridida [Accessed: 04 November 2019]

[4] Centre for Disease Control. Fasciola [Internet]. 2018a. Available from: https://www.cdc.gov/parasites / fasciola/biology.html [Accessed: 04 November 2019]

[5] World Health Organization. Neglected tropical diseases: Fascioliasis-infection with the "Neglected" neglected worms [Internet]. 2019b. Available from: https://www.who.int/neglected_ diseases /integrated_media /integrated_media_fascioliasis/en/ [Accessed: 04 November 2019]

[6] Mas-Coma S, Bargues MD, Valero MA. Fascioliasis and other plant-borne trematode zoonoses. International Journal for Parasitology. 2005;**35**:1255-1278

[7] Diyana JNA, Lokman IH, Fazila SHN, Latiffah H, Ibitoye EB, Hazfalinda HN, et al. A retrospective study on bovine fascioliasis in veterinary regional laboratories in Peninsular Malaysia. Journal of Parasitology Research. 2019;**2019**:7903682. DOI: 10.1155/2019/7903682

[8] Ashrafi K, Bargues MD, O'Neill S, Mas-Coma S. Fascioliasis: A worldwide parasitic disease of importance in travel medicine. Travel Medicine and Infectious Disease. 2014;**12**:636-649

[9] Vázquez AA, Alda P, Lounnas M, Sabourin E, Alba A, Pointier J-P, et al. Lymnaeid snails hosts of *Fasciola hepatica* and *Fasciola gigantica* (Trematoda: Digenea): A worldwide review. Centre for Agriculture and Bioscience International. 2018;**13**(62):1-15

[10] Taylor MA, Coop RL, Wall RL. Veterinary Parasitology. 3rd ed. London: John Wiley and Sons Ltd; 2007. p. 874

[11] Abunna F, Asfaw L, Megersa B, Regassa A. Bovine fasciolosis: Coprological, abattoir survey and its economic impact due to liver condemnation at Soddo municipal abattoir, Southern Ethiopia. Tropical Animal Health and Production. 2010;**42**:289-292. DOI: 10.1007/s11250-009-9419-3

[12] Kelly RF, Mazer S, Hartley C, Hamma SM, Ngwa VN, Nkongho EF, et al. Assessing the performance of a *Fasciola gigantica* serum antibody ELISA to estimate prevalence in cattle in Cameroon. BMC Veterinary Research. 2019;**15**:8

[13] World Health Organization. Zoonosis and veterinary public health: Trematodosis [Internet]. 2019c. Available from: https://www.who.int/zoonoses/diseases/trematodosis/en/ [Accessed: 16 November 2019]

[14] Moazeni M, Ahmadi A. Controversial aspects of the life cycle of *Fasciola hepatica*. Experimental Parasitology. 2016;**169**:81-89. DOI: 10.1016/j.exppara.2016.07.010

[15] Ishikawa T, Meier-Stephenson V, Heitman SJ. Biliary obstruction caused by the liver fluke, *Fasciola hepatica*.

CMAJ. 2016;**188**(7):524-526. DOI: 10.1503 /cmaj.150696

[16] Boray JC. Experimental fascioliasis in Australia. Advances in Parasitology. 1969;**8**:95-210

[17] Valero MA, Panova M, Comes AM, Fons R, Mas-Coma S. Patterns in size and shedding of *Fasciola hepatica* eggs by naturally and experimentally infected murid rodents. The Journal of Parasitology. 2002;**88**(2):308-313

[18] Nyindo M, Lukambagire A-H. Fascioliasis: An ongoing zoonotic trematode infection. BioMed Research International. 2015;**2015**:786195. DOI: 10.1155/2015/786195

[19] Andrews SJ. The life cycle of *Fasciola hepatica*. In: Dalton JP, editor. Fasciolosis. 3rd ed. Wallingford: CABI Publishing; 1999. p. 30

[20] Zhang X-X, Cwiklinski K, Hu R-S, Zheng W-B, Sheng Z-A, Zhang F-K, et al. Complex and dynamic transcriptional changes allow the helminth *Fasciola gigantica* to adjust to its intermediate snail and definitive mammalian hosts. BMC Genomics. 2019;**20**:729. DOI: 10.1186/s12864-019-6103-5

