Fasciolosis: a Non-attended Zoonosis

13

Carlos Ramón Bautista-Garfias[1*] and Astrid Rodríguez-Lozano[2]

ABSTRACT

In this chapter, recent aspects of fasciolosis are analyzed. The manuscript focusses mainly in Fasciola hepatica rather than other species of the Fasciola genera. Its biologic cycle, world geographical distribution, economic importance and importance on human health are reviewed. Fasciolosis, is one of the most important parasitic diseases of economically productive animals, particularly cattle, sheep and goats. This disease is caused by a flatworm, one to four centimeters long, similar to a small brown leaf and is scientifically known as Fasciola hepatica. It is also known by the names of orejuela, moth or liver fluke. The adult worm lives in the bile ducts of the liver and can parasitize different animal species, including man. F. hepatica parasitizes cattle and humans since at least 4,500 years. At present, fasciolosis is recognized as an important parasitic disease which affects humans too (zoonosis). Due to the climate change, fasciolosis is a serious health threat for human beings. The authors suggest that this disease may be controlled on the basis of the One Health approach.

Key words: Fasciola hepatica, Zoonosis, ruminants, economic losses, humans

CITATION

Bautista-Garfias CR and Rodrígez-Lozano A, 2023. Fasciolosis: a non-attended zoonosis. In: Abbas RZ, Hassan MF, Khan A and Mohsin M (eds), Zoonosis, Unique Scientific Publishers, Faisalabad, Pakistan, Vol 2: 159-169. https://doi.org/10.47278/book.zoon/2023.60

CHAPTER HISTORY Received: 23-April-2023 Revised: 12-June-2023 Accepted: 26-Aug-2023

[1]CENID-SAI, INIFAP, Jiutepec, Morelos, México
[2]Universidad Veracruzana, Veracruz, México
***Corresponding author:** foto.dibujo@gmail.com

1. INTRODUCTION

This chapter deals more with *Fasciola hepatica* rather than other species of the *Fasciola* genera. Among the most important parasitic diseases of economically productive animals, particularly cattle, sheep and goats, is Fascioliosis, a disease caused by a flatworm, one to four centimeters long, similar to a small brown leaf scientifically known as *Fasciola hepatica* (Sánchez Manzano et al. 1998, Rokni 2014). It is also known by the names of orejuela, moth or liver fluke. The adult worm lives in the bile ducts of the liver and can parasitize different animal species as indicated above, including man (Fig. 1). *F. hepatica* parasitizes cattle and humans since at least 4,500 years (Dittmar and Teegen 2003). At present, fascioliosis is known as an important parasitic disease which affects humans too (Carrada-Bravo 2007, Robinson and Dalton 2009, Mas-Coma et al. 2009, Nyindo and Lukambagire 2015, Mehmood et al. 2017, Caravedo and Cabada 2020, Bargues et al. 2022). In this context, and due the climate change, fascioliasis is a health threat for human beings (Anonymous 2020).

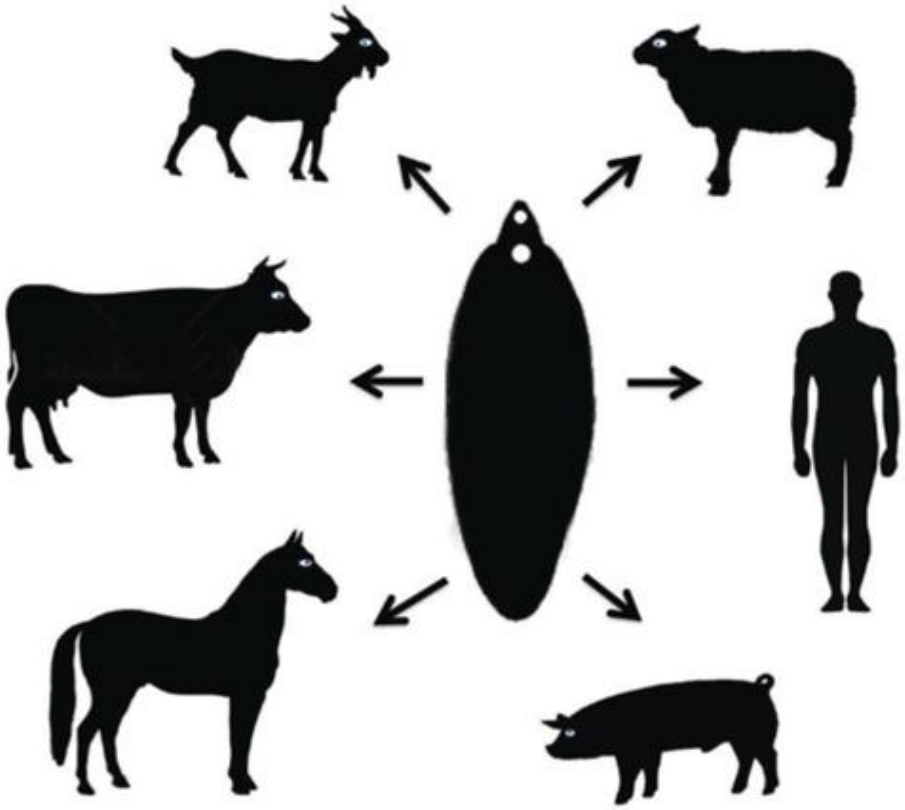

Fig. 1: Animal species of economic importance, including man, parasitized by *Fasciola hepatica* (Figure made by Carlos Ramón Bautista-Garfias).

