ORIGINAL ARTICLE

MACROMICROSCOPIC ARGUMENTATION OF THE PATHOGENETIC SCENARIO OF BABESIOSIS IN THE COORDINATE SYSTEM «PATHOGEN-CARRIER-RESERVOIR»

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ABSTRACT
The aim is to get a thorough argument for the babesiosis pathogenetic scenario in the coordinate system «pathogen (*Babesia spp.*) – carrier (ticks of the *Ixodoidea* superfamily of the *Ixodidea* family) – reservoir (a susceptible organism)» with the emphasis on the epizootic/epidemic role of the carrier.

Materials and methods: The macromicroscopic method of research was used in order to maximize the clarification of the babesiosis scenario, its pathogenetic links, the connection of the latter with attacks of active stages of ixodes ticks, types of circulation of ontogenetic forms of *Babesia spp.* in the body of carriers and their inoculation of the pathogen into an organism susceptible to it. The use of this method helped to strengthen the diagnostic potential of the study, and increase the reliability of the results obtained. Taking this into consideration it was focused on the epizootological/epidemiological aspects of babesiosis, the role and significance of the most vulnerable epizootic link – Ixodes ticks on the body of the vertebrate provider (mammal), poikilomorphism, anisomorphy.

The study of the monolithic idiosome and ticks salivary glands were carried out on activated (capable of attack) female individuals aged 2-3 months after molting. Ticks were dissected in a cool (t=4°C) Ringer's saline solution for arachnids. Ticks and prepared salivary glands were fixed in 12% formalin solution on 0.1 M phosphate buffer (pH=7.0-7.2) at t=4°C for 3 hours, washed with the buffer, and fixed again for 1 hour (t=4°C). To achieve tonicity, sucrose was added to the fixatives and the washing medium. Dehydration occurred due to a battery of alcohols of increasing concentration and absolute acetone. Microspecimens stained with hematoxylin and eosin were studied using an Olympus BX-41 microscope (Japan).

Results: Implementation of the leading stages of the babesiosis pathogenetic scenario is focused on the coordinate system «pathogen (*Babesia spp.*) – carrier (ticks of the *Ixodoidea* superfamily of the *Ixodidea* family) – reservoir (a susceptible organism)» in which carrier take the leading place.

The macromicroscopic specificity of the structure of the ticks (variability: ability to aniso-, poikilomorphism) is an evidence-based criterion for pathogens inoculation to the macroorganism of warm-blooded vertebrates. It determines the features of circulation and organ/cellular locations of *Babesia spp.* (intestines and its epithelium, hemolymph, gonads, salivary glands).

The species belonging of warm blooded vertebrates susceptible to babesiosis pathogens correlates with the species belonging of ticks and determines the tropicity of the latter. The simultaneous implementation of a complex of research procedures with the tick biological material samples is problematic taking into account the physical lack of material, which requires researchers to re-orient the diagnostic vector towards the use of additional methods for babesiosis diagnosing, including in vitro ones.

Conclusions: In the pathogenetic scenario of babesiosis, the carrier (Ixodes ticks) is the central figure in the epidemic/epizootic coordinate system.

KEY WORDS: babesiosis, pathogenetic scenario, epizootic/epidemic coordinate system, Ixodes ticks

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INTRODUCTION
Ontogenesis of *Babesia spp.* is impossible without the participation of biological vectors – Ixodes ticks [1]. Infection of the latter occurs in the case of ingestion of pathogens with the blood of an animal or a parasite carrier [2].

