PARASITISM INFLUENCE UPON THE PLASMATIC HAEMOSTASIS INDEXES IN CATTLE

Galina Melnic

Institute of Zoology of Academy of Sciences of Moldova
str. Academiei 1, or. Chişinău, MD – 2028, Republica Moldova

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Abstract: The accomplished research show that the acute alteration of plasmatic haemostasis does not exceed the pathologic conditions of parasitosis. The results of laboratory parasitologic investigations established an invasion extensity with *Eimeria* spp. of 68%, *S. papillosus* – 62%, *D. lanceolatum* – 29%, *E. granulosus larvae* – 45%.

The parasitic association between *S. papillosus* and *D. lanceolatum* induce in the host organism the diminishing of prothrombin index caused by gastrointestinal diseases and by hepatic injuries these modifications, at their turn, perturb the hepatic synthesis of the proteins, including coagulation factors, thus, inducing the deficiency of vit. K, that is necessary for the hepatocytes for prothrombin gama-carboxylation, as well as of factors III, IX and I. Fibrinogen increasing would indicate an acute stage of parasitic and infectious inflammations. The above mentioned modifications are the result of allergic processes, of tissular destruction and degradation, appeared as a consequence of mechanical and rapacious action produced by parasites and bacteria.

INTRODUCTION

In the process of parasitosis development in the animal organism complex correlation are creating between the parasite and the host that become apparent through morphologic-functional modifications in body organs and systems [19].

The quantity blood lost by the host organism doesn’t consist only in the blood ingested by parasites, although this quantity is not negligible. An important blood quantity is lost by hemorrhages caused by the traumatism, which take place during the parasite nutrition. They render mechanical, irritative and toxic-chemical actions, including through hystamine, hialuronidasis and other protein enzymes, and cease the blood coagulation [18].

The parasites act toxically continuously during all the parasitation period, as well as after their extermination. The exotoxins represent the excretions and the secretions and all the metabolism products during the infection. After the parasites death the endotoxins results, which act pathogenically upon the host organism. The substances inoculated by parasite pricks also act pathogenically. Among these substances there can be mentioned the anticoagulants from parasite saliva [21].

The penetration of tegument, of gastrointestinal mucous, of small blood vessel walls and of different tissues by the parasite larvae is realized by mechanical action, more or less expressed, as well as by chemical action of the larvae, using the hialuronidase secreted by them. However, not only the larvae secrete enzymes, the adult parasites also secrete hialuronidase that has a noxious influence upon the organism during the entire infection period [18].

The haemostatic complex represent the integrity of morphologic-functional and biochemical mechanisms. The importance of hemostasis consist in maintenance of blood
volume, of blood pressure and of blood flow through a harmed vessel, the final purpose being the maintenance of fluid-coagulant equilibrium [22].

There are many various pathologies associated with haemostasis alteration, their clinical manifestation consist in a hemorrhagic or thrombotic syndrome of different degrees or consist only in a pathologic modification of the special laboratory samples. In a healthy organism, in the absence of any pathological action, the blood fluidity is maintained by the equilibrium between the factors of coagulation and anticoagulation processes [17].

After any kind of vascular lesion the sequential events released with haemostatic aim include the following actions: local vessel constriction, activation of coagulation, forming of the blood thrombus (fibrin), retraction and lysis of thrombus (repairing of vascular ductus). The disequilibrium between the haemostasis phases leads to hemorrhages or thromboses with clinical or underclinical manifestations [3].

The main algorithm of the plasmatic haemostasis includes: Prothrombin Index (IP), Time of recalcificated-T. Howwell plasma coagulation (THw), Time of partially activated thromboplastine (TTPa), thrombin time (TT), Fibrinogen [20].

The Prothrombin Index characterized the Its stage (prothrombin forming) and stage II (thrombin forming) of plasmatic haemostasis and represent a method of coagulation factor exploration from the extrinsic pathway (VII, X, V, II) and of common pathway that constitute the prothrombin complex. The diminution of prothrombin index can be observed in the congenital and acquired deficiencies of prothrombin complex factors (II, VII, X), all of them synthesized in the liver under the indispensable presence of vitamin K. The K hypovitaminosis appears because of insufficient synthesis or absorption (sterilization of intestinal flora after antibiotic use, malabsorption, the deficiency of bile or of pancreatic juice in intestine etc.); in primary or secondary fibrinolysis results the exaggerate consumption of these factors; the cumulative anticoagulants competitively suppress the vitamin K; severe or chronic hepatocellular lesions affect the capacity of hepatic synthesis of the proteins, including the synthesis of coagulation factors II, VII, X, V [11].

