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Enzyme-linked immunosorbent assay for post-slaughter diagnosis of bovine leukosis

Arkif R. Mustafayev, Magomed O. Baratov

Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center, 88 Dakhadaeva str., Makhachkala 367000, Republic of Dagestan, Russia

ABSTRACT

Postmortem diagnosis of enzootic bovine leukosis is made on the basis of the results of tests of biological materials from emergently slaughtered or fallen animals using pathomorphological, histological and molecular genetic methods that have some disadvantages. Results of post-slaughter diagnostic tests for bovine leukosis with enzyme-linked immunosorbent assay are described in the paper. For this purpose, 83 swabs were collected from different carcass parts including 71 swabs from carcasses of the animals that were not pre-slaughter tested and 12 samples from the carcasses of the animals that were pre-slaughter tested with immunodiffusion assay and found bovine leukemia virus-seronegative (control samples). Sterile scalpels, cotton wool, 5 mL tubes with caps were used for swab collection. The samples were taken from incisions in carcasses and internal organs of slaughtered animals with sterile cotton-wool swabs and placed in single-use tubes. Distilled water (or isotonic solution – 0.85% NaCl) was added to the tubes with samples, 0.1 to 0.2 mL per tube depending on the sample size, and the tubes were left at room temperature (22–26 °C) for 1.5–2.0 hours and regularly shaken. Resulting homogeneous substrate was used for enzyme-linked immunosorbent assay carried out in accordance with the instructions for the test-kit for detection of antibodies against bovine leukemia virus. Specific antibodies to bovine leukemia virus gp51 antigen were detected in 6 (8.5%) out of 71 swabs subjected to the laboratory tests. Therewith, the antibodies were detected only in 3 swabs (4.2%) when the swabs were tested with immunodiffusion assay. All 12 control samples from animals that were pre-slaughter tested and found seronegative were negative when tested with enzyme-linked immunosorbent assay. Therefore, the above-said serological method can be used for post-slaughter diagnosis of bovine leukosis together with conventional methods.

Keywords: enzootic bovine leukosis, post-slaughter diagnosis, enzyme-linked immunosorbent assay (ELISA), swabs from carcasses and internal organs, specific antibodies, gp51 antigen of bovine leukemia virus

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For correspondence: Arkif R. Mustafayev, Cand. Sci. (Veterinary Medicine), Leading Researcher, Laboratory of Infectious Pathology of Farm Animals, Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center, 88 Dakhadaeva str., Makhachkala 367000, Republic of Dagestan, Russia, *e-mail: mustafaev_arkif@mail.ru*

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Применение иммуноферментного анализа в послеубойной диагностике лейкоза крупного рогатого скота

А. Р. Мустафаев, М. О. Баратов

Прикаспийский зональный научно-исследовательский ветеринарный институт – филиал ФГБНУ «Федеральный аграрный научный центр Республики Дагестан» (Прикаспийский зональный НИВИ – филиал ФГБНУ «ФАНЦ РД»), ул. Дахадаева, 88, г. Махачкала, 367000, Республика Дагестан, Россия

РЕЗЮМЕ

Послеубойный диагноз на энзоотический лейкоз крупного рогатого скота ставится на основании результатов исследований биологического материала, полученного от вынужденно убитых или павших животных, выполненных патоморфологическим, гистологическим и молекулярно-генетическим методами, обладающими рядом недостатков. В статье описываются результаты послеубойного диагностического исследования на лейкоз крупного рогатого скота с применением иммуноферментного анализа. Для этого с различных частей туш и органов было отобрано 83 пробы смывов, из них 71 проба – от прижизненно не исследованных животных, а 12 проб (контрольные образцы) – от прижизненно серонегативных в реакции иммунодиффузии к вирусу лейкоза особей. Для взятия проб были использованы стерильные скальпели, вата, пробирки с колпачком объемом 5 мл. С помощью

