OnLine Journal of Biological Sciences

Original Research Paper

Participation of *Dermacentor Reticulatus* Imago in the Reservation of Bovine Leukemia Virus

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Article history
Received: 06-06-2022
Revised: 31-08-2022
Accepted: 16-09-2022

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Abstract: Strict implementation of measures for the prevention and elimination of bovine leukemia does not allow completely getting rid of this disease. In all likelihood, the leukemia virus has additional transmission routes that have not been taken into account until today. In the Tyumen region, blood-sucking insects and ticks are widespread and take part in the transmission of some infectious and invasive diseases. The study aims to evaluate the possibility of reservation of the bovine leukemia virus in the imago of ixodid ticks. Studies on the spread of bovine leukemia were conducted from 1995 to 2019 based on veterinary reports. Laboratory and production experiments on feeding imago of ixodid ticks were carried out in disadvantaged farms of the Tyumen region and at the Department of Infectious and Invasive Diseases of the Federal State Budgetary Educational Institution of Higher Education, State Agricultural University of the Northern Trans-Urals. Detection of the bovine leukemia virus was carried out at the veterinary diagnostic center of the Ural Scientific Research Veterinary Institute (Yekaterinburg) and the Agrobiotechnology Center of the State Agricultural University of the Northern Trans-Urals (Tyumen). The experiments were carried out on Dermacentor reticulatus ixodid ticks, which were fed on infected and sick animals. It was found that the imago of ixodid ticks was capable of preserving the DNA molecule of bovine leukemia provirus in their body when feeding on hematologically ill animals in 92.9% of cases, whereas when feeding on seropositive animals, all the fed females retained bovine leukemia provirus in their body. It was found that the bovine leukemia virus could persist in the body of ixodid ticks for at least one year. The study allows determining the role of ixodid ticks in the persistence of the leukemia virus and the likely possibility of its transmission to subsequent generations of ixodid and infection of more susceptible animals in a grazing system.

Keywords: Bovine Leukemia Virus, Ixodid Ticks, *Dermacentor Reticulatus* Reservation Molecular Diagnostic Methods

Introduction

Ixodid ticks attracted attention as carriers of pathogens in the 10-11th century. In the 20th century, ixodid ticks, as vectors of pathogenic microorganisms and parasites, attracted the attention of not only epidemiologists but epizootologists. Despite the progress in science, the increase in the incidence of natural focal infections in people or animals does not decrease. All this indicates the lack of complete data on the mechanisms of transmission and the possibility of preserving the infectious origin in the body of the ixodid tick.

Tick-Borne Viral Encephalitis (TBVE), the causative agent which was discovered in 1937 in the Far East of Russia, was the first disease to attract attention. Another serious disease, in the maintenance of which ixodid ticks are involved, is ixodid tick-borne borreliosis. It is also known that ixodid ticks are involved in the transmission of Crimean-Congo hemorrhagic fever, being the main and long-term carriers of the causative agent of this disease and also support

natural foci of Q fever and transmit paroxysmal rickettsiosis and infectious nephritis to humans (Stańczak, 2006; Tijsse-Klasen *et al*., 2011; Walker *et al*., 2022).

A study of ticks on *Dermacentor reticulatus* widespread in Poland and the UK showed that these ticks in 96-99% of cases were the reservoirs of *Rickettsia slovaca*, *Rickettsia sibirica*, *Rickettsia honei*, and SFG *rickettsia* (Stańczak, 2006; Mierzejewska *et al*., 2015). In the Stavropol Territory of the Russian Federation, it was established that the *Hyalomma marginatum* tick was the main vector and reservoir of the Crimean-Congo hemorrhagic fever virus (Vasilyeva, 2006; Sivkov *et al*., 2009). It is also known that ticks are actively involved in the transmission of Omsk hemorrhagic fever, tick-borne typhus, and tularemia (Földvári *et al*., 2007).

