# STUDY OF IXODID TICKS ON EXISTENCE OF BLOOD PARASITES

Turganbayeva G. E., Akhmetsadykov N. N., Shabdarbaeva G. S., Khussainov D. M., Assylkhanov D. U., Akhmetzhanova M. N.

Scientific and Production Enterprise "Antigen" LLP, Kazakhstan,
Almaty region, Karasai district, v. Abay, Azerbayev street, 4.

**Abstract**

*The results of the identification of infestation ixodid ticks of blood parasites by preparing a homogenate of ticks, followed by the study are given as an antigen in the formulation prepared homogenate at statement of serological tests.*

**Key words:** *ixodid ticks, tick-borne diseases, homogenate, blood parasites, disintegrate, antigen, suspension of a tick, piroplasmosis, theileriosis, anaplasmosis, babesiosis, nuttaliosis*

## RELEVANCE

Blood-sucking ticks – is a major group of ectoparasites out of Ixodid ticks (Ixodidae) family (Ixodidae), damaging animal husbandry and locals, as bloodsuckers and as mechanical and biological vectors of vector-borne infectious and parasitic diseases. They are carriers of Siberian plague, deer fly fever, piroplasmosis, babesial infection (Redwater fever), anaplasmosis, nuttalliosis, animal theileriasis, Human Crimean-Congo hemorrhagic fever, human vernal tick-borne encephalitis, etc.

Among 70 species of ixodid ticks (Ixodidae) common within the country 23 species are vectors of 18 types of excitants of animal hemosporidial infections. Such situation results in complicated epizootic background, which manifests itself in forming different type foci, classification of which along with mapping of excitants and vectors spreading within different regions could have served as a basis for planning and effective implementation of measures to fight them. While dealing with epizootic chain of blood parasitic diseases it is vital to know what is the type of ticks' transmission of the activator of disease: transphasic (i.e. within one generation of ticks) or transovarian (i.e. through infested eggs to the next generations).

The ticks are characterized by the following biological peculiarity: once assimilating the blood parasitic disease vector into its organism, for example piroplasmosis (Piroplasma bigeminum), they are passing it to up to 60 generations, thus creating in their biotope a constant disease focus, able of breaking out any moment after bringing animals from blood parasitic disease favorable regions. The hemosporidial infections are characterized by distinct seasonality phenomenon and are preconditioned by areal of vectors and their activity, in other words the disease is manifested where there are vectors infected with this type of activator and susceptible animals. The disease season coincides with the active phase of ticks-vectors, being one of the main links in the epizootic chain: activator → vector → susceptible animal, revealing the significance of developing the integrated tick-combating approach.

Ixodic ticks (Ixodidae) family includes 6 classes: Ixodes, Hyalomma, Dermacentor, Boophilus, Rhipicephalus, Haemaphysalis, every of which having from 1 to 20 species. Every species of ticks resides in areas specific solely for them (biotope), exhibits activity only within definite time of year, has its own preferred range of hosts-providers, as well as its own biological peculiarities. All the measures taken without due consideration of the mentioned peculiarities of ticks, having no complex integrated approach will not have due effect, but on the contrary will cause the environmental degradation, due to random use of anti-tick medicinal products.

During the recent years, due to changes in economic conditions, predominance of small private business enterprises, lack of scheduled anti-tick arrangements, increase in noncultivated land areas, the nature evidences a tendency to sharp increase in ticks population and, as a result, appearance of new adverse foci of diseases. Example: annual break outs of lethal Crimean-Congo hemorrhagic fever among population within the eastern regions of the Republic of Kazakhstan; appearance of new blood

parasitic diseases foci, for example, in Almaty Region there was a break out of piroplasmosis with lethal outcomes among Przewalski's horses (Equus caballus) brought into the region; in 2013 a break out of animal theileriasis was registered among cattle in Almaty suburb [1].

Unsystematic use of anti-tick medicinal products not substantiated by screening in its turn gives rise to environmental problem, as many medicinal products contaminate the environment, slowly disintegrate, and are characterised by cumulative, allergic, teratogenic, embryogenetic, thyrocardiac and cancerogenic action. Their use requires definite restrictions in utilization of animal products, as far as traces of some acaricidal medicinal products are being revealed even in processed products, for example, in butter.

