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ORGANIZATION OF BIOLOGICAL MATERIALS COLLECTION ON STUDY OF THE NATURAL PROTOTYPE OF BABESIOUS INFECTION

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Ukraine's enzootic in accordance with natural focal diseases including babesiosis, makes the research undertaken relevant.

Aim of the study: *to determine the stages in organising the collection of biological materials in the study of the natural prototype of babesiosis.*

Materials and methods of research. *Objects were murine rodents of Muridae family of genera Myodes, Microtus, Apodemus, Sylvaemus of natural habitats of forest-steppe zone of Ukraine, their ectoparasites-carriers of babesiosis - ticks of family Ixodidae. Collection, accumulation and registration of biological materials was carried out in the conditions of scientific expeditions. Regional geography of them concerned Volyn, Zhytomyr, Kyiv, Poltava, Sumy, Kharkiv, Chernihiv regions of Ukraine.*

Results. *A total of 63 wild rodents were captured and used in the study. They were adult mice of both sexes, weighing 45-90 g, belonging to the Muridae family, genera Myodes, Microtus, Apodemus, Sylvaemus. Each group of animals gravitated to a specific geographical area of existence with appropriate attributes of flora and fauna. Collection and recording of those on rodent-feeding animals showed that Ixods differ according to phases of development, blood feeding, sex attributes and species affiliation.*

Conclusions. *Nosological profile of babesiosis as a natural focal obligate-transmissible protozoan blood parasitosis determined methodology of epizootic assessment of area, natural prototype of disease, collection of biological material samples (BMS). BMS collection activities for babesiosis are seasonally dependent. Conducted researches should be focused on 3 links of epizootic or epidemic chain of babesiosis. Invasion by babesia ticks in optimal natural-climatic conditions of development, annual contact with animals led to formation of a latent focus of babesiosis.*

Keywords: *mouses rodents, ecotopes, trapping, ectoparasites, babesiosis, natural prototype.*

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1. Introduction

Quite a large part of the territory of Ukraine is enzootic for various natural – focal diseases, including babesiosis (as obligate - transmissible, blood - parasitic anthropozoonosis [1]). Arthropod bloodsuckers of the *Ixodidea* family are considered to be carriers of babesiosis, and small murine rodents are a reservoir of infectious agents [2]. Animals not only ensure the circulation of pathogens in nature, but also serve as long-term and reliable storage for the latter. The study of ecological, epidemiological, epizootological features of medically significant vectors and reservoirs of babesiosis (by organizing the capture and accounting) is important not only in terms of modern anti-epidemic measures, but also the diagnosis of babesiosis infection. Exacerbation of the epidemic situation with the emergence of new and emergent infections, changes in the clinical profile of the latter leads to transformations of the spatial and biocoenotic structure of existing foci [3, 4]. Additional impetus to the processes is given by anthropogenic factors, dramatic changes in climatic conditions, and, consequently, the transformation of landscapes. Within one ecotope, foci of various infectious diseases (babesiosis, anaplasmosis, tayleriosis, and rickettsiosis) are capable of existence and functioning. On the other hand, the increase in the limit, marked increase in the epizootic activity of natural foci are due to changes in the habitats of vectors and reservoirs of pathogens of dangerous infections, respectively, – outbreaks of ticks. In such circumstances, the time-appropriate organization of catching murine rodents, collecting their ectoparasites with specific diagnostic measures, population control, determination of spatial distribution, genus-species relationships in the pathogen-vector-reservoir system remains relevant not only in the sense of epid review of the fundamental aspects of the nosology of modern infections (differential diagnosis of babesiosis, malaria, borreliosis). In addition, the analysis (based on objective accounting) of the specifics of vectors, reservoirs (systematics, ontogenetic component, quantity) within specific climatic conditions, trends, distribution of new detection methods, verification of the results remains an important component of the organizational process of collecting materials for study of the pathogenesis of babesia infection. Therefore, conducting an adequate organization for the collection of materials for a detailed study of the natural prototype of babesia infection is relevant.

The aim of the study: to determine the stages in the organization of the biological materials collection (murine rodents-feeders, vector ticks) in the study of the natural prototype of babesia infection.