тампонов из стерильной ваты из надрезов туш и органов послеубойных животных производили взятие смывов, которые помещали в одноразовые пробирки. В пробирки со смывами в зависимости от размера тампона добавляли от 0,1 до 0,2 мл дистиллированной воды (или изотонического раствора – 0,85%-го раствора NaCl), оставляли на 1,5–2,0 ч при комнатной температуре (22–26 °С) и периодически встряхивали. Полученный однородный субстрат использовали для проведения иммуноферментного анализа в соответствии с инструкцией по применению набора для выявления антител к вирусу лейкоза крупного рогатого скота. В результате проведенных лабораторных исследований 71 пробы смывов в 6 (8,5%) из них были выявлены специфические антитела к антигену gp51 вируса лейкоза, при этом при исследовании данных проб в реакции иммунодиффузии антитела выявили только в 3 (4,2%) пробах. Все 12 контрольных образцов от прижизненно серонегативных животных при постановке иммуноферментного анализа дали отрицательный результат. Таким образом, данный серологический метод может применяться в послеубойной диагностике лейкоза крупного рогатого скота наряду с общепринятыми методами.

Ключевые слова: энзоотический лейкоз крупного рогатого скота, послеубойная диагностика, иммуноферментный анализ, смывы с туш и органов, специфические антитела, антиген gp51 вируса лейкоза крупного рогатого скота

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Для корреспонденции: Мустафаев Аркиф Рамазанович, канд. вет. наук, ведущий научный сотрудник лаборатории инфекционной патологии сельскохозяйственных животных, Прикаспийский зональный НИВИ – филиал ФГБНУ «ФАНЦ РД», ул. Дахадаева, 88, г. Махачкала, 367000, Республика Дагестан, Россия, *e-mail: mustafaev_arkif@mail.ru*

INTRODUCTION

Enzootic bovine leukosis (EBL) is widely spread in many countries as well as in the Russian Federation. Animals infected with EBL virus are the source of the agent at all disease stages [1, 2, 3]. The peculiarity of the disease is that it occurs mainly in a chronic form without clinical symptoms and is characterized by rampant growth of neoplastic blood cells, that, through malignancy and proliferation, affect almost all organs of the animal [4, 5, 6]. The disease progresses through several stages from bovine leukemia virus entry to the animal's body to leukosis clinical manifestations:

1) incubation period (it lasts for 8 to 20 days);

2) asymptomatic virus-carrier state (seropositive animals);

3) hematological stage (changes in composition of blood formed elements);

4) clinical stage (tumor).

The disease is lifetime diagnosed with serological methods (immunodiffusion assay, enzyme-linked immunosorbent assay (ELISA), etc.) based on detection of antibodies to bovine leukemia virus antigens developed by the animals at all stages (except for incubation period). There are other methods that are used for lifetime diagnosis of EBL together with serological ones: clinical, cytomorphological, hematological, bioassay in animals (mainly in sheep), etc. [7, 8, 9, 10, 11, 12, 13]. Polymerase chain reaction (PCR) is also used for laboratory diagnosis of the disease [14, 15].

Post-mortem EBL diagnosis is made based on tests of biological materials collected from emergently slaughtered and fallen animals using pathomorphological, histological and molecular genetic (PCR) methods. The following lesions are detected during pathomorphological examination of carcasses and organs of emergently slaughtered or fallen animals with pathology: proliferative (tumor) masses, enlarged lymph nodes, changes in the internal organ size and organ tissue consistency. Pathological manifestations in organs and body systems vary depending on the bovine leukosis form. For example, in case of lymphoid, undifferentiated and myeloid forms of the disease, the lymph nodes are enlarged with gray-white, firm and waxy cut surfaces, and the spleen may be also enlarged. In case of myeloid form of leukosis, the spleen pulp is red-crimson in color with loose consistency and hemorrhages. In case of hematosarcoma (particularly lymphogranulomatosis), the spleen is enlarged in about 50% of affected animals. Focal (diffuse) proliferative masses of grey-pink or gray-white color in the affected organs (kidneys, liver, skeletal muscles, etc.) are found in animals with any of bovine leukosis forms. Histological analysis is carried out when pathomorphological picture is not obvious. For this purpose, sections of organ pieces (bone marrow, spleen, lymph nodes, etc.) and tissues (connective, muscle, etc.) are prepared using specified methods. The main disadvantages of pathomorphological and histological methods are as follows: these methods are not capable of detecting seropositive for bovine leukemia virus (BLV) animals at early stage and histological analysis and further post-slaughter diagnosis of bovine leukosis are time-consuming (3–4 days) that may affect the quality of tested meat and offal [16, 17].

