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Immunodiffusion assay as a method of bovine leukosis post-mortem diagnosis

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SUMMARY

New method of post-mortem diagnosis of bovine leukosis is proposed and it involves use of agar gel immunodiffusion assay. The proposed method allows for the detection of antibodies against bovine leukemia virus (BLV) antigen located in the muscle and tissue fluids (plasma and lymph) of meat and offal. Post-mortem sampling was performed by dragging sterile cotton swabs across different parts of carcass and organs of both animals known to be seronegative and animals not tested alive. The collected samples and accompanied documents were submitted to the laboratory. 0.5–0.7 mL of isotonic solution (0.85% sodium chloride solution) were added to the tube with the swabs and the tube was left for 3–5 hours at 18–26 °C until homogenous substance formation. The tube was occasionally shaken so that BLV antibodies moved to the normal saline solution for further immunodiffusion assay. The assay results were visually recorded by detection of precipitation lines. Testing of 175 samples collected from animals not serologically tested for bovine leukosis before slaughter demonstrated five positive results (2.9%). Immunodiffusion assay of the tissue (lymphatic) fluid swabs collected from 148 animals, declared BLV seronegative alive in the veterinary certificates, demonstrated negative results. Therefore, along with autopsy, histological, molecular and genetic methods the immunodiffusion assay can be one of the tools for post-mortem diagnosis of bovine leukosis.

Keywords: bovine leukosis, post-mortem diagnosis, immunodiffusion assay, serology, homogenous substance

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Применение реакции иммунодиффузии как один из способов послеубойной диагностики лейкоза крупного рогатого скота

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РЕЗЮМЕ

Предложен новый способ послеубойной диагностики лейкоза крупного рогатого скота с применением реакции иммунной диффузии в геле агара. Предлагаемый метод позволяет выявлять антитела к антигену вируса лейкоза крупного рогатого скота, находящиеся в мышечно-тканевой жидкости (в плазме и лимфе) мяса и субпродуктов. Послеубойный отбор проб производили стерильным ватным тампоном путем смыва из разных частей туши и органов как заведомо серонегативных, так и не исследованных прижизненно животных. Полученные образцы биологического материала доставляли с сопроводительными документами в лабораторию. В пробирку со смывом добавляли от 0,5 до 0,7 мл изотонического раствора (0,85%-й раствор хлорида натрия) и оставляли на 3–5 ч для перехода в однородную субстанцию, выдерживали при температуре 18–26 °С и периодически встряхивали, чтобы антитела к вирусу лейкоза крупного рогатого скота со смыва переходили в физиологический раствор для дальнейшей постановки реакции иммунодиффузии. Учет результатов при проведении реакции проводили визуально путем выявления линий преципитации. При исследовании 175 образцов биологического материала от животных, не исследованных прижизненно на лейкоз крупного рогатого скота, серологическим методом положительный на лейкоз результат был получен в 5 (2,9%) случаях. При постановке реакции иммунодиффузии с пробами смывов с тканевой (лимфатической) жидкости, отобранными от 148 животных, которые на основании ветеринарных справок были прижизненно серонегативными к вирусу лейкоза крупного рогатого скота, получили

отрицательные результаты. Таким образом, применение реакции иммунодиффузии может стать одним из способов послеубойной диагностики лейкоза крупного рогатого скота наряду с патолого-анатомическими, гистологическими, молекулярно-генетическими методами.

Ключевые слова: лейкоз крупного рогатого скота, послеубойная диагностика, реакция иммунодиффузии, серология, однородная субстанция