There is information in the literature about the possibility of preservation of the rabies virus for up to 21 days and its transmission by ixodid ticks (Bakulin and Fediakina, 2015). We should not forget about blood parasitic diseases that are transmissible, as ixodid ticks also participate in their transmission. The transmission of some diseases was already proved by E.N. Pavlovsky when he explored the biology and ecology of *I. ricinus* in the Novgorod province (Sivkov *et al*., 2009; Springer *et al*., 2021).

Almost 100 years later, it was proved that *Dermacentor reticulatus* ticks living in Slovakia were the main reservoirs of *Babesia canis* (Duh *et al*., 2006; Špitalská *et al*., 2021). Recent studies conducted in Hungary have proved that female *Dermacentor reticulatus* ticks retain *Babesia spp.* and are the main distributors of babesiosis among dogs (Levytska *et al*., 2019). Similar data have been obtained in Spain, Serbia, the Netherlands, and Belgium (Garcia-Sanmartin *et al*., 2008; Mihaljica *et al*., 2012; Koshkina and Goryachaya, 2013; Jongejan *et al*., 2015).

Polish scientists have established that *Ixodes ricinus* and *Dermacentor reticulatus* ticks are reservoirs of *Babesia microti*, *Babesia venatorum*, *Babesia divergens*, as well as unknown *Babesia* species that possibly transmit human babesiosis (Wójcik-Fatla *et al*., 2015). Besides, the pathogen of *Francisella tularensis* has been found in the body of *D. reticulatus*, while the pathogen of *F. tularensis* has not been found in *I. ricinus* ticks (Michelet *et al*., 2013; Matsuno *et al*., 2018).

When pathogens were detected in such ticks as *D. reticulatus, D. marginatus, and I. ricinus* collected from the territory of northwest France, it was found that in *D. reticulatus* ticks there were such pathogens as *A. marginale* (1%), *Bartonella spp.* (12%), *C. burnetii* (16%), *Borrelia spp.* (1.5%) and *F. philomiragia* (19%); in D. marginatus ticks *A. marginale* (0.5%), *Bartonella spp.* (9%), *C. burnetii* (12%), *F. philomiragia* (1.3%), *Theileria spp.*, and *Babesia bovis* (0.3%) were found, and the *I. ricinus* ticks were reservoirs of *A. phagocytophilum* (41%), *Bartonella spp.* (9%), *C. burnetii* (18%), *A. marginale* (1%), *Borrelia spp.* (4.5%), and *Babesia spp.* (7%) (Ondruš *et al*., 2020). *Francisella tularensis* and *Francisella spp* were also found in *Dermacentor reticulatus* ticks (Bonnet *et al*., 2013).

It can be seen from the cited literature sources that ixodid ticks are carriers of many well-known diseases related to viral, bacterial, and parasitic etiology. Identifying all new carriers in ixodid ticks is necessary to provide for the fact that certain territories are at higher risk for some infectious diseases, which, despite taking the necessary measures, have not yet been eliminated. Such a disease as bovine leukemia threatens advanced farms, not allowing them to increase capacity since the consequences of this disease negatively affect the breeding value of animals and decrease the quality of milk and meat (they contain a large amount of free tryptophan, whose metabolites have carcinogenic properties dangerous to humans).

Considering that about half of all cattle are still grazed on pastures where blood-sucking insects and ticks are widespread in the warm season, it is impossible to underestimate their role in the circulation of infectious diseases, including bovine leukemia. In the Tyumen region of the Russian Federation, three species of ixodid ticks are common, among which D. reticulatus (46.0±5.0%) and I. persulcatus (44.6±6.8%) are the most common ones and D. marginatus (9.4±3.3%) is found in steppe areas (Glazunov and Glazunova, 2018a, 2018b, 2020; Glazunov *et al*., 2021).

Taking into account the widespread and significant importance of *D. reticulatus* ticks in maintaining foci for viral, bacterial, and protozoal diseases, we set ourselves the goal of studying the possibility of reserving the bovine leukemia virus in the imago of *D. reticulatus.*

The research work is organized as follows. The first section presents a review of the literature; the second section describes materials and methods; the third section contains the obtained results; the fourth section briefly discusses the results in the context of other works; the fifth section presents the main conclusions of our study.

