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Neutralization test in mice (MNT) using effective dose$_{50}$ (ED$_{50}$) and International Units (IU) on bovine's sera vaccinated with an inactivated rabies virus vaccine

Teste de soroneutralização em camundongos utilizando dose eficaz$_{50}$ (ED$_{50}$) e unidade internacional (UI) em soro de bovinos vacinados com uma vacina anti-rábica de vírus inativado

Avelino Albas,* Paulo Eduardo Pardo,** José Giometti,** Fernanda Bernardi,*** Fumio Honma Ito***

Abstract

The results of neutralization test in mice (MNT) performed on bovine's sera that had been vaccinated previously with a commercial inactivated rabies virus vaccine were calculated and expressed either in logarithm of Effective dose$_{50}$ (ED$_{50}$) or in International Unit/ml (IU/ml). Statistical analysis revealed a Spearman nonparametric correlation of r = 0.6469, and when the use of IU/ml is impeditive, the MNT titers expressed in ED$_{50}$ is still a useful procedure to determine if the bovines have responded to antirabies vaccination.

Keywords: mouse serum neutralization, bovines, rabies antibody, vaccine.

Resumo

Os resultados do teste de soroneutralização em camundongos, aplicado em soro de bovinos que haviam sido vacinados previamente com uma vacina comercial de vírus inativado, foram calculados e expressos em logaritmo (base 10) da dose eficaz$_{50}$ (DE$_{50}$) ou em unidade internacional/ml (UI/ml). A análise estatística revelou uma correlação não-paramétrica de Spearman r = 0,6469, e quando do impedimento de expressar os resultados em UI/ml, a prova de soroneutralização em camundongos com resultados expressos em DE$_{50}$ é ainda um procedimento útil para determinar se os bovinos responderam à vacinação anti-rábica.

Palavras-chave: neutralização em camundongos, bovinos, anticorpo anti-rábico, vacina.

Introduction

The main application of rabies serology is to assess if a person or an animal has responded to rabies vaccination (Fitzgerald,1996), although in animals studies the neutralizing antibody titers have been shown to be imperfect markers of protection (Center for Disease Control and Prevention,1999).

The neutralization test in mice (MNT) is a test based on antigen-function assay, using living mice as the indicator system. Currently, the MNT and the rapid fluorescent focus inhibition test (RFFIT) using cell culture, described by Smith et al.(1973), are the most widely used potency tests for antirabies serum and immunoglobulin (Fitzgerald,1996), although several types of procedures to measure the rabies antibody have been published throughout the world.

The reliability and the reproducibility of the results using MNT is questionable, despite the efforts to standardize the test (Louie et al., 1975), and the OMS (1992) recommended that MNT or RFFIT results should be expressed in international unit/ml (IU/ml), but still there are laboratories using the results expressed in its reciprocal of the serum dilution (Center for Disease Control and Prevention, 1999).

To express the antibody titers in IU/ml (Cabasso et al., 1974), for each antibody assay there is a need to include a titration of positive reference serum. As the stock of international reference preparation is limited, this positive reference is a serum of known titer previously calibrated against International Standard Antirabies Immunoglobulin with a stated potency in IU/ml, and problems with unitage of the International Standard Rabies Immunoglobulin may occur (Fitzgerald and Rastogi, 1985). In

* Núcleo Regional de Presidente Prudente do Instituto Biológico.

** Faculdade de Ciências Agrárias da Universidade do Oeste Paulista, Presidente Prudente, SP;

*** Departamento de Medicina Veterinária Preventiva e Saúde Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, SP.

Address to: Avelino Albas, Núcleo Regional de Presidente Prudente/Laboratório de Sanidade Animal e Vegetal/Instituto Biológico. P.O. Box 298, 19001-970 Presidente Prudente, SP.
Telefax: (0xx18)222-8688

some laboratories, the results of the MNT were influenced by species differences between reference and test preparations (Meslin and Kaplan, 1996).

In many situations, in country like Brazil, the use of reference serum is impeditive, and the results are expressed in terms of the serum dilutions or in its reciprocals. In this work we analyzed the adequacy in using and expressing the results of MNT titers either using the reciprocal of serum dilution or IU/ml.

Material and method

During the year of 1995 to 1996, thirty weaned nelore crossbreds, 4-12 months old in age were vaccinated intramuscularly using 2 ml of RAI-LIQ* inactivated rabies virus vaccine, and had been maintained at Fazenda São José, Regente Feijó-SP, Brazil, and bled at different time intervals after the initial vaccination, and serum samples had been stored in vials of 2 ml at -20°C until use.

Serum neutralization (SN) test was conducted on 80 serum samples taken from the above described bovines. Samples were randomly divided into eight groups of 10 sera each and the test procedure was according to Fitzgerald (1996), adopting the 2-fold serial dilution and using $10^{2.398}$MICLD$_{50}$/0.03 ml of the CVS fixed strain in the testing system. The CVS virus was provided by the Laboratório de Referência Animal (LARA), Campinas-SP. The MNT titers were calculated by the method of Reed and Müench (1938), and results were expressed using the reciprocals of the dilutions. Results expressed in IU/ml were calculated according to Fitzgerald (1996), using a positive serum previously calibrated against the Rabies Antiserum Berna, Lot 137970103 - Swiss serum of human origin, from Institut Berne, having 200 IU/ml.

For statistical analysis, the results of MNT expressed in ED$_{50}$ titers were submitted to logarithmic transformation of Log$_{10}$(x+1). The descriptive statistics, the nonparametric test, and the correlation coefficient were calculated by using a computerized softwere GraphPad Instat tm v2.01.

