

SEROLOGICAL METHODS USED IN ROMANIA TO MONITOR RABIES VACCINATION IN DOMESTIC AND WILD ANIMALS

METODE SEROLOGICE UTILIZATE IN ROMÂNIA PENTRU MONITORIZAREA VACCINĂRII RABIEI ÎN RÂNDUL ANIMALELOR DOMESTICE ȘI SĂLBATICE

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ABSTRACT | REZUMAT

Monitoring the effectiveness of the rabies vaccination in human beings and animals, is done only by serological methods. Over time, in order to detect the level of rabies antibodies, many techniques have been used, such as fluorescent antibody virus neutralization test (FAVN), rapid fluorescent focus inhibition test (RFFIT), enzyme-linked immunosorbent assay (ELISA) and mouse neutralization test (MNT).

In the context of non-commercial movement of pet animals, the need for testing rabies vaccination emerged. The validation and standardization of methods as well as proficiency test for laboratories involved in this evaluation are only few duties of the European Union References Laboratories (EURL) for Rabies.

On the other hand, serological assays for rabies are also used in the countries where oral rabies vaccination (ORV) programs of wild animals are in progress, in order to evaluate the efficacy of the vaccination and each country could use any of the above mentioned methods.

Keywords: rabies, serology, vaccination FAVN, ELISA

Monitorizarea eficienței vaccinării antirabice atât la oameni cât și la animale, se realizează doar prin metode serologice. De-a lungul timpului au fost utilizate mai multe metode pentru determinarea titrului anticorpilor antirabici postvaccinali, precum: testul de virusneutralizare evidențiată prin fluorescență (FAVN) testul rapid de inhibare a fluorescenței (RFFIT), testul imunoenzimatic (ELISA) și testul de neutralizare pe șoareci (MNT).

În contextul circulației non-comerciale a animalelor de companie a apărut necesitatea testării vaccinării antirabice. Validarea și standardizarea metodelor, precum și evaluarea prin intercomparare a laboratoarelor implicate în această testare sunt doar câteva din atribuțiile Laboratorului European de Referință pentru Rabie.

Pe de altă parte, testele serologice pentru rabie sunt folosite de asemenea în evaluarea programelor de vaccinare orală a animalelor sălbatice în țări în care acestea sunt în desfășurare și fiecare țară poate utiliza în acest scop una dintre metodele menționate mai sus.

Cuvinte cheie: rabie, serologie, vaccinarea FAVN, ELISA

Serological methods are widely used to monitor the effectiveness of vaccination with parenteral or oral vaccines against rabies. By measuring the level of protective antibodies against rabies, these methods remain the only way to check the protection of domestic or wild animals, as well as humans (9,14).

From medical veterinary point of view, serological tests for rabies are used in two situations: 1) the evaluation of the efficacy of vaccination in the context of the non-commercial movement of pets and 2) the evaluation of the efficacy of vaccination campaigns in wild animals, in those countries conducting rabies eradication programs. For pets, one of three methods can be

used: the mouse neutralization test (MNT), rapid fluorescent focus inhibition test (RFFIT), and the fluorescent antibody virus neutralization test (FAVN) (12). All of these methods are recommended by the World Organization for Animal Health (OIE) (9). Laboratories conducting these tests must be authorized by the European Commission as a satisfactory result of in the inter-comparison test organized annually by the European Union References Laboratories for Rabies (4, 5).

Regarding the monitoring of the effectiveness of rabies vaccination in wildlife, the methods are not regulated by the EC so that the laboratory can use any of the above and/or immune-enzymatic techniques.

The aim of the following review was to provide an update of the situation regarding the non-commercial movement of pet animals and the evaluation of the

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ORV programs, at the international level as well as in Romania.

The non-commercial movement of pets

In order to avoid spreading rabies from infected countries into rabies-free territories, the non-commercial movement of pet animals is subject to strict legislation therefore international trade in domestic carnivores must meet specific requirements (6, 10).

The identity of the animal (by tattoo number or microchip) has to be mention in the passport of pet and the owners have to give a rabies vaccination with an inactivated vaccine and after that to check the antibodies titer against rabies by a blood test (11). The test is performing in 1-4 months after vaccination, according to legislation of each country. The methods used by laboratories involved in this test are approved and described by OIE Manual of diagnostic test and vaccines for terrestrial animals (1,8, 9). In the same time, laboratories must to be approved by official authority (for instance European Commission for European Union countries) to perform rabies serological assay for non-commercial movement of pets. In order to be approved laboratories must attend and succeed in the proficiency test, which are organized each year by the EURL for rabies. EURL for Rabies is located in Nancy, France, and belongs to French Agency for Food Safety (ANSES).

First run of proficiency test was initiated in 1999. At that time 14 laboratories attended, but the number of participants annually increased such that now there are about 50 laboratories. These laboratories are located all over the world: Europe, North and South America, Asia Australia. The list of approved laboratories is available and can be found on the European commission website: https://ec.europa.eu/food/animals/pet-movement/approved-labs_en.

The Institute of Diagnosis and Animal Health (IDAH) Bucharest introduced the FAVN test in 2003-2004 and since 2005 is on the list of approved laboratories, being the only laboratory like this from Romania. In the same time, since 2006 the method is accredited by Romanian Accreditation Body-RENAR according to ISO 17025:2005 standard.

Monitoring of the efficacy of the Oral Vaccination campaigns against Rabies

ORV programs and parenteral vaccination are the main tools for rabies eradication used in infected countries. The main reservoirs of rabies are: fox in Europe, skunk, fox, racoon in USA, dog in South America, Afri-

ca and Asia (13).

According to European Food Safety Administration (EFSA) recommendation, in order to monitor the efficacy of vaccination campaigns, the uptake of baits (detection of tetracycline biomarker in the teeth or bones of bait consumers) and the detection of rabies antibodies level (seroconversion) are performed (7).

The seroconversion is measured by different methods such as FAVN, RFFIT or ELISA ("in house" or commercial kits), with no harmonization of methodology as in the case of international trade in domestic carnivores. Not all techniques mentioned above are suitable to serological monitoring in wild animals., for instance MIT is very expensive, requires to use animals and several weeks are necessary to get the results. Live rabies virus strain and cell culture lines are necessary to perform FAVN test and RFFIT. In addition to these, the poor quality of field samples (hemolyzed serum, body fluids) are cytotoxic for these assays. Finally, these tests are not adapted to screening.

In the last years more and more laboratories use the immune-enzymatic assay (one of two ELISA commercial kits on the market, or even an "in house" ELISA) which much more suitable for these kinds of samples (2, 3). In Romania, a commercial ELISA kit is used, the test being performed in 3 laboratories (LNR, LSVSA IS and LSVSA SV) and for other two LSVSA (LSVSA GR and LSVSA SB) the accreditation process is in progress.

DISCUSSION AND CONCLUSION

In the context of non-commercial movement of pet animals, the need for testing rabies vaccination emerged and lead laboratories to apply in large number for approval and participation in proficiency testing under technical coordination of EURL for Rabies, Romania being one of the 43 countries all over the word in which the FAVN test is carried out.

Harmonization of rabies serological methods in wild life should be taken into consideration in order to increase the efficacy of ORV programs monitoring and to compare the efficacy of different vaccination protocols or strategies.

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