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INTRODUCTION

Bovine leucosis is induced by bovine leukemia virus (BLV) belonging to *Retroviridae* family. According to the International Committee on Taxonomy of Viruses (ICTV)[1], from 2020 *Retroviridae* family includes 68 species, 11 genera and two sub-families: *Orthoretrovirinae* and *Spumaretrovirinae*. Sub-family *Orthoretrovirinae* includes six genera: *Alpharetroviris*, *Betaretrovirus*, *Deltaretrovirus*, *Epsilonretrovirus*, *Gammaretrovirus*, *Lentivirus*; sub-family *Spumaretrovirinae* – five genera: *Bovispumavirus*, *Equispumavirus*, *Felispumavirus*, *Prosimiispumavirus*, *Simiispumavirus*. *Bovine leukemia virus*, or BLV, belongs to genus *Deltaretrovirus*, which, in addition to BLV, includes three more species: primate T-lymphotropic viruses (HTLV-I, HTLV-II, HTLV-III) [1–3]. BLV affects hematopoietic and lymphoid tissues of animals and involves bone marrow, spleen, lymph nodes, etc. into the pathologic process. At late disease stage, other organs are also affected (stomach, liver, intestines, lungs, etc.) due to proliferation and malignant degeneration of blast cells [4–6].

Veterinary laboratories make lifetime diagnosis of bovine leucosis using different methods, such as serological ones involving enzyme-linked immunosorbent assay (ELISA) and agar gel immunodiffusion (AGID), molecular-genetic tools involving polymerase-chain reaction (PCR), haemotological, clinical and cytomorphologic methods [7–9].

Post-mortem diagnosis of bovine leucosis is made on the basis of *post-mortem* examination and histological tests of fallen or emergently slaughtered animals. At necropsy, *post-mortem* lesions of organs and tissues are recorded depending on the leucosis lesion progression and nature. In case of lymphocytic leukemia, the spleen and lymph nodes are enlarged and bone marrow metaplasia is reported. On late stages, lymphoid hyperplasia is also observed in other organs. In case of monocytic leukemia, there are no *post-mortem* lesions on early stages, but on the later ones the lymph nodes get enlarged and often fused. In case of acute hemocytoblastic leukemia, spleen and lymph nodes are enlarged is size and weight. In case of myeloid bovine leukemia, the *post-mortem* lesions are reported in lymph nodes, spleen and liver; myelocyte foci are reported as well.

[1] https://talk.ictvonline.org.

Autopsy examinations do not always demonstrate lesions in animal organs, especially on early stages of the disease. Sometimes bovine leucosis cannot be successfully differentiated from many other diseases (actinomycosis, tuberculosis, paratuberculosis, brucellosis, etc.). In case the disease pathological pattern is not clear enough, the diagnosis should be confirmed by laboratory tools involving production of histological preparations of animal organs and muscles and their examination using light and electronic microscopes [10–12].

PCR-based molecular-genetic method is equally important for the *post-mortem* diagnosis of bovine leucosis. This method can be applied both for lifetime and *post-mortem* diagnosis of bovine leucosis. PCR can detect DNA of bovine leucosis provirus in animal blood or muscle tissue samples.

Due to the complexity of the above-mentioned tests (*post-mortem*, histological, molecular-genetic), new AGID tool can be relevant for the *post-mortem* diagnosis of bovine leucosis thus facilitating the efforts of the veterinarians. Therefore, the work was aimed at the use of AGID-based serological method for *post-mortem* diagnosis of bovine leucosis.

MATERIALS AND METHODS

The material used for bovine leucosis *post-mortem* diagnosis included 323 samples collected from bovine carcasses and offal (liver, spleen, kidney, etc.) on the market No. 2 in Makhachkala, Republic of Dagestan. Among these, 148 samples were collected from animals, which were declared seronegative when alive by the veterinary certificates; and 175 samples were collected from the animals not subjected to *ante-mortem* serological tests for BLV. Since BLV antibodies are detected in muscle tissue fluid (plasma and lymph) using AGID, the samples were collected as swabs taken by sterile cotton from the carcass surfaces as well as from the muscle tissues incised by sterile scalpel. The collected samples were delivered to the laboratory for serological testing. The samples were transported in sterile tubes with designation of the number, animal species, date, time and place of swab collection, etc.

Post-mortem AGID tests of animal carcasses and offal were performed in the laboratory using the test-kit for

bovine leucosis serological diagnosis manufactured by FKP "Kursk Biofactory" (Russia).

Samples (swabs) were collected from the carcasses and internal organs according to the "Rules for ante-mortem examination of slaughter animals and post-mortem inspection of meat and meat products" [13], and the serological tests were carried out using "Methodical guidance for bovine leucosis diagnosis" [14].