Molecular-genetic method is important for post-slaughter diagnosis; PCR is used when postmortem picture is not obvious and hampers diagnosis. This method allows for detection of BLV proviral DNA integrated in the host cell genome in organ tissues and muscles. However, PCR has some disadvantages: high cost of analyses, need for maintaining environment temperature, nonspecific reactions, etc.

Serological method was used for post-slaughter diagnosis of bovine leukosis during earlier studies. Antibodies

to BLV antigen were detected with immunodiffusion test in muscle-tissue fluid (plasma, lymph) collected from carcasses and offal of slaughtered animals [18, 19]. Despite of substantial benefits of the proposed method for post-slaughter diagnosis (low cost of the test-kit, easy test running, etc.) it has some disadvantages. The said disadvantages are as follows: time required for immunodiffusion assay (the test results are read after 48 hours), low sensitivity of the test, possible inconclusive results (cross-reactions) [20].

Considering the above, the study was aimed at use of a new technique for post-slaughter diagnosis of bovine leukosis with ELISA.

MATERIALS AND METHODS

Eighty-three samples collected from carcasses and offal of slaughtered animals on the Makhachkala universal market No. 2 were the main materials used for the tests for bovine leukosis. Twelve slaughtered animals used for the test were tested before slaughter and found BLV seronegative based on the veterinary certificates issued by the Veterinary Units and 71 slaughtered animals used for the test were not tested for bovine leukosis with immunodiffusion assay, ELISA, etc. before slaughter.

Swabs were collected from different parts of carcasses and organs for diagnostic tests. Sterile scalpels, cotton wool, 5 mL tubes with caps were used for swabbing. Small tampons were made from the cotton wool and used for taking swabs from incisions in carcasses and organs of the slaughtered animals. Then, the swabs were placed in single-use tubes, the tubes were properly labelled and accompanying documents were prepared for their transportation. The accompanying documents contained information on the sample collection time and place, sample number and other data. In the testing laboratory, distilled water (or isotonic solution – 0.85% NaCl solution) was added to the tubes with swabs, 0.1 to 0.2 mL per tube depending on the tampon size and then the tubes were left at room temperature of 22–26 °C for 1.5–2.0 hours and regularly shaken. Resulting homogeneous substrate from the tubes was used for ELISA testing in accordance with the Instruction on use of ELISA test-kit for detection of antibodies to BLV (Vetbiochem, Russia).

The swabs were taken from carcasses and internal organs (offal) of the slaughtered animals in accordance with Order of the Ministry of Agriculture of the Russian Federation No. 269 of 28 April 2022 on approval of the Veterinary Rules for animal slaughter and Veterinary Rules for veterinary and sanitary examination of meat and products derived from slaughtered (hunted) animals and intended for processing and (or) marketing[1]; serological tests were carried out in accordance with the Methodical Guidelines for bovine leukosis diagnosis approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation No. 1372/2130 of 23 August 2008[2].

RESULTS AND DISCUSSION

For ELISA tests of 71 samples collected from the cattle not subjected to lifetime tests for bovine leukosis, 100 µL of the buffer for sample dilution were added to each of 75 wells of a strip plate (96-well microplate coated with specific gp51 antigen of BLV). Control sera (C^+ and C^-) in duplicate were added to 4 out of 75 wells, 4 µL per well, and test homogeneous substrate (tissue fluid swab diffused with distilled water) was added to other 71 wells, 4 µL per well. The well contents were thoroughly mixed and the plate was coated with adhesive tape and incubated in a thermostat at temperature of 37 °C for 1 hour. After incubation the plate was washed thrice with preliminary prepared working phosphate-buffered saline (PBS) solution containing Tween-20, by filling the wells with the PBS solution to the top manually (300 µL per well). Then, liquid in the wells was decanted and the plate was dried by tapping against filter paper folded in several layers. The conjugate solution (peroxidase-conjugated anti-bovine IgG monoclonal antibodies) were added to the microplate wells, 100 µL per well, the plate was coated with adhesive tape and incubated in the thermostat at temperature of 37 °C for 1 hour. After incubation the wells were washed thrice with phosphate-buffered saline solution containing Tween-20 (300 µL per well) and dried by tapping against folded filter paper. Then, tetramethylbenzidine solution containing hydrogen peroxide was added to the plate wells, 100 µL per well, and the plate was left at temperature of 22 °C for 10 minutes in a dark place. The reaction was stopped by adding stop-solution (1 N H_2SO_4), 50 µL per well. ELISA results were read by measuring absorbance with a spectrophotometer at wave length of 450 nm.

