

ORIGINAL ARTICLE

VARIATION OF CALCIUM DISTRIBUTION IN DIFFERENT ORGANS OF LIVER FLUKE *FASCIOLA HEPATICA*

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Summary

Introduction: Livestock, including sheep, are infected with many types of parasitic diseases, and among these diseases is fascioliasis, or the so-called liver rot, which is caused by the *Fasciola hepatica* worm. This disease is widespread in many regions of the world, especially those with moderate climatic conditions. This disease harms the state's economy and livestock breeders due to the high rates of infection, which greatly affects animal production.

Materials: Wax sections were made from *F. hepatica*, cut with a thickness of 7 microns, and stained with Hematoxylin and Eosin stain and Von Kossa technique to investigate the calcium sites in different organs of the worm.

Results: There was a clear discrepancy in the density of calcium present in several internal organs of *F. hepatica*.

Conclusion: We infer from this research that there is a clear variation in the density of calcium as well as the size and shape of the calcareous corpuscles in their different locations in the worm's organs.

Key words: parasites; Fasciola hepatica; calcium; minerals; Calcareous corpuscles

Introduction

Livestock, including sheep, are infected with many types of parasitic diseases, and among these diseases is fascioliasis, or the so-called liver rot, which is caused by the *F. hepatica* worm. This disease spreads in many regions of the world, especially those with moderate climatic conditions (1, 2, 3).

Some types of parasites are distinguished by their ability to accumulate minerals inside their bodies, and among these parasites are flatworms, *Platyhelminthes*, including *Cestodes* and *Trematodes* (4). Organic substances in the form of concentric rings may be formed and accumulated within the parasite, containing in their composition lipids. Proteins, polysaccharides, nucleic acids, and minerals work to support these assemblies by depositing them on these assemblies (5).

These minerals including calcium assemblies in *Taenia* may be known as calcareous corpuscles. These particles vary in size, shape, and diameter according to the parasite, as well as the difference in the parasite's species (6).

Opinions vary about the role played by these calcareous corpuscles inside the parasites' bodies, but it is believed that they have an important role as a buffer and as a storehouse of calcium and inorganic ions, which contribute to preserving the parasite's tissues from organic acids that result from the metabolism of the intermediate host or through its passage through the stomach of the final host (7, 8).

It has been shown through experimental research on the parasite *Taenia taeniaeformis* that these particles may be a storehouse of phosphate, where this element enters and exits the larvae of this parasite through passive transport. This mineral may precipitate depending on the varying environmental conditions (9). The calcareous particles may act as an excretory device, as they excrete metabolic waste as well as protect the parasite from calcification processes (10, 11). It also acts as a secondary messenger that contributes to controlling physiological processes such as reproduction, differentiation, and migration (12). The lime particles of the larvae of some parasites such as *T. saginata*, *Spirometra masonoides*, and *Diphyllobothrium latum* showed a difference in the main components of these particles, which include (Ca, Mg, P, CO₂) and secondary components (Al, B, Cu, Fe, Pb, Mn, Si, Sn, Na), the preparation of crystallization and the nature of the formed the absence of calcareous corpuscles in *Schistosoma japonicum* as compared to the other three species is the most notable of these differences (4).

There are several controversies and opinions about the formation of calcareous corpuscles in *Hymenolepis*, represented by various processes, including ossification of the nuclei of some cortical cells, then continuous deposition of minerals in concentric layers in a granular manner, and calcium deposition in "vacuoles" or "vesicles" of the cytoplasm in concentric rings (13, 14). By researching and investigating scientific research sites on this topic, we found a lack of research done in this field. Several investigations have been carried out with the results demonstrating the availability of calcium and calcareous corpuscles. The first to analyze the existence of calcium corpuscles in the tissues of two species of adult tapeworms, (15) while (16) was the first to discover calcium in the larval stage of *Echinococcus granulosus*. While (17) looked at calcium in verified the existence of calcareous corpuscles in the tissues of the goat liver worm *F. gigantica*.

Other studies followed, with their researchers discovering calcareous corpuscle localization in the tissues of the bovine liver worm (18-20) concluding the polymorphism of calcareous corpuscles in the tapeworm *Bothriocephalus acheilognathi*. Hence this study aimed to shed light on this topic by verifying the presence of these calcareous corpuscles and their locations within the tissues and organs of this parasitic worm.