Materials and Methods

Monitoring of the epizootic situation for bovine leukemia was carried out based on the analysis of veterinary reports from 1995 to 2019. Production and laboratory tests on feeding imago of ixodid ticks were carried out from 2009 to 2019 at enterprises with unfavorable bovine leukemia situations located in the Tyumen region. The most common ticks in the region are ticks of the Dermacentor genus, in particular *D. reticulatus*, so we decided to conduct our study on the participation of ticks of this species in the reservation and the transmission of the bovine leukemia virus (Vasilyeva, 2006; Tijsse-Klasen *et al*., 2011).

Ticks collected from animals and vegetation were used to extract the culture. The ticks were placed in chemical test tubes and tightly closed with a cotton-gauze stopper. Up to 50 adult hungry ticks were placed in one test tube (Fig. 1).

Fig. 1: Test tubes for cultivation and maintenance of ticks

Fig. 2: Feeding of female *D. reticulatus* ticks

Fig. 3: Bags with ticks fixed on the auricles

Test tubes with ticks were stored in the refrigerator at t +4°C, as well as at room temperature (18-20°C).

To study the possibility of preserving the bovine leukemia virus in the body of *D. reticulatus* ticks, three groups of cattle were formed and previously subjected to laboratory hematological and serological (immune diffusion reaction) studies. The first experimental group (n = 9), consisted of hematologically sick animals, the second experimental group (n = 9), consisted of infected animals, and the third control group (n = 6) was formed from animals with a negative reaction. Ixodid ticks were planted on animals of all experimental groups during the summer period (June to July).

Experiments on feeding ixodid ticks on sick, infected, and healthy animals were carried out on laboratory lines of hungry imagos (Fig. 2). To do this, bags of dense fabric were tied to the auricle, with ticks placed in them in the amount of 20 individuals (ten females and ten males) per animal (Fig. 3).

Well-fed individuals fed on seropositive, hematologically ill, and healthy animals were divided into three groups, each well-fed female was stored in an individual test tube.

The 1st group, where the ticks were fed on seropositive animals, included a total of 11 individuals, of which six were sent for subsequent metamorphosis and five for laboratory studies to detect the DNA of the bovine leukemia virus

The 2nd group, where the ticks were fed on hematologically ill animals, included a total of 22 individuals, of which eight were sent for subsequent metamorphosis and 14 were subjected to laboratory tests to detect the bovine leukemia virus.

The 3rd group, where the ticks were fed on healthy animals, included a total of nine individuals, of which four were intended for subsequent metamorphosis and five were subjected to laboratory tests to detect the DNA of the bovine leukemia virus.

Further metamorphosis of experimental ixodid ticks was carried out to study the possibility of transovarial and trans-phase transmission of the bovine leukemia virus in the body of ixodid ticks in the egg, larva, and nymph stages.

Detection of the bovine leukemia virus was carried out at the veterinary diagnostic center of the Ural Scientific Research Veterinary Institute (Yekaterinburg) and the Agrobiotechnological Center of the State Agricultural University of the Northern Trans-Urals (Tyumen).

Two methods were used to isolate the proviral DNA of bovine leukemia.

A 10% suspension was prepared, for which the ticks were ground to a homogeneous state with a pestle in a porcelain mortar, then a sterile saline solution was added. To isolate DNA from the test material, we used a set of DNA-sorb-B reagents produced by the Federal State Research Institution (FGUN) "Central Research Institute of Epidemiology" of the Russian Federal State Agency for Health and Consumer Rights (Rospotrebnadzor). During this process, after treatment with a lysing solution, the DNA-protein complex is destroyed and nucleic acid is deposited on the sorbent, followed by its transfer to the eluting buffer.

To indicate the DNA of the leukemia provirus, we used the "Leukemia of cattle-provirus" test system produced by the FGUN "Central Research Institute of Epidemiology" of Rospotrebnadzor.

The second method had differences and consisted of the following stages. The isolation of total DNA was carried out

by phenolic and salt methods with the lysis of proteinase K, using a set of Diatom DNA Prep 100 (Genlab LLC Research and Production Company (NPF), Russia).

The results were visualized using the Night Hawk Berthold gel documentation system (Berthold Technologies, Germany).

