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OUR EXPERIANCES WITH HUMAN AND VERERINARY FASCIOLIASIS IN MONTENEGRO

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ABSTRACT

Fascioliasis, a cosmopolitan parasitic disease, has been classified as a neglected tropical disease NTDfor many years. (1) Over the last 20 years, the importance of this disease has been on rise, both in human and veterinary pathology, especially in regions ***with developed livestock and developing countries****. (2) According to WHO estimates, 2.4 million people are infected with fascioliasis, and another 180 million are at risk of this contagious disease. In Montenegro there is no data on number of patients with facioliasis, but the field workers and veterinarians detect the disease occassionaly. Thus, we know that is is present here and should be thought of, especially because of the wide range of clinical manifestations(4), frequent coinfections of numerous infectious agents (5). There is noticable increasingly significant development of resistance to almost successful antiparasitic therapy and vaccine deficiency (6).All of mentioned, complicates and increases the severity of the disease.*

We presented our experiences with two cases of human, and 12 cases of veterinary fascioliasis (in 7 cattle and 5 sheep), and the possibilities of using phytotherapy for the treatment and prevention of this parasitosis.

KEYWORDS: *Fascioliasis, in Humans, Animals, Therapy, Prevention*

Article History

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INTRODUCTION

The presence of Fasciolahepaticae in humans and cattle was first registered during archaeological excavations at a site in the Saale-Unstrut valley in Germany. Eggs of Fasciolehepaticae and capilariae spp. were found in a soil sample in the pelvis of a 4,500-year-old human skeleton, as the first evidence of a direct link between the infection and Fasciola hepatica, (7) which clearly indicated an existing infectious cycle involving humans as final hosts but main hosts being ruminants(sheep, goats, buffaloes).Slightly rarer in pigs, camels, ungulates, domestic rabbits, as well as in wild animals of ungulates and rabbits Fascioliasis is the reason for significant economic losses in the livestock industry (9, 10). Recently,

global losses in animal productivity due to fascioliasis have been estimated at over $ 3.2 billion per year. (3)

In the last 20 years, this cosmopolitan parasitic zoonosis has attracted more attention. The incidence of human fascioliasis is increasing in 51 countries on five continents. (3, 4, 5) It is ranked among the significant public health problems of human pathology. (2). In humans, it occurs accidentally in developing countries. According to WHO estimates, 2.4 million people are infected with Fasciola, and another 180 million are at risk of this contagious disease. [8].

The causative agent of the disease is the **digenetic** trematode of the class Fasciola (flukes), with the two most common genera Fasciola hepatica (F.hepatica) (11) and Fasciolagigantica (F.gigantica) (12) (Figure 1 A and B). They parasitize in the bile ducts of the liver and gallbladder of the end hosts of vertebrates (animals and humans). (13) In massive invasions it can have atypical localizations: in the lungs (14), eye (15), nervous system (16), skin (17) in abdomen, primarily liver, pancreas, intestines, spleen, kidneys, lymph nodes (18,19).

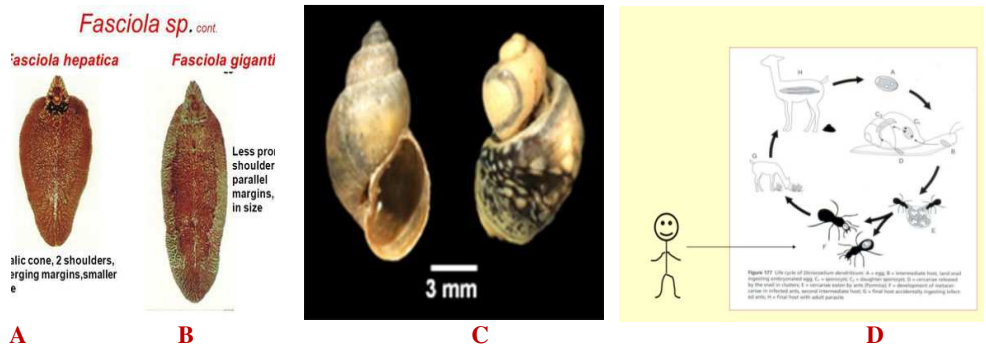


Figure 1: The Causes of Fascioliasis are two Digenetic Trematodes, F. Hepatica (A) and F. Gigantica (B). The Parasites are also known as "Flukes". Transitional Hosts in the Biological Cycle of Parasites are bar Snails of the Genus Lyminae, Galba in Africa (C) and other Carcasses (Formica) (D)

Fascioliasis is common in wetlands, where it is available to a transitional hosts: the bar snail of the genus Lymnae and Galba spp., the ant (formica). (20, 21) Two entomologists Krulli and Marpes in 1952. found that some ants are second intermediate hosts in the life cycle of trematodes - flukes. After traveling through the true middle host-snail, the flukes reach the ants and return to the main vertebrate to complete their life cycle (Fig. 1 C and D).

Animals got infested on pasture (9, 12). Humans become infected accidentally by alimentary ingestion of infectious forms of metacercariae, via contaminated water or plant foods.

Infections of people with F. hepatica depend on the presence of transitional hosts - bar snails (20.21), domestic herbivores (23), climatic conditions (24, 25, 26) and eating habits (27).

Several types of aquatic vegetables that are used for human consumption are important for transmitting the infection to humans. In Europe, these are Nasturtium officinale (common watercress), Nasturtinumsilvestris, Rorippa amphibia (wild watercress), Taraxacum den leonis (dandelion leaves), Valerianellaolitoria (Lamb salad) and Mentaviridis (spreamint), lettuce and other garden products. In northern Bolivia there are several aquatic plants important in pathogenisis of the disease, such as bero-bero (watercress), algae, kjosko and tortora. It has been experimentally proven that people who consume raw dishes from fresh liver infested with young flukes can also be infected.

The primary hosts and reservoirs of parasites are domestic animals (sheep, cattle, goats). (9, 10) Other infected animals are not of major importance for disease transmission to humans. Donkeys and pigs are said to contribute to the transmission of the disease in Bolivia. (23) Among wild animals, it has been found that the peridomestic rat (Rattus rattus) may play an important role in the transmission and spread of the disease in Corsica (28). In France, the nutria (Myocastor coypus), is confirmed as a wild host and reservoir of F. hepaticae, is important for human infections (29) In India, the species F. jacksoni has been found in elephants. (30).

ETIOLOGY

F.hepatica and F.gigantica are the causes of Fascioliasis. The flat body of the parasite is soft, has a leaf shape and pink in color. There is no body cavity and the organs are packed into the parenchyma. The body is covered with a protective mesenchymal sheath (tegument), through which the absorption of nutrients is performed by microvilli.Under the sheath are placed mitochondria, pinocytes, endoplasmic reticulum and other structures that enable absorption. (Figure 2) Below the thin basement membrane, there is a layer of oblique, circular, longitudinal muscles, which surround the mesenchyme.