In compliance with literature data, within the territory of the South Kazakhstan 35 species of blood-sucking ticks are registered. The cattle are diagnosed with 13 species of ixodic ticks (Ixodidae) [2, 3]. Incidence of ticks in animals is unfavorable households reaches up to 100%, while their contamination with Theileria, transmitted by ticks, mounted to 80%. Invasion intensity mounted to 15-30 of ticks per one animal. Within the period of its parasitizing on a host ticks cause reduction in productiveness and quality of raw material on the one hand, and constitute obligate hemophages and specific biological vectors of human and animal dangerous illnesses (Crimean-Congo hemorrhagic fever and human tick-born vernal encephalitis; Mediterranean fever (brucellosis), encephalitides, animal theileriasis, hemosporidial infections, anaplasmosis, and other animal diseases; plague, panleukopenia, avian spirochetosis).

Ticks of family Hyalomma have epidemiological significance, being the vectors of Crimean-Congo hemorrhagic fever, especially Hyalomma asiaticum, Hyalomma anatolicum, Hyalomma plumbeum. [2]. Ticks retain in their organisms the activator of Crimean-Congo hemorrhagic fever for the life term [3]. In southern Kazakhstan lethal outcomes of this disease are registered annually. Nuttalliosis of horses and anaplasmosis of cattle, spread by ticks, are certifiable diseases while importing animals, what enhances the significance of the problem.

The main measures aimed at destroying ticks include processing of live-stock animals and cattle yard with acaricidal medicinal products [4, 5, 6, 7]. Desinsection of animals at different times was recommended to be carried out with the use of organophosphorous medicinal products (chlorophos, trichlorometafos-3, ciodrin, and other), carbamate compounds (sevin, dicresil, and other).

For clarification of degree of contamination of ixodid ticks of blood parasites is of great importance for an assessment of an epizootic situation on tick-transmitted blood parasitic disease. To determine ticks' invasiveness with hematozoons (blood parasites) autopsy of ticks, preparation and examination of swabs from haemolymph. The haemolymphs and salivary glands of dissected ticks were harvested and stained by Giemsa. Also the cultivation method in the thermostat at a temperature is known 37°C well become impregnated ticks removed with animal, receiving from them the laid eggs, preparation from eggs of a tick of dabs, fixing, staining of dabs and research under a microscope, definition of a type of blood parasites on morphological features and their calculation under a microscope [8].

The disadvantage of this method to identify infested of ticks of blood parasites is low efficiency and high labor input, the difficulty in differentiating blood parasites, need of a wide experience at a smear microscopy of dabs.

The aim of the present work is increase of efficiency and decrease in labor input of identification of an infestation of ticks of blood parasites. These objectives were achieved by us by preparation of a homogenate from ticks with the subsequent statement of serological tests, with use as the studied antigen from homogenate a tick.

Process of identification of an infestation of ticks with blood parasites includes gathering of the studied well become impregnated ticks on animals and in biotopes, preparation of a homogenate from ticks by the technique patented by us, receiving specific to defined to an antiserum of blood parasites by immunization of animals, susceptible to blood parasites, and statement of serological tests.

## MATERIALS AND METHODS

*Collection of sample*. Gathering of ticks from animals and in biotopes was carried out by the standard techniques [8]. Tick collection was performed by descriptions Lorusso V et al. [18] using blunt steel forceps, by thorough examination of the entire body surface of the animals. Ticks from each animal were stored separately in vials containing 70% ethanol, labelled with information on the host (i.e., sample number, age), village, and date of sampling. Age of the animals was estimated on the basis of the dentition score method developed for cattle under a low plane of nutrition [16, 18] and on information provided by their owners. Once quantified, each animal's age was recorded either as 'calf' (0–6 months), 'juvenile' (6–24 months), or 'adult' (older than 24 months).

*Isolation whole homogenate antigen*. Receiving a homogenate from ixodic ticks was carried out by the technique patented by us [9].

