ORIGINAL ARTICLE

Productivity and health markers for large cattle

I. M. Donnik[1], A. S. Krivonogova[2], A. G. Isaeva[2], A. G. Koshchaev[3], O. P. Neverova[1], O. A. Bykova[1]

[1]Department of Agriculture, Federal State Budgetary Educational Institution of Higher Education "Ural State Agrarian University" (FSBI HE Ural State University), Yekaterinburg, Russia, [2]Department of Biological Sciences, Federal State Budgetary Scientific Institution "Ural Scientific Research Veterinary Institute" (FSBSI the Ural SRVI), Yekaterinburg, Russia, [3]Department of Biological Sciences, Federal State Budgetary Educational Institution of Higher Education "Kuban State Agrarian University" (FSBEI HE Kuban SAU), Krasnodar, Russia

Abstract

Aim: For the first time, the Ural region reported positive results on the study of syncytial test diagnostic value at an early detection of the cattle leukemia virus carriers. **Method:** Study includes an intraperitoneal infection of laboratory animals (small ruminants) by the leukocyte suspension containing viral material. To perform the comparative diagnostic studies, they developed a group of animals kept in isolation, weekly studied by serological (infectious disease requisition [IDR] and enzyme-linked immunosorbent assay [ELISA]), molecular genetic (polymerase chain reaction [PCR]), cultural (ST) diagnostic research starting from the 6th day after infection. **Results:** Performed laboratory studies confirmed the diagnostic value of direct method identification concerning the causative agent of cattle leukemia. Thus in the earliest period (13 days) after the artificial infection of laboratory animals, they revealed a positive reaction in the syncytial method of research, which is confirmed by the controls. Positive PCR was revealed in 21 days, during the third study, and IDR and ELISA were revealed only on the 37th day (week 5). **Conclusion:** The developed early diagnosis of leukemia will improve the efficiency of agricultural enterprise recovery from the infection as the isolation of infected animals during the earliest period is one of the priority tasks during the implementation of health programs.

Key words: Cattle leukemia, cell culture, diagnosis, polymerase chain reaction, syncytial test

INTRODUCTION

The population provision with food requires the progressive production of agricultural raw materials. Nowadays, meat supply in Russia makes 80%, milk supply makes 75%, bakery product supply makes 100%, and vegetable supply makes 50% of the demand. If in recent years, the production of grain, vegetables, and poultry products has a pronounced positive dynamics, then, unfortunately, the production of milk and meat is inadequate. Thus, the growth of meat production makes 3-4%, milk production remains almost at the same level - 0.3-0.6%, despite the intensification of the livestock sector.[1]

This is primarily due to the steady decline of productive animal number, especially dairy cattle, and the reduction of their economic use period. The analysis of productive animal disposal shows that the term of the so-called productive longevity of cows differs significantly from the physiological life expectancy. Since the main determining factor of productive animal value in livestock production is economic expediency, the greater part of them (even especially valuable in genetic and tribal relations) is eliminated during the early life. It is noted that the higher the productivity, the shorter the period of animal use [Figure 1]. The most expedient disposal of animals is after four lactations.

The longer an animal retains its potential, the more profitable to keep it. At present, there is the tendency to reduce the use of farm

Address for correspondence:
I. M. Donnik, Federal State Budgetary Educational Institution of Higher Education "Ural State Agrarian University" (FSBI HE Ural State University), 42, Karla Liebknechta Street, Yekaterinburg, Russia.
E-mail: rector@urgau.ru

Received: 09-09-2017
Revised: 15-09-2017
Accepted: 19-09-2017

animals.[2] This is especially noticeable in dairy cattle breeding of breeding herds. Their productive longevity makes 2.2-3.2 of lactation, which is extremely unprofitable economically. The main reasons for this are the deterioration of animal health, the development of infectious diseases [Figure 2].

However, the transfer of dairy farming to industrial technology necessitates an increased need for healthy, highly productive dairy cattle well suited for such technologies.[3]

In this regard, the issue of productive animal health preservation is a priority for the agro-industrial complex of the country.[4]

Modern technologies of animal keeping, intensive technologies for the selection of dairy products, a significant dependence on the quality and the quantity of feed cause a high predisposition of productive animals to diseases of both infectious and non-contagious genesis.[5,6]

The adaptive potential of cows depends on the genetic, morphological, and physiological characteristics of their organism in the conditions of existing technological factors. The maximum realization of the genetic potential of animals is possible in certain technological conditions.