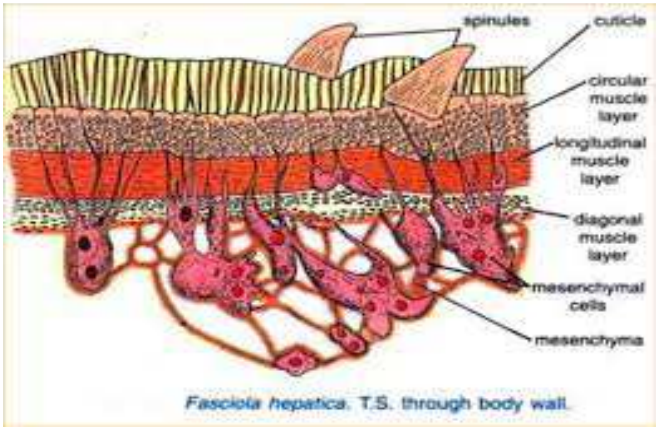


Figure 2: Protective Sheath of F. Hepaticae (Tegument), through which Nutrients are Absorbed

The parasite contains sclero protein (31), resistant to gastrointestinal juices. The senses are poorly developed. Tactile- and chemo-receptors have been developed in larvae, with the help of which it finds a transitional host.. The hermaphroditic reproductive system is well developed, occupying most of the interior of the body. Fertilized eggs are released into the external environment. Development takes place over a series of larval stages, and the life cycle is very complex and includes at least two hosts - the host in which the adult parasite is the final host . The host in which the larva parasitizes is a transitional host (snail, ants).

Life Cycle of Fascioliasis Figure 3

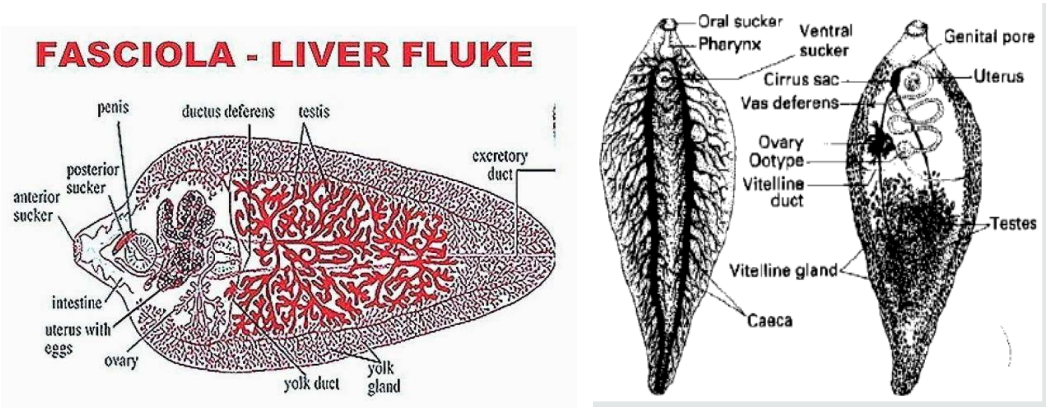


Figure 3: Structure of the Fasciola Hepatica in the Host Organism, the Predilection Site for Adult Parasites of both Species are the Bile Ducts of the Liver or Gallbladder. F. hepatica is smaller in Size from 2 to 3 cm and is Characterized by a Cosmopolitan Distribution. F. Gigantica is 4 to 10 cm Long.

Unlike in nematodes, in trematodes hundreds of adults can develop from a single egg. This is due to the phenomenon of pedogenesis in the middle host mollusk. Adult flukes are always oviparous. Eggs with an operculum (lid) lay on one pole. In the egg, the embryo develops into a piriform (pear-shaped), ciliated miracidium. Infected animals excrete ciliated miracidia into the environment through feces. They move through the water with the help of cilia, do not feed and for their further development must find a suitable transitional host - a snail within a few hours. They are believed to use the chemotactic responses of the snail, and upon contact it clings to the snail by suction and penetrates its soft tissues, aided by the cytolytic enzyme. (Figure 4)

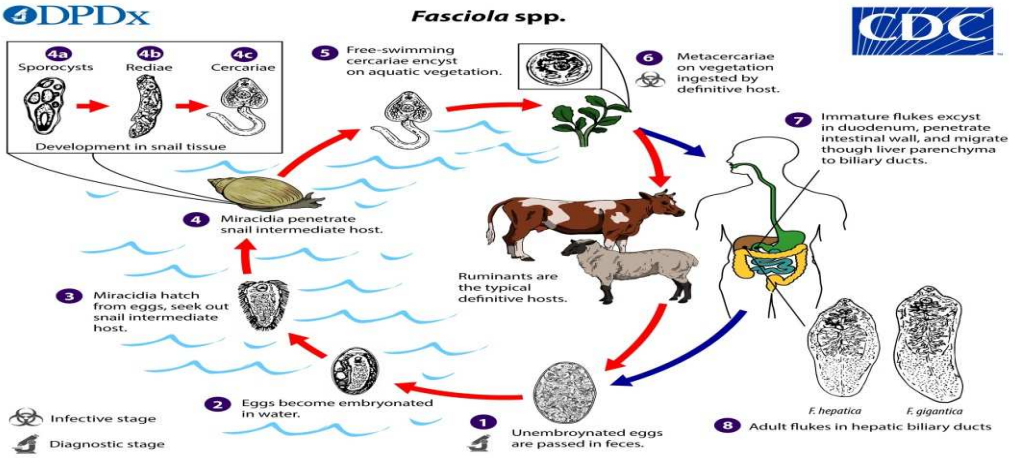


Figure 4: Life Cycle of F.hepaticae.

In the transitional host (snail), they lose their **lashes**. They develop in the sporocyst, which contains numerous cellular embryos. These cells develop into larval forms - redies, which migrate to the hepato-pancreas of the snail. This is where the final stages, the cercariae, occur, although in unfavorable conditions for the snail, the second generation of daughters is often produced. Cercariae are young **butterflies** with a long tail. In the infected snail, they are produced in significant numbers, they actively come out of the snail, although most infected snails die prematurely due to the rough destruction of the hepatopancreas. Cercariae swim in water, using even a water film, and within an hour or more, they

attach to the vegetation, drop their tails and encist. Encyclosedmetacercariae have great potential for survival in nature, up to several months. (Figure 5).

Figure 5: Developmental Stages of Fasciole Hepaticae: Egg, Miracidia, Sporocysts, Redia, Cercariae, Encrusted Metacercariae, Adult Forms

After the infection is established in the vertebrae, young fascioles are released from the metacercariae, which penetrate the intestinal wall, migrate to the abdominal cavity, and from there to the predilection site of the liver in 4-6 days. They reach the surface of the liver, penetrate the capsule and penetrate the parenchyma. In a few weeks (average 6), they reach the bile ducts, where in the next few weeks (4-6), they mature into sexually mature (adult) forms. The prepartent period lasts 8-12 weeks, they can live for several years.

Epidemiology

The prevalence of fascioliasis is limited to the tropics of Africa, the Middle East, Eastern Europe, South and East Asia, and South America [8].

In Africa, cases of fascioliasis in humans, except in the northern parts, are uncommon. The highest prevalence was recorded in Egypt, in communities living in the Nile Delta (32). In Asia, the highest number of cases was recorded in Iran, where more than 10,000 cases of human disease were detected, especially in the province of Gillan on the Caspian Sea (33). In East Asia, human fasciolosis is sporadic. Few cases have been reported in Korea, Vietnam and Thailand. In Japan, diploid (2n = 20), triploid (3n = 30) and chimeric flukes (2n / 3n) have been described in domestic cattle, many of which reproduce parthenogenetically. Analysis of mitochondrial genes showed that the Japanese Fasciola spp. closely related to F. gigantic and not to F. hepatica. [34] Human fasciolosis is very rare in Australia. In India, the species F. jacksoni has been found in elephants. [35].