*Serological tests*. The specific to a particular blood parasites antiserum was received step by step. In the beginning from infected with this or that blood parasites received invasiveness of blood from a vein, centrifugation with physiological solution separated invasiveness of erythrocytes. By N. I. Stepanova's method prepared a specific antigene [10]. The prepared series of a specific antigene controlled on sterility, using special nutrient mediums, checked activity of an antigene a titration in LCFR, IHA and ELISA. For definition of blood parasites to the ELISA methods carried out by the Atif F.A et al method. [11]; Figueroa J.V et al. [12].

The rabbits prepared for immunization (weight not less than 2,5 kg) were immunized a specific antigene with The Complete Freund's Adjuvant (CFA) by the Fey et al method. [13].

Statement of serological reactions (LCFR) was carried out by the standard methods [14], using as the studied antigene suspension of ticks, and as a diagnosticume positive serum, specific to certain blood parasites. Statement of serological reactions (IHA) was carried out mo to the Weiland G method. [15].

*Microscopic examination*. Once in the laboratory, all collected ticks were counted and identified to the genus and species level using a stereomicroscope (up to 100× magnification) and following the morphological keys in Walker et al.[17].

*Staining of salivary glands*. Dissection of ticks was done according to the method of Purnell and Joyner [19].

## RESULTS

A total of 833 adult ticks were collected from animals. In collecting the following species of ixodic ticks are registered: Rhipicephalus rossicus, Ixodes ricinus, Hyalomma marginatum, Hyalomma scupense, Hyalomma anatolicum, Dermacentor pictus and Dermacentor marginatus (Figure 1).



**Figure 1.** Ixodic ticks on a body of cattle

In biotopes taking into account character of the surveyed territory and ecological features of ticks applied various ways of their collecting and the account.

On steppe sites of collected ticks on "Active tick dragging" method, i.e. on a cut (1,5 x 2,0 m) of monophonic light fleecy fabric (wafer, flannel). Inserted into seams of the opposite narrow sides of a cut on a lath. Attached a cord for which slowly stretched "Active tick dragging" (sideways from themselves) on a site to the top lath. Ticks cling to fabric from which they were removed tweezers and transferred to a test tube or on bandage.

On meadow sites and the timberland with a high grass and a bush of ticks collected on a flag from the same fabric. The piece of matter 60x100 cm was attached the narrow party to a stick. Dragged the developed flag on vegetation before themselves or sideways, periodically performing inspection of a flag. The flag has to slide on a grass the most part of the surface. In case of gathering of ticks from high bushes the panel of a flag was lifted vertically and from a windward side pressed to the ends of branches. It is convenient to pieces to conduct calculation of length of a route on 20 - 25 m, in advance having defined the number of couples of steps corresponding to them. In intervals between pieces did stops for records, survey of clothes of the collector. Total extent of a route at the account made about 1 km.

Accurate ticks were placed in glass test tubes with a wadded and gauze stopper or in plastic test tubes with the screwing-up cover. For maintenance of humidity threw into a test tube usually a leaf of a cereal. Test tubes placed in a linen sack and transported in a metal case. In a test tube poured water on 1/3 - 1/4 volumes and the smooth movement closely to water entered a wadded tampon. Then placed a wadded and gauze tampon, having densely put them to test tube walls. The distance between these tampons made 5 mm. Tied a strong thread which one end acted from a test tube on 3 - 4 cm to the end of a wadded and gauze tampon. It serves for extraction of a wadded and gauze tampon and the pincers which are on it outside. In a test tube it is possible to place an accordion from filter paper. The test tube was closed a wadded and gauze stopper. Test tubes and material have to be sterile.

Long preservation of ixodic ticks live in the cool place provides their collecting in wide (14 cm) unsterile bandage. The bandage was plentifully moistened with clear water and slightly wrung out. The end of bandage several turns was wound on a wooden stick with a ridge or rough surface length slightly more widely than bandage (of the size of a pencil). The ticks placed on bandage were fixed two-three turns of a gauze. After each separate collecting under bandage put the label written by a simple pencil. The filled bandage was not hardly connected by an elastic band and placed in a bound sack. In such state we kept separate species of ticks in the refrigerator about several days.

Collected ticks from animals and in natural biotopes of blood-sucking arthropods brought to laboratory live (Figure 2).