In the ticks organs there are separate stages of circulation of various ontogenetic forms of *Babesia spp.*, and it, in the end, leads to the entry of the latter into the gonads and salivary glands. Merogonia with the subsequent formation of merozoites, which are invasive to a susceptible vertebrate host, are extremely dangerous phenomena that objectively cause infection of the host with *Babesia spp.* pathogens by inoculation into the blood of merozoites with the tick saliva [3, 4]. However, the pathogenic effect of pathogens begins from the moment the latter enter a susceptible organism. Its skin is the first to face the attack of *Ixodes* and responds with a cascade of structural and functional changes. It was observed not only skin damage, but also affection the lymph nodes, internal organs lymphoid formations, liver, spleen etc. They are evidence-based criteria for inoculating pathogens into the body of susceptible vertebrates [5, 6].

The delayed observation period thoroughly demonstrates the presence of *Babesia spp.* in red blood cells [4, 7]. Intensive destruction of the latter, enhanced by the toxicity of accumulated metabolic products of protozoans, contributes to the launch of mechanisms for reproducing clinical prototypes of babesiosis. Their full comprehension, the introduction of effective therapeutic measures in relation to them, and

Table 1. The number of collected ticks (n=162) the Ixodidea family (in absolute and relative indicators, %) according to the species and groups of providers

Provider	The number of ticks of the *Ixodidea* family (in absolute and relative indicators)	
Species of animals	**Species of ticks: *Ixodes ricinus, Ixodes persulcatus* (n=34)**	**Species of ticks: *Dermacentor marginatus, Dermacentor pictus* (n=128)**
Cattle *(Bos taurus taurus)*	34 (20.99 %)	–
Horses (*Equus caballus*)	–	21(12.96 %)
Domestic dogs (*Canis familiaris*)	–	107 (66.05 %)

Table 2. The shape variability (poikilomorphism) of the monolithic idiosome of ticks of the Ixodidea family (n=162), the number of samples with the biological material (in absolute and relative indicators)

The number of ticks of the *Ixodidea* family	Poikilomorphism of the monolithic idiosome of ticks of the *Ixodidea* family					
Species of ticks	***Ixodes ricinus, Ixodes persulcatus* (n=34)**			***Dermacentor marginatus, Dermacentor pictus* (n=128)**		
The form type (by number)	1	2	3	1	2	3
Absolute and relative indicators	27 (79.41%)	5 (14.71%)	2 (5.88%)	23 (17.97%)	31 (24.22%)	74 (57.81%)

Table 3. The size variability (anisomorphy) of the monolithic idiosome of ticks of the Ixodidea family (n=162), the number of samples with the biological material (in absolute indicators)

The number of ticks, their taxonomy	Anisomorphy of the monolithic idiosome of ticks of the *Ixodidea* family					
Species of ticks	***Ixodes ricinus, Ixodes persulcatus* (n=34)**			***Dermacentor marginatus, Dermacentor pictus* (n=128)**		
The form type (by number)	I	II	III	I	II	III
Absolute indicator, $\times 10^{-3}$m	2-5	3-5	12-15	3-5	6-10	11-16

prevention are impossible without structural and functional grounding of the moments associated with the ontogenesis of babesiosis pathogens exactly in the tick's body [8].

Therefore, in view of all the above, the importance of the macromicroscopic argumentation of the babesiosis pathogenetic process through the coordinate system «pathogen (*Babesia spp.*) – carrier (ticks of the *Ixodoidea* superfamily of the *Ixodidea* family) – reservoir (a susceptible organism)» with the emphasis on the epizootic/epidemic significance of the carrier is an obvious fact.

THE AIM

The aim is to get a thorough argument for the babesiosis pathogenetic scenario in the coordinate system «pathogen (*Babesia* spp.) – carrier (ticks of the *Ixodoidea* superfamily of the *Ixodidea* family) – reservoir (a susceptible organism)» with the emphasis on the epizootic/epidemic role of the carrier.