The life cycle of *F. hepatica* is summarized in Fig. 2.

This cycle, which can last between four and six months, takes place in two hosts, one terrestrial vertebrate (mammal) and the other aquatic invertebrate (gastropod mollusk) as described in Fig. 2: 1. The eggs of the parasite hatch in the manure of the definitive bovine host, mature and hatch in a humid place. 2. A miracidium hatches from each egg and swims in the water, seeking out and infecting an intermediate host aquatic snail (*Lymnaea*) that lives on the banks of puddles, lagoons or irrigation canals. 3. In this, the parasite becomes a sporocyst, a mother redia and then a daughter redia that gives rise to another phase called the cercaria. 4. The cercariae is mobile, swims in the water and attaches itself to the surrounding vegetation or to the surface of the water, then loses its tail and encysts. 5. Now the parasite is called metacercaria and constitutes the

infective phase that can remain like this for several months waiting for the plant to which it is attached (grass, alfalfa, watercress) to be ingested. 6. The animal or man ingests the vegetation along with the metacercariae. 7. These pass through the digestive tract to the duodenum, where they are excysted, releasing newborn flukes that cross the intestine, travel through the peritoneal cavity and enter the liver, perforating the Gleason capsule and migrating through the parenchyma. 8. The parasite proceeds to the bile ducts where it matures and after eight to 10 weeks produces eggs. 9. These travel through the intestine in the bile along with the feces. 10. The eggs come out. A single fluke can oviposit up to 25,000 daily (Fig. 3).

2. GEOGRAPHICAL DISTRIBUTION OF *FASCIOLA* SPP

Fasciolosis in animals and man is distributed worldwide. The flatworms in the *Fasciola* genera originated in the African continent, then dispersed inside their hosts to other continents (Fig. 4).

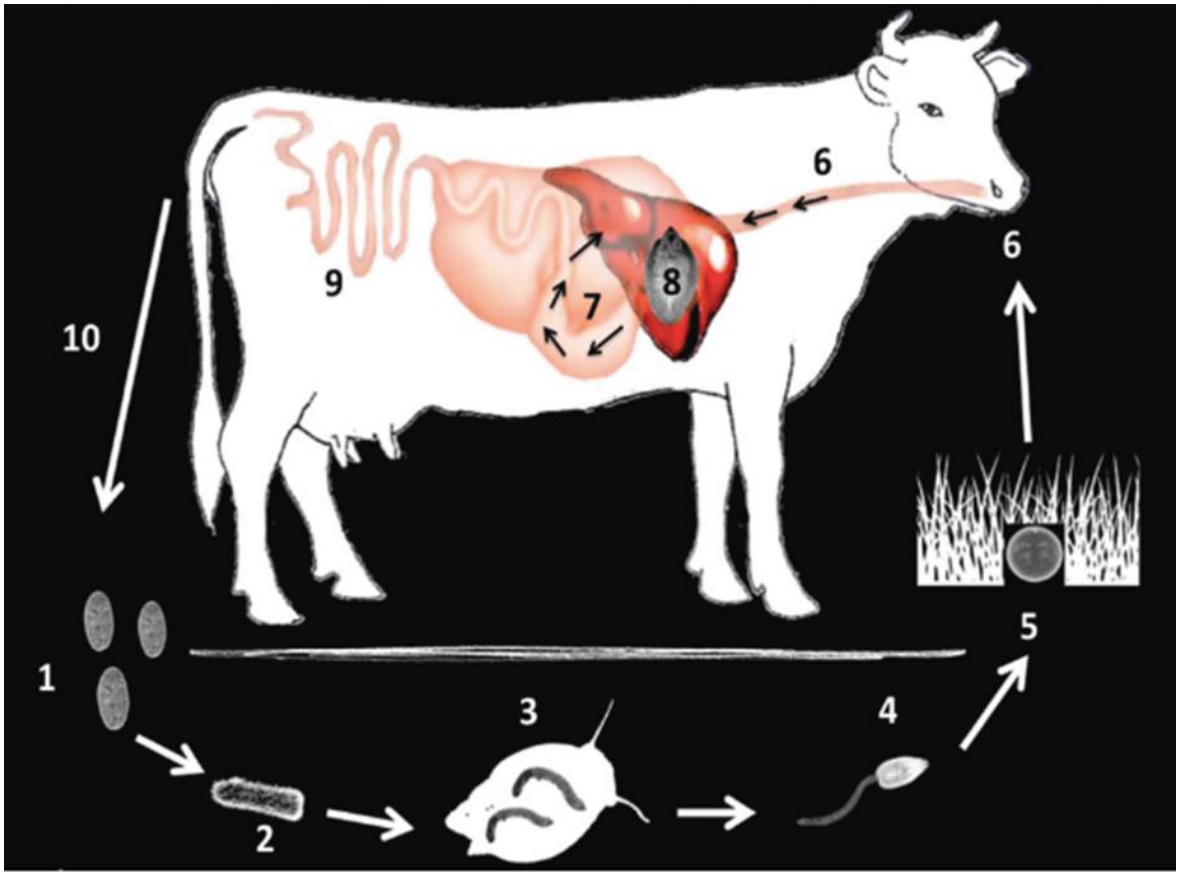

Fig. 2: *Fasciola hepatica* life cycle (Figure made by Carlos Ramón Bautista-Garfias)