2. Materials and methods

The research was conducted from 2015 to 2020 inclusive (in the seasonal period for ticks from April to June and from August to September, annually). The objects of material collection were methods of catching / collecting murine rodents (feeders) of natural habitats of the forest-steppe zone of Ukraine and their ectoparasites-carriers of babesiosis – ticks. Catching of certain groups of rodents and selection of arthropods was determined by their position in the epidemiological / epizootic babesiosis coordinate system (chain) of the pathogen-carrier-reservoir, strategy and tactics of detection of babesiosis pathogens – the simplest genus of Babesia spp. -vectors) sampling of biological material (BMS). The regional geography of expeditions to collect rodents and ticks concerned Volyn, Zhytomyr, Kyiv, Poltava, Sumy, Kharkiv, Chernihiv regions (Fig. 1).

The accounting and catching of rodents (feeders) was carried out in the locations of their natural existence with the use of traps (mousetraps) on a wooden platform with triggers and hooks for food baits (Fig. 2). The traps were scattered in lines of 10–15 (∑=25) pieces in each, 5 m apart. Their installation took place in the second half of the day, closer to the evening. In order to facilitate the collection of traps, the first of them was placed near a visible landmark, bait to the next. The exception was the last trap, the bait of which was oriented towards the penultimate mousetrap. The distance between the lines was 100–500 m. Traps and rodents were collected from 6 to 7 o'clock in the morning.

Fig. 1. Regional geography of rodents catch expeditions, collecting ticks used in the initiated study (the red line indicates the boundaries of the areas where the expeditions took place)

Fig. 2. The trap (reusable/permanent) with triggers on a wooden platform for catching rodents. Photographic drawing. Scale 1: 2

The maximum preservation of ectoparasites was achieved by minimizing manipulations in the habitat. Each of the captured feeders underwent a detailed parasitological examination. For this purpose, a standard set of equipment, tools, reagents and medicines was used (Table 1).

Ectoparasites localized on the walls of the bag for transporting rodents combed from the hair (in the opposite direction of its overall growth), removed from the auricles of the latter, carefully loaded with tweezers into microtubes with 70 % alcohol. After delivery to the laboratory of new and little-studied infectious diseases (LNLSID), a macroscopic analysis of the external structure of the tick, dissection of the bloodsucker and preparation of relevant for the initiated study areas of the monolithic idiosome were performed.

In addition to the above-mentioned sources of collection of *Ixodes* ticks, methods of obtaining bloodsuckers on routes laid in heterogeneous natural habitats were used. For this purpose, the areas that were visited by people and cattle with different frequency were surveyed in turn. After that, the areas where ticks could permanently wait for a feeder were carefully inspected. In summer, the most effective collection of ticks was in the forest area. The study began with a single count of ticks (1–3 times during the period of greatest activity to assess the species composition and dispersion in the survey area). In the presence of several species of ticks in different areas with different periods of activity, the meeting was held, taking into account the phenomenological features of each of them. In sunny weather, ticks were collected in the morning (at 8–9 a.m.), in the evening (at 7–9 p.m.) in the absence of dew and wind. In cloudy weather, the collecting was held in the afternoon.

Table 1

Standard set of equipment, tools for accounting and capture of ecoparasites (ticks-vectors of babesia infection pathogens)

№ in order	Equipment/ reagents / medicines	Number (in absolute terms)	Tools	Number (in absolute terms)
1	"Drag" (in the form of a cut of a monophonic light manufactory (flannel) fabric in the size, S=1.5×2.0 m) with plug-in rails in seams of opposite (narrow) perimeters (used in steppe zones)	1	Cuvette	1
2	Manufactory flag of flannel fabric, in the size, S=60×100 cm, fixed with a wooden handle (used in conditions of high vegetation)	1	Eye anatomical/ surgical tweezers	4
3	Ethyl alcohol (*spiritus vini*), 70 % 100 ml in standard tinted glass vials	1	Toothbrush	4
4	Bags for rodents transportation	20	Microtubes	20–40
5	Hydrogen peroxide solution 3 % 100 ml in standard tinted glass vials	1	Plastic tripod	2
6	Potassium iodide solution, 5 % 20 ml in standard tinted glass vials	1	Suitcase for tools, equipment and reagents	1

The timing of tick collection period had to be consistent with the temperature conditions, which depended on the geographical coordinates (latitude, altitude zonation and degree of continentality of the climate of a particular area). The nature of the territory, the ecological specificity of ticks determined the means of collecting and accounting for the latter. Under the conditions of steppe areas, "drag" was used. Ticks were removed using anatomical tweezers and loaded into test tubes. No more than 3 well-fed and/or 30 hungry individuals were loaded into one sample (capacity – a sterile test tube with a screw crust, with a piece/leaf of a cereal plant to maintain humidity) to streamline procedures and facilitate accounting. In case of detection of nymphs – 15 fed and/or 50 hungry forms, larvae – no more than 30 fed individuals. Studies of ticks must be carried out on the day of collection of the latter [2].