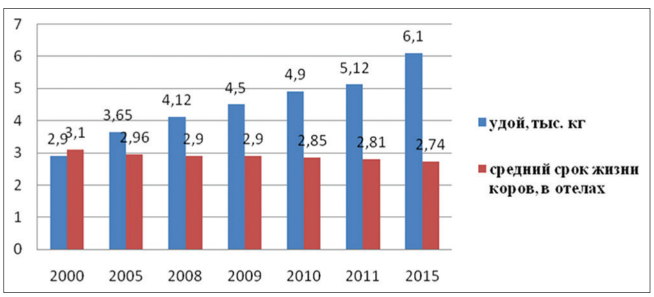

Figure 1: Dynamics of cow productivity and the period of economic use. Удой, тыс. кг. - The yield, thousands of kg, средний срок жизни коров, в отелах - Average life of cows, calving

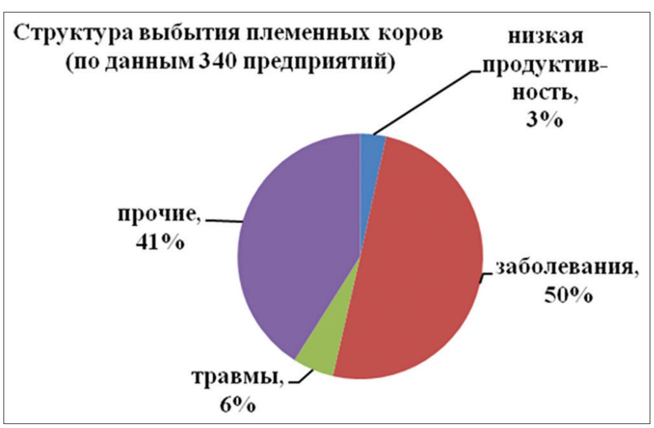

Figure 2: Structure of breeding cow disposal at agricultural enterprises (2015, *n* = 340). низкая продуктивность - Low productivity, заболевания – Diseases, травмы – Injuries, прочие - Other

Biogeochemical, technological and financial heterogeneity of agricultural enterprises determines the various mechanisms of cow adaptation to specific production conditions and develops a specific pathology structure for each enterprise. The effectiveness of therapeutic and prophylactic measures directly depends on the degree of a pathological process development.[6] Therefore, a timely donor zoological diagnostics of deviations in the conditions of a particular production comes to the forefront.[7,8]

LABORATORY DIAGNOSTICS OF ANIMAL DISEASES

Biochemical testing is one of the main links concerning the diagnostic algorithm for the study of highly productive animal health status, as well as an important component of the outpatient study of cattle. Metabolic indicators of blood are the reflection of biochemical processes occurring in an animal body.[9] Along with the approved methods of animal health evaluation, there is a need to search for new criteria and biochemical markers based on the donor zoological diagnosis of metabolic abnormalities. Unfortunately, often used the narrow list of biochemical indicators does not always allow a full assessment of productive animal organ functional state. The use of modern diagnostic methods will allow to conduct timely corrective measures, as well as to predict possible changes in productivity and, ultimately, to increase the useful life of highly productive animals.

Laboratory studies were conducted among 38,587 cows with the productivity of more than 6000 kg of milk/year from 28 dairy enterprises of the Urals region.

The biochemical blood test included a list of basic biochemical indicators (total protein, albumins, urea, creatinine, bilirubin total, cholesterol, alkaline phosphatase, alanine aminotransferase, aspartate amino transferase, calcium, phosphorus, magnesium, potassium, sodium, and chlorides), as well as additional indicators to assess the general state of metabolic processes, the functional state of liver, kidneys, heart muscle, and electrolyte blood balance (lactate dehydrogenase [LDG], α-hydroxybutyrate dehydrogenase, creatine phosphate kinase (CPK), CPK-MB, glutamate dehydrogenase, γ-glutamyltranspeptidase, cholinesterase, copper, zinc, etc.). The range included 26 blood indices.

The main pathological conditions associated with the violation of metabolic processes and the pathology of internal organs are the violation of protein, mineral, micro elemental metabolism, the violation of acid-base balance and electrolyte balance of blood, as well as pathology of liver, cardiovascular system, and skeletal musculature.

Studies showed that, despite the absence of clinical disorders among studied animals, 28% showed protein metabolism disorders, 58% demonstrated mineral metabolism disorders, and 41% demonstrated acid-base and electrolyte imbalance. The major share in the structure of mineral disorders was represented by the imbalance of total calcium and inorganic phosphorus, against the background of alkaline phosphatase increased activity, pathological changes in magnesium - 1.8% were less frequent. First of all, the deficiency of a complex of microelements in a body was negatively manifested in the digestive tract. At that, the assimilation of micronutrients from feeds decreases, while the decrease of microelement concentration in the blood serum is noted. Thus, the biochemical signs of hypomicroelementosis development were revealed among 16.3% within the group of studied animals.

