VETERINARSKI ARHIV 76 (4), 281-289, 2006

Vaccination against rabies and protective antibodies - comparison of ELISA and fluorescent antibody virus neutralization (FAVN) assays

Mirjana Stantić-Pavlinić[1]*, Peter Hostnik[2], Snežna Levičnik-Stezinar[3], and Lijana Zaletel-Kragelj[4]

[1]Community Health Centre Ljubljana, 1000 Ljubljana, Slovenia
[2]Veterinary Faculty, Department of Virology, University of Ljubljana, 1000 Ljubljana, Slovenia
[3]Blood Transfusion Centre of Slovenia, 1000 Ljubljana
[4]Medical Faculty, Cathedra for Public Health, University of Ljubljana, 1000 Ljubljana, Slovenia

STANTIĆ-PAVLINIĆ, M., P. HOSTNIK, S. LEVIČNIK-STEZINAR, L. ZALETEL-KRAGELJ: Vaccination against rabies and protective antibodies - comparison of ELISA and fluorescent antibody virus neutralization (FAVN) assays. Vet. arhiv 76, 281-289, 2006.

ABSTRACT

The aim of the study was monitoring the efficacy of primary vaccination against rabies and the need for booster doses. These studies validate at the same time recent technological improvements in laboratory diagnostics of the level of rabies protection in human sera. Research was carried out into the level of antibodies, considering that an antibody titer ≥0.5 IU/mL is protective. We used Platelia rabies ELISA kit (BIO-RAD Laboratories) for the detection of rabies virus anti-glycoprotein antibodies in 41 human sera of previously healthy veterinarian students. Neutralisation rabies virus antibodies were also measured simultaneously by fluorescent antibody virus neutralization (FAVN) test. Two to eight years prior to entering the study subjects had received rabies treatment with human diploid cell vaccine (HDCV, Rabivac, Chiron Germany) according to the schedule: one vaccine on days 0, 7, 21 and 365. Mean level of rabies antibody detected by ELISA was 19.6 (SD 18.8 minimum 1 maximum 56). Results were higher in the groups vaccinated recently. No subject had titer ≤0.5 IU/mL in ELISA, as well as in FAVN. In FAVN test the average titer was higher, reaching 54.4 (SD 44.3 minimum 0.7 maximum 152.5). An immune-complex-like reaction occurring after administration of the booster doses of rabies vaccine is the reason for reconsideration of the needs for administration of booster rabies vaccines. At the same time, the need for mass protection of professionals exposed subjects to rabies virus is real everywhere. Results of these studies indicate that HDCV is highly protective in both FAVN and ELISA tests. A high level of protection lasts at least 8 years in human sera. Average levels of detected rabies antibodies were lower in ELISA in comparison with FAVN test. A correlation between the two tests was found.

Key words: rabies, vaccination, enzyme-linked immunosorbent assay (ELISA), fluorescent antibody virus neutralization test (FAVN), antibody formation

* Contact address:
Dr. Mirjana Stantić-Pavlinić, MD, MS, Specialist in Infectious Diseases and Epidemiology, Community Health Centre Ljubljana, Metelkova 9, 1000 Ljubljana, Slovenia, Phone: +386 1 300 39 41; Fax: +386 1 300 39 11; E-mail: stantic@bigfoot.com