Manipulations on selection, preparation, carrying out of researches of BMS were carried out with observance of asepsis rules for the maximum possible prevention of additional contamination by foreign microflora [5]. Mortalization was performed by hypernarcosis with chloroform. Conditions for keeping and caring for animals met the requirements of current international and national documents [6-8] of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986), the provisions of the Council of Europe Convention on Bioethics (1997), First National Congress of Ukraine on Bioethics (Kyiv, 2001).

Determination of species affiliation was performed in the laboratory using the resources of the light-optical microscope MBS-1 (x7; x9) [2]. Photographing of native preparations of ticks of *Ixodes ricinus*, *Ixodes persulcatus*, *Dermacentor marginatus* and *Dermacentor pictus* species was performed using a digital camera "Canon EOS-3000".

Verification of the pathogen in the blood of animals was performed by polymerase chain reaction, PCR [9]. Statistical processing of the obtained data was performed using Microsoft Excel 2016 and its additional component "Analysis Package".

3. Research results

Preparation of BMS for the study of the natural prototype of babesiosis infection consisted of the following stages: organization of activities for catching rodents, collection of Ixodes ticks. Relevant studies were conducted on the basis of the State Institution "Institute of Microbiology and Immunology named after I. I. Mechnikov of the National Academy of Medical Sciences of Ukraine".

To objectify the results, the latter should focus on all three links (coordinates) of the epizootic/epidemic chains of babesiosis. The most optimal period for the collection of STDs for babesiosis (epidemic/epizootic) was spring-autumn (May-June, August-September). Geographical locations of natural existence (characteristic ecotopes) of carriers and reservoirs (Volyn, Zhytomyr, Kyiv, Poltava, Sumy, Kharkiv and Chernihiv regions) corresponded to wildlife (mixed forests and forest belts, shrubs, abandoned areas with bushes, etc.). 578 animals were caught in the surveyed areas with the help of traps. The general predominance of genus and species taxonomy concerned individuals (∑=464) who did not belong to the objects of scientific interest. As a result, the latter were rejected (if possible, released, ∑=241). Of the 114 rodents selected, ectoparasites (of the *Ixodidea* family) could not be identified in 51 individuals. Such feeding rodents were also removed from the experimental sample. Finally, the number of wild rodents used in the study was 63 individuals. The animals obtained in the case of expeditionary catches were mature murine rodents of both sexes, weighing 45–90 g, belonging to *Muridae* family of the *Myodes, Microtus, Apodemus, Sylvaemus* genera. Each individual group of animals tended to a certain geographical area of existence (eco-, biotopes) with the appropriate attributes of flora (thickets, shrubs) and fauna of the latter (minimization of skirmishes with natural enemies of small rodents). The general characteristics of the study materials with a focus on endemic/epizootological catch zones are presented in Table 2. Based on the obtained data, the number of wild rodents (family *Muridae* of *Myodes* genus), group I, which conquered the areas of Zhytomyr, Kyiv, Poltava, Sumy and Kharkiv regions, was 18.0±4.0 (28.57±3.0 %) individuals. Rodents (*Muridae* family of *Microtus* genus), group II, inhabitants of Volyn, Zhytomyr, Poltava, Sumy, Kharkiv and Chernihiv regions were 27.0±7.0 (42.86±6.0 %), respectively. The third group of surveyed mice from catching loci (Zhytomyr, Poltava, Sumy and Kharkiv regions) reached 11.0±3.0 (17.46±2.0 %) in numerical parameters. The fourth group had in absolute and relative indicators 7.0±1.5 (11.11±1.0 %) individuals from the total number of all caught ticks.