3. SIGNS OF THE DISEASE IN RUMINANTS

As in most parasitic infections caused by worms, the signs of fasciolosis in ruminants are not specific. Thus, for example, weakness, anemia (blood deficiency), submandibular edema (soft swelling in the lower part of the jaw) can be observed (Fig. 5), sometimes jaundice (yellowing of the mucous membranes and eyes), diarrhea or constipation, abortions in pregnant females, chronic weight loss (Fig. 6) and sometimes death.

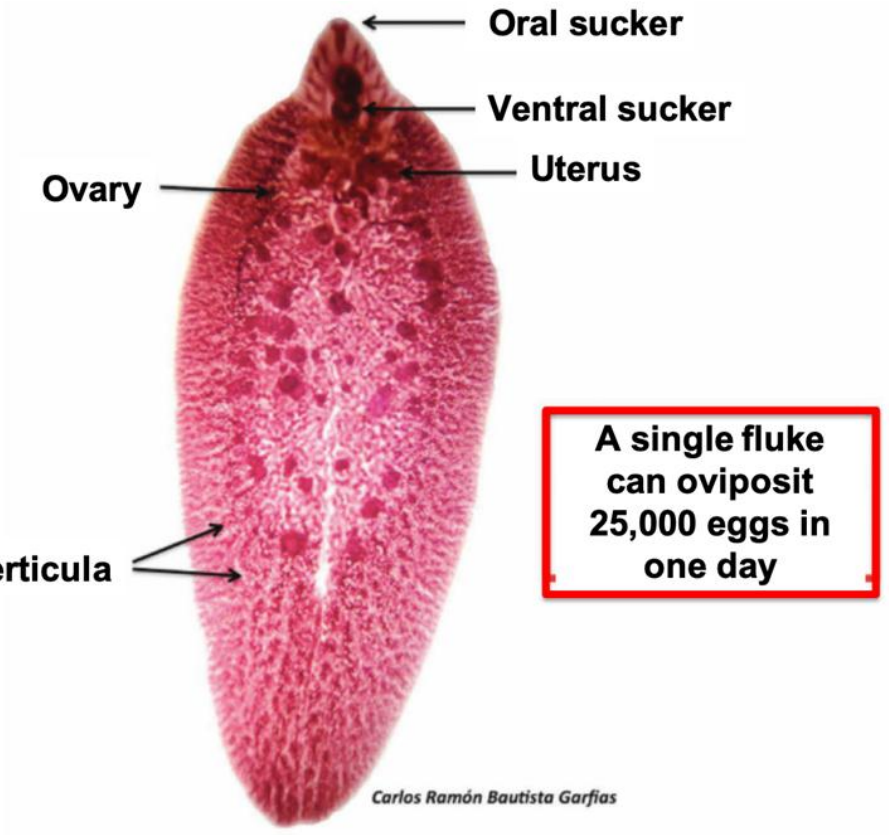

Fig. 3: Adult *Fasciola hepatica* (Figure made by Carlos Ramón Bautista-Garfias).

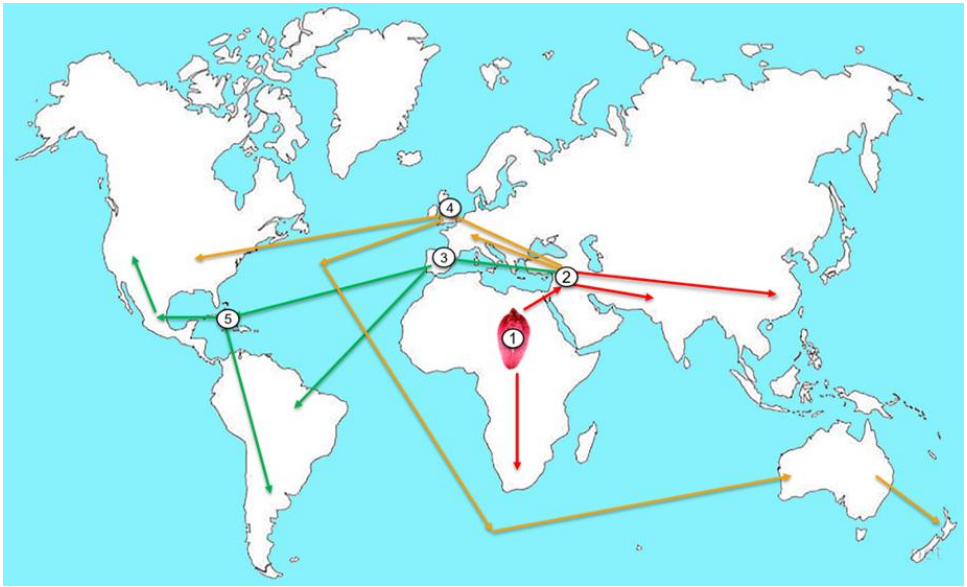

Fig. 4: Dispersion of *Fasciola* spp. 1. *Fasciola* genera originated in Africa. 2. Moved to the Middle East, then to Asia. 3, 4. Infected ruminants transported the trematode to Europe, and then, 5, to the American Continent. In the world. Based on the following references: Dittmar and Teegen 2003, Mas-Coma et al. 2009, Mehmood et al. 2017, Caravedo and Cabada 2020, Bargues et al. 2022.