ISSN 0372-5480
Printed in Croatia

Introduction

Published data on the longevity of lasting specific immunity after primary vaccination against rabies have proved that antibody level after vaccination is protective for at least two upcoming years (BRIGGS and SCHWENKE, 1992). A sufficient level of antibody is, according to the recommendations of the World Health Organization and Centre for Disease Control, appointed at 0.5 IU/mL (WHO, 2002; ANONYMOUS, 1999). For persons continuously professionally exposed to rabies virus, it is recommended that they have a serum sample tested for rabies antibodies from between 6 months and two years. The testing interval depends on level of exposure. Rabies researchers need to be laboratory tested every 6 months, employees in rabies diagnostics laboratories once a year, and veterinarians every two years. If the titers proved by the rapid fluorescent focus inhibition test (RFFIT) are below 0.5 IU/mL, a booster vaccination with one vaccination dose is indicated (WHO, 2002). Pre-exposure immunization for rabies is necessary for spelunkers, bat researchers, animal control and wildlife workers in rabies epizootic areas, veterinary students, and travellers visiting areas where rabies is enzootic and where immediate access to appropriate medical care, including biologics, is limited (CDC, 2003). Several serological tests for the detection of rabies virus neutralization antibodies have been described. The first mouse neutralization test (MNT) has been developed (WEBSTER and DAWSON, 1935). The most commonly used technique for detection of protective level of rabies antibodies in sera of animals and humans is the rapid fluorescent focus inhibition test (RFFIT) developed by Smith (SMITH et al., 1973). Another cell culture-based technique, the fluorescent antibody virus neutralization (FAVN) test, has been shown to be more specific than RFFIT (CLIQUET et al., 1998). The FAVN test, for the quantification of rabies antibodies, is based on neutralization of rabies virus using cell culture. The reading and interpretation of results is less subjective than RFFIT, because it uses an "all or nothing" method of reading. Several indirect ELISA tests (Enzyme-Linked Immunosorbent Assay) which incorporate rabies glycoprotein/anti-human immunoglobulin/enzyme conjugates have been described for human and animal post-vaccination rabies antibodies titration (PIZA et al., 1999). The Platelia Rage kit incorporates protein A but has rabies virus glycoprotein as the coating antigen.

The aim of our study was to detect the level and duration of rabies antibody in the sera of pre-exposure treated persons and to compare two laboratory tests for that purpose: FAVN and ELISA.

Materials and methods

Immunogenetic property of a human diploid cell vaccine (HDCV) was evaluated using veterinary medical students. Forty-one healthy adults were enrolled in our trial. A person was excluded from enrolment if he/she had a previous history of additional rabies vaccination or had a history of any immunosuppressive disease or chronic disorders,

immunosuppressive therapy. Therefore, no subject entering the study had received booster doses of rabies vaccine. The Ethic Commission of the Ministry of Health of the Republic of Slovenia approved this research.

Mean age of subjects at the time of entering the study (in 2004) was 25.3 SD 2.6 median 25 minimum 22 maximum 33. At the beginning of the vaccination subjects were aged between 20 and 29 years (mean 21.1 SD 1.4). We tested 12 male and 29 female subjects. Sixteen subjects had received pre-exposure treatment against rabies 2 years prior to entering the study, 9 subjects 4 years before, 4 subjects 5 years before, 5 subjects 6 years before, and 7 subjects 8 years before the research commenced. Intervals between the start of treatments and entering the study were calculated beginning on zero days of pre-exposure treatments. The Human Research Board at the Ministry of Health of the Republic Slovenia approved the study protocols and prepared consent forms, which were signed by all subjects.

Four 1.0 mL injections of human diploid cell vaccines HDCV (Rabivac, Chiron Vaccines, Germany) were administered intramuscularly in the deltoid area on days 0, 7, 21 and 365 during the course of pre-exposure treatment.

Blood samples were collected from subjects and sent for laboratory testing of rabies virus antibodies. Rabies virus neutralizing antibody levels in the sera were measured using Fluorescent Antibody Virus Neutralization (FAVN) (CLIQUET et al., 1998) in the Department of Virology, Veterinary Faculty of the University of Ljubljana. The same samples were tested by ELISA test in the Blood Transfusion Centre of Slovenia.

Subjects were considered to be protected against rabies virus infection if they achieved FAVN or ELISA titers of $\geq$ 0.5 IU/mL. FAVN test, measuring neutralizing antibodies, was used as the reference test. The test assay was PLATELIA ELISA (BIO-RAD, France). The PLATELIA rabies kit is an immunoenzymatic technique for the detection of rabies virus anti-glycoprotein antibodies in human serum and plasma.

All statistical analyses were carried out before the code was broken. Statistical analyses were performed using the version SPSS System for Windows.