Table 2

General characteristics of the study materials with a focus on endemic/epizootological areas of murine rodents capture (∑=63), collection of ticks (∑=162)

№ in order	Name of objects	Administrative and geographical location of catch /collection zones	Number (in absolute and relative (%) indicators)
1	Wild murine rodents (*Muridae* family of the genus *Myodes*), I group	Zhytomyr, Kyiv, Poltava, Sumy, and Kharkiv regions	18.0 (28.57±3.0 %)
2	Wild murine rodents (*Muridae* family of the genus *Microtus*), II group	Volyn, Zhytomyr, Poltava, Sumy, Kharkiv and Chernihiv regions	27.0 (42.86±6.0 %)
3	Wild murine rodents (*Muridae* family of the genus *Apodemus*), III group	Zhytomyr, Poltava, Sumy, and Kharkiv regions	11.0 (17.46±2.0 %)
4	Wild murine rodents (*Muridae* family of the genus *Sylvaemus*), IV group	Zhytomyr, Poltava, Sumy, and Kharkiv regions	7.0 (11.11±1.0 %)
5	Ticks (superfamily *Ixodoidea,* family *Ixodi-dea, Ixodes ricinus, Ixodes persul-catus spp*.), I group (IR) females : males	Volyn, Zhytomyr, Kyiv, Poltava, Sumy, Kharkiv and Chernihiv regions	27.0 (79.41±10.3 %): 7.0 (20.59±4.0 %)
6	Ticks (superfamily *Ixo-doidea,* family *Ixodidea, Dermacentor marginatus, Dermacen-tor pictus spp.*), II group (DC) females : males	Volyn, Zhytomyr, Kyiv, Poltava, Sumy, Kharkiv and Chernihiv regions	112.0 (87.50±11.0 %) *: 16.0 (12.5±3.0 %) *

*Note: * – the difference is significant relative to other groups of wild rodents, P <0.05; * – the difference is likely relative to other groups of ticks, p<0.05*

Murine rodents-feeders were subjected to a thorough parasitological examination. After that, strictly following the rules of the anti-epidemic regime, an autopsy was performed. The last manipulation was carried out in the next hours of capture, due to the need to obtain blood smears and homogenates of the spleen of animals in order to further detect the causative agents of babesiosis – the simplest genus *Babesia spp*. Obtaining positive results made it possible for further conduction the biological stage of experiments to study the pathogenesis of both the model of the natural prototype of babesia infection and the example of the "wild reservoir" [10].

Given the fact that Ixodes ticks are vectors of babesiosis, the latter were collected (animals who were small rodents-feeders, previously caught in the above eco- / biotopes).

According to the results of tick collection, it was found that *Ixodes* differed in stages of development (n=136 – not included in the sample size, taking into account the program objectives of the study, which concerned only adults), blood supply, sex, genus and species. The research collection included well-fed (mostly) and hungry bloodsuckers. It was found that out of 162 arthropods infested (by PCR results) with babesia in accordance with the taxonomy, the collected ticks belonged to the *Ixodidea* family of *Ixodes ricinus*, *Ixodes persulcatus, Dermacentor marginatus* and *Dermacentor pictus spp*. Females in the first group of ticks numbered 27.0±9 (79.41±10.3 %) individuals, males – respectively 7.0±3.0 (20.59±4.0 %). The group of ticks of the *Dermacentor marginatus*, *Dermacentor pictus spp.* included 112.0±12.0 (87.50±11.0 %) females and 16.0±4.0 (12.5±3.0 %) males.

Morphological differentiation was focused on the typical features of the structure of arthropods. Thus, individuals of the species *Ixodes ricinus, Ixodes persulcatus*, were characterized by small size, yellowish/pale color, the presence in females of a rounded brown color of the dorsal shield. The idiosome of such ticks had a constriction, an elongated, elongated proboscis. In the peritrema of *Ixodes ricinus, Ixodes persulcatus* ticks are rounded, eyes are absent. The anal sulcus (generic differential criterion) is located in front of the anus. The coke of the first pair of legs had no cleavage. The morphology of ticks *Dermacentor marginatus, Dermacentor pictus* testified in favor of the indicated size of these bloodsuckers, the presence of gray-marble color of the dorsal shield in females, short proboscis. In individuals of these species, the eyes were poorly developed. The ticks had wide, well-marked cocci of the fourth pair, thick expressive legs.

Invasion of ticks by babesia (n=162) in optimal natural and climatic conditions of development, annual contact with animals (probable immunization) contributed to the development of latent foci of babesiosis.