Pathogenesis

Trematodes of the genus Fascioliasis are among the most successful globally distributed helminths, with a negative impact on humans and livestock. Many physiological adaptations allow them to become infected and develop within the host mammal. (36). In the final host, the mechanism of infection development is divided into two phases: parenchymal (migratory) and biliary (biliary phase).

The parenchymal phase begins when young (NEJ) flukes penetrate the intestinal wall, migrate within the abdominal cavity, and penetrate the liver or other organs. F. hepatica has a predilection predisposition to liver tissues, [37] but can also attack ectopic sites such as the lungs, diaphragm, intestinal wall, kidneys, CNS, subcutaneous tissue.

[14,15,16,17,18,19] During migration, flukes mechanically destroy inflammatory tissues around migratory pathways.

The second (bile phase) begins when flukes enter the bile ducts of the liver. They mature in them, feed on blood and produce eggs. (38) Biliary duct hypertrophy is associated with bile duct lumen obstruction, with consequent development of icterus.

The main adaptive mechanisms of methyl are cysteine peptidases (39, 40, 41,42). Fasciola secretes them directly into the tissues of the host in order to open in a relatively short time and ensure the passage of parasites from the duodenum to the bile duct. (Figure 6). When the relative safety of the bile duct and the growth / maturity of the parasite is complete, the secreted peptidases facilitate the provision of nutrients contained in the protein part of the blood that provide the fascioli with essential building blocks for egg synthesis.

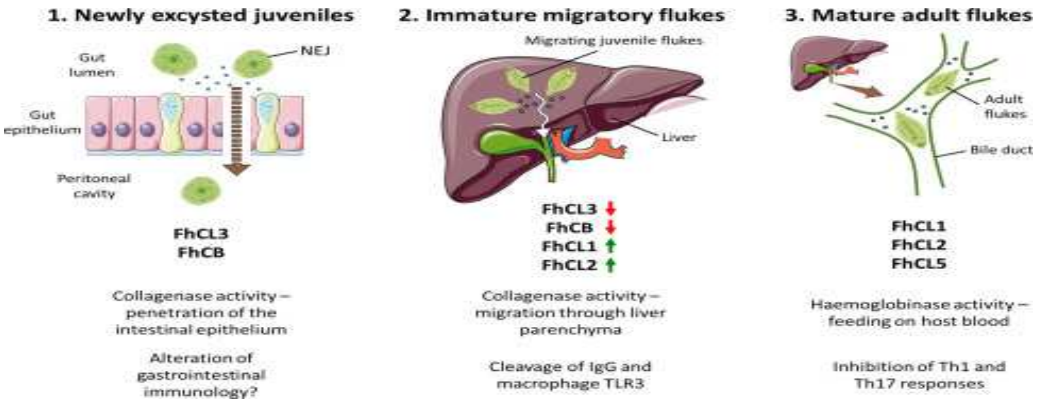


Figure 6: Fasciola Hepatica goes through a Complex Life Cycle in the host Mammal, Which includes Transitions Between the organ System and the Host Tissue. This allows it to Coordinate the action of Specific Cathepsin L and B Peptidases with Different but Complementary Substrate Specificities

Cathepsins act similarly to collagenase in degrading the extracellular matrix. During the migration of immature flukes through the host liver parenchyma, protein degradation is mainly performed by Fasciola clade 1 and 2 cathepsin L peptidases. FhCL2 enzymes also have the ability to break down collagen which is crucial for tissue breakdown at this stage of the parasite's life cycle. Upon arrival in the bile ducts, the parasites mature and receive nutrients created by the breakdown of the host's hemoglobin. At this stage, members of clades 1, 2, and 5 of cathepsin L peptidase are the only proteases secreted by adult flukes with FhCL1 that are most dominant. The effects of secreted cathepsin peptidases on the host immune response are indicated for each phase. The importance of peptidases for parasite survival is reflected in the efforts made to spread cathepsins by positive selection, especially in their active sites, as well as their constant adaptation to changes in hosts over the past 25 million years. The coevolution of an elaborate peptidase activation system has been discovered in the simultaneous proliferation of their legumain trans-activators, and the development of a number of other regulators (statins and CT inhibitors), as evidence of their key countermeasures against the physical barrier of tissues and macromolecules. break through without causing excessive damage and pathological changes in the host.

The main obstacle that parasites must overcome is the immune response of the host-vertebrate to the presence of parasites and soluble cellular components of the innate and adaptive immune system. (43) Many studies on the immune-modulatory capacity of Fasciola, especially molecules contained in the adult (ES), suggest that peptidase secretion contributes to the regulation of host proinflammatory responses by altering the activity of innate immune cells, T cells, and antibody function. 44).However, many of these studies have been performed in vitro, and thus confirm in vivo the role that

cysteine peptidases play in immune modulation, and the mechanisms they use to influence the activation and function of a number of immune cells associated with parasitic infection (mast cells, eosinophils, NK cells, γδT-cells and innate lymphoid cells) are not known.Cysteine peptidases are not known to directly induce or proliferate Th2 or Treg cells. (45, 46, 47) Inhibition of classical DC maturation and activation of M1 macrophages are thought to be enhances the Th2 / Treg immune response to other parasite molecules and / or host signals. contribute to the range of host signals (DAMPs) that mediate the immune response as the infection progresses

As an example, mass spectrometry (MS) analysis of sheep peritoneal fluid 18 days after infection is reported, which revealed that many peptides derived from liver tissue are systemically released by parasite tunnel activity. Collagen-derived peptides, for example, produced by hydrolysis of the interstitial matrix via FhCL3 / FhCL2, could be potent regulators of inflammation. Continuous use of MS-based proteomics allows the detection of multiple physiologically relevant substrates for parasitic cathepsin peptidases in vivo. Although the expression patterns and biology of cathepsins secreted in the life cycle stages of F. hepaticae in mammals are well characterized, especially in adult flukes, because these parasites and their ES products are easily obtained, our knowledge of their use in parasite life cycle stages is limited. . Cathepsins were not detected during proteomic analysis of F. hepaticae embryos and in vitro transformed F. hepaticae parent sporocysts.

The central role of cathepsin-like cysteine peptidases in Fasciola biology has made them major targets for the development of molecular vaccines. could be accepted as a commercial vaccine.

Resistance to Infection

Cattle were found to acquire resistance to infection with F. hepatic and F. gigantic when they were sensitized to the primary infection, which was shortened due to treatment. Natural resistance to fascioliasis has also been documented in rats. [28] In contrast, cattle, sheep, and goats are not resistant to re-infection with F. hepatic. There is also evidence that two breeds of sheep (Indonesian sheep with a thin tail and red maasai) are resistant to F. gigantica. No human resistance reports are available.

Coinfections

Coinfections of pathogens in the same host are common. An example is given of numerous coinfections with parasites, chronic infections with Mycobacterium tuberculosis (49) and viruses such as HIV, which can affect one third of the human population in some developing countries (50). There is growing evidence that coinfections with these pathogens may alter susceptibility to other important pathogens and / or adversely affect the efficacy of drugs and vaccines, by their effects on the host immune response. (52).