[21] Sustainable Control of Parasites (SCOPS). Internal parasites: Liver fluke [Internet]. 2019. Available from: https://www.scops.org.uk/internal-parasites/liver-fluke/lifecycle/ [Accessed: 18 November 2019]

[22] Graczyk TK, Fried B. Development of *Fasciola hepatica* in the intermediate host. In: Dalton JP, editor. Fasciolosis. CABI Publishing; 1999. pp. 31-46

[23] Rondelaud D, Belfaiza M, Vignoles P, Moncef M, Dreyfuss G. Redial generations of *Fasciola hepatica*: A review. Journal of Helminthology. 2009;**83**:245-254

[24] Valero MA, Perez-Crespo I, Periago MV, Khoubbane M, Mas-Coma S. Fluke egg characteristics for the diagnosis of human and animal fascioliasis by *Fasciola hepatica* and *F. gigantica*. Acta Tropica. 2009;**111**(2):150-159

[25] Carrique-Mas JJ, Bryant JE. A review of foodborne bacterial and parasitic zoonoses in Vietnam. EcoHealth. 2013;**10**(4):465-489

[26] Rahman MH, Begum N, Alim A. Factors for the development of Furcocercus and other cercariae in snails and their release. Bangladesh Veterinarian. 1997;**14**:29

[27] Dodangeh S, Daryani A, Sharif M, Gholami S, Kialashaki E, Moosazadeh M, et al. Freshwater snails as the intermediate host of trematodes in Iran: A systematic review. Epidemiol Health. 2019;**41**:e2019001. DOI: 10.4178/epih.e2019001

[28] Hotez PJ, Savioli L, Fenwick A. Neglected tropical diseases of the middle east and north africa: Review of their prevalence, distribution, and opportunities for control. PLoS Neglected Tropical Diseases. 2012;**6**(2):e1475

[29] Centre for Disease Control. *Fasciola*: Epidemiology and risk factors [Internet]. 2018b. Available from: https://www.cdc.gov/ parasites/fasciola/epi.html [Accessed: 19 November 2019]

[30] World Health Organization. Foodborne trematode infections: Fascioliasis epidemiology [Internet]. 2019d. Available from: https://www.who.int/foodborne_trematode_infections/fascioliasis/fascioliasis_epidemiology/en/ [Accessed: 19 November 2019]

[31] Elelu N, Ambali A, Coles GC, Eisler MC. Cross-sectional study of *Fasciola gigantica* and other trematode infections of cattle in Edu

local government area, Kwara state, North-Central Nigeria. Parasites & Vectors. 2016;**9**:470. DOI: 10.1186/s13071-016-1737-5

[32] Dida GO, Gelder FB, Anyona DN, Matano A-S, Abuom PO, Adoka SO, et al. Distribution and abundance of schistosomiasis and fascioliasis host snails along the Mara River in Kenya and Tanzania. Infection Ecology & Epidemiology. 2014;**4**:24281. DOI: 10.3402/iee.v4.24281

[33] Mochankana ME, Robertson ID. A retrospective study of the prevalence of bovine fasciolosis at major abattoirs in Botswana. Onderstepoort Journal of Veterinary Research. 2016;**83**(1):a1015. DOI: 10.4102/ojvr.v83i1.1015

[34] Nyirenda SS, Sakala M, Moonde L, Kayesa E, Fandamu P, Banda F, et al. Prevalence of bovine fascioliasis and economic impact associated with liver condemnation in abattoirs in Mongu district of Zambia. BMC Veterinary Research. 2019;**15**:33. DOI: 10.1186/s12917-019-1777-0

[35] Keiser J, Sayed H, El-Ghanam M, Sabry H, Anani S, El-Wakeel A, et al. Efficacy and safety of Artemether in the treatment of chronic fascioliasis in Egypt: Exploratory phase-2 trials. PLoS Neglected Tropical Diseases. 2011;**5**(9):e1285. DOI: 10.1371/journal.pntd.0001285

[36] Elshraway NT, Mahmoud WG. Prevalence of fascioliasis (liver flukes) infection in cattle and buffaloes slaughtered at the municipal abattoir of El-Kharga, Egypt. Veterinary World. 2017;**10**(8):914-917. DOI: 10.14202/vetworld.2017.914-917