Results

100% of the subjects in each tested group had post-vaccination rabies antibodies titers >0.5 IU/mL using both FAVN and ELISA test methods. For surveillance of exposed subjects, WHO consider that so high a level of rabies antibody in RFFIT protects subjects exposed to risks of rabies. Results of these studies indicate that HDCV administered intramuscularly to healthy adults according to schedule 1, 7, 21 and 365 is excellently immunogenic on both glycoprotein and neutralizing antibodies. Extraordinarily high levels of antibodies were detected in both tests. Correlation between values of titers measured

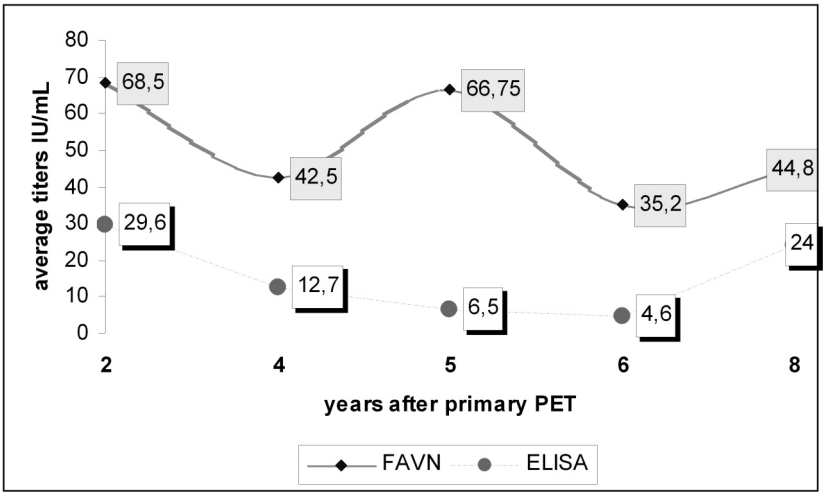
in ELISA and FAVN tests using Spearman's Correlation Coefficient was significant at a level of 0.01 (2-tailed), indicating correspondence of results in both tests.

Table 1. Comparison of the level of rabies antibodies detected by ELISA and FAVN

	Number	Minimum	Maximum	Mean	Std. Deviation
Vaccinated (years before)	41	2	8	4.3	2.2
FAVN	41	0.7	152.5	54.4	44.3
ELISA	41	1.0	56.0	19.6	18.8

Absolute values in each group (FAVN and ELISA) were somewhat different. Table 1 and Figure 1 shows that average means of antibodies levels were usually higher in the groups vaccinated recently, as well as in the FAVN test. Comparison of the average level of antibodies in the years after primary pre-exposure vaccination revealed that FAVN and ELISA tests were capable of detecting lower levels of antibodies in years after the start of vaccination. An increasing level of rabies antibodies in both tests 8 years after primary vaccination was observed in subjects who were eventually professionally exposed as veterinarians to rabies virus

Fig. 1. Average level of rabies antibodies in ELISA and FAVN tests - years following primary pre-exposure vaccination



HDCV vaccine met the immunogenicity goal of producing rabies virus neutralization and glycoprotein antibodies titers. Comparative study showed that all subjects had neutralizing, as well as anti-glycoprotein antibodies levels in human sera, well above the satisfactory level even 8 years after the commencement of pre-exposure treatment.

Discussion

We consider that different kinds of rabies vaccines and different vaccination schedules have an influence on the achieved level and the duration of protective antibody levels against rabies. The kind of laboratory assays used for detection of the levels and sort of rabies antibodies are also important. Different laboratory assays are recognized as tools for clinical decisions for many infectious diseases, although their interpretation is sometimes a difficult task. The assay PLATELIA used in ELISA test is carried out on the sera or plasma of many animal species, but rarely in humans. Rabies antibody virus response in our study was so high in both laboratory assays that any concern about protective levels of rabies pre-exposure treatment can only be speculative.

In our study we very often detected a much higher protective level of anti-rabies antibodies as officially recognized as lower protective level by WHO and Center for Diseases Control, Atlanta (WHO, 2002; CDC, 1999). Takayama and his co-workers (TAKAYAMA et al., 1999) published similar observations some years ago. Oelofsen and Smith, however, did not obtain positive results during the course of serological testing of bats by ELISA and concluded that bats are unlikely to play an important role as hosts of rabies in southern Africa (OELOFSEN and SMITH, 1993). In both studies anti-rabies antibody titers were measured by the ELISA method, with Platelia rabies kit (Diagnostic Pasteur, France) or "Trousse Platelia Rage" ELISA kit, Diagnostic Pasteur. The tests were used for diagnoses of rabies in animals and humans.

