

окситетрацикліну (МІК $\leq 0,06$ мкг/мл). Помірний рівень чутливості був виявлений до азітроміцину (МІК ≤ 3 мкг/мл), гентаміцин (МІК $\leq 0,25$ мкг/мл). На відміну від *B. anthracis* усі інші випробувані штами були стійкими до пеніциліну та амоксициліну з клавулановою кислотою (МІК ≥ 8 мкг/мл).

Ключові слова: антимікробна чутливість, мікроорганізми роду *Bacillus*, *Bacillus anthracis*, антимікробні речовини.

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THE ROLE OF THE FAT IN LABORATORY DIAGNOSIS OF RABIES

The article presents analysis of accompanying notes for the specimens of pathological materials from animals diagnosed rabies and other reporting documentation. It was proved the importance of using fluorescent antibody test for laboratory diagnosis of rabies and shown the advantages and disadvantages of this method.

Keywords: rabies, laboratory diagnosis, FAT.

Introduction. Rabies has a great importance among infectious diseases. Sensitivity regard this diseases of all domestic and wild animals species and extreme danger to humans determine its social and economic importance and focus attention of veterinary, medical science and practice [1].

An important link in the control of rabies is laboratory diagnosis, because in veterinary laboratories of Ukraine about 1500 cases of rabies in animals confirmed annually [2].

Rabies is a major zoonosis for which diagnostic techniques have been standardized internationally [2]. So, **the goal of the work** was determination of role of the FAT for laboratory diagnosis of rabies in Ukraine and in foreign countries.

Materials and methods of research. In this work we used laboratory research expertise, reports of regional departments of veterinary medicine of the State Scientific Research Institute of Laboratory Diagnostic and Veterinary Sanitary Expertise of the State Veterinary and Phytosanitary Service of Ukraine. Also we used the accompanying notes to the pathological material of animals diagnosed with rabies.

The results of research and discussion. At present, the rabies diagnosis in animals is based on complex epizootic, clinical, pathological and laboratory studies. But, as there are neither gross pathognomonic lesions nor specific and

constant clinical signs for rabies, accurate diagnosis can only be made in the laboratory [1].

For immunochemical identification of rabies virus antigen used the fluorescent antibody test (FAT), immunochemical tests, enzyme-linked immunosorbent assay (ELISA) and a rapid immunodiagnostic test (RIDT). For detection of the replication of rabies virus after inoculation used a cell culture test and mouse inoculation test. In view of the usually short delay in obtaining a result, isolation of rabies virus in cell culture should replace intracerebral mouse inoculation whenever possible, as it avoids the use of live animals, and is less expensive and gives more rapid results. Various molecular diagnostic tests, e.g. detection of viral RNA by reverse transcription PCR (RT-PCR), PCR-ELISA, hybridisation *in situ* and real-time PCR are used as rapid and sensitive additional techniques for rabies diagnosis. For typing the virus used DNA sequencing of genomic areas [4].

Now, the direct FAT is a basic technique. It is fast and available for rabies diagnosis, which use in 96 % of countries, including Ukraine [5, 6].

FAT is recognized WHO as a gold standard in rabies diagnosis. It is based on microscopic examination of sections or impressions smears of brain or nervous tissue after incubation with anti-rabies polyclonal globulin or monoclonal antibodies conjugated with fluorescein isothiocyanate [6 – 10]. FAT was first developed in 1958 by Goldwasser R. and Kissling R. Many of variations have been developed in the procedure for immunofluorescent staining, however, the direct method is the most practical, and now it is the most widely used procedure for rabies laboratory diagnosis in medicine and veterinary medicine [5, 6, 8, 11].

According to the reporting documentation in recent years in Ukraine the number of diagnostic tests for rabies in the regional veterinary laboratories increases. During 2001–2005, about eight thousand pathological material samples suspected of rabies have been examined annually, while during 2010–2014 it was made 11–15 thousand diagnostic tests.

After analyzing accompanying notes to pathological materials, found that the main method of diagnosis of rabies in the regional veterinary laboratories in Ukraine is FAT. In case of doubtful results, mouse inoculation tests (MIT) is carried out. From available 1360 accompanying notes to pathological materials when MIT was used there was only 75, which is about 5.5 %, thus 94.5 % of cases for the definitive diagnosis in Ukraine is established using FAT.

The direct FAT is a rapid, sensitive, specific method for rabies diagnosis in animals and humans and results can often be obtained within several hours [8, 12, 13]. However, the accuracy of the test depends on factors such as experience laboratory workers, quality of diagnostic conjugate, unsatisfactory or inadequately adjusted equipment (fluorescence microscope) and the appropriate working dilution labeled antibodies [14].

Also, the accuracy of rabies diagnosis is depend on the area of the brain that is selected for setting reaction and the quality of the sample. Sylvia G. Whitfield and others (2001) have found that distribution of the rabies virus antigen has been

observed to depend on several variables, including the virus strain, the dose of the inoculum, and the site of inoculation in the experimentally infected animals [11].