Materials and Methods

The livers of *F. hepatica*-infected sheep were collected. To maintain the integrity and uprightness of the worm, it was isolated with a small brush, cleaned with 0.9 % physiological saline, placed between two glass slides, and secured with threads. They were placed in neutral 10 % formalin and then poured into paraffin wax molds and sliced longitudinally to a thickness of 7 µm using a microtome (Thermo-Fisher Company, UK). Hematoxylin-Eosin stain (Figure 1) and Von Kossa (Figure 2) techniques were used to stain the tissues slices. We used an optical microscope to detect calcium at different magnifications. Pictures were taken with a TOUPCAM camera. Colour intensity has been quantified using Image J software.

Results

It is noted that calcium is present in the form of small particles scattered inside the uterus, which is branched and in the form of many coils in the frontal area of the worm and front of the genital pore. It is filled with immature and mature eggs and is surrounded by a layer of smooth muscle and is embedded in the parenchymal tissue. The testis with many branches located in the back half of the worm and extending from each testis a vas deferens that fuses to open in the genital pore, which mediates the space between the oral and ventral suckers with the presence of calcium in the form of small particles scattered inside the testis. Calcium also appeared in the form of scattered minutes inside the ovary, which is characterized by its many branches and is surrounded by a layer of smooth muscle and contains mature vitelline cells. As for the tegumental syncytium surrounding the worm, from which the straight

and oblique muscles extend inward, a clear aggregation of relatively large calcium particles appeared, compared to the above-mentioned organs. Results have shown a difference in the accumulation of calcium between small particles and accumulations in the form of large particles of calcium scattered within the parenchyma tissue in which the internal organs of the worm are embedded and characterized by the presence of many vacuoles (Figure 1). As for the vitelline follicles scattered on both sides of the worm, a dense presence of calcium appeared in the form of small particles. While a very small amount of calcium was observed in the anterior organs of the worm (Figure 2).

