QUANTIFICATION AND EVOLUTION OF RABIES ANTIBODIES LEVEL BY ELISA IN DOG SERA WITH DIFFERENT NUMBER OF VACCINATIONS

Cuantificación y evolución del nivel de anticuerpos de rabia por Elisa en sueros de perros con diferente número de vacunaciones

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SUMMARY

In the present study have been analyzed 253 sera samples from dogs with different number of anti-rabies vaccinations. The aim of this work has been to obtain dates that allow to detect and quantify the level of antibodies against rabies virus to know its evolution in refering to the numbers of vaccination. The method used has been ELISA, by considering that is rapid and safe and we can analize a great number of samples, therefore its sensitivity is comparable at seroneutralization methods. By simple correlation analysis ($\alpha = 0.05$), the results obtained show no significant differences between vaccinated dogs in the first time and those vaccinated several times, but a difference between the antibodies level (optical densities) in sera from vaccinated dogs and those non vaccinated animals, was found.

Key words: ELISA, dog, rabies, vaccine.

RESUMEN

En el presente estudio se han analizado 253 muestras de suero de perros que han recibido diferentes números de vacunaciones anti-rabia, con objeto de detectar y cuantificar anticuerpos contra el virus rábico y controlar la evolución de la tasa de anticuerpos en función del número de vacunaciones. El método elegido ha sido la técnica ELISA, por considerar que reune las ventajas de seguridad y rapidez, junto a la posibilidad de analizar un número elevado de muestras, y poseer una sensibilidad comparable a la seroneutralización. Los resultados obtenidos nos permiten concluir que no existen diferencias significativas mediante un análisis de correlación simple ($\alpha = 0.05$) entre los animales vacunados por primera vez y aquellos que han recibido más de una dosis, si existiendo, en cambio, entre el grupo de los no vacunados y los grupos de los vacunados.

Palabras clave: ELISA, perro, rabia, vacuna.

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INTRODUCTION

To determine the level of protection of anti-rabies vaccines, WHO recommends to quantify the level of neutralizing anti-bodies by seroneutralization in mice or by fluorescent focus inhibition tests. These widespread techniques, however, are long, tedious, and entail a risk inherent in the manipulation of rabies virus. These drawbacks have prompted researchers to find more rapid and reliable methods.

ELISA are rapid and safe, they can be used in a great number of samples, and their sensitiveness is comparable to that of seroneutralization techniques. ELISA have, thus, prevailed for diagnosis not only in detecting rabies virus antigen (ATANASIU *et al.*, 1979) but also in assessing post-vaccination humoral responses (ATANASIU *et al.*, 1977, NICHOLSON and PRESTAGE, 1982; SUREAU *et al.*, 1982). ELISA have been mainly used in the detection of rabies antibodies in humans (GRASSI *et al.*, 1989; KAVAKLOVA *et al.*, 1984), and have proved to be as sensitive as seroneutralization methods, and superior to methods such as latex particles agglutination tests (PERRIN *et al.*, 1988).

In applying ELISA for the detection of rabies post-vaccination antibodies, the glycoprotein of the viric capsid must be used as antigen, since the protecting antibodies act against the capsid spikes of the rabies virus (ATANASIU, 1974; ATANASIU *et al.*, 1974; ATANASIU and PERRIN, 1979; BLANCOU *et al.*, 1983; COX *et al.*, 1977; PERRIN *et al.*, 1985; SMITH *et al.*, 1973; WIKTOR *et al.*, 1973). However, in using tissue culture vaccines, a high correlation exists between the level of anti- glycoprotein antibodies and that of the antibodies directed to all viral particles (PERRIN *et al.*, 1986), thus permitting the using of the whole virus (SZKUDLAREK *et al.*, 1982) without resorting to the purification of glycoprotein.

In this work were quantified the rabies antibody level in dog sera from vaccinated animals in several times and non vaccinated animals by ELISA.

MATERIAL AND METHODS

Sera (253 samples) were collected in Murcia Region (Spain) from dogs of different ages and analyzed by ELISA.