Fasciolagigantica and Mycobacterium bovis (bTB), the causative agent of bovine tuberculosis, is a major livestock and veterinary health problem in underdeveloped countries. (49) There is currently no effective vaccine against this serious disease. Control of naturally infected bovine populations relies on regular testing and removal of positive animals, but surveillance and control are hampered by a lack of diagnostic tests. Recent studies of cattle with bovine tuberculosis, co-infected with Fasciola, have raised concerns in areas with a high incidence of flukes. The large influence of parasitic coinfections on Th1 and Th2 immune responses, with simultaneous severe forms of the disease, and increased susceptibility of cattle to mycobacterial infections has been shown. In co-infections of bovine tuberculosis with Fasciolagigantea, complex interactions at the immune level have been registered.

Escherichia coli O157 and Fasciola hepatica The zoonotic bacterium Escherichia coli O157 can cause hemorrhagic diarrhea in humans and is a public health problem worldwide. Cattle are considered the main reservoir for human infection. Fasciola hepatica is a globally important ruminant parasite, and is known to modulate the host's immune response and affect susceptibility to bacterial pathogens such as Salmonella Dublin. (50) The pathogenetic mechanisms of coinfection between E.coli O157 and fasciola are triggered by unknown events, but the immune system is thought to play an important role in this. The hypothesis that the severity of E.coli O157 infection was associated with co-infection with F. hepatic in cattle. It was checked by examination of 334 cattle, intended for the food chain, at the end of 2915. E. coli O157 was detected by immunomagnetic separation and bacterial load control. F. hepatitis infection status was assessed by copro-antigen ELISA. A significant association ($p = 0.01$) was found between the percentage positivity of F. copro-antigen ELISA and E. coli O157. These results suggest that the control of F. hepatic infection may affect the attenuation of E. coli O157 potential in cattle for human consumption. (51)

F.hepatica and Echinococusgranulosus Simultaneous infection of animals with multiple parasites (polyparasitism) is a common but insufficiently studied phenomenon. In wild and domestic animals. Fasciola hepatica and Echinococcus granulosus, are helminths of ungulates. The metastasis Echinococcus granulosus is surrounded by an adventitial layer, which responds to the host's immune response to the parasite. This layer in cattle is formed by a granulomatous reaction and the fertility of the echinococcal cyst (EC) depends on it. Thanks to the systemic ability of the immune system to modulate coinfections with F. hepatic, it is possible to create a favorable environment for EC growth. A total of 203 cysts of Echinococcus granulosussensustricto were found in 82 cattle, of which 42 EC were found in 31 animals infected with Fasciolahepatic. It has been determined that coinfection with Fasciola hepatic + Echinococcus granulosus has a detrimental effect on EC: it reduces the medium intensity of infection, affects the reduction of the size of echinococcal cysts, especially infertile cysts. (52).

CLINICAL CHARACTERISTICS OF HUMAN FASCIOLIASIS

In 50% of human cases, the infection is asymptomatic. When the infection is symptomatic, it goes through several stages. Incubation phase: lasts from several days to 3 months. After ingestion, the first symptoms of the disease depend on the number of ingested metacercariae as well as the immune status of the host. Invasive or acute phase: is a consequence of the migration of metacercariae (immature flukes) into the bile ducts. There is mechanical destruction of the peritoneum and liver tissue, due to local or generalized toxic damage and allergic reactions. Manifested: fever; abdominal pain, loss of appetite, bloating and gas, diarrhea, ascites. Urticaria, respiratory symptoms may occur: cough, dyspnea, chest pain, hemoptysis (very rare), hepato-splenomegaly, anemia, icterus. Adult flukes can cause obstructive jaundice or make the patient vulnerable to cholelithiasis, which is a risk factor for cholangiocarcinoma (53), calculosis, fibrosis, cirrhosis (71). It follows

Latent phase: with fewer and milder symptoms, and finally

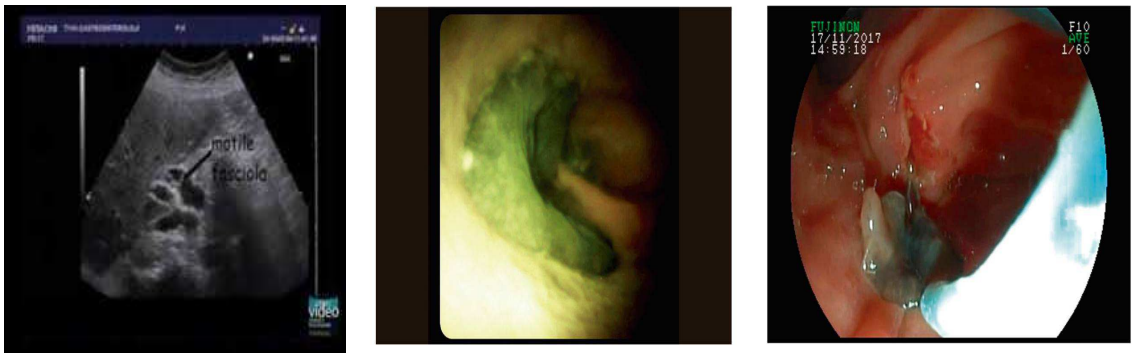
Chronic or obstructive phase: Occurs several months to years after infection. The symptoms of inflammation of the bile ducts, vesicles of the feleae, and possibly with the formation of gallstones, or fibrosis, dominate. Although chronic inflammation is associated with an increased rate of malignancy, fasciola has not been shown to be associated with this risk.

ECTOPIC FILARIASIS IN HUMANS

Fascioliasis in Choledochus

A 60-year-old woman, with two weeks of constant abdominal pain. Four years ago she had a cholecystectomy diagnosed with symptomatic gallbladder disease. On physical examination, it appeared to be acute hepatitis, but high eosinphilia 1,160 / µl, alkaline phosphatase of 198 IU / l and gamma glutamyl transpeptidase level 103 IU / l did not match. On examination of the abdomen, dilated bile ducts were found and choledochus, without clear causes.

Magnetic resonance imaging, cholangiopancreatography (MRCP) showed probable distal choledochus stricture. Endoscopic ultrasound showed an actively moving tubular structure in the gallbladder. (Figure 7 A, B. C)



A B C

Figure 7: Endoscopic Ultrasound shows a Tubular Structure in the bile duct (A), Cholangioscopy Reveals a Leaf-like Trematode, Fasciola Hepatica, in the Common bile Duct (B), Endoscopic Extraction of Fasciola Hepaticae from the Common Bile Duct (C).

ERCP was performed to assess and treat obstruction. Spy glass cholangioscopy revealed a trematode, Fasciola hepatica, which was extracted and then antiparasitic drugs were administered (Figure 8 A, B, C).