**Figure 2.** Collected ixodid ticks

A total of 833 adult ticks were collected from animals. When carrying out cameral processing accurate ticks were defined to a sort and a look, distributed on development stages (to an adult, nymphs, larvae), distributed on degree of fatness (Table 1).

**Table 1.** Species composition of ixodid ticks

| № | Species of ticks | Total | The number of ticks | | | | Percentage |
|---|---|---|---|---|---|---|---|
| | | | Female | Male | Nymph | Larva | |
| 1 | Rhipicephalus rossicus | 14 | 5 | 4 | 3 | 2 | 1,7% |
| 2 | Ixodes ricinus | 33 | 10 | 7 | 9 | 7 | 4,1% |
| 3 | Hyalomma marginatum | 131 | 30 | 42 | 47 | 12 | 15,7% |
| 4 | Hyalomma scupense | 202 | 66 | 58 | 66 | 12 | 24,2% |
| 5 | Hyalomma anatolicum | 247 | 100 | 35 | 92 | 20 | 29,7% |
| 6 | Dermacentor pictus | 62 | 30 | 15 | 6 | 11 | 7,4% |
| 7 | Dermacentor marginatus | 144 | 80 | 20 | 24 | 20 | 17,2% |
| **Total:** | | **833** | **321** | **181** | **247** | **84** | **100,0** |

In collecting the following species of ixodic ticks are registered: Rhipicephalus rossicus - 14 specimens or 1.7% of the total number of ticks; Ixodes ricinus - 33 specimens (4.1%); Hyalomma marginatum - 131 specimens (15.7%); Hyalomma scupense - 202 specimens (24.2%); Hyalomma anatolicum - 247 specimens (29.7%); Dermacentor pictus - specimens (7.4%); Dermacentor marginatus - 144 specimens (17.2%).

Females of 321 specimens; from them Rh. rossicus - 5 specimens (1,5%); I. ricinus - 10 specimens( 3,1%); H. scupense - 66 specimens (20,5%); H. anatolicum - 100 specimens( 31,1%); H. marginatum - 30 specimens (9,3%); D. pictus - 30 specimens (9,3%); D. marginatus - 80 specimens (24,9%).

Males of 181 specimens; from them Rh. rossicus - 4 specimens (2,2%); I. ricinus - 7 specimens (3,8%); H. scupense - 58 specimens (32,0%); H. anatolicum - 35 specimens (19,3%); H. marginatum - 42 specimens (23,2%); D. pictus - 15 specimens (8,2%); D. marginatus - 20 specimens (1,1%).

Nymphs - 247 specimens; from them Rh. rossicus - 3 specimens (1,2%); I. ricinus – 9 specimens (3,6%); H. scupense – 66 specimens (2,67%); H. anatolicum – 92 specimens (37,2%); H. marginatum - 47 specimens (19,0%); D. pictus – 6 specimens (2,4%); D. marginatus – 24 specimens (9,7%).

Larva - 84 specimens; from them Rh. rossicus - 2 specimens (2,3%); I. ricinus – 7 specimens (8,3%); H. scupense – 12 specimens (14,2%); H. anatolicum – 20 specimens (23,8%); H. marginatum - 12 specimens (14,2%); D. pictus – 11 specimens (13,0%); D. marginatus – 20 specimens (23,8%) (Table 1).

Very well-fed ticks totaled 215 specimens (25.8%), moderately well-fed ticks totaled 325 specimens (39.0%) and the hungry not become impregnated ticks totaled 293 specimens (35.1%).

The next step was to obtain a homogenate ticks.Ticks before study were sorted by specific, sexual and age signs. Well become impregnated ticks were washed out 1 time in ethyl alcohol and 2 - 3 times in solution with use of 0,01 M of phosphatic and buffer solution, pH 7,2 - 7,4 with antibiotics (penicillin of 500 units/ml and streptomycin of 1000 units/ml). Each tick was transferred to the small cooled porcelain mortar and pounded. For preparation of suspension of a tick added 0,2 ml 0,01 M of phosphatic and buffer solution, pH 7,2 - 7,4 with antibiotics (penicillin of 500 units/ml and streptomycin of 1000 units/ml).