The deficiency of vitamin K appears as a result of reduced alimentary contribution, or by the reduction of microbial normal intestinal flora caused by gastrointestinal affections, being well known the fact that the main source of vitamin is the intestinal microbial synthesis [1].

Vitamin K is necessary to the liver cells for prothrombin gamma – carboxilation, to factors III, IX and I and of protein C and S. The absence of this final synthesis result that these factors remain functionally inactive, although they continue to produce and remain in blood in a sufficient quantity. The main symptoms of K hypovitaminosis are the hemorrhages, the prothrombin time last longer than TTPa [23].

The malabsorption induced by some parasitosis of vitamins and mineral elements provoke serous malfunctions of the metabolism. Along with the general clinical manifestation, the pathologies show specific symptoms which are characteristic for the insufficiency of each element [12].

THw – is an index of general orientation and it is equivalent with the determination of blood coagulation time, a test of global intrinsic coagulability and of the coagulation stage where all the plasmatic factors are involved (except factor VII) and the cellular factors, thus appreciating the plasmatic and cellular haemostatic activity. THw of long duration is significant in the cases of deep alterations of fluid-coagulant equilibrium, trombocitopeny and anticoagulants excess in blood [4].

TTPa – is the test that exclusively elucidates the defect of plasmatic factors, which participate at the internal mechanism of thromboplastin formation. It is a test of global plasmatic
coagulation that involve in its development all the plasmatic factors and the factors of common way of coagulation. Its long duration is significant and marks the global deficit of plasmatic factors, by appearance of these factor inhibitors, as well as to the anticoagulant excess in the plasma [3].

TT represent the time necessary for the fibrin thrombus genesis in the plasma by adding the thrombin. It is dependent on the fibrinogen concentration and on the thrombin inhibitors activity (antithrombin III, heparin, paraproteins); it is used at the reevaluation of blood coagulation stage III – fibrin and content of natural and pathologic anticoagulant creation. Prolongation of TT reveal a fibrinogen anomaly, the presence of antibodies in thrombin, the presence of paraproteins in plasma, which prevent the fibrin monomer polymerization, the presence of exceeded antithrombin, severe hepatitis [6].

The fibrinogen (factor II) is a protein, it is synthesized mostly in the liver. In the blood it is in soluble state, but as result of fermentative process under the action of thrombin and of factor XIII it turns in unsolvable fibrin. The fibrinogen is an indicator of severe stage of inflammations of infectious, parasitic allergenic origin and alteration of tisular integrity. The hyperfibrinogenemy proves the existence of infection, inflammation or the appearance of malign cells in the blood. The diminution of fibrinogen content – in hypofibrinogenemias, of the liver chronic pathologies, cirrhosis, uterine-placental hemorrhages, at primary and secondary fibrinolisis with fibrinolitic products accumulation in blood, blockage of fibrinogen coagulation under the action of fibrin [10].

Lungs are the source of tissular thromboplastin, of factors and activators of anticoagulant system. The alveolar mastocytes produce 70%-90% of heparin in the circulating blood, they actively synthesize the prostaglandins, metabolize the fibrinogen and take away the products of fibrin degradations from the plasma [8].

Although the studies concerning the deep implications on the morphophysiology of the host organism, including at the level of metabolism, chemistry, blood elements, components of the immune system, are rather ample, nevertheless, in the special scientific there are no data regarding the investigations in haemostatic indexes at cows, especially at infected animals.

MATERIALS AND METHODS

The basis of the studies was the methodology of plasmatic haemostasis basic algorithm exploration with normal results at the cows from the control group, practically healthy and the interpretation of the abnormal results at mono- and poliparasitated animals.

For the accomplishment of these tasks there were selected calf of 6-8 months old from breed Holstein and distributed in 5 groups: group I consisted of non-infected calf and was the control group. Group II were cows infected with S. papillosus. Group III were animals infected with D. lanceolatum. Group IV were calf poliparasitated with S. papillosus and D. lanceolatum. Group V were cattle infected with Eimeria spp. S. papillosus, D. lanceolatum and E. granulosus larvae.

The studies were accomplished in the Parasitology and Helminthology laboratory of Institute of Zoology of A.S.M. and in the diagnostic clinic laboratory of University Clinic of Family Medicine Aid of State University of Medical and Pharmaceutical „N. Testemițanu“. To obtain the experimental material samples were collected from the farm of Colonița village, Chișinău city, from cattle kepeed in stabulation and the invasion intensity (II) and invasion extensity (IE) was determined by coprologic analyses.
The studies were based on the scientific elaboration and argumentation reported in the papers of well known scientists in the field of plasmatic haemostasis physiology and pathology: Enache F., Ellison N., Barcagan Z.S., Baluda V.P., Zubairov D.M., Mamot A.P., Menișicova V.V. and other.