The technique used coincides basically with the protocol described by GOMEZ- LUCIA *et al.* (1989): The ready-for-use microplates (Flow EIA microplates; 96 wells) were coated with 100µl per well of a rabies virus suspension, obtained in BHK-21 monolayer cells, inactivated by binary ethylen amine (Cooper Zeltia 14/89, 7.05 DL50 lactant mouse/ml) in 0.05 M carbonate buffer, pH 9.6, and were incubated for 24 h at 4°C. A 1:20 dilution in phosphate-buffered saline (PBS), with 5% skimmed milk, 100 µl per well was used for serum dilution. The mixture was incubated for 1 h at 37°C. Dog anti-globulin horseradish peroxidasa labeled antibody (Biosys) diluted to 1:2000 in PBS, with 5 % skimmed milk, 100µl per well was added to the plate. The mixture was incubated for 1 h at 37°C. As substrate of the reaction, 2,2'azinobis 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) in substrate buffer, pH 4 (30.7 ml, 0.1M citric acid; 19.3 ml, 0.2M $PO_4HNa_2.12H_2O$, in 50 ml distilled water) with 200 µl of H_2O_2 solution (100 µl H_2O_2 30 vol in 3 ml distilled water), was used. This mixture was distributed 150 µl per well, and was incubated for 4 h at room temperature. After each incubation the microplates were washed three times with PBS- Tween 20 solution.

Controls for serum, antigen, conjugate, and substrate were added to every plate. A mixture of three sera from one year old, non vaccinated dogs served as negative control serum. A mixture of three sera from three year old, twice vaccinated dogs served as positive control serum. A suspension of non-infected BHK-21 cells was used as negative control antigen.

The optical density values were read on a 8-channel Tikerket Multiscan photometer at 450 nm.

RESULTS AND DISCUSSION

The serum samples were grouped according to the number of vaccines. These results are shown in table 1 and figure 1.

The optical density values shown in Table 1 correspond to the optical densities calculated according to the formula:

		OPTICAL DENSITIES	
NO. VACCINE	NO. SAMPLES	MEAN	STAND DESVIATION
Non vaccinated	71	0.156	0.011
1 vaccine	59	0.248	0.019
2 vaccine	28	0.266	0.029
3 vaccine	28	0.252	0.036
4 vaccine	31	0.256	0.027
5 vaccine	14	0.286	0.040
6 vaccine	9	0.237	0.044
7 or more	13	0.307	0.066

TABLE 1. ELISA results in corrected optical densities. The serum samples are grouped according to the number of vaccines.

$$DO = \frac{(DOa) - (DOb)}{(DOc)}$$

where:
- DO a: optical density of the serum tested.
- DO b: optical density of the negative control serum.
- DO c: optical density of the highest positive serum.

Though the simple correlation analysis shows no significant differences between the mean values of the optical densities obtained in vaccinated animals, our results show a difference between the antibodies levels (optical densities) found in sera from vaccinated animals (mean value of 0.260), and those found in non vaccinated animals (mean value of 0.156). These results can be interpreted as showing that the vaccine was immunogenic.

Our results differ from those obtained by ATANASIU and PERRIN (1979) (optical densities mean value of 0.085, for the non vaccinated; and 0.516 for the vaccinated animals), who in contrast to our experiment, analysed 14 samples, and used purified G protein as antigen. These experimental differences prevent possible comparisons between the two studies.

Nevertheless, the hight correlation between ELISA and seroneutralization test results (ATANASIU *et al.*, 1977; BLANCOU *et al.*, 1983; KAVAKLOVA *et al.*, 1984; SUREAU *et al.*, 1982) leads us to think that ELISA are greatly useful in detecting rabies antibodies in dogs immunized with vaccines obtained by tissue culture (PERRIN *et al.*, 1986). Furthermore, we consider that ELISA methods are technically more simple, and allow more samples to be assayed in a shorter time than the neutralization assays.

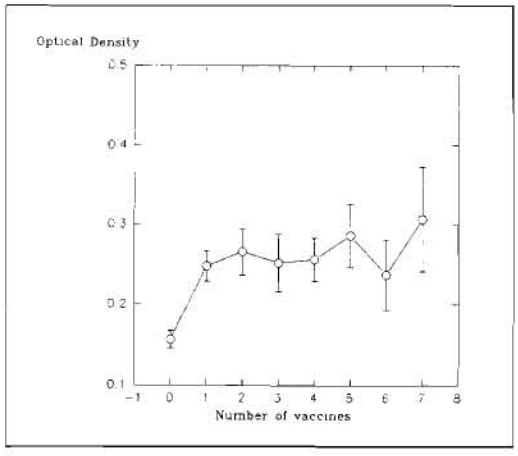

FIGURE 1. Mean values with confidence intervals (95%).

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