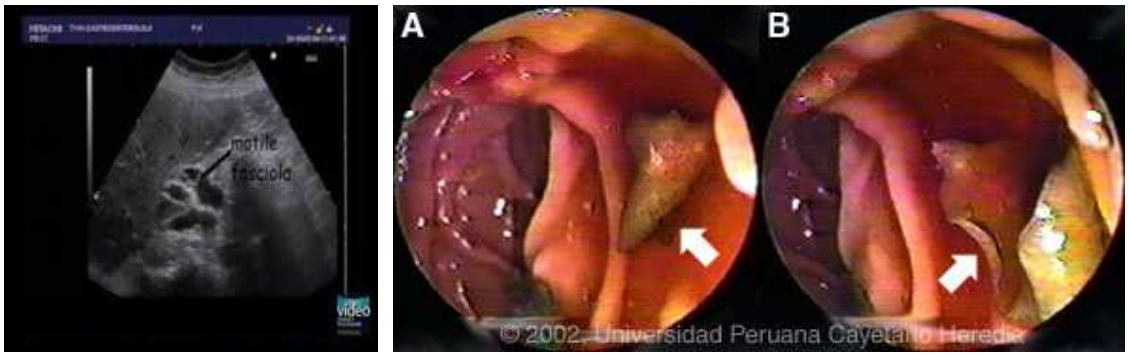


Figure 8: On Ultrasound Examination, Dilated bile Ducts and Choledochus (C). ERCP was Performed, Which Showed a lack of Filling Compatible with CBD Cholelithiasis. Papillotomy was Performed and CBD was directly Visualized. The Left (A) and Right (B) Images Show Disturbances, at Low and High Magnification.

Neurofilariasis and Ocular Fascioliasis

The registered case of neurofilariasis and ophthalmofilariasis included neurological, meningeal and psychiatric manifestations and eye disorders. The first case in the medical literature of ectopic neurofilariasis (in the brain and right eye) was registered in school-age boys, in whom two intracranial aneurysms also manifested. Four months after consuming

Potamondenticulata (fresh crabs), a ten-year-old boy from Xuan-Han County, Sichuan Province, China, had a six-month headache, nausea and vomiting. At the same time, he suffered recurrent pain in his right eye, followed by exophthalmos and paralysis of the facial nerve. More than one cerebral hemorrhage and hematoma in the brain were detected by computed tomography. Digital subtraction angiography revealed 2 intracranial aneurysms. Twenty-six days after admission to the hospital, a squamous parasite erupted from the patient's right eye, identified as F. hepatica, based on morphological characteristics. The final diagnosis of this case was confirmed by morphological identification of the parasite, and based on the results of laboratory findings. After treatment with praziquantel, he fully recovered. The two aneurysms were not surgically treated, but were followed for a long time. Following the DSA, one aneurysm was found to have disappeared while the other remained unchanged (Figure 9).

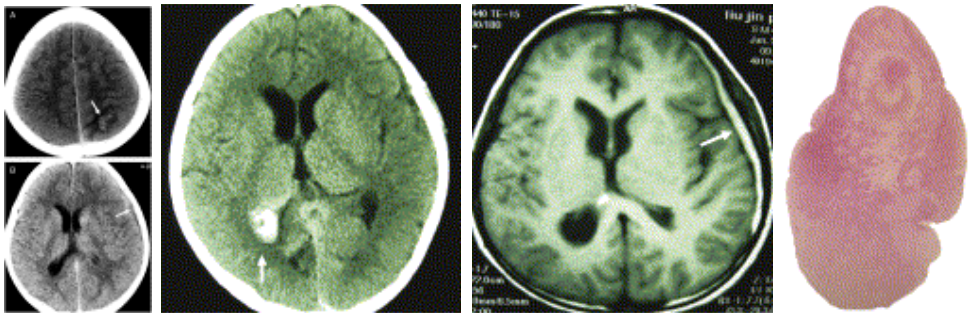


Figure 9: Multiple Hemorrhages in the Brain and Hematomas may be Associated with Ectopic Fascioliasis in the Brain and eye. Intracranial Infection Occasionally caused by F hepatica or other Parasites may be Indicated by Fever of Unknown Origin, Eosinophilia, and Recurrent Intracranial Pain.

Figure 10: F. Hepatica in the Anterior Chamber of the Eye in a Woman from Guilan Province, Northern Iran. Serological Tests and Examination of the Stool on the fasCiola are Negative. It is Recommended that in Endemic areas of Infection with F, Hepatitis in case of Uveitis, Eye Involvement should be Considered, Despite no other Systemic Interventions.

Among the atypical localizations, ocular (Figure 10), pulmonary manifestations, heart and blood vessel involvement were registered. In mass invasions, fascioles can also be found in mesenteric lymph nodes, kidneys and other organs in yellow-green necrotic nodules 5-10 mm in size.

CLINICAL CHARACTERISTICS OF FASCIOLIASIS IN ANIMALS

Fasciola hepatica is common parasite in various species of animals. (54). How intensely the animals get infestated is also shown by the data of slaughterers that the presence of this parasite is registered in 20-70% of slaughtered sheep and

10-40% of slaughtered cattle (cows). In animals, the clinical course depends on the degree of invasion, the type and age of the animals, the diet, the way of upbringing.

In sheep and goats the course can be acute and chronic. The acute form occurs in late summer and early autumn. Diseased animals are depressed, and excitation and agitation occur less frequently, with elevated body temperature values(41-41.5 C). Coronary dysfunction occurs with consequent rapid fatigue. The animals lag behind the herd and often lie down. Appetite is reduced and they often completely refuse food, whilepolydipsia is pronounced. Profuse diarrhea and pain on palpation of the liver often occur, and the conjunctiva is icteric.

The chronic form occurs throughout the year. The sheep are losing in the weight, they get tired quickly, their appetite is preserved, diarrhea turns into constipation. The eyes are inflamed in the eye sockets, the conjunctivae are anemic (porcelain pale). Edema occurs in the submandibular, thoracic and abdominal regions as well as around the eyes, which disappear when the animal moves and reappear when the animal is at rest. Milk yield is significantly reduced. Wool is brittle and easily torn and often falls spontaneously. When the liver is palpated, the animal reacts painfully and the organ is enlarged. One of the common symptoms is progressive anemia. In pregnant animals, miscarriage may occur.

In cattle, the acute form occurs rarely, only in cases of massive invasion. The chronic course is more frequent and the first symptoms appear in late autumn and winter. The animals are thin, appetite is reduced, polydipsia is present. The mucous membranes are anemic and the conjunctiva edematous. Salt deposition in the bile ducts in cattle is a characteristic feature of the disease.

In pigs, the disease has an easier course. Symptoms usually include thinness, edema, general weakness, and in rare cases, sudden death.

The number and variety of symptoms depends on how many parasites there are and at what stage of the infection. The mortality rate is significant in both cattle (67.55%) and goats (24.61%), but is low in humans.

DIAGNOSTICS

Fasciolosis is a difficult diagnostic problem. 50% of infected people have no symptoms of the disease. (70) In clinically manifest human filariasis, epidemiological data, clinical and radiological, pathohistological examinations are important for diagnosis. (55, 56) Stool examination is generally unreliable because parasite eggs can rarely be detected in chronic phase of infection. (3) They appear in the feces between 9-11 weeks after infection. The cause is unknown. It is also difficult to distinguish between different types of fascioliosis, as well as to distinguish them from Echinostomes and Fasciolopsis. (ELISA and Western blot test. (57)

Most immunodiagnostic tests detect infection with a susceptibility above 90% during all stages of the disease. In addition, the antibody concentration decreases rapidly after therapeutic treatment, and one year after treatment, no antibodies are found, which is a significant diagnostic / prognostic finding. Radiological and laparoscopic diagnostics are modern methods of choice for the diagnosis of fasciolar lesions. Occasionally both eggs and live parasites (58). In case of suspicion of the disease, it is useful to obtain data on the patient's diet, which is also important for the differential diagnosis. Clinically, it is often not possible to distinguish fascioliasis from other diseases, especially liver and bile.