[37] Magaji AA, Ibrahim K, Salihu MD, Saulawa MA, Mohammed AA, Musawa AI. Prevalence of fascioliasis in cattle slaughtered in Sokoto Metropolitan Abattoir, Sokoto, Nigeria. Advances in Epidemiology. 2014;**2014**:247258. DOI: 10.1155/2014/247258

[38] Karshima SN, Maikai B, Kwaga JKP. Helminths of veterinary and zoonotic importance in Nigerian ruminants: A 46-year meta-analysis (1970-2016) of their prevalence and distribution. Infectious Diseases of Poverty. 2018;**7**:52. DOI: 10.1186/s40249-018-0438-z

[39] Ekong PS, Juryit R, Dika NM, Nguku P, Musenero M. Prevalence and risk factors for zoonotic helminth infection among humans and animals - Jos, Nigeria, 2005-2009. The Pan African Medical Journal. 2012;**12**:6

[40] Yatswako S, Alhaji NB. Survey of bovine fasciolosis burdens in trade cattle slaughtered at abattoirs in north-Central Nigeria: The associated predisposing factors and economic implication. Parasite Epidemiology and Control. 2017;**2**:30-39

[41] Isah UM. Studies on the prevalence of fascioliasis among ruminant animals in northern Bauchi state, North-Eastern Nigeria. Parasite Epidemiology and Control. 2019;**3**:e00090

[42] Shinggu PA, Olufemi OT, Nwuku JA, Baba-Onoja EBT, Iyawa PD. Liver Flukes Egg Infection and Associated Risk Factors in White Fulani Cattle Slaughtered in Wukari, Southern Taraba State, Nigeria. Advances in Preventive Medicine. 2019;**2019**:2671620. 5p. DOI: 10.1155/2019/2671620

[43] Ibironke AA, Fasina FO. Socio-economic implications of bovine liver rejection in a major abattoir in South-Western Nigeria. Revista de Ciências Agrárias. 2010;**33**:2

[44] Afolabi OJ, Olususi FC. The prevalence of Fascioliasis among slaughtered cattle in Akure, Nigeria. Molecular Pathogens. 2016;**7**(1):1-5. DOI: 10.5376/mp.2016.07.0001

[45] Abraham JT, Jude IB. Fascioliasis in cattle and goat slaughtered at Calabar abattoirs. Journal of Biology, Agriculture and Healthcare. 2014;**4**(18):34-40

[46] Eze NC, Briggs AA. Prevalence of Fascioliasis and histopathology of the liver in cattle slaughtered in Port Harcourt Abbatoir, Rivers state Nigeria. World News of Natural Sciences. 2018;**16**:105-116

[47] Njoku-Tony RF, Okoli GC. Prevalence of fascioliasis among slaughter sheep in selected abattoirs in Imo State, Nigeria. American Journal of Science. 2011;**7**(2):361-366

[48] Anyaegbunam LC, Ajana U. *Fasciola hepatica* infestation in slaughtered cow liver in Eke Uli market abattoir, Ihiala local government area of Anambra State, Nigeria. Tropical Journal of Applied Natural Sciences. 2017;**2**(1):21-24

[49] Malkhazova SM, Mironova VA, Kotova TV, Shartova NV, Orlov DS. Natural-focal diseases: Mapping experience in Russia. International Journal of Health Geographics. 2014;**13**:21. DOI: 10.1186/1476-072X-13-21

[50] Pavlovsky EN. Fundamentals of the theory of natural focality of transmissible human diseases. Zhurnal Obshcheĭ Biologii. 1946;**7**(1):3-33

[51] Korenberg EI. Natural focality of infections: Current problems and prospects of research. The Biological Bulletin. 2010;**37**(7):665-676. DOI: 10.1134/S1062359010070010

[52] Reisen WK. Landscape epidemiology of vector-borne diseases. Annual Review of Entomology. 2010;**55**:461-483. DOI: 10.1146/annurev-ento-112408-085419

[53] World Health Organization. Food-borne trematode infections [Internet]. 2019e. Available from: https://www.who.int/foodborne_trematode_ infections/en/ [Accessed: 19 November 2019]