Pre-exposure vaccination against rabies is usually done by HDCV, human diploid cell vaccine; PCEC, purified chick embryo cell; RVA, rabies vaccine adsorbed. HDCV is recognized as a gold standard for other rabies vaccines (WHO, 2002). This is possibly the reason for such high titers of antibody found in our study. Another possible reason is that we apply the fourth dose of HDCV rabies vaccine one year after the start of treatment. The schedule used in our study is recognized by producers of vaccine and certain authors (BRIGGS et al., 2000; BRIGGS et al., 1996) as one of possible schedules for pre-exposure treatment. We should accept the reality that existence of individual immune reactions on rabies or other vaccines is obvious, and that non-responders could also be expected after rabies vaccination. Therefore, decisions should be made individually, case by case. An average level of protection is more interesting for research purposes. After a new exposure to the rabies virus a new dose of rabies vaccine is obligatory despite a probably high level of antibodies.

Further studies are necessary in order to provide evidence if a high level of achieved protection with HDCV, and with presented vaccination pre-exposure schedules, is connected to immunity lasting even more than 8 years.

We consider that a fourth dose in pre-exposure schedule is definitely not obligatory, but if a fourth dose is given as booster after one year from the start of vaccination, substantial prolongation of protection could be expected. Persons at continuing risk of rabies exposure should also consider acceptance of the presented regimen and regular boosting with rabies vaccine.

Serological testing may be useful for reducing the number of rabies vaccine doses in the course of boosters. Enhanced surveillance of the necessity for the start of pre-exposure protection and booster is advisable. In this way the total number of professionally exposed persons, who regularly need the boosters, could be reduced, as well as undesirable side-effects of vaccinations. We predict that the use of pre-exposure rabies vaccine could, in such a way, even increase the demand for pre-exposure preventive treatment. Accordingly, total protection of professionally exposed subjects will be elevated to a higher level. An immune-complex-like reaction occurs after administration of booster doses of HDCV (ANONYMOUS, 1984). Local reactions (JONES et al., 2001) and systemic hypersensitivity reactions after booster vaccinations with HDCV (FISHBEIN et al., 1993) have also been reported. These reactions are an additional reason to very carefully take into consideration the needs for administration of booster rabies vaccines. However, rabies treatment saves lives and should be accepted as being obligatory.

PLATELIA rabies kit is an immunoenzymatic technique for the detection of rabies virus antibodies in serum or plasma of animal species (CLIQUET et al., 2004). Some authors have also used ELISA tests for detection of rabies virus antibodies in human sera (PIZA et al., 1999). In this way it can be used for monitoring the efficiency of vaccine testing on laboratory animals, for possible confirmation of rabies diagnosis, and also as a research tool for monitoring the titer of antibody of vaccinated subjects. The present research revealed that the ELISA method (kit BIO-RAD France) is useful for subjects with protective, mostly high level, of antibodies in FAVN and also that a correlation exists between the results achieved in these two assays. Correlation between neutralization and ELISA antibody titers were proved in recently published studies of Arai et al. with higher neutralization than ELISA titers for most of the samples as well as (ARAI et al., 2002). Results obtained by this Japanese researcher are in accordance with the results obtained in our study.

Cliquet, together with co-workers (CLIQUET et al., 2004) found that although the ELISA has a lower sensitivity than the FAVN test, it is a useful tool for rapidly screening serum samples from vaccinated companion animals, and that the ELISA test compared favourably with data generated using the FAVN test. The major advantages of the ELISA test are that it can be completed in a few hours, does not require the use of live virus, and

can be performed without the need for specialised laboratory containment. This contrasts with 4 days using conventional rabies antibody virus neutralization assays. According to some authors, the ELISA assay would be a valuable screening tool for the detection of rabies antibodies from vaccinated domestic animals, in combination with other accepted serological tests. We suggest that the same consideration could be accepted for human sera as well. Comparison with RFFIT or equally worthwhile FAVN (MEISNER et al., 1997) will be welcomed.

Prevention of diseases in professionally exposed persons is one of the priorities in public health (STANTIC-PAVLINIC, 2003; STANTIC-PAVLINIC, 2002). In our opinion and in the opinion of certain other authors (SIMANI et al., 2004), monitoring the titers of antibodies with good laboratory assays could be a useful contemporary method for making decisions for the purpose of whether or not to give boosters to professional persons exposed to rabies. At the same time, it could be the best way of lowering the number of boosters required for long-term professionals.