Results

The descriptive statistics corresponding to the MNT results of bovine's sera, vaccinated previously with an antirabies vaccine, are presented as the follow: the mean ED$_{50}$ titer was 0.7004, and 0.8096 in IU/ml; with standard deviation of 0.3287 for ED$_{50}$ titers, and 1.445 for IU/ml values. The minimum titer found was 0.3010 and 0.10, respectively for ED$_{50}$ and IU/ml, and the maximum,1.920 and 10.220. The median ED$_{50}$ was 0.6990, and 0.4200 for the IU/ml, and the 95% confidence interval found for ED$_{50}$ ranged from lower 0.6272 to upper 0.7737, and for IU/ml values, from lower 0.4875 to upper 1.132. Among the sera expressed in ED$_{50}$ titers, 48.75% were found with titers <5 (0.3010 in log$_{10}$), and 58.75% when considering <0.5 IU/ml. Titers in IU/ml did not pass the normality test, and then the Spearman nonparametric correlation coefficient was applied after logarithmic transformation of ED$_{50}$, showing r = 0.6469, indicating a significant correlation between the columns (Figure 1).

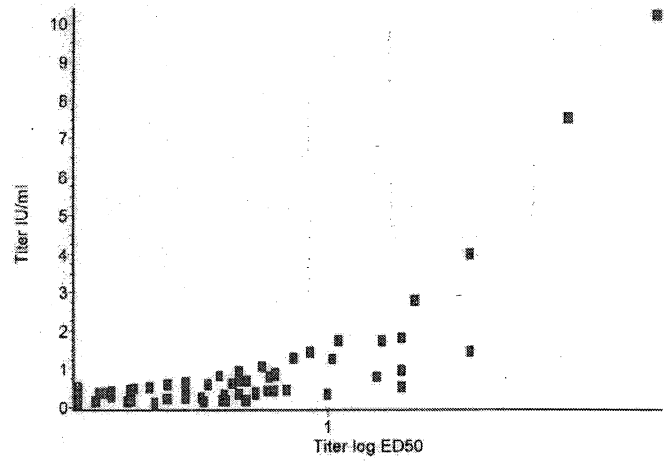

Figure 1: SN titers of bovine's sera previously vaccinated with a commercially available inactivated rabies virus vaccine and Spearman nonparametric correlation (r = 0.6469) between the titers expressed in log$_{10}$ ED$_{50}$ and IU/ml.

Discussion

The MNT is still a very useful and frequently used test in Brazil. The MNT is an "in vivo" method of antibody assay and uses the death of the indicator animal as an endpoint, thus resulting in imprecisions of the many uncontrollable factors. Although antibody levels alone do not define an animal's immune status, they are markers of continuing immune response and according to Atanasiu (1966), a titer ^{3}5 (0.699 in log$_{10}$) in MNT is an indication of the immune response, and the OMS (1992) stated that titer ^{3}0.5 IU/ml following vaccination is a protective antibody titer in humans. For testing of bovine's serum, an International Standard Rabies Immunoglobulin like the human or dog origin is not available, and using the criterion of ^{3}0.5 IU/ml to interpret as an indication of immune response in this species is questionable.

The serum samples tested in this experiment were all taken from bovines undergoing an immunization experiment with a commercially available antirabies inactivated virus vaccine. Bleedings were made at different post-vaccination days, and the SN titers determined were in a range of 0.3010 to 1.920, and 0.10 to 10.220 in IU/ml, indicating the humoral response of those bovines, because the serology conducted on the sera taken shortly before vaccination were all <2 (0.3010 in log$_{10}$). The maximum titer found in ED$_{50}$ was 83.5 (1.92 in log$_{10}$), however, the corresponding titer in IU/ml was 10.22. The second highest titer was 46.88 (1.67 in log$_{10}$), and 7.5 in IU/ml, so, the reliability and the reproducibility of MNT depend on careful attention to technical details.

Because multiplicative nature of serial dilutions contribute to a skewed distribution of titer values (Louie et al., 1975), the titers in ED$_{50}$ were transformed into log$_{10}$ values for all statistical calculations. The distribution of titers in ED$_{50}$ after the log$_{10}$ transformation has narrowed the original ED$_{50}$ range of 2.00 to 83.50, making the distribution closer to normal. However, the IU/ml data failed the normality test, then a nonparametric Spearman correlation test was applied with a significant r = 0,6469, as illustrated in Figure 1. Although the OMS (1992) recommends the expression of MNT results in IU/ml, many

*RAI-LIQ, lot number 001-95, IRFA - Química e Biotecnologia Ltda., Porto Alegre, RS - Brazil

laboratories still use the serum dilution or its reciprocal to express the titer in ED_{50} (Center for Disease Control and Prevention, 1999).

The variation in the titers of challenge virus used for the MNT testing system is another factor that makes the reproducibility of results difficult, in MNT titrations the challenge virus varied from 10 to 447 $MICLD_{50}$ (LOUIE et al., 1975) and according to Côrtes and Nilsson (1974), the increase in the dose of challenge virus terminates in decrease of antibody titer. In this experiment, the virus dose was calculated to $10^{2.398}$ $MICLD_{50}$/0.03 ml, however the dose effectively used had varied from 10 to 10^2 MICLD50/0.03 ml.

Although the recommendation of the World Health Organization to express the results of MNT testing in IU/ml, we conclude that expressing the results in ED_{50} is still a useful and reliable method to indicate that vaccinated animals have responded to vaccination. The definition and acceptance of a minimum antibody titer (>0.5 IU/ml) considered as providing protection against rabies in bovines deserves further investigation.

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