Immunological methods ELISA and Western blood 2 - 4 weeks after infection are reliable for the diagnosis of infection (56, 57, 58). They are based on the detection of specific antibodies in serum. The diagnostic antigens used are

mainly derived from extracts of secreting / secretory products of adult fasciols or with partially purified fractions thereof. Coprologic examinations of the stool alone are generally inadequate. Butterfly eggs often cannot be found in the feces, even after multiple stool examinations.

Biochemical and hematological tests of human sera support an accurate diagnosis (eosinophilia, elevated liver enzymes, alkaline phosphatase). Ultrasonography, liver biopsy and gallbladder puncture may be used. In animals, intravital diagnosis is based on faecal examination and immunological methods. However, clinical signs, biochemical and haematological findings, season, climatic conditions, epidemiological situation and snail examinations must be taken into account. Similar to humans, fecal testing is not reliable. Nevertheless, fecal examination is still the only diagnostic tool used in some countries. Although coprologic diagnosis of fasciolosis is possible 8 to 12 weeks after infection (WPI),

Evaluation of the MM3-COPRO method for the detection of Fasciola coproantigen in human stool samples and the utility of the new preservative / diluent Copro Guard, developed for the preservation of Fasciola coproantigen. The MM3-COPRO test was performed on 213 samples from healthy patients, 30 samples from fecal-positive Fasciola (according to the Kato-Katz method) and 83 samples from patients with other parasitic infections. All Fasciola positive samples were detected by the MM3-COPRO test (100% sensitivity). There was no cross-reactivity with other common parasites present in the analyzed clinical samples (100% specificity). The use of CoproGuard improved the extraction of coproantigen without affecting the limit of detection of the test, and the antigenicity of Fasciola coproantigen in faecal samples stored at 37 ° C was maintained throughout the observation period (120 days). The conclusion is that MM3-COPRO ELISA in combination with the use of CoproGuard can be a very useful method for the diagnosis of human fasciolosis.

THERAPY AND PREVENTION OF FASCIOLIASIS

Therapy and Prevention of Fascioliasis in the Human Population

Treatment options for Fascioliasis are limited. Old effective therapies (emetin and bitionol) are no longer used due to toxicity and unavailability. Another problem is the fact that the most commonly used anthelmintics have become ineffective. Clinical trials over 25 years have shown that tricarbendazole is highly effective for the treatment of human fasciolosis in adults and children and in all stages and forms of infection. Triclarbendazole has been very effective for a long time (59). Resistance of F. hepaticae to triclabendazole was reported in Australia in 1995 and Ireland in 1998 (60, 61). Nitazoxanide has been used successfully to treat human fasciolosis in Mexico. In addition to the established efficacy, its use is currently not recommended due to severe side effects.

Praziquantel has not been shown to be effective, and older drugs such as bitionol are moderately effective, but also cause more side effects.

Secondary bacterial infections that cause cholangitis are treated with antibiotics, and toxemia with prednisolone. A vaccine to protect people from Fasciola infection is not yet available, although intensive work is underway. Preventive measures primarily include treatment and immunization of livestock. Veterinary vaccines are under development, and many countries are considering their use, due to the risk to human health and economic losses as a result of livestock infection. [62] Educational methods have been shown to reduce the consumption of wild watercress and other plants in areas with a high disease burden.

In some areas, special prevention and control programs exist or are planned. Like epidemiological, ecological and cultural. Strict control of the growth and sale of watercress and other edible aquatic plants is also important. Travelers to areas with poor sanitation should avoid food and water that may be contaminated. Vegetables grown in fields that have been irrigated with contaminated water should be cooked thoroughly, as should the offal of potentially infected animals.

Phytotherapy

The earliest records of the use of medicinal herbs for the treatment of parasitic diseases are found in the medicine of ancient civilizations.

For the treatment of fesciolosis and other intestinal parasites, phytotherapy can also be used successfully. The antiparasitic phytotherapeutic program contains numerous possibilities for successful treatment and prevention of alimentary parasitic diseases. , or in the form of capsules with oil and garlic. As a spice it can be added to many dishes. It can also be used in pet food to protect them from parasite infestations.

Pumpkin seeds can be used to cleanse the intestines of parasites. It is one of the powerful foods. They can be eaten raw, every morning on an empty stomach and during the day. From pumpkin seeds, you can make a drink Preparation: 50 grams of chopped pumpkin seeds are poured with a glass of water, sweetened with stevia. Pumpkin seeds can be ground and added to any dish as a spice

If pineapple is regularly included in the diet, it will help break down proteins in the digestive tract, which is the reason why it is equally effective for the treatment of plathelminth (tapeworms and flukes) as well as worms. A few pieces of raw pineapple a day will be enough to protect the digestive tract from many parasites. Coconut leaves are effective against parasites because they contain lauric acid, which the body uses to expel parasites. Raw cranberry juice is also able to improve the defense against parasites. Regular intake of this juice changes the Ph value in the intestines, and the acidity of the environment, helps eliminate parasites. Ren is an effective antiparasitic. Fresh grated rhubarb from which the juice can be squeezed, or a spoonful of fresh rhubarb can be added to the soup.

Our experience shows that the preparation, which is composed of 22 different medicinal plants, gives excellent results that can be used not only for treatment, but also for preventive purposes. One of these mixtures, which based on our experience gives excellent results, contains plants: shower, walnut leaf, anise seed, cluster, basil, cinnamon, green tea, cloves, wormwood, ginger, buckthorn, hay, buckthorn fruit, breccia fruit , chamomile, flaxseed, bekunis, celery root, honey, royal jelly, pollen, propolis.

Through many years of work on the treatment of diseases in a natural way (phytotherapy), it has been established that there is a cure for all diseases in nature. (64, 65). The path to healing with natural preparations is longer than the therapy offered by the official pharmaceutical industry and medicine, but with fewer side effects, without toxic damage to tissues, organs and without contraindications, of course if medicinal herbs are carefully selected and prepared appropriately. The preparation from the combination of these herbs should be drunk three times a day 20-30 minutes after a meal.

In the prevention of parasitosis in some areas of the world, special control programs exist or are planned. The types of control measures depend on the environment (they include epidemiological, environmental and cultural factors). Strict growth control and the sale of watercress and other edible aquatic plants are important. Individuals can protect themselves by not eating raw watercress and other aquatic plants, especially from endemic grazing areas. Travelers to areas with poor sanitation should avoid food and water that may be contaminated. Vegetables grown in the fields, which were irrigated with contaminated water, should be cooked thoroughly, as well as the offal of potentially infected animals. [68]

Therapy of fasciolosis in domestic animals

Drugs to control Fascioliasis in Animals

(Fasciolicides) belong to five main chemical groups: [61] Halogenated phenols: bithionol (Bitin), hexachlorophene (Bilevon), nitroxynil (Trodax) Salicylanilides: closantel (Flukiver, Supaverm, Frafaxine) Ranizol) Benzimidazoles: triclabendazole (Fasinex), albendazole (Vermitan, Valbazen), mebendazole (Telmin), luxabendazole (Fluxacur) Sulfonamides: chlorsulone (Ivomec Plus) Phenoxyalkans: diamfenetide (Corbendazine) (most effective) against adult and young flukes. However, long-term veterinary use of triclabendazole has caused resistance in F. hepaticae. From this, work began on the development of a new drug. Recently, a new fasciolicide has been successfully tested on naturally and experimentally infected cattle in Mexico. This new drug, called “Compound Alpha,” is chemically very similar to triclabendazole. [66]. It is applied by many countries in the world.