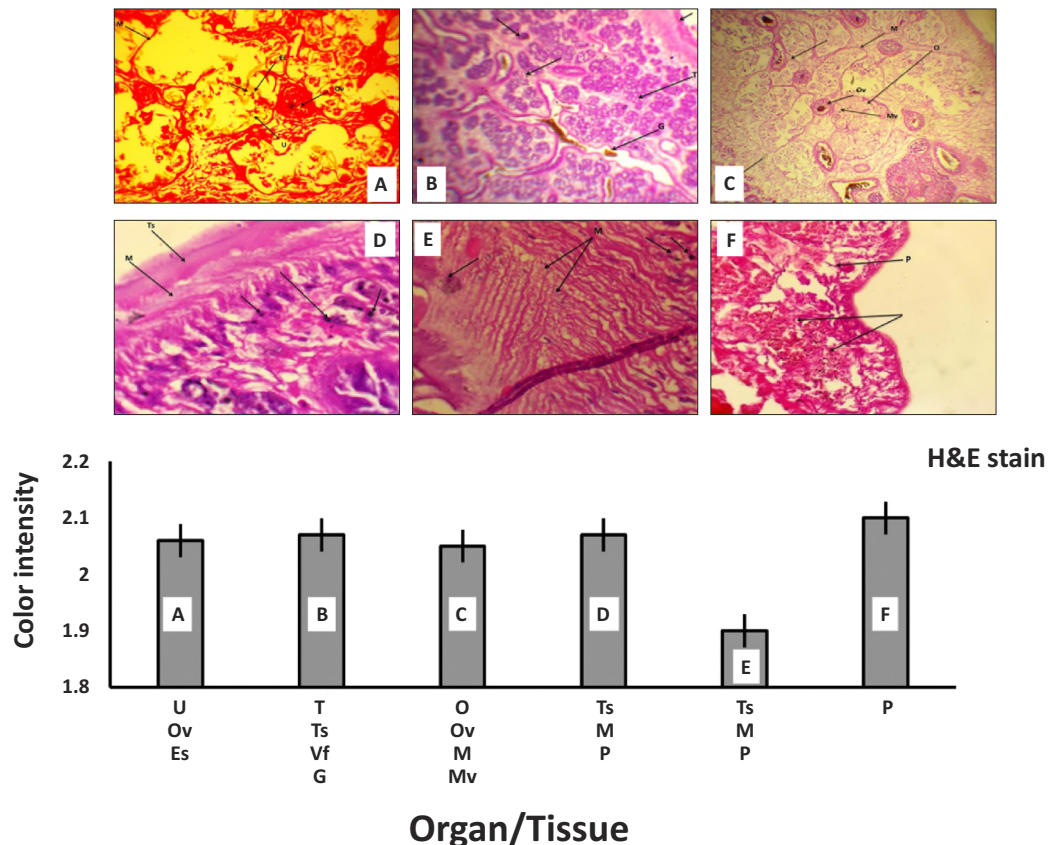


Figure 1. A representative image of section slices of different parts of the parasite. The histogram shows the color intensity of the stain which reciprocally represents calcium concentration. H-E= hematoxylin and eosin stain, U=uterus, Ov=Ovaries, Es= egg-shell, T= testis, Ts= Tegumental syncytium, Vf= vitelline follicles, G=Gut, O=Ovary, Ov=Ovum, M=Muscles surrounded the ovary, Mv=Mature vitelline cells, P=Paranchyma tissue.

Discussion

The results of the current study showed a variable present in the size, shape, and density of calcareous corpuscles in the *F. hepatica* tissues, including the reproductive and digestive organs, as well as the parenchymal tissue, and this indicates the role of these particles in regulating the vital functions of these tissues.

This is in line with the findings of Von-Brand and Weinbach 1965, who discovered that calcium distribution is variable in the different organs of several parasite larvae in different geographical locations of the world. The size, shape, and proportion of phosphate involved in the composition of these corpuscles were all different. Variation in feeding components, as well as the difference in parasite growth phases, were cited as reasons for this. The results of the current study showed the concentration of calcareous corpuscles play vital functions such as inorganic ion stores (8, 15, 18, 19, 20, 21)