[54] Mas-Coma S, Esteban JG, Bargues MD. Epidemiology of human fascioliasis: A review and proposed new classification. Bulletin of the World Health Organization. 1999;**77**:340-346

[55] Mas-Coma S, Bargues MD, Valero MA. Human fascioliasis infection sources, their diversity, incidence factors, analytical methods and prevention measures. Parasitology. 2018:1-35. DOI: 10.1017/S0031182018000914

[56] Mas-Coma S, Valero MA, Bargues MD. Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis. Veterinary Parasitology. 2009;**163**:264-280

[57] Houghton JT, Jenkins GJ, Ephraums JJ, editors. Scientific Assessment of Climate Change. Report of Working Group I of the Intergovernmental Panel of Climate Change (IPCC). Cambridge: Cambridge University Press; 1990. p. 357

[58] Lafferty KD, Porter JW, Ford SE. Are diseases increasing in the ocean? Annual Review of Ecology, Evolution, and Systematics. 2004;**35**:31-54

[59] Cattadori IM, Haydon DT, Hudson PJ. Parasites and climate synchronize red grouse populations. Nature. 2005;**433**:737-741

[60] Olsen A, Frankena K, Bødker R, Toft N, Thamsborg SM, Enemark HL, et al. Prevalence, risk factors and spatial analysis of liver fluke infections in Danish cattle herds. Parasites & Vectors. 2015;**8**:160. DOI: 10.1186/s13071-015-0773-x

[61] Marcos LA, Maco V, Samalvides F, Terashima A, Espinoza JR, Gotuzzo E.

Risk factors for Fasciola hepatica infection in children: A case-control study. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2006;**100**(2):158-166. DOI: 10.1016/j.trstmh.2005.05.016

[62] Rahman AKMA, Islam SKS, Talukder MH, Hassan MK, Dhand NK, Ward MP. Fascioliasis risk factors and space-time clusters in domestic ruminants in Bangladesh. Parasites & Vectors. 2017;**10**:228. DOI: 10.1186/s13071-017-2168-7

[63] Esteban JG, Flores A, Angles R, Mas-Coma S. High endemicity of human fascioliasis between Lake Titicaca and La Paz valley, Bolivia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1999;**93**:151-156

[64] Esteban JG, Gonzalez C, Curtale F, Muñoz-Antoli C, Valero MA, Bargues MD, et al. Hyperendemic fascioliasis associated with schistosomiasis in villages in the Nile Delta of Egypt. The American Journal of Tropical Medicine and Hygiene. 2003;**69**:429-437

[65] Zumaquero-Rios JL, Sarracent-Perez J, Rojas-Garcia R, Rojas-Rivero L, Martinez-Tovilla Y, Valero MA, et al. Fascioliasis and intestinal parasitoses affecting schoolchildren in Atlixco, Puebla State, Mexico: Epidemiology and treatment with nitazoxanide. PLoS Neglected Tropical Diseases. 2013;**7**(11):e2553

[66] Oladele-Bukola MO, Odetokun IA. Prevalence of bovine fasciolosis at the Ibadan Municipal Abattoir, Nigeria. African Journal of Food Agriculture Nutrition and Development. 2014;**14**(4):9055-9070

[67] Kiran S, Asma'US T. Prevalence of fascioliasis among animals slaughtered at Sokoto state Abattoir. International Journal of Malaria and Tropical Diseases. 2017;**1**(11):059-062

[68] World Health Organization. The management of bloody diarrhoea in young children (unpublished document). Bulletin of the World Health Organization. 1995;**73**(3):397-401

[69] Sharma RL, Godara R, Thilagar MB. Epizootiology, pathogenesis and immunoprophylactic trends to control tropical bubaline fasciolosis: An overview. Journal of Parasitic Diseases. 2011;**35**(1):1-9. DOI: 10.1007/s12639-011-0025-8

[70] Beesley NJ, Caminade C, Charlier J, Flynn J, Hodgkinson JE, Martinez-Moreno A, et al. Fasciola and fasciolosis in ruminants in Europe: Identifying research needs. Transboundary and Emerging Diseases. 2018;**65**(Suppl 1):199-216. DOI: 10.1111/tbed.12682