In the United States, only chlorsulone and albendazole are approved for use in the treatment of domestic animals, but fluocidides are also available and used worldwide. However, the list of available drugs has certain shortcomings. [56] Closantel, nitroxynil and oxyclozanide are not effective against young liver flukes and should only be used to treat subacute and chronic infections. Triclabendazole is effective against flukes of any age, but only those that cause acute infections; flukes that have remained in the body for a long time become resistant to this drug. [57] Treatment time is crucial for success, and is determined by environmental factors and analysis of the expected distribution and prevalence of the disease. For example, in European countries that have large numbers of sheep, computerized systems predict when fascioliasis will have the greatest impact on sheep populations and how many sheep will be affected. Predictions depend on and are related to predictions of when the environmental conditions that are most favorable for the reproduction of parasites will occur (the amount of rain, evapotranspiration and the ratio of wet and dry days in a given month). If severe infections are expected to occur, treatment of sheep should begin in September, then again in January and finally in April; the amount of fluke eggs released is minimal at this time, because they need a warm, wet environment, which makes the treatment more effective.

MATERIALS AND METHODOLOGY

In Montenegro, at the Clinic of Infectious Diseases in Podgorica, two cases of human fascioliasis were diagnosed. In 2005, a 56-year-old patient from Pljevlja (north of Montenegro), who was suspected of acute viral hepatitis due to fever, icterus, pain in upper right abdomen, was referred to the Clinic. During 2017another case of fasciolosis was diagnosed at the same Clinic in the boy, aged 9 years. He was admitted due to gastrointestinal problems, weight loss, abdominal cramps, especially in the lower abdomen with occasional false calls for defecation, alternating constipation / diarrhea, occasionally with the presence of small amounts of blood in the stool. A flat parasite, in the shape of a "butterfly", was discovered during the stool examination.

The Private Veterinary Clinic "Grandov" from Bijelo Polje, Montenegro,has been authorized to perform health inspections for two past years in the local slaughterhouse. The vetereniansaccidentaly found fascioliasis total of twelve animals: 5cases in cattle and 7 cases in sheep. None of the animals have shown symptoms of general health disorders during the ante - mortem examination. The age of the animals in which we found fasciolosis ranged from 3 to 12 years for cattle and from 3 to 5 years for sheep.

RESULTS

Fasciolosis in humans

The first patient diagnosed in 2005, aged 56, a farmer from the Plevalja, was referred to the Infectious Diseases Clinic in Podgorica on suspicion of hepatitis, due to the appearance of icterus, pain under the right costal arch, fever and weakness. In laboratory findings, moderate leukocytosis, anemia, severe eosinophilia, elevated total bilirubin (direct and indirect), elevated serum transaminases (AST, ALT) and alkaline phosphatase (AF) were found. Serological tests exclude a wide range of infectious agents, potential etiological factors, which could be the cause of these clinical and laboratory disorders (viral hepatitis, brucellosis, coxielosis, leptospirosis, trichinosis, leischmaniasis, babesiosis.) No parasitic eggs were found in the stool. Ultrasound of the abdomen showed moderate enlargement of the liver, without more detailed changes in the structure of the parenchyma, with pronounced hilus. In the gallbladder described thick, muddy contents, bile ducts easily dilated, without peculiarities. The patient performed serological tests for hepatitis fasciola, outside of our Institution due to the fact that we do not have these serological tests. The diagnosis was set up at the Institute for Infectious and Tropical Diseases in Milan, Italy, from where he returned with a positive enzyme-linked immunosorbent assay, finding specific antibodies to hepatitis fasciola in the diagnostic titer. In the same institution, the diagnosis was confirmed by an immuno-blood test. Treatment and controls were performed at the Clinic for Infectious Diseases in Podgorica with triclarbendazole, according to the recommended scheme with hepatoprotective therapy. After completing the therapeutic treatment, he continued treatment with antiparasitic phytotherapy, obtained from the private clinic "Zelenkada" in Bijelo Polje. After a year, he stopped reporting for check-ups, explaining that he felt well and healthy. Up to this day, immunological diagnosis of fasciolosis in Montenegro is not performing.

During 2017another patient, a 9-year-old boy, came to see the Clinic of Infectious Diseases in Podgorica, due to gastrointestinal problems that have lasted for one to two months, lower abdomen pain, occasional false calls to defecate, alternating constipation / diarrhea. In the stool, occasionally traces of blood. At one point, he noticed a flattened "butterfly-shaped" parasite in his stool, which his mother brought for inspection. (Figure 11). Morphological identification of parasites, performed at the Department of Biology, Faculty of Science in Podgorica, had revealed fasciola hepatica. Laboratory findings were within referent ranges, except for moderate eosinophilia. However, after the therapeutic treatment with tricarbendazole, eosinophilic count dropped to normal.

Figure 11: Flat Parasite in the form of a Butterfly Isolated from the Patient's Stool. The Parasite was Morphologically Identified as Fasciola Hepatica at the Department of Biology, Faculty of Science in Podgorica (Fotodocumentation of Prof dr B. Andric)

Fascioliasis in Domestic Animals

In Montenegro, as well as in the world, fascioliasis in domestic animals is a significant problem in both the breeding and slaughter industry because it can cause large-scale damages and economic losses. Damages and losses are related to deaths, treatment costs, reduced utilization of animals and their products. In our conditions, due to the lack of awareness of the need for regular deworming in domestic animals, fascioliasis is occasionally found as an accidental finding during the examination of animal bodies in slaughterhouses, and would probably be found more often if therewould be complete data or access at home slaughter, the slaughter of animals in households for personal use.

The changes found in the liver corresponded to the changes characteristic of the chronic form of fascioliasis in both cattle and sheep. (Figure 12 A, B, C).

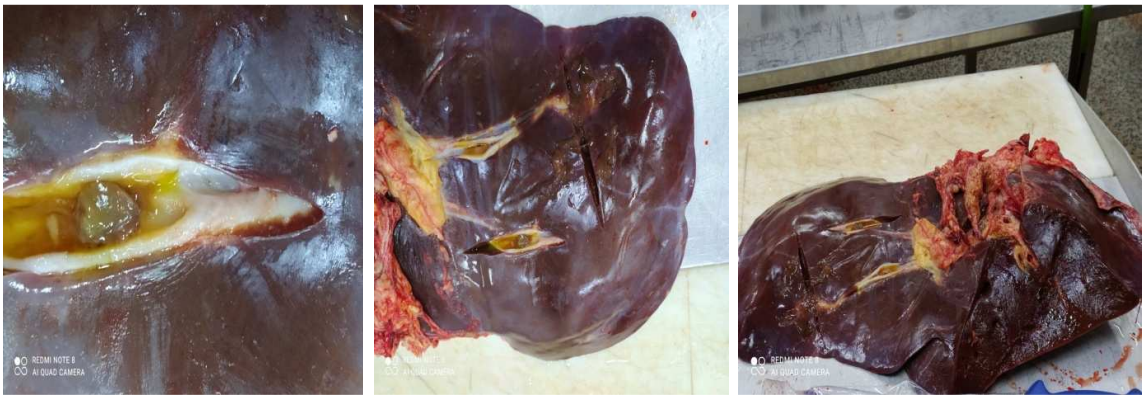


Figure 12: Fasciola Hepatica in the Bile Ducts of Cattle at the Slaughter Line. The Liver is Enlarged, Hyperemic with Bleeding below the Capsule and in the Parenchyma. Focal Yellow-Brown Foci about 2 cm in Size are Present, which protrude above the Surface (Fasciola Hepatica). Changes in the Bile Ducts are Characterized by a Chronic Inflammatory Process Accompanied by Swelling or Growth of Connective Tissue, which results in Thickening of the Bile Ducts. . (Thanks to Dr. Vet. Davor Korunić)