Statistical analysis of the obtained results was carried out according to the student's method using the Biostat program (Analyst Soft Inc., USA).

Results

The study was carried out on the territory of the Tyumen region. It had been established that the region was at great risk of bovine leukemia since 1968. In 1995, the proportion of animals with leukemia was at the highest level (3.5% of the examined animals). Such significant indicators of animals with leukemia were recorded until 1997. Later, the proportion of sick animals decreased to 2% and currently, hematological studies are continuing in the region, but indicators below 2.0% of the livestock have not been registered for 20 years. From the moment of fixing the highest indicator (1995) to the present (2019), the proportion of hematologically ill cattle has decreased to 1.6%, that is, by 54.3%.

The maximum rates of virus transmission among cattle in the region were recorded from 1989 to 1997. During this period, the values of infection in the herd were fixed at the level of 24.0-37.5%. Currently, the seropositivity of the herd is decreasing and in 2019 this indicator reached 7.0%, which is 81.3% lower than in 1989. In 2020, 160 unfavorable points for bovine leukemia were registered in the region. Monitoring of the epizootic situation of leukemia shows that the implementation of standard methods of prevention and elimination of leukemia is not effective enough, therefore it is necessary to study additional ways of spreading the pathogen to stop the disease.

Analyzing the methods of livestock management, we found that a significant part of the animals spent the period from April to October on pastures and was attacked by blood-sucking insects and ticks. According to our observations, 31.96% of all livestock in the Tyumen region (Russia) grazed in the warm season. Bloodsuckers are widespread in the region, which may be a mechanism for the transmission of infectious diseases, including bovine leukemia. Transmission of infection can vary from mechanical to complex preservation of the leukemia virus in the body of arthropods and transovarial and trans-phase transmission to the next generations and infection of livestock in a safe area a few years after the elimination of the disease.

We have to remember the wide distribution of *Dermacentor reticulatus Fabricius* in the Tyumen region, which is active from the third decade of March to the first decade of April (depending on meteorological conditions). In some years, the first cases of ixodid tick suction were recorded in the third decade of February. The first peak of activity is recorded from the third decade of April to the first of May, the second peak is recorded from the middle of August to the first decade of September. The last imagos of *Dermacentor* ticks on animals are detected until October 29.

The period of spring activity of *Dermacentor reticulatus* ticks lasts 52.1±1.27 days and is characterized by a high Abundance Index (AI), namely, 84.4±5.43 individuals with the duration of the mass population of 20.1±1.57 days. The summer-autumn period of parasitization lasts 48.2±2.31 days and is characterized by AI of 38.2±1.61 individuals and the mass population last 16.0±0.6 days. The duration of parasitization of ixodid ticks of the *Dermacentor* genus in the conditions of the Northern Trans-Urals is 100.3±1.79 days.

Taking into account the widespread and long period of activity, laboratory culture of *Dermacentor reticulatus* was used to reach this objective. The ticks were fed on animals with leukemia, infected (seropositive), and healthy animals.

After saturation (for 9-12 days), well-fed females of each group were used for two purposes: One for further metamorphosis and study of the preservation and transmission of the leukemia virus in the preimaginal stages of ticks and the other one for laboratory testing for the detection of leukemia provirus in them.

Adult ticks were also sent for laboratory testing.

Before the start of the study, all phases of tick development were kept in the laboratory at a temperature of minus 18°C.

The results of molecular genetic studies of imago ticks are shown in Table 1. As a result of laboratory tests, it was found that a DNA molecule of bovine leukemia provirus was found in the body of imago ticks fed on seropositive animals (n-5). Studies by the polymerase chain reaction of ixodid ticks taken from hematologically ill animals (n-13) in 92.9±1.9% of cases confirmed the presence of DNA of bovine leukemia provirus. A study of ixodid ticks fed on healthy animals (n-13) confirmed the absence of the causative agent of bovine leukemia in the body of ticks throughout the entire experiment.