The coprologic analyses were accomplished after the traditional methods of Popova, Baermann, Fulleborn, Darling, and by successive wash in the Parasitology and Helminthology laboratory of Institute of Zoology of A.S.M. The sample collection was accomplished individually and by groups of three samples in different periods of the day.

The prothrombin level determination was accomplished after method of Quik. It is a method of coagulation factor exploration from the extrinsic way (VII, X, V, II) and of the common way that constitute the prothrombin complex. It is the time of recalcification realized in the presence of tissular thromboplastin excess (III). The analysis express the prothrombin activity in percents, determined after the calibration chart, build on the basis of measurement of prothrombinic time in the diluted solution of normal plasma [14].

The test aim is: creation of exogenous thromboplastin from the interaction of tissular thromboplastin factors with plasmatic factors VII, X, V and ionic Calcium, thrombin synthesis from the test plasma prothrombin, fibrin forming, consecutively to thrombin action.

The prothrombin index express the coagulation time in seconds of the test plasma divided with that of normal plasma, considered as 100% [15].

The coagulation time of recalcification plasma (T. Howwll – THw) shows the coagulation time after the recalcification of citrated plasma, reach in placoid cellular (trombocites). It is a test of intrinsic global coagulability and of the coagulation phase where all plasmatic and cellular factors are involved, and, thus, evaluate the haemostatic, plasmatic activities and the cellular haemostatic function [2].

The test of partial activated thrombinic time is based on the recalcification of placoidless cellular plasma, in the presence of erytrofosfatin (substitute of placoid cellular) and of coalin (standardized activation of F.XII). Therefore the intrinsic global coagulability and the final coagulation phase is exploited (XII, XI, IX, VIII, X, V, II, I). F. VII and F.XIII don’t evaluate [9].

The thrombin time is a simple test that gives valuable information on fibrinogenesis. In presence of a standard quantity of thrombin normal plasma with a normal fibrinogen level is coagulating in a defined and constant time period. The test consists in determining of necessary time for decalcified plasma coagulation after adding an excess of thrombin [7]. The prolongation reveal: hypofibrinogens, use of streptokinase, urokinase, aspariginase, excess of antithrombins in blood and of fibrin degradation products, in some fibrinogen anomalies, severe liver affections [5].

The fibrinogen determination in plasma was accomplished according to gravimetric method (Rutberg method). The method principle consists in fibrinogen coagulation from plasma with adding citrate of Na, at adding Ca chloride, quick dryness and weight of fibrin thrombus. To accelerate the coagulation processes or when THw change significantly, we used the thrombin solution or the mixture of tromboplastin with Ca chloride. Determination of Ca was accomplished after the kinetic Arsenazo, (ELITECH, Franța). The hypocalcicemia can be observed in liver cirrhoses, hypoalibuminemin, atresia of bile ducts etc. [16].
The studies concerning the pathogenesis of secondary consequences of the parasitic invasions with strongiloides, dicrocelium, eimeris and echinococcus upon the animal organism are one of the main concern of the actual science.

The enlargement of the research possibilities of the pathogenic implications that specify these infections is indispensable connected to the elaboration and perfection of some methodological access modalities in laboratory test accomplishment, but also in the explanation of investigation result.

Furthermore, the clearing up of the support of physiologic-pathologic mechanisms in the accomplishment of biologic correlation between the invaded organism and the parasites, by which the evolution of pathologic processes is initiate, represent an objective of high theoretical and practical value for the veterinary medicine.

At the existence of hemoragipar syndrome many questions appear: if there exists a congenital deficiency, is it consequence of trauma, is it iatrogen, is it involve an immune reaction, if there exists a severe organo-systemic formation, if there exists a chronic organo-systemic affection?

The research results (tab. 1) indicate the decrease of prothrombin index by comparing with the control group at cows infected with strongiloides (group II) with 7,5% (p>0,05), at calf (group III) infected with dicrocelium – 10,7% (p>0,05), at the animals infected with strongiloides and dicrocelium (group IV) – 12,8% (p<0,05), at calf infected with eimeria, strongiloides, dicrocelium, echinococcus (group V) – decrease with 17,1% (p<0,05). The modifications of this index in group II are caused probably by K hypovitaminosis due to the impairment of intestinal absorption, as consequence of digestive disturbance provoked by strongiloides; in group III because of bile stasis in the liver and its insufficiency in the intestine hypovitaminosis K is induced, and this disturb the final phase of prothrombine complex factor synthesis; in group IV and V probably due of these deficiencies there were recorded: severe hepatocellular lesions that affect the capacity of hepatic protein synthesis, including the synthesis of coagulation factors II, VII, X, V as well as the K avitaminosis.