It is the most convenient to pound separate specimens of ticks in plastic test tubes for microprobes. The tick was transferred tweezers to a test tube and lowered it in liquid nitrogen for 10 - 15 seconds, homogenized a pestle from stainless steel (a core with a diameter of 3 - 4 mm with the edges rounded in an internal form of a test tube). Added 0,2 ml 0,01 M of phosphatic and buffer solution to a test tube, rn 7,2 - 7,4 with antibiotics (penicillin of 500 units/ml and streptomycin of 1000 units/ml). The pestle after use was burned over a torch flame, cooled in liquid nitrogen and reused. Use of liquid nitrogen for deep freezing of ticks gives the chance to keep biological activity of material and promotes thinner crushing of tissues of tick.

From the prepared suspensions took 0,05 ml for research. For preparation of an antigene for research of suspension of ticks before research clarified centrifugation at 2000 rpm within 10 minutes.

The remained material was stored at -40 °C or at -70°C in the refrigerator or in liquid nitrogen for repeated research. Period of storage has to be no more than a year.

Receiving antiserum, specific to a certain type of blood parasites, which process consists of several stages was the following research problem.

The first stage was receiving an infested of blood parasites of erythrocytes. From a vein of a sheep - the carriers of blood parasites was taken by 20 $cm^3$ of blood, added to it 40 mg trilone-B (2 $mg/cm^3$) and entered it intramuscularly into the area of a croup non-invasive blood parasites to a sheep. For 21-30 day to this animal deleted with an operational way a spleen for strengthening of a parasitemia. After operation and to a total bleeding to an animal for the purpose of decrease in influence of anemia fed an iron preparation sulfate (200-300 mg a day). At the parasitemia height when 80-90% of erythrocytes are struck with blood parasites (usually for 20-30 day), an animal a la carte or it is total exsanguinated for the purpose of receiving an invasiveness blood parasites of blood.

Similar researches were conducted also on cattle. From a vein of a cow (bull-calf) - the carriers of blood parasites was taken by 100 $cm^3$ of blood, added to it 40 mg trilone-B (2 $mg/cm^3$) and entered it intramuscularly into the area of a croup non-invasive blood parasites to a bull-calf (age of 6-12 months). For 21-30 day to this animal deleted with an operational way a spleen for strengthening of a parasitemia. After operation and to a total bleeding to an animal for the purpose of decrease in influence of anemia fed an iron preparation sulfate (300-500 mg a day). At the parasitemia height when 80-90% of erythrocytes are struck with blood parasites (usually for 20-30 day), an animal a la carte or it is total exsanguinated.

The second step in the process of obtaining specific antiserum were prepared from infested blood antigen.The technology of receiving an antigene from blood parasites shows as follows. The blood received from infested blood parasites was processed by trilone - B (2 $mg/cm^3$). Erythrocytes were washed physiological solution (pH 7,0-7,2) at 5000 about., within 15 minutes, 3-5 it is multiple, before full clarification of supernatant liquid. One volume of the washed erythrocytes mixed with five volumes of the sterile, neutral distilled water. Mix was maintained by 2 hours at + 4 C, then filtered via the sterile double gauze filter and centrifuged at 7000 rpm, within 30 minutes. The received rainfall connected in one bottle to a beads, carefully stirred up and washed the distilled water at repeated centrifugation, then subjected to freezing thawing, and 3-fold centrifugation washing to a full enlightenment of supernatant liquid. After the last centrifugation the deposit was collected in a bottle, parted with sterile physiological solution (rn 7,0 - 7,2), in half volume from the number of the washed parasitic weight, and within 30 minutes subjected to processing by ultrasound at 22 Khz. Received desintegrat used as an antigene for immunization of producers intramuscularly. The part of a dizintegrate was centrifuged at 7000 rpm./ min., during 30 minutes, the deposit was used for hypodermic immunization, supernatant liquid for intravenous immunization.

The antigen was monitored for sterility using special nutrient mediums (absence of bacterial and fungal contamination microflora indicates the purity of the drug). Activity of an antigen was checked a titration in LCFR, IHA and ELISA.