Considering that laboratory tests were carried out throughout the calendar year, that is, from the moment the biomaterial was provided to the laboratory where the ticks were stored at low temperatures, even after a long period after their nutrition, the causative agent of leukemia was detected in the body of ixodid ticks, which indicates its high stability when located in a biological object.

The conducted studies give grounds to assert that the bovine leukemia virus can persist in the body of ixodid ticks for at least one year.

Table 1: Results of molecular genetic studies of imago ticks fed on healthy, infected, and leukemic animals

Group	Total female ixodid ticks fed, individuals	Transferred for laboratory diagnostics, individuals	The leukemia provirus was present in the body of an ixodid tick in…	
			Individuals	%
1st, experimental, imago, fed on seropositive animals	11	5	5	100.00000
2nd, experimental, imago, fed on hematologically sick animals	22	14	13	92.9±1.9
3rd, control, imago, fed on healthy	9	5	0	0.00000

Discussion

Researchers, acarologists, and protozoologists noted that the *Dermacentor reticulatus* tick dominated among other tick species, preserving the infectious origin as much as possible (Levytska *et al*., 2019; Fischer *et al*., 2020). It has been confirmed that *Dermacentor reticulatus* is actively involved in the transfer and preservation of protozoa in its body. The ability to reserve *Rickettsia slovaca*, *Rickettsia sibirica*, *Rickettsia honei*, and SFG *rickettsia* has been established (Levytska *et al*., 2021; Masika *et al*., 2021; Tian *et al*., 2021). The possibility of not only the preservation of *Babesia canis and B. caballi* in the body of ixodid ticks but also the spread of the pathogen among the urban population of dogs has been observed (Bonnet *et al*., 2013). The most extensive pathogen detection studies have revealed a wider range of protozoa and bacteria in the body of *D. reticulatus* ticks. Thus, the presence of *A. marginale, Bartonella spp., C. burnetii, Borrelia spp., and F. philomiragia* has been demonstrated. It was also found that *Dermacentor reticulatus* ticks were carriers of *Francisella tularensis and Francisella spp.* (Michelet *et al*., 2013; Springer *et al*., 2021). There is currently no data on the possibility of leukemia virus transmission. The preservation of leukemia provirus in the body of ixodid ticks has been proved by the use of two techniques that may not be suitable for further research in the study of the transovarial and trans-phase transmission of the virus. The absolute preservation of the provirus of leukemia in the body of ixodid ticks feeding on seropositive animals indicates the infectivity of infected animals and their main role in maintaining a dysfunctional focus of leukemia. There are fewer and fewer hematologically ill animals in the region every year, as they are culled from the herd as quickly as possible. Their participation in the spread of the virus is also great, but only 92.9±1.9% of ixodid ticks retained the virus in the body, which indicates either false-negative results or a decrease in the viability of the virus during the long course of the disease. The preservation of the provirus in the body of ixodid ticks does not yet prove the fact of the transmission of the infectious origin to subsequent generations, but only its presence. Taking into account the gonotrophic harmony of ixodid ticks, the transmission of imago provirus of leukemia in females is excluded. To clarify the participation of ixodid ticks in the transmissible spread of bovine leukemia, it is necessary to conduct model experiments and passage of the leukemia virus through all phases of the development of ixodid ticks.

Conclusion

In the Tyumen region, the problem of bovine leukemia has been urgent since 1968. Although the level of sick and infected animals has significantly decreased, it has not been possible to eliminate this disease until now. It has been established that the imago of ixodid ticks can preserve the DNA molecule of the bovine leukemia provirus in their body in 92.9-100% of cases when feeding on hematologically ill and seropositive animals, respectively. It has been found that the bovine leukemia virus can persist in the body of ixodid ticks for at least one year.

There is a possibility of transmissible transmission of leukemia virus transphasically, which would greatly increase the risk of the spread of infected ixodid and transmissible transmission of leukemia among susceptible animals. To isolate the DNA provirus of leukemia, it is advisable to use additional methods of genetic research, including real-time methods using new primers.

Funding Information

The study is funded by the Ministry of Agriculture of the Russian Federation, registration number 1022040800309-3-4.3.1;1.6.12;1.6.14

Author's Contributions

All authors contributed equally to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

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