Seemingly small variations in test procedures may have dramatic effects on sensitivity. According to Sylvia G. Whitfield and others (2001), the evaluation of rabies status between fresh and formalin-fixed tissues was in agreement with more than 99,8 % of the cases examined by the FAT [11]. Dean D.J. and others (1996) argue that the sensitivity of the test using formalin-fixed specimens has been reported to be 90–100 % of that obtained using fresh specimens. However, it is recommended that fresh tissue be examined where possible [6].

If the specimen was fixed in formalin, it should be treated with proteolytic enzymes before staining to unmask the antigenic sites. However, the FAT on formalin-fixed and digested samples is always less reliable and more cumbersome than when performed on fresh tissue [4, 6, 11].

A prospective study of 8,987 canine, feline, human, and other mammalian brains was undertaken by Veera Tepsumethanon and others (1997) and there were no false negative results. They conclude, therefore, that post exposure rabies treatment is not requisite in all cases, provided that the fluorescent antibody test is performed without delay in a laboratory experienced with the procedure and microscopy results are fluorescent negative [14].

Using the FAT for rabies diagnosis also has some significant drawbacks. Contraindicated use FAT for the rabies diagnosis in animals that were vaccinated by rabies vaccine – for three months after vaccination (presence of vaccine antigen in nerve tissue), and application of this test to find rabies antigen in the pathological material which has undergone autolysis, because of possible non-specific glow of autolyzed tissue. Tissues that have been fixed by ethanol or other means also can not be investigated by this method as they cause denaturation and coagulation of antigens or create additional non-specific luminescence [6]. Another drawbacks of this technique is that it cannot be applied a large number of samples and the considerable cost of fluorescence microscope equipment is also a drawback [15].

Conclusions and prospects for further research:

1. FAT is recognized WHO as a gold standard in rabies diagnosis. It is a rapid, sensitive, specific method for rabies diagnosis and results can often be obtained within several hours.

2. The direct fluorescent antibody test is a basic technique in Ukraine. Every year in the laboratories of Veterinary Medicine of Ukraine held about 14 thousand diagnostic tests, and in 94.5% of cases a definitive diagnosis is established using FAT.

3. FAT is the most widely used method for laboratory diagnosis of rabies in Ukraine, but national diagnostic test-system still not developed. It is required to develop of effective national diagnostic test-system for immunofluorescence diagnosis of rabies, which will help eliminate dependence of Ukraine on imported diagnostic products.

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РОЛЬ МФА В ЛАБОРАТОРНОЙ ДИАГНОСТИКЕ БЕШЕНСТВА /

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В статье предоставлен анализ сопроводительных писем к образцам патологических материалов от животных с подтвержденным диагнозом на бешенство и прочих отчетных документов, доказана важность постановки МФА для лабораторной диагностики бешенства, приведены преимущества и недостатки данного метода.

Ключевые слова: бешенство, лабораторная диагностика, МФА.

РОЛЬ МФА В ЛАБОРАТОРНІЙ ДІАГНОСТИЦІ СКАЗУ / Мазур Н.В., Недосеков В.В., Полупан І.М.

На даний час діагностика сказу тварин проводиться на основі комплексу епізоотологічних, клінічних, патологоанатомічних і лабораторних досліджень. Але, враховуючи небезпечність хвороби, яка обумовлена абсолютною летальністю, постановка остаточного діагнозу здійснюється тільки в лабораторії.

В роботі використовували експертизи лабораторних досліджень, звіти обласних управлінь ветеринарної медицини, Державного науково-дослідного інституту лабораторної діагностики і ветеринарно-санітарної експертизи, Державної ветеринарної та фітосанітарної служби України, супровідні листи до зразків патологічних матеріалів від тварин з підтвердженням діагнозом на сказ, які були надіслані в лабораторію сказу Інституту ветеринарної медицини НААН.

Згідно звітних документів, в останні роки в Україні кількість діагностичних досліджень на сказ в обласних лабораторіях ветеринарної медицини збільшується. Протягом 2001–2005 рр. щорічно досліджували близько восьми тисяч патологічних матеріалів з підозрою на сказ, в той час як протягом 2010–2014 рр. здійснено 11–15 тисяч діагностичних досліджень.

Провівши аналіз супровідних листів до патологічних матеріалів від тварин з підтвердженням діагнозом на сказ, встановлено, що основним методом діагностики сказу в обласних лабораторіях ветеринарної медицини України є МФА. У випадку отримання сумнівних результатів, проводиться постановка біологічної проби на білих мишах. Із наявних 1360 супровідних листів до патологічних матеріалів вказують на постановку остаточного діагнозу за біологічною пробой лише 75, що складає близько 5,5 %. Тобто, 94,5 % встановлених остаточних діагнозів на сказ в Україні здійснюється постановкою РПФ, що свідчить про високу діагностичну цінність цього методу.

Ключові слова: сказ, лабораторна діагностика, МФА.