4. ECONOMIC IMPORTANCE AND ZOONOSIS

In general, the losses caused by *F. hepatica* in production are not noticed by the producer and depend on the number of flukes that parasitize the animals, as well as their age, diet and health status. The main losses are due to: 1) delay in growth, 2) reduction in body weight, 3) decreased

production of meat, milk or wool, 4) reproductive disorders, 5) abortions, 6) predisposition to contract other diseases, 7) increased food consumption due to poor digestion. In short, fasciolosis negatively affects all sectors that make a livestock business profitable. In addition, we must add the risk of death of animals that are not adequately cared for and at the end of their reproductive life, the confiscation of the liver (loss) in the slaughter, since said viscera is not suitable for human consumption. In this context, it is estimated that in Mexico 100 million kg of beef production per year are lost due to fasciolosis (Bautista, unpublished data). Based on the price per kg of carcass meat, the annual loss in bovines amounts to 6,600 million pesos (393.6 million dollars, MDD). Worldwide, it is estimated that 46,958 million pesos are lost per year (2,800.5 million dollars). On the other hand, *Fasciola hepatica* is found on all continents with almost 180 million people at risk of acquiring the parasitosis and an estimated 17 million people already infected worldwide (World Health Organization 2007, Mas-Coma et al. 2009). Regarding Mexico, in Atlixco, Puebla, in 2013, of 865 examined children, a prevalence of fasciolosis was found that ranged between 2.94 and 13.33% (Zumaquero-Rıos et al. 2013).

5. FACTORS THAT FAVOR THE SPREAD OF *F. HEPATICA*

The most important are:
• The long life of the parasite in animals. In sheep it can live between eight and 11 years.
• The large number of eggs that each adult fluke is capable of producing daily (between 8,000 and 25,000).
• The biotic potential of the parasite inside the snail, since each egg can form between 400 or more cercariae.
• The great resistance of the infecting metacercariae. In the environment they can survive for up to 12 months and up to eight in wet harvested hay
• Vertebrate animal species in which *F. hepatica* can live include cattle, sheep, goats, horses, pigs, dogs, deer, rabbits, hares, squirrels, and wild rats, among others; which hinders the effective application of fasciolicide treatments.
• The high concentration of grazing animals per hectare in humid areas and the fertilization with recent or unfermented manure from sick animals are actions that contribute to the contamination of pastures with *Fasciola* eggs.
• The ease of reproduction of snails (Lymnaea) that are infected with Fasciola and the great number of eggs removed by each host vertebrate (up to 25,000).
• The great resistance of the snails that can stay alive, even when infected, for more one-year-old, buried in the dried mud.
• Inadequate drainage of the pastures, which gives rise to permanently humid areas, in which the snails live and reproduce easily.
• Periodic flooding that contributes to the spread of both snails and parasites.
• Temperatures, between 10 and 30ºC, and abundant rain that favor the development of the parasite, both in the external environment and in the intermediate host snails.

6. HOW ANIMALS AND MEN ARE INFECTED WITH *F. HEPATICA*

In animals, infection occurs by ingesting encysted metacercariae in grass, grass, and water. Most infections occur when animals graze in areas where snails have been or are eliminating parasites. However, they can also occur in the barn due to the ingestion of metacercaria attached to fresh or poorly ensiled fodder.
Man is infected mainly by ingesting raw plants (for example, watercress) or undercooked, contaminated with metacercariae

Fig. 5: Submandibular edema in a cow infected with *F. hepatica*. (Figure made by Carlos Ramón Bautista-Garfias).