Immunization producers of animals was the last stage of preparation of specific antiserums. The rabbits prepared for immunization (weight not less than 2,5 kg) were immunized by specific antigen

according to the following scheme: the 1st day - the antigen prepared from a deposit of a disintegrate in mix with the equal volume of the Complete Freund's Adjuvant (CFA) in small pillows of paws of rabbits on 0,25 $cm^3$ in everyone, hypodermically in six points: at the left and on the right to the area of shovels, the center of a back and a tailbone, protein content there have to be not less than 5 $mg/cm^3$; for the 7th day - the antigen prepared from a disintegrate intramuscularly, at the left and on the right in gluteus's - on 1,0 $cm^3$ (20 mg of protein); for the 14th day - intravenous immunization by the antigen prepared from supernatant part of a disintegrate according to the scheme: at first 0,02 mg of protein, in 30 minutes - 0,2 mg, and in 30 minutes - 20 mg for an exception of anaphylactic shock. In 21 days this manipulation was repeated. Blood sampling was begun from the 3rd day after the last injection of the 1st block of immunizations, carried out collecting on 40-50 ml of blood, it is triple, with an interval of 72 hours. Repeatedly blood was taken since 3rd day after intravenous immunization, according to the same scheme. Further the cycle of immunization and a blood withdrawal repeated for the purpose of an operating time as it is possible bigger amount of antiserums. On ways of additional immunization of laboratory animals for receiving the maximum quantity of antiserums the Kazakhstan patents are held.

Positive serum was controlled on sterility, using special nutrient mediums (lack of bacterial pollution and fungal microflora indicates purity of a preparation). Activity of serum was checked a titration in LCFR, IHA and ELISA.

Study on infested ticks of blood parasites by statement of serologic reactions was the end result of preliminary researches.

Statement of serologic reactions (LCFR) was carried out by the standard methods, using as the studied antigen suspension of ticks, and as a diagnostic kit - positive serum.

The process of identification of an infested ticks of blood parasites into LCFR with suspension of ticks, for example in an anaplasma infections.

In reaction used the following components:

- the suspension of the studied tick divorced physiological solution of sodium of chloride in the ratio 1:5;
- control anaplasma antigen, with the subsequent cultivation by physiological solution of sodium of chloride in the ratio 1:5;
- negative serum;
- serum positive in a working caption;
- complement - serum of blood of a guinea pig (fresh, preserved with addition of 4% boric acid or freeze-drying);
- hemolytic serum (hemolysin) - in the trebled caption;
- erythrocytes of a ram (a suspension of erythrocytes, 3% from a deposit, in physiological solution of chloride sodium).

The test and control (positive) prior to assay anaplasma antigen subjected inactivation in a water bath on the day setting reaction at 60-62°C for 30 minutes.

When using as a complement of fresh or tinned serum when receiving each new series of a dry complement carried out titration it in hemolytic system for determination of activity. The dry bio factory complement was dissolved in physiological solution as it is specified on a label. Took such number of ampoules (bottles) which contain quantity of a complement, necessary for carrying out all experience. Contents of ampoules were merged in one test tube, carefully mixed, took 0,5 $cm^3$ or parted with physiological solution 1:20 for titration. Other quantity of a complement was kept in the refrigerator at 2-4 °C;

To prepare the respective slurry mixtures hemolytic erythrocytes and in the serum hemolytic working dilution were mixed in equal volumes and incubated at 37 ° C for 15-20 minutes. When mixing poured in a hemolysine in a suspension of erythrocytes.

The long reaction complement fixation (LCFR) on cold was carried out in test tubes in volume of the divorced suspension or a control antigen and a complement on 0,2 $cm^3$, hemolytic system - titration in working dose (on 0,4 $cm^3$).

Carried out statement of LCFR in the following sequence.

*First day*. Flood of examinees and control cultivations of suspension or a control antigen for the main experience and for titration of hemolytic mix.

Inactivation of tests of an antigen.

Control of components of reaction to an anti-complementarity and hem toxicity.

Spill of suspension or control antigen and complement. Preparation of hemolytic mix. The room of test tubes in the refrigerator.