By comparing with noninfected cows, the $THw$ increasing can be met both in monoinvasions – 9,4% (p>0,05), and in polyinvasions – 11,7% (p>0,05), the possible cause would be the coagulation factor deficit (XII, XI, IX and VIII) and the presence of anticoagulants in blood, as well as of other coagulation inhibitors in hepatic, gastrointestinal, lung (parasitologic origin) affections.

$TTPa$ is greater than in the control group, at group II with 7,5% (p>0,05), in group III – 10,8% (p>0,05), and in group IV and V with the mean of 10,8% (p>0,05).
modifications are induced probably by the deficit of plasmatic factors appeared as a result of gastrointestinal disturbances, as well as the presence of these factor inhibitors to the anticoagulants (hialuronidase) exceed, both caused by parasites.

By comparing with the control group the tested TT is longer, in group II with 14,3% (p>0,05), in group III – 21,1% (p<0,05), in group IV – 16,7% (p>0,05), in group V – 21,3% (p<0,05), which fact assume the presence of thrombin inhibitors (heparin, degradation products of fibrin and of circulating pathologic anticoagulants), as well as of paraproteins induced probably by sophisticated mechanisms by the parasitic factor.

The laboratory studies that of fibrinogen content emphasize a growth of this index in group II with 16,5% (p>0,05), in group IV – 18,9% (p>0,05), in group V – 22,3% (p<0,05), but in group III decrease with 12,5% (p>0,05). The low fibrinogen level in group III is influenced by the perturbancies of protein metabolism mechanic icterus, by the deregulation of the final stage of prothrombin complex factor syntheses. The increased fibrinogen content in groups II, IV and V revels the persistence of inflammation processes (pneumonia, abscess), chronic hepatitis, conditioned by parasitoses.

The content of Ca++ ions is lower in all the groups by comparing with the control group: with 20,0% (P>0,05) – in group II and III; with 16,0% (p>0,05) – in group IV and with 24,0% (p>0,05) – in group V. The low level of Ca ions is caused by the gastrointestinal disturbances associated with diarrhea, maldigestion and malabsorption, induced by the parasitic factor.

From all the mentioned above we can see that the parasites by their toxic, chemical and mechanical activity induce deep changes in the plasmatic haemostasis, which occurs with severe deregulation of coagulation factors.

CONCLUSIONS

- The accomplished researches denote the fact that the severe alteration of the plasmatic haemostasis doesn’t exceed the pathological conditions of the parasitoses.
- The results of laboratory pathologic investigations emphasize and invasion with Eimeria spp. of 68%, S. papillosus – 62%, D. lanceolatum – 29%, E. granulosus larvae – 45%.
- Strongiloidosis in our case is characterized by the diminution of prothrombin index caused probably by the gastrointestinal deregulation followed by the protein K vitamin and Ca malabsorption, and the fibrinogen increasing is an indicator of the acute stage of the inflammation process as well as of tissular destruction and its degradation, induced by strongiloides.
- Dicroceliosis appeared by the decreasing of prothrombin and fibrinogen indexes which is due to the incapacity of hepatocytes to use vit. K, and the bile stasis in the liver and its insufficiency in the intestine induce K hypovitaminosis. This one perturbs the final stage of prothrombin complex factor synthesis.
- The parasitic association between S. papillosus and D. lanceolatum provoke in the host organism the decreasing of prothrombin index because of the gastrointestinal affections and hepatic lesions, and these modification their turn deregulate the hepatic protein synthesis, including of coagulation factors, thus inducing K vitamin insufficiency, that is necessary to hepatocytes for gamma-carboxilation of prothrombin, as well as of factors III, IX and I. The increasing of fibrinogen would indicate the severe phase of parasitic and infectious inflammation. The appropriate
modifications are associated with allergies, tissue destruction and degradation appeared as a result of mechanical and spoil activity of parasites and bacteria.

- The gastrointestinal and hepatic-bile deregulations are deeper, if the polyparasite structure is richer (*Eimeria spp. S. papillosus, D. lanceolatum, E. granulosus larvae*), and the modifications of plasmatic haemostasis index are more emphasized by comparing with other animal groups.

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