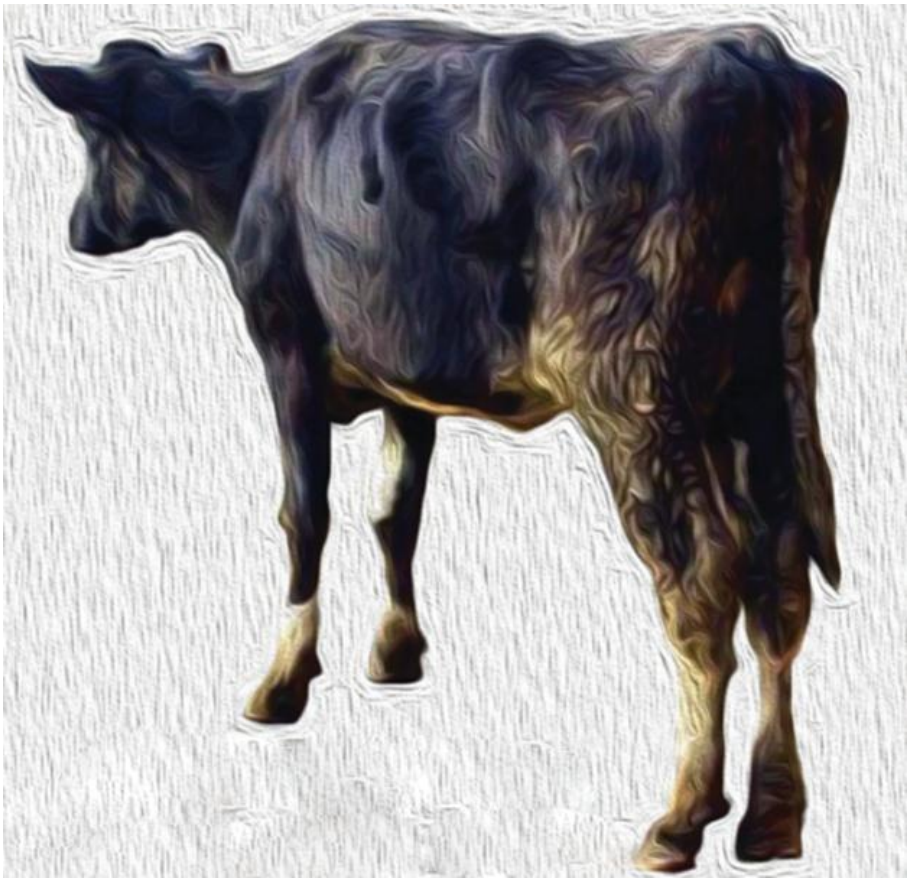

Fig. 6: Fasciolosis signs in cattle: diarrhea, chronic weight loss. (Figure made by Carlos Ramón Bautista-Garfias).

7. PLACES AND TIMES OF GREATEST RISK FOR ACQUIRING THE INFECTION WITH *F. HEPATICA*

The most propitious places for acquiring *Fasciola* are those in which the aquatic snail (Lymnaea) lives, such as:

- Slow watercourses on whose banks different types of plants grow.
- Wet meadows or those that are periodically flooded.
- Muddy terrain with small holes filled with water.
- Surroundings of fountains and cattle troughs.
- Land with insufficient drainage.

The most dangerous times to acquire the infection are those in which the snails release cercariae as they encyst and rapidly transform into metacercariae. These are already capable of infecting animals and humans 24 hours after being encysted.

The studies carried out during the last 10 years in various slaughterhouses in Mexico indicate that fasciolosis in cattle and sheep has not diminished; rather it shows an increasing trend. It is important to take into account that environmental conditions are not the same throughout the country and that they vary from year to year. In the same way, important meteorological phenomena (climate change) that affect different countries should be considered, such as "El Niño" (Trenberth 1997), which occurs every two to seven years and causes droughts and areas of atypical high humidity. "El Niño" of 2015 was one of the worst in 15 years (Milton 2017), which it has caused intense rains in much parts of Mexico, which will favor parasites such as *F. hepatica*. Typically, “El Niño” events reach their maximum effect between October and January, but often continue to wreak havoc during the first four months of the year. In this context, it has been stated that climate change contributes to the emergence or re-emergence of parasitic diseases (Short et al. 2017).

8. HOW FASCIOLOSIS MANIFESTS ITSELF IN CATTLE AND SHEEP

The disease is classified as acute or chronic. The acute one occurs more frequently in sheep and occasionally in cows. It is due to a massive ingestion of metacercariae found in the green grass of irrigation canals. In the acute form, anemia, digestive disorders, loss of appetite, pale mucous membranes and little movement are observed in the animals; signs that evolve until death generally rapid. Chronic fasciolosis in cows and sheep results in listless and anemic animals. Weight loss, diarrhea, submandibular edema, wool loss, abortions and, at slaughter, increased liver volume are observed, a condition known as hepatomegaly. When the infection is light, only a decrease in production and in the general physiological state is observed. If there are no complications, the course of bovine fasciolosis is benign. However, in some young animals or those subjected to intensive production, considerable weight loss is detected. It's important pointing that some cattle with fasciolosis give negative reactions to tuberculin.

9. DIAGNOSIS OF FASCIOLOSIS

The clinical diagnosis, both in the acute and chronic forms, is carried out based on the signs described above. Additionally, in chronic fasciolosis, the diagnosis is made by examining fecal material (coproparasitoscopic examination), to verify the existence of *F. hepatica* eggs in it. However, it must be taken into account that eggs will only appear when the cow or sheep already have adult flukes in the liver, approximately three months after the first infection. For this reason, all animals in the herd should be examined, including the apparently healthy ones. Feces obtained directly from the rectum should be sent to the nearest diagnostic laboratory for analysis. In cases

where no clinical signs or parasite eggs observed in the feces, serological diagnosis can be used. In this case serum samples must be taken from the animals for analysis by means of immunological tests such as the standard immunoenzymatic assay (ELISA) and its variants (DIG-ELISA, Dot-ELISA), passive hemagglutination Test (Fig. 7) and others that are practiced in some research institutions (Arriaga de Morilla et al. 1989, Bautista-Garfias et al. 1989, Bautista Garfias 1991, Ruíz-Navarrete et al. 1993, Fernández et al. 1995, Arriaga and Bautista Garfias 1997, Álvarez Rojas et al. 2014) or the parasitological diagnosis can be practiced after the sacrifice of the animals. It is important to note that *F. hepatica* infection depress the immune response of the host (Bautista and Lebrija 2008) and dairy cattle infected with this parasite may not respond to the tuberculin test (Claridge et al. 2014). Today, by using molecular techniques it is possible to identify Fasciola species (Baran et al. 2017, Tang et al. 2021, Saadatnia et al. 2022, Levy et al. 2023).