The assessment of the internal organs of cow functional state revealed the pathological disorders of the liver among 38% of animals, heart muscle among 15% of animals, and skeletal muscles among 8.4% of animals [Table 1].

The hepatodepression syndrome characterizes the decrease of liver functioning parenchyma mass. With this syndrome, the content of albumins, cholesterol, urea, and cholinesterase activity was lowered in blood.

The syndrome of cholestasis was characteristic of 16.3% among examined cows. The syndrome characterized by impaired secretion or the outflow of bile from the liver. This group of animals included the animals that showed the increase of bilirubin, the activity of gamma-glutamyl transferase, and alkaline phosphatase.

Cytolysis syndrome is characterized by increased permeability of hepatocyte cellular membranes or their destruction. The indicators of cytolysis are represented by the enzymes that are located in the cytoplasm of the LDG cell and are found both in the cytoplasm and in the mitochondria of a cell - aspartate aminotransferase and glutamate dehydrogenase. At that, the appearance of the second group of enzymes in blood indicated a stronger degree of dystrophic changes in the liver. According to the obtained data, cytolysis syndrome was recorded among 14.9% of examined cows. Thus, taking into account comorbidities, liver damage syndromes were detected among 37.6% of the animals under study.

The performed studies showed that 15% of examined cows showed biochemical changes, which indicate the development of pathology in respect of cardiovascular system. The changes in the biochemical profile of animals were noted: An increase in LDG due to the isoenzyme LDG 1,2-alpha hydroxybutyrate dehydrogenase, as well as the increase in the activity of CPK, with the increase of CPK-MB activity.

During the study of CPK activity, it was found that 8.4% of the cows under study had the increase in enzyme activity due to the muscle fraction (CPK-MM), which indicates the damage to skeletal muscles: Trauma and progressive muscular dystrophy. Moreover, the increase of total CPK activity indicated the effects of fodder poisoning.

In addition to non-contagious diseases, infectious diseases cause significant damage to livestock. The situation in Russia is quite good for most especially dangerous animal diseases (foot and mouth disease, tuberculosis, brucellosis, salmonellosis, and others). However, in recent years, the epizootic situation has been complicated by the diseases among farm animals: African swine fever, nodular dermatitis and sheep, goat pox, Siberian anthrax, and others.

PECULIARITIES OF LEUKOSIS INFECTION DISTRIBUTION IN RUSSIAN FEDERATION

The disease leukosis bovine (leukosis of cattle) causes significant damage to dairy cattle. Unfortunately, this disease is widespread in the country; the epizootic situation does not change for the better.[10] In some regions, up to 50-80% of dairy cows are infected with the causative agent of this disease [Table 2].

Table 1: Cow pathology structure in the case of donor zoological biochemical diagnostics

Pathological impairments	Amount of animal with syndrome, %	Total number of pathologies, %
Liver: Syndrome of hepatodepression (decrease of albumins, urea, cholesterol, cholinesterase)	18.8	37.6
Cytolysis syndrome (increased activity of AST, GulDG, LDG)	14.9	
Cholestasis syndrome. Increased activity of GTP, alkaline phosphatase, increased total bilirubin	16.3	
Heart: Increased activity of LDG, by increasing alpha-GbDG, KFK activity increase due to CPK-MB increase	15.0	15.0
Muscle tissue: Increased activity of CPK at CPK-MB within the limits of physiological norm	8.4	8.4

AST: Aspartate aminotransferase, LDG: Lactate dehydrogenase, GulDG: Glutamate dehydrogenase, GGT: Gamma-glutamyl transferase, CK: Creatine kinase, GbDG: Hydroxybutyrate dehydrogenase

The elimination of this disease at dairy farms lasts more than 30 years. The data on virus biology that causes leukemia were obtained during the 80s of the last century and allowed to develop the necessary legal and regulatory documents that allow to conduct recreational activities among cattle. However, the disease was completely eliminated only in three regions of Russia (Sverdlovsk, Leningrad region and YaNAO).