For final assessment of ELISA results, mean optical density values of positive and negative controls were determined. The relative amounts of anti-BLV antibodies expressed in international ELISA units (EU) in the negative control (C^-) and in test samples were calculated according to the formula:

$$EU = \frac{OD\ (test\ sample)}{OD\ (positive\ control)} \times 100.$$

Specific antibodies to BLV gp51 antigen were detected in 6 (8.5%) out of 71 homogeneous substrate (swab) samples subjected to laboratory tests with ELISA.

Twelve samples from carcasses and offal of the slaughtered animals subjected to lifetime testing for bovine leukosis with immunodiffusion assay and found BLV-seronegative (control samples) were similarly tested with ELISA. All samples were tested negative.

At the next stage, ELISA-tested samples (71 samples) were comparatively tested with immunodiffusion assay and antibodies to BLV antigen were found in 3 (4.2%) samples (swabs). Results of post-slaughter bovine leukosis diagnosis with immunodiffusion assay and ELISA given in the table below show that ELISA is more sensitive as compared to immunodiffusion assay.

Thus, ELISA is able to detect specific antibodies in tissue fluids (plasma and lymph) to BLV gp51 antigen that simplifies and may facilitate post-slaughter bovine leukosis diagnosis.

CONCLUSION

Based on the results of post-slaughter tests of swabs from the animal carcasses and offal for antibodies against BLV antigen contained in tissue fluids (plasma and lymph), bovine leukosis was diagnosed with ELISA in 6 (8.5%) out of 71 swabs and was diagnosed with immunodiffusion assay in 3 (4.2%) out of 71 swabs. Twelve (12) swabs from carcasses and offal of the slaughtered animals subjected

[1] https://www.garant.ru/products/ipo/prime/doc/404684483
[2] https://docs.cntd.ru/document/1200118749

Table
Post-slaughter diagnosis of bovine leukosis with ELISA and immunodiffusion assay

Serological diagnosis of EBL in animals	Number of samples	Specific anti-BLV antigens were detected	No specific anti-BLV antigens were detected
No lifetime tests for bovine leukosis were carried out			
Tested with immunodiffusion assay	71	3 (4.2%)	68 (95.8%)
Tested with enzyme-linked immunosorbent assay		6 (8.5%)	65 (91.5%)
Animals were lifetime tested with immunodiffusion assay and found negative			
Tested with enzyme-linked immunosorbent assay after slaughter	12	0	12 (100%)

to lifetime testing for bovine leukosis with immunodiffusion assay and found BLV-seronegative served as control samples and the said animals were also post-slaughter tested negative with ELISA.

Thus, post-slaughter ELISA tests showed that this test-system can be used for bovine leukosis diagnosis together with conventional methods (postmortem examination, histology, etc.) [21].

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INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Arkif R. Mustafayev, Cand. Sci. (Veterinary Medicine), Leading Researcher, Laboratory of Infectious Pathology of Farm Animals, Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center, Makhachkala, Republic of Dagestan, Russia; *https://orcid.org/0000-0002-5142-8360, e-mail: mustafaev_arkif@mail.ru*

Magomed O. Baratov, Dr. Sci. (Veterinary Medicine), Chief Researcher, Head of Laboratory of Infectious Pathology of Farm Animals, Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center, Makhachkala, Republic of Dagestan, Russia; *https://orcid.org/0000-0002-8261-5038, e-mail: alama500@rambler.ru*

Мустафаев Аркиф Рамазанович, канд. вет. наук, ведущий научный сотрудник лаборатории инфекционной патологии сельскохозяйственных животных Прикаспийского зонального НИВИ – филиала ФГБНУ «ФАНЦ РД», г. Махачкала, Республика Дагестан, Россия; *https://orcid.org/0000-0002-5142-8360, e-mail: mustafaev_arkif@mail.ru*

Баратов Магомед Омарович, д-р вет. наук, главный научный сотрудник, заведующий лабораторией инфекционной патологии сельскохозяйственных животных Прикаспийского зонального НИВИ – филиала ФГБНУ «ФАНЦ РД», г. Махачкала, Республика Дагестан, Россия; *https://orcid.org/0000-0002-8261-5038, e-mail: alama500@rambler.ru*

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