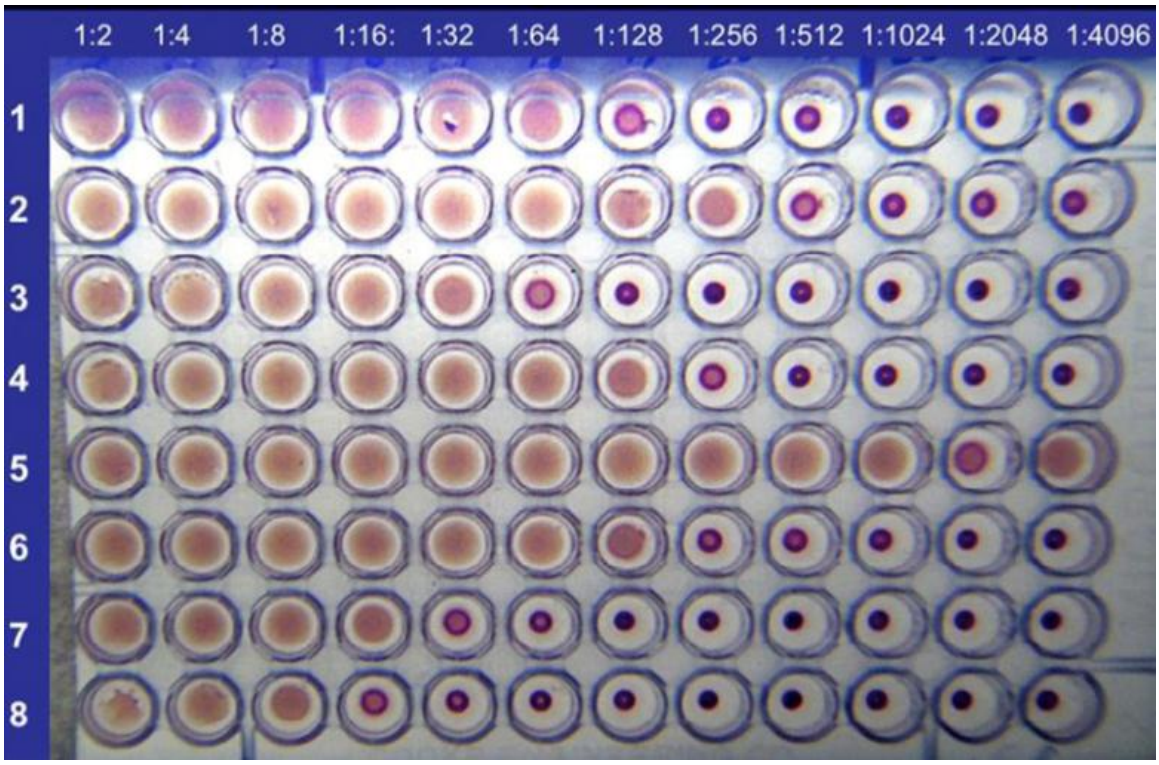

Fig. 7: Passive hemagglutination test for the detection of anti-*Fasciola hepatica* antibodies in sheep sera. The figures at the top indicate the serum dilution. The numbers on the left indicate the serum number of the animal examined. Number 5 is from a positive sheep with a very high titer (1:4096), while number 8 is from an animal negative to *F. hepatica* (1:8), the other sera are positive. (Figure made by Carlos Ramón Bautista-Garfias)

10. TREATMENT

Once the diagnosis has been made, fasciolicide treatment should be applied to the entire herd, including those animals that appear to be healthy or those that shed small numbers of parasite eggs. It is advisable to repeat the treatment every three months.

When cows are of reproductive age, dry cows and those that have not yet calved should be treated. It is not convenient to use fasciolicides in cows in production since the chemical products are eliminated in the milk and can harm the health of man and calves.

To determine the difference between what is spent and what is earned, when deworming is carried out, the following points must be taken into account:

1) Fasciolicide (route of administration, presentation, ease of obtaining it, side effects and cost).
2) Animals to be treated (cattle, sheep or goats and number to be treated, cost of handling them, production period).
3) Time of the year
4) Production system

To select the most effective fasciolicide (considering the increase in parasite resistance to fasciolicides such as Albendazole, Triclabendazole and Clorsulon) and its proper use, the Veterinarian should be consulted. It must be taken into account that *F. hepatica* has developed resistance to Albendazole and to Triclabendazole (Álvarez-Sanchez et al. 2006, Daniel et al. 2012, Brockwell et al. 2014, Kelley et al. 2016).

11. VACCINES AGAINST *FASCIOLA*

One of the more attractive technologies for controlling parasites is vaccination. In this context, several experiments have been carried out through the years to develop vaccines against *Fasciola hepatica* with different degrees of success (Bautista Garfias et al. 1987, Turner et al. 2016, Wesolowska et al. 2018, Silvane et al. 2020, Zafra et al. 2021, Cwiklinski and Dalton 2022, Cwiklinski et al. 2022)

12. SUGGESTIONS TO PREVENT FASCIOLOSIS

The animals must be kept away (even with the use of fences) from places that favor the presence of the aforementioned aquatic snail (*Lymnaea*).