[71] Dixit AK, Pooja D, Sharma RL. Immunodiagnostic/protective role of Cathepsin L cysteine proteinases secreted by Fasciola sp.—A review. Veterinary Parasitology. 2008;**154**:177-184

[72] Garg R, Yadav CL, Kumar RR, Banerjee PS, Vatsya S, Godara R. The epidemiology of fasciolosis in ruminants in different geo-climatic regions of North India. Tropical Animal Health and Production. 2009;**41**:1695-1700

[73] Jezek J, El Ridi R, Salah M, Wagih A, Aziz HW, Tallima H, et al. *Fasciola gigantica* cathepsin L proteinase-based synthetic peptide for immunodiagnosis and prevention of sheep fasciolosis. Biopolymers. 2008;**90**(3):349-357

[74] Martınez-Perez JM, Robles-Perez D, Rojo-Vazquez FA, Martınez-Valladares M. Immunological features of LPS from *Ochrobactrum intermedium* on sheep experimentally infected with *Fasciola hepatica*. Research in Veterinary Science. 2014;**97**:329-332

[75] Martınez-Sernandez V, Muino L, Perteguer MJ, Garate T, Mezo M, Gonzalez-Warleta M, et al. Development and evaluation of a new lateral flow immunoassay for serodiagnosis of human fasciolosis. PLoS Neglected Tropical Diseases. 2011;**5**:e1376

[76] Sarkari B, Khabisi SA. Immunodiagnosis of human fascioliasis: An update of concepts and performances of the serological assays. Journal of Clinical and Diagnostic Research. 2017;**11**(6):OE05-OE10. DOI: 10.7860/JCDR/2017/26066.10086

[77] Martınez-Perez JM, Robles-Perez D, Rojo-Vazquez FA, Martınez-Valladares M. Comparison of three different techniques to diagnose *Fasciola hepatica* infection in experimentally and naturally infected sheep. Veterinary Parasitology. 2012;**190**:80-86

[78] Robles-Perez D, Martınez-Perez JM, Rojo-Vazquez FA, Martınez-Valladares M. The diagnosis of fasciolosis in feces of sheep by means of a PCR and its application in the detection of anthelmintic resistance in sheep flocks naturally infected. Veterinary Parasitology. 2013;**197**:277-282

[79] Martınez-Valladares M, Rojo-Vazquez FA. Loop-mediated isothermal amplification (LAMP) assay for the diagnosis of fasciolosis in sheep and its application under field conditions. Parasites & Vectors. 2016;**9**:73-77

[80] Soliman MFM. Epidemiological review of human and animal fascioliasis in Egypt. Journal of Infection in Developing Countries. 2008;**2**(3):182-189

[81] Fairweather I. Reducing the future threat from (liver) fluke: Realistic prospect or quixotic fantasy? Veterinary Parasitology. 2011;**180**(1-2):133-143. DOI: 10.1016/j.vetpar.2011.05.034

[82] Elelu N, Eisler MC. A review of bovine fasciolosis and other trematode infections in Nigeria. Journal of Helminthology. 2018;**92**(2):128-141. DOI: 10.1017/S0022149X17000402

[83] Fairweather I, Boray JC. Fasciolicides: Efficacy, actions, resistance and its management. The Veterinary Journal. 1999;**158**:81-112

[84] Boray J, Crowfoot P, Strong M, Allison J, Schellenbaum M, Von Orelli M, et al. Treatment of immature and mature *Fasciola hepatica* infections in sheep with triclabendazole. The Veterinary Record. 1983;**113**:315-317

[85] World Health Organization. Report of the WHO Informal Meeting on use of triclabendazole in fascioliasis control [Internet]. 2007. Available from: http://www.who.int/neglected_diseases/preventive chemotherapy/WHO_CDS_NTD_PCT_2007.1.pdf [Accessed: 18 December 2019]

[86] Mas-Coma S, Bargues MD, Valero MA. Diagnosis of human fascioliasis by stool and blood techniques: Update for the present global scenario. Parasitology. 2014;**141**:1918-1946

[87] Olaechea F, Lovera V, Larroza M, Raffo V, Cabrera R. Resistance of *Fasciola hepatica* against triclabendazole in cattle in Patagonia (Argentina). Veterinary Parasitology. 2011;**178**(3-4):364-366