MATERIALS AND METHODS

The macromicroscopic method of research was used in order to maximize the clarification of the babesiosis scenario, its pathogenetic links, the connection of the latter with attacks of active stages of Ixodes ticks, types of circulation of ontogenetic forms of *Babesia* spp. in the body of carriers and their inoculation of the pathogen into an organism susceptible to it. The use of this method helped to strengthen the diagnostic potential of the study, and increase the reliability of the results obtained. Taking this into consideration it was focused on the epizootical/epidemical aspects of babesiosis, the role and significance of the most vulnerable epizootic link – Ixodes ticks (registration of the number (table 1) on the body of the vertebrate provider (mammal), poikilomorphism (table 2), anisomorphy (table 2) caused by the state of blood saturation, location in the organs/cells of the tick body.

At the same time, morphological features of the monolithic idiosome structure, potentially tropical to *Babesia* spp. organs of the ticks' body were studied.

The study of the monolithic idiosome and ticks salivary glands were carried out on activated (capable of attack) female individuals aged 2-3 months after molting. Ticks were dissected in a cool (t=4°C) Ringer's saline solution for arachnids. Ticks and prepared salivary glands were fixed in 12% formalin solution on 0.1 M phosphate buffer (pH=7.0-7.2) at t=4°C for 3 hours, washed with the buffer, and fixed again for 1 hour (t=4°C). To achieve tonicity, sucrose was added to the fixatives and the washing medium. Dehydration occurred due to a battery of alcohols of increasing concentration and absolute acetone.

Microspecimens stained with hematoxylin and eosin were studied using an Olympus BX-41 microscope (Japan).

RESULTS AND DISCUSSION

The analysis of poikilomorphism and anisomorphy (changes in the body shape and parameters of its size) of these invertebrate arthropods proved the heterogeneity of the latter. Depending on the phase of ontogenesis and blood saturation the body shape varied from flattened in the upper -lower direction to oval, rounded/spherical. The ticks with oval and rounded/spherical shapes had the quantitative advantage, which objectified the meaning of using these parameters as an undoubted macroscopic criterion for acarian danger. The size of the monolithic idiosome of bloodsuckers by groups varied from (2-5)$\times10^{-3}$m to (11-16)$\times10^{-3}$m. Mimicry phenomena were moderate. There were no abnormalities in the development of ticks, a clear delay in molting. In bloodsuckers, after successful blood saturation, there was a clear loss of activity followed by anfastenization.

Morphological examination of the tick's body showed that parasites had a complete monolithic idiosome without defects in the chitinous cover (scutum) and the loss of six-membered tarsi (four pairs), distinct sexual dimorphism. Sexual differentiation of individuals was accessible, understandable and focused on somatic parameters, location, and the chitinous cover size. The body size of females exceeded that of males which were protected by a denser (harder, thicker) chitinous cover. In males, the chitinous cover completely covered the body and genitals (located ventrally, behind). In the anterior part of the body of arachnid arthropods, a gnathosoma (proboscis) is visualized, with the basis, two pairs of palps and chelicerae, a hypostome. Proboscis (the ticks mouthparts) was a complex structure of a piercing-sucking type. The tick palps were covered with numerous, well-marked, somewhat misdirected bristles (sensory structures). Chelicerae armed with teeth were located between the palps. In the vast majority of our samples, a hypostome, which due to teeth fixed ticks on the animal/human skin, and clothing, was preserved. On the anterior part of the scutum the visual apparatus (eyes) of ticks was visualized.