Drain, when possible, the land to eliminate excessive soil moisture and achieve the eradication of the snail.

For the same reason, surround the troughs with a wide strip of material permeable to water such as sand, small stones, tezontle, etc.

The forage must be adequately tied, avoiding harvesting it with humidity. It is convenient to add two percent of common salt to the hay grass, or even delay its consumption for two or three months, to be sure that the metacercariae die.

It is advisable to ensile grass from wet areas to avoid the risk of infection of the animals. Do not allow animals with fasciolosis to graze

in irrigated meadows and alfalfa fields to avoid contamination with F. hepatica eggs.

The manure that is going to be used for fertilizing must first be dried, properly fermented or treated with copious amounts of lime, to destroy the eggs and miracidia of the parasite.

12.1. ONE HEALTH APPROACH CONTROL OF FASCIOLOSIS

Taking into account several factors, including climate change, the best system for controlling fasciolosis is by using the One Health approach (Cwiklinski et al. 2016, Mas-Coma et al. 2020).

12.2. PERSPECTIVES

At the present, man has effective tools for controlling fascioliasis such as simple practices for avoiding infection of cattle (pasture handling, draining accumulation of rainwater, avoiding to cattle pasture in water plants) promising vaccines, one Health approach, among others. Government health authorities (medical and veterinary) must cooperate to control Fasciolosis in animals and

man. However, as is the case of México, cattle Fasciolosis is a neglected parasitic disease. As an example, condemned cattle livers infected with F. hepatica during a period of 11 years (from 1977-1987), in one of the main abattoirs in the Country, showed a frequency similar each year, except in the years 1979, 1981, and 1982 (Encinas et al. 2020).

REFERENCES

Álvarez Rojas CA et al., 2014. Techniques for the diagnosis of *Fasciola infections* in animals: Room for improvement. Advances in Parasitology 85: 65-107.

Álvarez-Sanchez MA et al., 2006. Resistance of *Fasciola hepatica* to triclabendazole and albendazole in sheep in Spain. Veterinary Record 159: 424-425.

Anonymous, 2020. Human fascioliasis emergence risks in developed countries: from individual patients and small epidemics to climate and global change impacts. Enfermedades Infecciosas y Microbiolgía Clinica 38(6):253-256.

Arriaga de Morilla C et al., 1989. Comparison of Dot enzyme-linked immunosorbent assay (Dot-ELISA) with passive haemagglutination and thin layer immunoassay for the diagnosis of Fasciola hepatica natural or experimental infections in sheep. Veterinary Parasitology 30: 197-203.

Arriaga C and Bautista Garfias CR, 1997. Literature review on the serological diagnosis of some helminth infections in ruminants. Técnica Pecuaria en México 35: 105-114.

Baran A et al., 2017. Molecular determination of *Fasciola* spp. isolates from domestic rumnants fecal samples in the Northwest of Iran. Iranian Journal of Parasitology 12: 243-250.

Bargues MD et al., 2022. Paleobiogeographical origins of *Fasciola hepatica* and *F. gigantica* in light of new DNA sequence characteristics of *F. nyanzae* from Hippopotamus. Frontiers in Veterinary Science DOI 10.3389/fvets.2022.990872

Bautista Garfias CR et al., 1987. Inmunización intraperitoneal de ovinos contra *Fasciola hepatica* con antígenos del parásito adulto. Técnica Pecuaria México 25: 365-373.

Bautista-Garfias CR et al., 1989. A new method for serodiagnosis of sheep fascioliasis using helminth excretory secretory products. Parasitology Research 76: 135-137.

Bautista Garfias CR, 1991. Immunodiagnosis of fasciolasis. In: Quiróz RH, editor. Diagnosis and control of parasites and animals and man: UNAM, Mexico; pp: 298-313.

Bautista GCR and Lebrija RA, 2008. The *Fasciola hepatica* excretory-secretory products reduce the production of cells producing antibodies against thymus-dependent antigens in mice. Veterinaria México 39: 429-433.

Brockwell YM et al., 2014. Confirmation of *Fasciola hepatica* resistant to triclabendazole in naturally infected Australian beef and dairy cattle. International Journal for Parasitology 4: 48-54.

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

Cwiklinski K et al., 2016. A prospective view of animal and human fasciolosis. Parasite Immunology 38: 558-568.

Cwiklinski K and Dalton JP, 2022. Omics tools enabling vaccine discovery against fasciolosis. Trends in Parasitology doi.org/10.1016/j.pt.2022.09.009.

Cwiklinski K et al., 2022. Targeting secreted ptotease/anti-protease balance as a vaccine strategy against the helminth *Fasciola hepatica*. Vaccines doi.org/10.3390/vaccines10020155.

Claridge J et al., 2014. Fasciola hepatica is associated with failure to detect bovine tuberculosis in dairy cattle. Nature Communications 3: 853. doi:10.1038/ncomms1840.