For many years, the causative agent can persist in a herd without causing symptomatic signs and seroconversion, which indicates both the lack of diagnosis and the possibility of leukemia causative agent (cattle LCA) to bypass an immune response in some individuals. Until now, the data of LCA cattle three strains of provirus consequences were obtained from various geographical regions.[11,12]

Having studied the genetic structure of the leukemia virus, it is easier to optimize the diagnostic methods of research by developing specific primers for polymerase chain reaction (PCR). This will make it possible to identify virus carriers at an early age (15 days) and in healthy farms with confidence, as well as at the final stages of farm recovery from leukemia.

Table 2: Epizootic situation in cattle leukemia in the Federal Districts (Gulyukin *et al.*, 2016)

Name of subjects	Number of unfavorable places (%)
Russian Federation	2216 (100)
Central Federal District	607 (27.33)
North-Western Federal District	73 (3.2)
Southern Federal District	161 (7.2)
North-Caucasian Federal District	26 (1.1)
Volga Region Federal District	570 (25.7)
Ural Federal District	375 (16.9)
Siberian Federal District	281 (16.8)
Far Eastern Federal District	124 (12.6)
Crimean Federal District	-

The studies of samples from various parts of the country showed that the samples from the Urals region represent the American version of the leukemia virus. The sequences are very closely related to one another, and were grouped in the genetic group A, along with some samples from Poland and Ukraine [Table 3].

The study of structure peculiarities and virus/provirus genome functioning may help to understand and control the course of the infectious process in the future.[11,12] We can also detect infection before the appearance of a definite antibody titer. Besides, one may distinguish active infection from passive maternal immunity, when newborn calves with colostrum receive colostral antibodies to cattle LCA.

Kuzmak (2008) in his works points out that the genetic variants of cattle LCA with certain amino acid substitutions in the epitopes of gp51 protein were found in 7.5% of cattle infected with LCA, but among seronegative animals such variants may avoid the detection of antibodies and will interfere significantly with the serological diagnosis of cattle LCA infection.[13]

Based on the results of the sequencing, we performed the phylogenetic analysis and, according to the international classification, the dendrogram of the territorial distribution of cattle LCA strains was developed. Phylogenetic analysis allowed to classify the experimental strains within the group and the subgroup, and also to compare with known world isolates. During this analysis the differences between the groups and the distance between the known genotypes of the virus were determined. During comparison, the main

Table 3: The results of cattle LCA genotyping from isolates obtained in various regions of Russian Federation (the studies were carried out jointly with SRVI [the city of Pulawy, Poland])

No.	Accession No.	Geographic origin	Restriction enzymes and fragments			Classification
			BanHI	BclI	PvuII	Beier *et al.*, 2001
1S	JF720349.1	Western Siberia Tyumen region	315/129	226/218	444	Australian
2S	JF720350.1		315/129	226/218	444	Australian
3S	JF720351.1		315/129	226/218	281/163	Not classified
5S	JF720353.1	Western Siberia Tyumen region	315/129	226/218	281/163	Not classified
1C	JF720354.1	South Ural Chelyabinsk region	444	226/218	281/163	Belgian
7C	JF720355.1		444	226/218	281/163	Belgian
8C	JF720356.1		444	226/218	281/163	Belgian
9C	JF720357.1		444	226/218	281/163	Belgian
10C	JF720358.1		444	226/218	281/163	Belgian

LCA: Leukemia causative agent

mutational changes are located in the epitopes 2 ND and CD8 + (the subgroup of cytotoxic lymphocytes), the latter of which is responsible for an immune response suppression in a body, namely, the production of T lymphocytes. These animals have a large amount of provirus in blood. Leukocytosis is observed, the immunological response is suppressed.[14]

This "aggressive" type of virus is predominantly defined in Group III (B subgroup) and its existence is explained by the evolutionary stage of the virus adaptation in a host organism during territorial migration.

We compared 18 samples taken from different regions with 62 known world strains taken from GenBank (NCBI). Four groups of LCA cattle were classified, the genetic distance between which was not so great; the performed genotyping was confirmed - the dendrogram has clearly visible distinctions between the Australian and Belgian types of LCA cattle, defined by us in each of the isolates under restriction fragment length polymorphism (RFLP). The first group, along with the isolates from Chile, Poland, and Ukraine, had all samples from the Sverdlovsk region (2, 3, 4, 5), one from the Krasnodar Territory (3), one from the Kurgan region (4K), and three samples from Tyumen region. The third group along with samples from Poland, Belgium, Ukraine, Germany, and Chile had four isolates from the Krasnodar Territory (1, 2, 4, 5), two from the Chelyabinsk region (4Z, 5Z), one sample from the Kurgan region (3K), and one from the Tyumen region (Yalutorovsky district (6T)). One isolate from the Kurgan region was located somewhat apart from the others, at the border of the second and third group. According to RFLP result it was classified as A/B.