RESULTS AND DISCUSSION

All veterinary and sanitary requirements applicable to biomaterial collection from the carcasses and offal for further *post-mortem* examination were strictly followed. The collected swabs were transferred to the numbered sterile tubes and delivered to the Laboratory of farm animal infectious pathologies in the Caspian Zonal Veterinary Research Institution. The samples were accompanied with relevant documents. Before AGID, depending on the size of the swab 0.5–0.7 mL of isotonic solution (0.85% sodium chloride solution) were once added to the tubes with the swabs and the tubes were left for 3–5 hours until homogenous substance was formed. After that the tubes were kept at room temperature (18–26 °C) and occasionally shaken (2–3 time) so that antibodies contained in the swabs collected from BL-infected carcasses could transfer to the saline solution.

AGID was carried out according to the following scheme: 0.04–0.06 mL of the tested substance were inoculated in the wells punched in the agar gel. The tested substance was inoculated in wells No. 1, 3, 4 and 6 using automated pipette (dosing device); BLV antigen was inoculated in central well No. 7 and opposite peripheral wells No. 2 and 5 were inoculated with precipitating serum (Fig.). Then, the Petri dishes were incubated in thermostat at 20–26 °C, and the results were visually recorded in 48 hours.

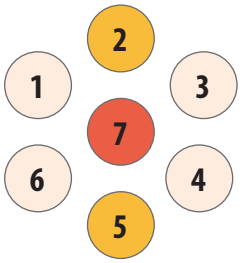
Ante-mortem BL diagnosis using AGID involves detection of the anti-BLV-specific precipitating antibodies in animal sera.

To assess AGID as a *post-mortem* BL diagnosis tool, laboratory tests of 148 isotonic solution-diffused tissue (lymphatic) fluid samples collected from carcasses and offal of animals confirmed to be BL-negative by AGID as well as 175 samples randomly collected from animals not subjected to *ante-mortem* BL testing were performed (see Table)

The Table demonstrates that all 148 samples from animals confirmed BLV seronegative during lifetime demonstrated negative results during *post-mortem* BL diagnosis using AGID.

AGID-tests of 175 biological samples collected from animals not subjected to *ante-mortem* testing for BL demonstrated 5 (2.9%) positive results on Day 1 and 2 post sample collection. On Day 5 post swab collection, the antibodies were detected only in 4 (2.3%) of the tested samples. The key task of the *post-mortem* tests performed at different time-points post sample collection (Day 1, 2, 5) was to identify decrease (maintenance) of the level of antibodies against BLV antigen in muscle tissue fluids (lymph and plasma) using AGID.

Serological tests of the samples collected from the surfaces as well as from the incised muscle tissues of the animal carcasses and offal demonstrated that AGID is an inexpensive and user-friendly tool for *post-mortem* diagnosis of bovine leucosis.



1, 3, 4, 6 – tested substance (solution with the swab);
2, 5 – specific precipitating serum;
7 – freeze-dried BLV antigen

Fig. ID assay of test substance for post-mortem diagnosis of bovine leucosis

Table
***Ante-mortem* and *post-mortem* BL diagnosis using serological method (AGID)**

Ante-mortem bovine leucosis diagnosis	Total AGID-tested, animals	*Post-mortem* diagnosis using AGID, terms of tests		
		Day 1	Day 2	Day 5
BLV-negative in AGID	148	–	–	–
Serological (other) diagnostic tests for bovine leucosis were not carried out	175	5	5	4
Total	323	5 (2.9%)	5 (2.9%)	4 (2.3%)

CONCLUSION

The foregoing prompts the conclusion that immunodiffusion test can be used for *post-mortem* bovine leucosis diagnosis during meat inspection along with other recognized methods – pathological and histological tests as well as molecular-genetic PCR.

Therefore, *post-mortem* serological testing of carcasses and offal for bovine leucosis demonstrated AGID suitability as one of the test-systems for detection of antibodies to BLV antigen in tissue fluids (plasma and lymph) and offal of animals during *post-mortem* examination [15].

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