*Second day*. Keeping of test tubes of the main experience and hemolytic system at the room temperature of 20 minutes. Titration of hemolytic system. Definition of a working dose of hemolytic system.

*Chief experience*.

Flood of hemolytic system in test tubes with the studied tests of an antigen.

Account and assessment of results of reaction.

The test sample of suspension or control antigen investigated in cultivations 1:5 and 1:10 with positive serum and 1:5 without serum (control on an anti-complementarity of suspension or a control antigen), at mass research carried out reaction in one test tube in cultivation of suspension 1:5 about an antigen.

At the same time spilled suspension of a tick and a control antigen for titration of hemolytic system. Necessary cultivations of suspension of a tick and control prepared an antigen as it should be as it is stated above. The inactivation of the divorced tests of suspension of a tick and a control antigen of blood was carried out at 62-64 °C.

The complement was applied in working cultivation 1:25 or 1:30 (at a caption in the gem system specified by the bio venture - 0,19 - 0,22).

Flood of components and sequence of statement of reaction carried out according to the scheme specified in Table 1.

*Reaction control*:

- positive anaplasma antigen in cultivations 1:5 and 1:10 with positive serum and 1:5 without serum;
- hemolytic system without suspension of a tick, positive serum and a complement.

Before flood of hemolytic system with the studied tests of suspension of a tick carried out titration of hemolytic system on three tests of suspension of a tick to test tubes - negative (without suspension of a tick), positive (an anaplasma antigen) and test of suspension of a tick of the studied party (Table 2).

**Table 2.** Scheme of statement of the long reaction complement fixation (LCFR)

| The reaction components | At mass study | | For repeated study | | | |
|---|---|---|---|---|---|---|
| | Test tubes with examinees tests of suspension of a tick | Control of hemolytic system | Test tubes for each studied test suspensions of a tick | | | Control of hemolytic system |
| | | | 1 | 2 | 3 | |
| The test sample of suspension of A tick | 0,04 | - | 0,04 | | 0,04 | 0,02 |
| Saline | 0,16 | 0,6 | 0,36 | 0,16 | 0,18 | 0,6 |
| Water bath of 63-64°C - 30 minutes | | | | | | |
| Serum positive in a working caption | 0,2 | - | - | 0,2 | 0,2 | - |
| Complement in a working dilution | 0,2 | - | 0,2 | 0,2 | 0,2 | - |
| The refrigerator + 2 - 6 °C - 16 - 18 hours and 20 minutes at the room temperature | | | | | | |
| Hemolytic system in working capture | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 |
| Water bath of 37-38°C-20 minutes | | | | | | |

For studying of diagnostic efficiency of this scheme we conducted researches of 48 tests of suspensions from pincers to a zone, unsuccessful on an anaplasmosis, and 48 tests of suspensions from ticks to a zone, safe on an anaplasmosis. Studying conducted researches of a lymph of ticks in comparison with a classical microscopic method. The obtained data are provided in table 3.

**Table 3.** Results of test of a way of identification of an infested of ticks of an anaplasmosis in LCFR in safe and unsuccessful zones

| The studied arthropod | Wellbeing of economy on an anaplasmosis | The number of the investigated serological tests of ticks | | Results of microscopic study of tests of ticks | |
|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Negative |
| Ixodid ticks – Dermacentor marginatus | Unsuccessful | 7 | 41 | 5 | 43 |
| Ixodid ticks– Dermacentor marginatus | Safe | - | 48 | - | 48 |

Apparently from data of table 3, as a result of the carried-out tests high activity and specificity of a way of identification of an infested of ticks of an anaplasma infection, in particular, in LCFR is established at research of suspension of a tick.

At the ticks which gave positive reaction from suspension of a tick anaplasma infection were microscopically revealed that testifies to reliability of results of research of suspension of a tick and to his higher sensitivity.

Thus, the way of identification of an infested of ticks of an anaplasma by research of suspension of a tick in serologic reactions on the example of LCFR was sensitive and specific. With its help an opportunity to reveal ticks of carrier anaplasma infection is represented.

## CONCLUSIONS

Serologic reactions on the example of LCFR are a sensitive method for identification infested blood parasites of ticks at application as an antigene of a homogenate from ticks.

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