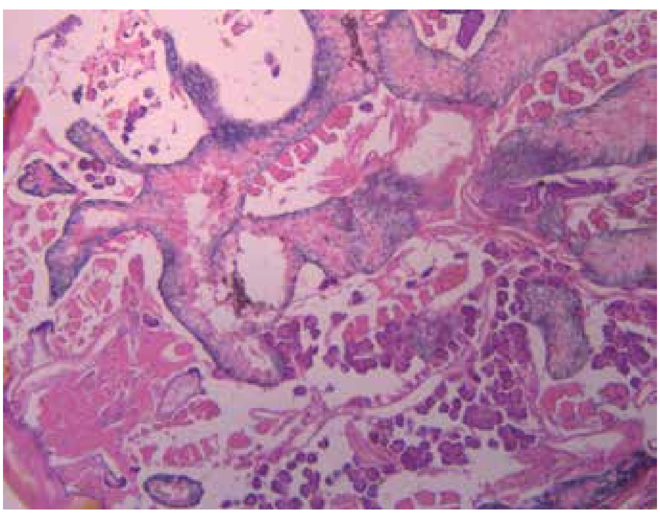

Fig. 1. A histological section of the monolithic idiosome of a sexually mature female tick of the Dermacentor genus of the Dermacentor marginatus species with a fragment of chelicera, racemose salivary glands. Stained with hematoxylin and eosin, × 100.

The ventral surface of the idiosome (less protected) was equipped with four pairs of six-membered tarsi, which last segment ended in a suction pad, two tarsal claws (the functional mobility and fixation of ticks). On the ventral side, at the level of the location of the second pair of cowies, a small genital pore covered with a chitinous plate was visualized. The genitals were in the lower (ventral) part of the tick's body, behind (a tool for internal/spermatoform fertilization of individuals). At the level of the fourth pair of cowies in the antero-posterior direction, the anal groove was located (a landmark of generic differentiation of ticks). The digestive system was developed, and it was a target locus (salivary glands, intestines) for babesiosis pathogens.

In the lateral areas of the monolithic idiosome from its anterior edge (the level of the posterior end of the pharynx) to the stigmas/spiracles, sometimes to the posterior end of the body, racemose paired salivary glands were localized (fig. 1). Each of the glands was formed by several hundred glandular vesicles – alveoli localized on clearly developed excretory ducts and their numerous primary, secondary, tertiary branches. Adult sexually mature mites were armed with two-lobed glands (corresponding to two primary branching of excretory ducts) and a large number of small lobes connecting alveoli associated with the tertiary, quaternary branches. The main excretory ducts were marked, directed to the salivary reservoir of the preoral cavity. The alveoli, in turn, opened directly into the excretory ducts of the salivary glands with their own short, well-contrasted ducts. The structure of the main, primary, secondary, and tertiary ducts was identical in each of the cases analyzed. The presence of cuticular expulsion was considered as a structurally specific aspect of the latter. A prognostically important fact was the detection of contacts (plexuses) between the salivary glands and numerous tracheae of ticks.

The traditional microscopic examination visualized type I alveoli. They were represented by pear-shaped, spherical structures (vesicles) localized on the walls of the main excretory ducts and primary branches. On histologic specimens stained with hematoxylin and eosin, type I alveoli were transparent, pink, pink-lilac in color, without inclusions, and secretory granules. These structures have the osmoregulatory function (secretion of hygroscopic hypertonic saline solution into the preoral cavity; it adsorbs water vapor from the atmosphere and replenish water loss by the tick's body in anabiosis and the absence of a nutrient substrate) [1, 10].

Type II-III alveoli contained heterostructural cells and heterogeneous secretory granules. It was obvious that the functional load of the alveoli, and their secretory activity

Table 4. Localization of ontogenetic forms of *Babesia spp.* in the organs/cells of the ticks body of the Ixodoidea superfamily of the Ixodidea family

Organs/cells of the tick's body	Ontogenetic forms of *Babesia spp.*	
	meronts	merozoites
intestines	+	+
intestinal epithelial cells	+	+
tick's body cavity	–	+
hemolymph	–	+
salivary glands	–	+

were determined by the specificity of cells. In view of the lack of basic structural criteria and ambiguity of conceptual approaches it was problematic to homologize these cell types among the ixodid species studied using the resources of available methodological approaches.