In mass invasions, fasciolas can also be found in mesenteric lymph nodes, kidneys and other organs in yellow-green necrotic nodules 5-10 mm in size. (Figure 13 A, B, C)

Figure 13: In Mass Invasions, Fasciolas can be Found in Mesenteric Lymphatics (B), and other organs in Yellow-Green Necrotic Nodules 5 - 10 mm in size (A, B). In Intensive Invasion, Hypertrophic or Atrophic Cirrhosis (C) often Occurs. (Thanks to Dr. Vet. Davor Korunić)

Hepatomegaly in cattle can be so severe that the weight of the organ reach up to 21 kg.

During a long-term chronic process, the affected parts of the liver atrophy, gaining a firm consistency and an uneven surface.

Autopsy: Dilated and thickened bile ducts protrude above the surface of the liver, resembling thick hard ropes. Calcium salt deposits in the walls (in cattle), as a result of which the bile ducts resemble thumb-thick tubes. On palpation of the organs, these deposits can be felt under the fingers and strong resistance and creaking can be felt by cutting. Bile in such altered channels resembles a fatty, greenish-brown fluid containing fascioles, their eggs, desquamated epithelium, erythrocytes and leukocytes.

In sheep, there is no calcification of the bile ducts. Intensive invasion often results in hypertrophic or atrophic cirrhosis.

This indicates the need for better education of pet owners, especially the need for regular deworming to protect their animals and thus have greater economic benefits, and reduce the risk of fascioliasis in humans, reduce the likelihood of parasite eggs spreading to the enviornment, and reduce the risk of closing the parasite development chain.

DISCUSSIONS

Our modest experiences with fascioliasis, which for many years was classified in the group of neglected tropical parasitic diseases, are presented (1, 69). For the last 20 years, this cosmopolitan zoonosis has been attracting attention (2, 3, 4). The incidence of human and animal fascioliasis is on the rise worldwide. Recently, global losses in animal productivity due to fascioliasis have been estimated at over $ 3.2 billion per year. [3] In human pathology, according to WHO estimates, 2.4 million people are infected with Fasciola, and another 180 million are at risk of this contagious disease. [4,5,6] Based on the above facts, it has been declared an important public health problem and the cause of large economic losses. In addition to its wide distribution and trend of further spread in the world, fascioliasis is a major diagnostic and therapeutic problem. (8,9,10,11)

About 50% of infected people have no symptoms of the disease (70). In manifest cases, the enigmatic polymorphism and multifocality of clinical manifestations as well as their changes during the evolution of the disease in the same patient, stand out.

Clinically, it is often not possible to distinguish fasciolosis from other diseases of the liver and bile, but also from many other infectious, neurological, psychiatric, hematological, and surgical diseases. In massive infections, supplemented by numerous possibilities of coinfections, atypical presentations and localizations of damage are common. Parasitological examinations of the stool are generally not adequate for diagnosis, because discrete and important clinical signs can be registered, long before the eggs are found in the stool, if this disease is thought of. Moreover, in many human infections, fluke eggs often cannot be found in the feces, even after multiple stool examinations. [3] Also, eggs of F. hepaticae, F. giganticae and Fasciolopsisbuski do not differ morphologically. [3].

Immunodiagnostic tests (Elisa, Immuno-blod and others) are a valuable help in the diagnosis of fascioliasis (45, 50, 57). They can detect infection with susceptibility above 90% during all stages of the disease, but in the absence of epidemiological data, a variety of clinical presentations, and a lack of immunodiagnostic tests, the disease is often overlooked. Immunodiagnostics in one of our cases was done abroad (in Italy). In the second case, it was not done because the fasciola hepatica from the stool was available for inspection and morphological identification.

Antibody concentration decreases rapidly after therapeutic treatment, and one year after treatment antibodies are not found, which is a good diagnostic / prognostic finding, with the established etiological diagnosis [11, 12]

If this severe parasitic disease is meant, human biochemical and hematological examinations

serum, will direct the diagnosis. Eosinophilia, elevated liver enzymes, are important for diagnosis. In vivo confirmation of elevated cysteine peptidase (11, 41), suggests its importance in immunosuppressive

modulation of the mechanisms used by the fasciola to affect activation / inactivation and function numerous immune cells, to defend the body. On mission models with eosinifil deficiency,infected with Schistosoma mansoni and Nippostrongulusbrasiliensis, high levels of eosinophils may play a role in tissue remodeling rather than as agents for parasite damage (57). The peritoneal cavity is thought to be a reservoir for eosinophils during helminth infection, and to they are not protective in the primary responses to parasitosis. The fact that most parasites are F. hepaticae present in the liver, means that the peritoneal cavity acts as a reservoir for inactivated eosinophils, which are distributed from there to other sites. We had blood in our two patients elevated eosinophil levels

In domestic animals, subclinical infections are even more frequent than in humans. How intensely the animals get sick is also shown by the data of slaughterers that 20-70% of slaughtered sheep and 10-40% of slaughtered cattle (cows) register the presence of this parasite. Our experiences are modest, for making such conclusions, but the fact is that we years registered fascioliasis in 5 cattle and 7 sheep. All ante mortem animals did not show symptoms of the disease, ie disorders of general health, and during slaughter, the changes found in the liver corresponded to the changes characteristic of the chronic form of fascioliasis.

In relation to therapy, it has been shown that a major modern problem is the resistance of parasites to the almost effective drug Triclarbendazole (59 - 62) and that intensive search for new anthelmintics.

For the treatment of fesciolahepaticae and other intestinal parasites, phytotherapy (68), which we have forgotten about, can provide valuable help in therapy and prevention, and the time has come to remind ourselves of it as well. The antiparasitic phytotherapy program contains numerous possibilities for successful treatment and prevention of parasitic diseases, some of which we have listed (63 - 67). diseases. The path to healing with natural herbal preparations is longer compared to official pharmaceutical products, but without side effects, and toxic damage to tissues, organs and without

contraindications, of course if medicinal herbs are expertly selected and prepared in an adequate way. Natural preparations for the treatment of flukes, ie flatworms, give good results. By combining several types of medicinal herbs, even better results were achieved. Our patients were treated with Triccarbendazole and phytotherapy, with satisfactory results. (68)

CONCLUSIONS

Parasitic diseases, especially flukes, are widespread throughout the world. The problem is the fact that human and veterinary medicine, for now, are not able to monitor and influence these severe systemic diseases, in terms of diagnosis, therapy and prevention. On the other hand, the WHO warns that they represent an important and growing public health and economic problem. In this paper, we have shown that fascioliasis is a problem in our environment as well. Despite the fact that there are a small number of registered cases in human and veterinary pathology, the stated facts should be a warning. To think about this severe parasitosis and make efforts to improve the diagnosis and therapy.

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