[88] Ortiz P, Scarcella S, Cerna C, Rosales C, Cabrera M, Guzman M, et al. Resistance of *Fasciola hepatica* against Triclabendazole in cattle in Cajamarca (Peru): A clinical trial and an *in vivo* efficacy test in sheep. Veterinary Parasitology. 2013;**195**(1-2):118-121. DOI: 10.1016/j.vetpar.2013.01.001

[89] Winkelhagen AJS, Mank T, de Vries PJ, Soetekouw R. Apparent

Triclabendazole-resistant human *Fasciola hepatica* infection, the Netherlands. Emerging Infectious Diseases. 2012;**18**(6):1028-1029

[90] Keiser J, Utzinger J. Emerging foodborne trematodiasis. Emerging Infectious Diseases. 2005;**11**(10):1507-1514

[91] Parkinson M, O'Neill SM, Dalton JP. Endemic human fasciolosis in the Bolivian Altiplano. Epidemiology and Infection. 2007;**135**(4):669-674

[92] Torgerson P, Claxton J. Epidemiology and control. In: Dalton JP, editor. Fasciolosis. 1st ed. Wallingford: CABI Publishing; 1999. pp. 113-149

[93] Gboeloh LB. Seasonal prevalence of *Fasciola gigantica* in slaghter cattle in major abattoirs in port-Harcourt. Advances in Agriculture, Sciences and Engineering Research. 2012;**2**:336-340

[94] Aliyu AA, Ajogi IA, Ajanusi OJ, Reuben RC. Epidemiological studies of *Fasciola gigantica* in cattle in Zaria, Nigeria using coprology and serology. Journal of Public Health. 2014;**6**:85-91

[95] Damwesh SD, Ardo MB. Detection of *Fasciola gigantic* antibodies using Pourquier ELISA kit. Sokoto Journal of Veterinary Sciences. 2013;**11**:43-48

[96] Molina-Hernández V, Mulcahy G, Pérez J, Martínez-Moreno Á, Donnelly S, O'Neill SM, et al. Fasciola hepatica vaccine: We may not be there yet but we're on the right road. Veterinary Parasitology. 2015;**208**(1-2):101-111. DOI: 10.1016/j.vetpar.2015.01.004

[97] Jayaraj R, Piedrafita D, Dynon K, Gramsc R, Spithill TW, Smooker PM. Liver fluke vaccines: Vaccination against fasciolosis by a multivalent vaccine of recombinant stage-specific antigens. Procedia in Vaccinology. 2010;**2**(1):82-85. DOI: 10.1016/j.provac.2010.03.015

[98] Sajid M, McKerrow JH. Cysteine proteases of parasitic organisms. Molecular and Biochemical Parasitology. 2002;**120**(1):1-21

[99] Grams R, Vichasri-Grams S, Sobhon P, Upatham ES, Viyanant V. Molecular cloning and characterization of cathepsin L encoding genes from *Fasciola gigantica*. Parasitology International. 2001;**50**(2):105-114

[100] Irving JA, Spithill TW, Pike RN, Whisstock JC, Smooker PM. The evolution of enzyme specificity in Fasciola spp. Journal of Molecular Evolution. 2003;**57**(1):1-15

[101] Beckham SA, Law RHP, Smooker PM, Quinsey NS, Caffrey CR, McKerrow JH, et al. Production and processing of a recombinant Fasciola hepatica cathepsin B-like enzyme (FhcatB1) reveals potential processing mechanisms in the parasite. Biological Chemistry. 2006;**387**:1053-1061. DOI: 10.1515/BC.2006.130

[102] Dowd AJ, Smith AM, McGonigle S, Dalton JP. Purification and characterisation of a second cathepsin L proteinase secreted by the parasitic trematode *Fasciola hepatica*. European Journal of Biochemistry. 1994;**223**(1):91-98

[103] Toet H, Piedrafita DM, Spithill TW. Liver fluke vaccines in ruminants: Strategies, progress and future opportunities. International Journal for Parasitology. 2014;**44**(12):915-927. DOI: 10.1016/j.ijpara.2014.07.011