Conclusions

Protective levels of rabies antibodies in all tested human sera were present even 8 years after the commencement of the pre-exposure treatment with 4 doses of HDCV. Results were positive in ELISA as well as the FAVN test, both lower in the former. A correlation between results of compared tests was found.

Acknowledgements

The authors are indebted to all volunteers who participated in this study, as well as the staff of each site, for their excellent level of assistance. We are grateful also to Metka Paragi, Institute of Public Health of the Republic Slovenia, and to Jerica Tovornik and Eva Grilc, Institute of Public Health of Ljubljana, for their technical assistance and support in our research.

References

ANONYMOUS (1984): Systemic allergic reactions following immunization with human diploid cell rabies vaccine. Morbid. Mortal. Week. Rep. 1133, 185-187.

ANONYMOUS (1999): Human Rabies Prevention - United States, 1999 Recommendations of the Advisory Committee on Immunization Practices (ACIP). Morbid. Mortal. Week. Rep. 48 (No RR-1), 1-21.

ARAI, Y. T., M. KIMURA, Y. SAKAUE, A. HAMADA, K. I. YAMADA, M. NAKAYAMA, T. TAKASAKI, I. KURANE (2002): Antibody responses induced by immunization with a Japanese rabies vaccine determined by neutralization test and enzyme-linked immunosorbent assay. Vaccine 20, 2448-2453.

BRIGGS, D. J., J. R. SCHWENKE (1992): Longevity of rabies antibody titre in recipients of human diploid cell rabies vaccine. Vaccine 10, 125-129.

BRIGGS, D. J., D. W. DREESEN, P. MORGAN, J. E. CHIN, C. D. SEEDLE, L. CRYZ, R. GLUCK, S. J. CRYZ (1996): Safety and immunogenicity of Lyssavac Berna human diploid cell rabies vaccine in healthy adults. Vaccine 14, 1361-1365.

BRIGGS, D. J., D. W. DREESEN, U. NICOLAY, J. E. CHIN, R. DAVIS, C. GORDON, A. BANZHOFF (2000): Purified chick embryo cell culture rabies vaccine: interchangeability with human diploid cell culture rabies vaccine and comparison of one versus two-dose post-exposure booster regimen for previously immunized persons. Vaccine 9, 1055-1060.

CLIQUET, F., M. AUBERT, L. SAGNE (1998): Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies-neutralising antibody. J. Immunol. Methods 212, 79-87.

CLIQUET, F., L. M. MAC ELHINNEY, A. SERVAT, J. M. BOUCHER, J. P. LOWINGS, T. GODDARD, K. L. MANSFIELD, A. R. FOOKS (2004): Development of a qualitative indirect ELISA for the measurement of rabies virus-specific antibodies from vaccinated dogs and cats. J. Virol. Methods 117, 1-8.

FISHBEIN, D. B., K. M. YENNE, D. W. DREESEN, C. F. TEPLIS, N. MEHTA, D. J. BRIGGS (1993): Risk factors for systemic hypersensitivity reactions after booster vaccinations with human diploid cell rabies vaccine: a nationwide prospective study. Vaccine 11, 1390-1394.

JONES, R. L., J. E. FROESCHLE, R. L. ATMAR, J. S. MATTHEWS, R. SNADERS, J. PARDALOS, L. MOELLER, J. E. CHIN, M. FAMULA, D. J. BRIGGS, J. LANG (2001): Immunogenicity, safety and lot consistency in adults of a chromatographically purified Vero-cell rabies vaccine: a randomized, double-blind trial with human diploid cell rabies vaccine. Vaccine 19, 4635-4643.

MEISNER, F. L., R. D. DAVIS, M. K. BROWN, C. E. RUPRECHT, J. S. SMITH, D. J. BRIGGS (1997): Rabies Serological Testing in Dogs and Cats Exported to Rabies-free Countries: Does the Choice of Test Make a Difference? United States Animal Health Association. Proceedings.

OELOFSEN, M. J., M. S. SMITH (1993): Rabies and bats in a rabies-endemic area of southern Africa: application of two commercial test kits for antigen and antibody detection. Onderstepoort J. Vet. Res. 60, 257-260.

PIZA, A. S., J. L. SANTOS, L. B. CHAVES, C. R. ZANETTI (1999): An ELISA suitable for the detection of rabies virus antibodies in serum samples from human vaccinated with either cell-culture vaccine or suckling-mouse-brain vaccine. Rev. Inst. Med. Trop. Sao Paulo 41, 39-43.