CONCLUSIONS

The conducted laboratory tests confirmed the diagnostic value of direct identification methods concerning the causative agent of LCA cattle. At that, during the earliest possible time (13 days), after an artificial infection of laboratory animals, they revealed a positive reaction in the syncytial method of the study, which was confirmed by controls. Positive PCR appeared in 21 days, with the third study, and infectious disease requisition, enzyme-linked immunosorbent assay, appeared only in 37 days (5 weeks).

At the moment practically syncytial method can be used as the method of biomaterial preparation for PCR diagnostics according to a proposed scheme, with the cultivation of the CC81 cell culture within the Eagle MEM medium. With the given parameters, the level of viral load increases, which increases the efficiency of PCR diagnostics and allows to detect virus carriers among the calves of embryonic infection at the age of 15-30 days, reducing the cost of herd improvement.

SUMMARY

Thus, we carried out the studies that allow us to classify LCA cattle isolates from Russia. Molecular-genetic and immune-biochemical markers are the indicators of productive animal health and they make it possible to increase the efficiency of pathological condition early diagnosis and to correct them in time.

ACKNOWLEDGMENTS

The study was funded by Russian Science Foundation (project No. 16-16-00071) and supported by the FSBEI of the Ural State Agrarian University in the city of Yekaterinburg.

REFERENCES

1. Ushachev IG. Import substitution in the agro-industrial complex: Trends, problems, ways of development. Econ Agric Process Enterp 2016;1:2-10.
2. Shabunin SV, Nezhdanov AG. Systemic solution of the problem concerning the preservation of reproductive ability and productive longevity of dairy cattle. Vet Farm Anim 2014;8:3-13.
3. Fisinin VI. Success and the problems of Russian livestock breeding. Livest Breed Russ 2008;1:4-6.
4. Donnik I, Voronin B, Loretts O. Production of organic agricultural products is an important area of "Green" economy. Indian J Sci Technol 2016;9. DOI: 10.17485/ijst/2016/v9i14/91512.
5. Smirnov AM, Butko MP. The provision of veterinary and sanitary well-being within the territory of Russian federation. Russ J Probl Vet Sanit Hyg Ecol 2011;1:7-11.
6. Strekozov NI, Chinarov VI, Chinarov AV. Strategic trends of dairy cattle development. Econ Agric Process Enterp 2016;4:11-4.
7. Romanenko G.A. Genetic markers in the selection of the Ural black-and-white cattle. Agrar Herald Urals 2009;4:82-3.
8. Yaryshkin AA, Shatalina OS, Novitskaya KV, Puzanova IA. Manufacturing of reagents for immunogenic examination of cattle. Bull Biotechnol 2017;3:???.
9. Bykova OA. Morphological composition and metabolites of young cattle blood. Agrar Bull Urals 2017;5:5-12.
10. Gulyukin MI, Barabanov II, Ivanova LA, Trukhachev VI, Selionova MI, Chizhova LN, *et al*. Monitoring of epizootic situation in leukemia of cattle at commodity and breeding farms of Russian federation during 2014-2015. Vet Med Feed 2016;4:5-41.
11. Lomakina NF, Gulyukin MI. Genotyping of cattle leucosis viruses circulating in farms of Moscow, Kaluga and Rostov regions. Mol Diagn 2010;2:140-2.
12. Vinogradova IV, Gladyr EA, Kovalyuk NV,

Petropavlovsky MV, Ernst LK, Zinovieva NA, *et al*. The study of genotypic diversity, molecular genetic structure and phylogenetic analysis of cattle leucosis causative agent circulating in the animal population from different regions of Russia. In: Modern Molecular Genetics and Immune - Physiological Approaches to the Elimination of Animal Hemoblastoses. Collection of Scientific Works. Yekaterinburg: Ural Publishing House; 2014. p. 184.
13. Rola-Luszczak M, Pluta A, Olech M, Kuzmak J, Petropavlovskiy M, Gerilovych A, *et al*. The molecular characterization of leukemia virus isolates from Eastern Europe and its impact on phylogeny. PLoS One 2013;3:e58705.
14. Donnik IM, Petropavlovsky MV, Krivonogova AS, Shkuratova IA, Rola-Łuszczak M, Kuzrmak J. Revisiting the Issue of the molecular-genetic structure of the causative agent of the bovine leukemia virus in the Russian federation. Indian J Sci Technol 2016;9:15-20.

Source of Support: Nil. **Conflict of Interest:** None declared.