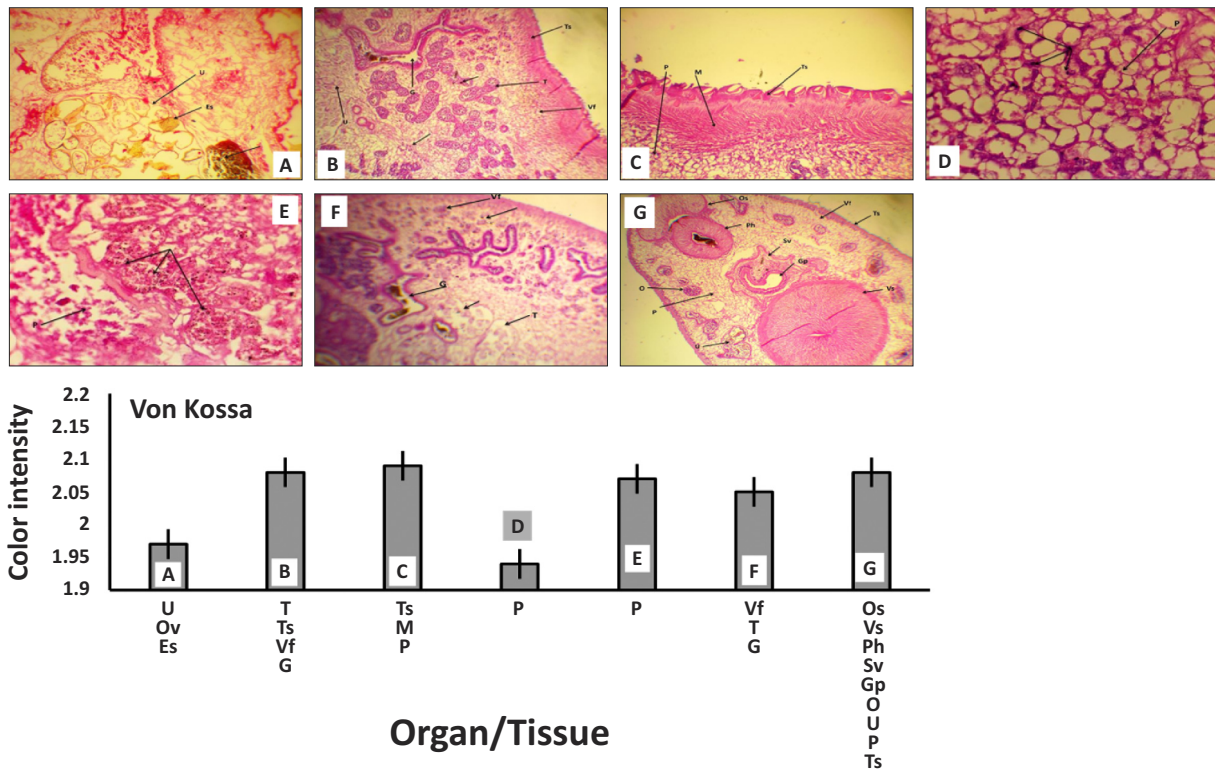


Figure 2. A representative image of section slices of different parts of the parasite. The histogram shows the color intensity of the stain which reciprocally represents calcium concentration. Von Kossa stain, U=uterus, Ov=Ovaries, Es= egg-shell, T= testis, Ts= Tegumental syncytium, Vf= vitelline follicles, G=Gut, O=Ovary, Ov=Ovum, M=Muscles surrounded the ovary, P=Paranchyma tissue, Os= oral sucker, Vs=ventral sucker, Ph=pharynx, Sv=seminal vesicle, Gp=genital pore.

However, these calcareous particles are beginning to form in the form of organic assemblages (lipoprotein) that fuse into parenchymal cells, according to a histochemical study of *Mesocostoides vogae*. Then there's an accumulation of organic and inorganic crystalline elements in the form of center circles, which leads to the production of brittle calcareous bodies. But the calcareous corpuscles may play a vital function as inorganic ion stores or depots in all of these compartments (8, 21). Depending on the parasite, differences in the mineral components of calcareous corpuscles were identified (9).

Calcareous corpuscles are frequently uneven in shape, transparent or granular, and vary widely in size, and the scolex has smaller corpuscles than the pregravid proglottid in *Hymenolepis microstoma* (16-20, 22), this is what we found from a lack of calcium in the oral cavity, pharynx, and anterior organs of the worm, which is assumed to be related to metabolism and/ or storage in the area. These corpuscles are assumed to play a significant role in the healing processes that the embryos go through at different phases of development in the early and late post-embryonic stages. Phospholipids are hypothesized to play Ca^{2+} and other ions transport, deposition, and solidification (22) while (14, 15, 17, 18, 19, 20, 23, 24) all of them found that the calcareous corpuscles are of cellular origin and vary in shape between an irregular spherical shape (16-19, 22-25). It can also be ovoid and come in a variety of sizes (26).

Calcareous corpuscles come in a variety of forms and sizes, the current study's findings revealed a formal multiplicity of calcareous corpuscles in this regard. This might be the outcome of metabolic processes and tissue physiology, as well as the nature of the content of its important component, and it could be varied depending on what is required by its tissue physiology and the parasite's adaptability and development. It varies according to the host or environment, so the ventral suckers and reproductive organs appeared to have distinct calcareous corpuscles availability, which highlights the significance of minerals present in such active organs, assuming calcium plays a key role in the biological parasites (17-20, 26, 27).

These calcareous corpuscles can be seen in the parenchymal cells of the larval and adult tapeworms and can be utilized as a diagnostic marker of tegument cestodes containing polysaccharides in it, such as potassium, sodium, magnesium, calcium, phosphate, and sulfate (28). The musculature system of *F. hepatica* is made of two layers and is surrounded by non-striated muscles. The outer layer is a circular layer with a thick layer of longitudinal muscle beneath it (Figure 1). Muscle contraction is fundamental in several aspects of worm movement physiology, and the muscles under the tegument depend on calcium for their contraction (17-20, 29), therefore, it's apparent that these corpuscles have a variety of roles, including serving as a source of reserve nutrients for the embryo and protecting the worm from the digestive juices of the final host's infective. Because they supply buffer anions, they may help to balance the acidic effect of carbon dioxide accumulation in worm tissue (30), this is what we found in our study of the abundance of calcium in large and clear sizes in the parenchymal tissue of the *F.* worm. The activity of calcareous corpuscles varies substantially depending on the parasite. In nematodes, these calcareous corpuscles may have two functions: the embryo shields the worm against the digestive juice of the definitive host and stabilizes metabolic waste products (7), and It could potentially be used as a source of insulating anions to prevent corrosion if the carbon dioxide buildup in tissues has an acidic effect (31).