Carrada-Bravo T, 2007. *Fasciola hepatica*: biological cycle and biotic potential. Revista Mexicana de Patología Clínica 54: 21-27.

Fernández RM et al., 1995. Evaluation of the DIG-ELISA test for the detection of anti-*Fasciola hepatica* antibodies in cattle. Parasitology 19(1-2): 4-8.

Daniel R et al., 2012. A composite faecal egg count reduction test to detect resistance to triclabendazole in *Fasciola hepatica*. Veterinary Record 171: 153–157, doi: 10.1136/vr.100588.

Dittmar K and Teegen WR, 2003. The presence of *Fasciola hepatica* (liver-fluke) in humans and cattle from a 4,500 year old archaeological site in the Saale-Unstrut Valley, Germany. Memorias do Instituto Oswaldo Cruz, Rio de Janeiro 98(1): 1|41-143.

Encinas GR et al., 2020.Frecuencia de fasciolosis hepática e impacto económico en bovinos sacrificados en Ferrería, México, D.F. Veterinaria México OA DOI: http://dx.doi.org/10.22201/fmvz.24486760e. 2020.3.928.

Kelley JM et al., 2016. Current threat of tricabendazole resistance in *Fasciola hepatica*. Trends in Parasitology doi: 10.1016/j.pt.2016.03.002.

Levy S et al., 2023. First molecular identification of *Fasciola gigantica* in salaughtered cattle in Cape Verde: prevalence, gross pathological lesions, genetic identification and coprological analysis. Pathogens doi.org/10.3390/ pathogens12010075.

Mas-Coma S et al., 2009. *Fasciola,* lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Advances in Parasitology 69: 41-146.

Mas-Coma S et al., 2020. Sheep and Cattle Reservoirs in the Highest Human Fascioliasis Hyperendemic Area: Experimental Transmission Capacity, Field Epidemiology, and Control Within a One Health Initiative in Bolivia. Frontiers in Veterinary Science doi: 10.3389/fvets.2020.583204

Mehmood K et al., 2017. AS review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. Microbial Pathogens 109: 253-262.

Milton D, 2017. El Niño 2015-26. Geo Factsheet 365. https://www.thegeographeronline.net/ uploads/2/6/6/2/26629356/365_el_ni%C3%B1o_2015-16

Nyindo M and Lukambagire AH, 2015. Fascioliasis: An ongoing zoonotic trematode infection. BioMedical Research International doi.org/10.1155/2015/786195

Robinson MW and Dalton JP, 2009. Zoonotic helminth infections with particular emphasis on fasciolosis and other trematodiases. Philosophy Transactions of the Royal Society B 364: 2763-2776.

Rokni MB, 2014. Helminth-Trematode: *Fasciola hepatica* and *Fasciola gigantica*. Encyclopedia of Human Nutrition, Volume 2 doi:10.1016/B978-0-12-378612-8.00154-2

Ruíz-Navarrete A et al., 1993. *Fasciola hepatica* Characterization of somatic and excretory secretory antigens of adult flukes recognized by infected sheep. Revista Latino-Americana de Microbiología 35: 301-307

Saadatnia A et al., 2022. Molecular identification of Fasciola isolated from the liver of meat animals in Fars Province, Iran. Journal of Parasitology Research doi.org/10.1155/2022/4291230.

Sánchez Manzano RM et al., 1998. *Fasciola hepatica* staining without glycocalyx, an alternative for the observation of internal structures of the parasite. Técnica Pecuaria en México 32: 159.

Short EW et al., 2017. Climate change contribution to the emergence or re-emergence of parasitic diseases. Infectious Diseases: Research and Treatment 10: 1–7.

Silvane L et al., 2020. A vaccine base don Kunitz-type melecule confers protection against Fasciola hepatica challenge by inducing IFN- and antibody immune responses through IL-17A production. Frontiers in Immunology doi: 10.3389/fimmu.2020.02087.

Tang M et al., 2021. Molecular identification and genetic polymorphism abalysis of *Fasciola* flukes in Dali prefecture, Yunnan Province, China. Parasitology International doi.org/10.1016/j.parint.2021.102416 .

Trenberth KE, 1997. The definition of El Niño. Bulletin of the American Meteorological Society 78: 2771-2777.

Turner J et al., 2016. A model to assess the efficacy of vaccines for control of liver fluke infection. Nature (Scientific reports) 6: 23345. DOI: 10.1038/srep23345.

Wesolowska A et al., 2018. Vaccination against Fasciola hepatica using cathepsin L3 and B3 proteases delivered alone or in combination. Veterinary Parasitology 250: 15-21

World Health Organization, 2007. Report of the WHO Informal Meeting on use of triclabendazole in fascioliasis control. WHO/CDS/NTD/PCT/2007.1. Geneva: WHO, Headquarters.

Zafra R et al., 2021. Efficacy of a multivalent vaccine against *Fasciola hepatica* in sheep. Veterinary Research doi.org/10.1186/s13567-021-00895-0.

Zumaquero-Rıos JL et al., 2013. Fascioliasis and Intestinal parasites affecting schoolchildren in Atlixco, Puebla State, Mexico: Epidemiology and treatment with Nitazoxanide. PLoS Neglected Tropical Diseases 7(11): e2553. doi:10.1/371 journal.pntd.0002553