The content of secretory vacuoles and granules was observed in the alveolar cavities (saliva). The latter contributed to the infection of vertebrates with babesiosis pathogens under the conditions of inoculation of merozoites into their blood (since the appearance of a characteristic color of the inclusion was noted in the alveolar cells). A colorless hemolymph, intestinal epithelial cells, and the surface of the salivary gland ducts sometimes contained pairs of cells/single forms with a pink-lilac cytoplasm and blue inclusions marginally located. Detection of the later (full implementation of traditional histological procedures) in other organ structures of the ticks was complicated by a number of factors, including the state of blood saturation.

Ontogenetic stages of *Babesia* spp were characterized by certain tropism to the organs and cells of the body ticks (table 4). The trophism consisted in the predominant localization of parasites in the intestine and its epithelial layer, salivary glands. An intermediate stage of pathogen migration was hemolymph, which acted as a transport system for Babesia spp. It should be noted that the cells that played the role of substrate for the pathogens reproduction were of mesenchymal origin.

Ixodes ticks occupy a leading place in the babesiosis pathogenesis. Blood supply for them is a significant physiological moment [9, 10]. In the case of blood sucking by a tick (the *Ixodoidea* superfamily of the *Ixodidea* family) merozoites of the pathogen are inoculated into the blood, causing the fact of infection [3, 8]. Tick organs/cells (including a well-developed digestive system) are the known locations of individual stages of circulation of various ontogenetic forms of *Babesia* spp. In the intestines of arthropods, meronts are soon formed, in which merozoites are formed. The breakdown of meronts initiates the penetration of merozoites into the intestinal epithelium where the process repeats. Since then, merozoite inoculation is targeted at the host's body cavity and hemolymph. In the hemolymph the introduction of pathogens into the gonads and salivary glands takes place. The ontogenetic cycle of merozoites associated with the concentration in the salivary glands repeats. Transovarian transmission of the pathogen (merozoites) consistently accompanies the ongenetic stages of the tick's development [7-10].

In the wild, especially in pastures, animals and people can be attacked by ticks in all ontogenetic stages (larvae, nymphs, sexually mature individuals) [6]. Technically, they are armed with everything they need to do this. Three-four pairs of tarsi (larvae – nymphs – adult ticks) with a suction pad, two tarsal claws allow ticks to move easily and hold firmly on any surface. Compared to other representatives of the Ixodidea family the complex and prolonged mouthparts – gnathosome provides them with intensive unhindered nutrition (e.g., a female tick is able to suck up to 3 mL of blood, then changing somatometrically from 2-7 to 10-15 and even 35-40 mm) [3]. Attachment of the tick occurs painlessly due to a specific anesthetic secret that completely fills the resulting defect and localizes the pain from the bite. The effect is enhanced by simultaneous penetration of secretions with an anticoagulant and toxic substances into the wound [5]. The host's defense reaction (immunological reactivity manifestation) is triggered precisely at the moment when the tick wounds the skin and inserts the proboscis (oral apparatus) into the epidermal layer. The occurrence of local inflammatory and allergic phenomena is natural in this case.

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CONCLUSIONS

1. Implementation of the leading stages of the babesiosis pathogenetic scenario is focused on the coordinate system «pathogen (*Babesia* spp.) – carrier (ticks of the

Ixodoidea superfamily of the *Ixodidea* family) – reservoir (a susceptible organism)» in which carrier take the leading place.
2. The macromicroscopic specificity of the structure of the ticks (variability: ability to aniso-, poikilomorphism) is an evidence-based criterion for pathogens inoculation to the macroorganism of warm-blooded vertebrates. It determines the features of circulation and organ/cellular locations of *Babesia* spp. (intestines and its epithelium, hemolymph, gonads, salivary glands).
3. The species belonging of warm blooded vertebrates susceptible to babesiosis pathogens correlates with the species belonging of ticks and determines the tropicity of the latter.
4. The simultaneous implementation of a complex of research procedures with the tick biological material samples is problematic taking into account the physical lack of material, which requires researchers to re-orient the diagnostic vector towards the use of additional methods for babesiosis diagnosing, including in vitro ones.

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The Author declare no conflict of interest

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