SIMANI, S., A. AMIRKHANI, F. FARATHAI, B. HOOSHMAND, A. NADIM, J. SHARAFIAN, N. HOWAIZI, N. ESLAMI, A. GHOLAMI, A. HANANI, A. FAYAZ (2004): Evaluation of the effectiveness of preexposure rabies vaccination in Iran. Arch. Iranian Med. 7, 251-255.

SMITH, J. S., P. A. YAGER, G. M. BAER (1973): A rapid reproducible test for determining rabies neutralising antibody. Bull. World. Health. Organ. 48, 535-541.

STANTIC-PAVLINIC, M. (2002): Rabies treatment of health care staff. Swiss Med. Wkly. 132, 129-131.

STANTIC-PAVLINIC, M. (2003): How dangerous is European Bat Lyssa virus? Wien. Klin. Wochenschr. 115, 3-5.

TAKAYAMA, N., K. OKUMA, H. SAKUMA (1999): A case received pre-exposure immunization against rabies by intradermal injection of rabies vaccine because of allergic reaction to the component of the vaccine. Kansenshogaku. Zasshi 73, 600-601 (Japanese).

WEBSTER, L. T., J. R. DAWSON (1935): Early diagnosis of rabies by mouse inoculation. Measurement of humoral immunity to rabies by mouse protection test. Proc. Soc. Exp. Biol. Med. 32, 570.

WHO (2002): Current Strategy for Human Rabies Vaccination and WHO Position. Rabies Bulletin Europe 26, 14-16.

Received: 21 July 2005
Accepted: 27 June 2006

STANTIĆ-PAVLINIĆ, M., P. HOSTNIK, S. LEVIČNIK-STEZINAR, L. ZALETEL-KRAGELJ: Cijepljenje protiv bjesnoće i zaštitna protutijela - usporedba imunoenzimnog i fluorescentnog neutralizacijskog testa**. Vet. arhiv 76, 281-289, 2006.**

SAŽETAK

U radu je uspoređena djelotvornost primarnoga cijepljenja protiv bjesnoće i potreba za »booster« dozama. Istovremeno su istražene suvremene tehnološke mogućnosti laboratorijske dijagnostike usmjerene na praćenje zaštitne razine protutijela u uzorcima seruma ljudi. Titar protutijela ≥0,5 IU/mL smatra se zaštitnim. Upotrijebljen je Platelia rabies ELISA komplet (BIO-RAD Laboratories, Francuska) za određivanje glikoproteinskih protutijela za virus bjesnoće u uzorcima seruma uzetima od 41 studenta veterinarske medicine. Određivana su i neutralizacijska protutijela za virus bjesnoće rabeći fluorescentni neutralizacijski test (FAVN). Ispitanici su bili 2 do 8 godina ranije preventivno cijepljeni protiv bjesnoće humanim diploidnim cjepivom (HDCV, Rabivac, Chiron, Njemačka) po shemi 0., 7., 21. i 365. dan. Prosječna razina protutijela za virus bjesnoće određena ELISA-om bila je 19,6 (SD 18,8, minimum 1, maksimum 56). Ta je razina bila viša u osoba, koje su bile nedavno cijepljene. Nijedan ispitanik nije imao razinu protutijela nižu od ≥0,5 IU/mL u ELISA, a takodjer niti u FAVN testu. Rezultati polučeni FAVN testom pokazali su više vrijednosti: 54,4 (SD 44,3; minimum 0,7; maksimum 152,5). Tvorba imunokompleksa razlog je utvrđivanja stvarnih potreba za »booster« dozama pri cijepljenju protiv bjesnoće. Potreba za masovna zaštitna cijepljenja profesionalno izloženih skupina stanovništva svugdje je aktualna. Rezultati dobiveni pretragom imunoenzimnim i FAVN testom pokazuju visoku imunogenost HDCV cjepiva protiv bjesnoće. Visoka razina zaštite u ljudi traje najmanje 8 godina. Prosječna razina protutijela bila je niža u ELISA u usporedbi s FAVN testom. Ustanovljena je korelacija između ta dva testa.

Ključne riječi: bjesnoća, cijepljenje, imunoenzimni test, FAVN test, tvorba protutijela