The position of calcium in the oral sucker, pharynx, genital pores, and tegument of *F. gigantica* (CaBP4) was revealed by an immunohistochemical investigation of calcium-binding proteins in *F. gigantica* (CaBP4), these findings add to the growing body of evidence suggesting calcium-binding proteins play a key role in the parasite-host connection, they also play an immune role through the formation of calcium-associated antigens, calcium-binding proteins have been discovered in helminth species including *F. hepatica* (32), *F. gigantica* (33), *S. mansoni* (34), *Clonorchis Sinensis* (35), *Echinococcus multilocularis* (36, 37).

The fasciola's tegument serves a range of roles, including structural support, transmembrane osmosis regulation, nutrition uptake, signal transmission, and immunological modulation (38, 39).

Calcium signaling is critical in all eukaryotes, and recent findings of novel calcium-binding proteins in liver flukes (and similar trematodes) imply that this group of animals may have calcium signaling processes that are unique to them. If that's the case, this may contribute to the opening of extensive horizons to develop new antihelminthic medications (40).

The three main groups of calcium-binding proteins discovered in *F. hepatica* are the FH8 family, the calmodulin family (FhCaM1, FhCaM2, and FhCaM3), and the EFhand/dynein light chain family (FH22, FhCaBP3, and FhCaBP4). These chemicals' sequencing, predicted structures, and biological properties have all been thoroughly investigated. The next stage is to determine how they function within the body (41, 42).

The predicted amino acid sequence was 96.3 percent identical to that of *F. hepatica* Fh22CBP. During development in the mammalian host, FgCaBP1 RNA was identified in metacercariae, juveniles, and adults, and was entirely limited to the tegumental cell bodies (43). The tegument is closely linked to the host tissues and responsible for a variety of functions, such as substance synthesis and secretion, nutrition absorption, osmoregulation, and defense against host enzymes (40). Densely packed posteriorly pointed spines scattered throughout the tegument keep the fluke in place within the tissues and bile duct. By eroding the epithelium and puncturing microscopic blood arteries, the spines also aid the blood-feeding adults in their eating (40). The shed tegumental coat likely gets into the bloodstream, and anti-tegumental antibodies have been discovered in the serum of infected animals (43).

The liver fluke, *F. hepatica*, is a trematode that causes fasciolosis in ruminants and humans. *F. hepatica*'s outer tegumental coat (FhTeg) is a complex metabolically active biological matrix that is constantly exposed to the host immune system of the host, making it a good candidate for vaccine development. *F. hepatica*'s tegumental coat is extensively glycosylated, and immunogenic oligosaccharide motifs and glycoproteins are produced by helminths, such as proteases, protease inhibitors, and paramyosin, are currently being investigated as prospective vaccine candidates with known immune-modulatory capabilities (44).

The liver fluke's surface tegument *F. hepatica* is a syncytial cytoplasmic layer delimited on the outside by a plasma membrane and coated on the inside by a glycocalyx that serves as the parasite's interaction with its ruminant host.

During the fluke's lengthy migration from the gut lumen through the peritoneal cavity and liver parenchyma to the lumen of the bile duct, the tegument's contact with the immune system plays a vital role in the fluke's establishment or removal. Tegument surface proteins and secretions, on other hand, are little understood (45).

A glycocalyx protects the tegument's outermost surface, which is mostly made up of glycoconjugates (46). Parasitic helminths' glycoproteins and glycolipids frequently contain a combination of oligosaccharide motifs that are similar or identical to those found in their hosts, as well as structurally unique, pathogen-specific patterns (47). By interacting with C-type lectin receptors, these glycoconjugates can play a major role in the parasite's immunoregulatory action. C-type lectin receptors (CLRs) interact with the parasite (CLRs), those that are expected to play critical roles at the parasite-host interface have been discovered by the FhTeg (48). Even though calcareous corpuscles are found everywhere, most notably in parenchyma and nematodes, their function is still a mystery. Even though its function remains a mystery, using X-ray diffraction and infrared light, it was determined that calcium carbonate, monohydrocalcite, is the primary mineral component of calcareous corpuscles. This piques curiosity concerning the role of calcareous particles and biomineralization mechanisms in these organisms (47-49).

Conclusion

The current study suggests that further research be done on parasitic worms' calcareous particles in general, as well as at different stages of their larval and adult lives, to gain a better knowledge of their function and ascertain the nature of their mechanisms. Of course, the answer to this question is of considerable scientific relevance, and it may be viewed in a variety of ways as a key component in the development of pharmacological therapeutic techniques for a variety of diseases produced by these larval and adult stages.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Adherence to Ethical Standards

Not applicable.

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