4. Discussion of research results

The research methodology of the natural prototype of babesiosis (organization of procedures for material collection, accounting, analysis) in Ukraine, as well as in the world, is focused on its nosological profile, which corresponds to the natural focal obligate protozoan blood parasitic invasion, with characteristic biological vectors – Ixodes ticks [11]). The above causes a close dependence of the area (forest-steppe zones rich in mixed vegetation, including shrubs, pastures, bushes etc.) of this disease on the spread of these species of ticks and their feeders [warm-blooded vertebrates, often mammals [12]). Studies have shown that in the wild, small rodents (families *Muridae* of the genera *Myodes, Microtus, Apodemus, Sylvaemus*) most often become ticks feeders, however, the mobility and location of the latter change significantly over time. Land development or, conversely, agronomic negligence requires adequate methods of catching animals (equipment: traps, mousetraps, baits, etc.), careful monitoring of the migration of small rodents (clear consideration of biorhythms: nocturnal and lifestyle: burrow animals) in heterogeneous habitats (Volyn, Zhytomyr, Kyiv, Poltava, Sumy, Kharkiv, Chernihiv regions), the presence of parasites – vectors of babesiosis. Collecting ticks from feeders is quite an effective measure, given the study of the epizootic chain (coordinate system: pathogen-vector-reservoir) in the wild "in miniature". However, we should not forget about the tendency of arthropods to a certain kind of "independence" from the latter (conditions and specifics of the ontogenesis of ticks). These circumstances require the optimization of guidelines for the use of reliably tested zooentomological methods of epizootic assessment of the area of their collection and tick danger. According to the conclusions of previous experts [13, 14], according to the presented work, the fundamental approaches of traditional classics in the epizootic assessment of the area for Ixodes ticks (“on dry ice”, “on themselves”, “flag”, “drag”) remain a priority.

Research limitations. Limitations of the study include technical (lack of modern equipment of world brands for catching rodents and collecting ticks, which is used for personal and collective safety by scientists in Europe, USA, Australia, Canada) and climatic aspects (seasonal nature of this hemoparasitosis, natural biorhythms of murine rodents-feeders of ticks), which makes it impossible to collect arthropods that carry babesiosis during the year. In some cases, the research work of specialists is litickd by the lack of funds associated with chronic underfunding of the medical field in the medical field in Kharkiv and Ukraine as a whole.

Prospects for further research are to apply the developed measures for the organization of collection and accounting of materials in conducting field research on other transmissible-mediated parasitic diseases (borreliosis, tick-borne encephalitis) as a technical algorithm. The obtained results are the basis and signaling data for determining the epizootic/epidemic type of the center of transmissible hemoparasitosis and carrying out preventive measures on its territory.

5. Conclusions

1. The nosological profile, babesiosis as a natural focal obligate-transmissible protozoan blood parasitosis, determined the appropriate methodology of epizootic assessment of the area (use of traps-baits; “flag”, “drag”), and the collection of SBM (feeders, rodents, rodents).

2. Measures of BMS collection for babesiosis is a seasonal (spring-autumn: April-June, August-September) dependent procedure, which should focus on all three links (coordinates: pathogen (Babesia spp.) - vector (family Ixodidea) - reservoir (murine) rodents of the family Muridae)) epizootic/epidemic chains of babesiosis.

3. The central figure of the epizootic/epidemic chains of babesiosis are ticks (superfamilies Ixdoidea of the family Ixodidea, species Dermacentor marginatus, Dermacentor pictus and Ixodes ricinus, Ixodes persulcatus). Quantitative advantage * of the studied population of arthropods by species parameters was Dermacentor marginatus, Dermacentor pictus (according to the sex differences of individuals: females – 112.0 (87.50±11.0 %) and 16.0 (12.5±3.0 %) males). Quantitative parameters of Ixodes ricinus, Ixodes persulcatus species were: 27.0±9.0 (79.41±10.3 %) females and 7.0±3.0 (20.59±4.0 %) males (* with a probable difference relative to other groups of ticks, $p<0.05$).

4. According to numerical indicators * (27.0 – 42.86±6 % – with a probable difference relative to other groups of wild rodents, $P<0.05$) the greatest level of danger (as a reservoir for the location of ticks -

vectors of BI) are wild murine rodents-feeders of the *Muridae* family of the genus *Microtus* (inhabitants of natural habitats of Volyn, Zhytomyr, Poltava, Sumy, Kharkiv and Chernihiv regions).

Conflict of interests

The authors declare that they have no conflicts of interest.

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