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Original article

Detection of rabies antibodies in dog sera

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Abstract

In the presented work, we compared the results of determination of rabies antibodies using three *in vitro* methods: rapid fluorescent focus inhibition test (RFFIT), fluorescent antibody virus neutralisation test (FAVNT) and the immunoenzymatic assay (ELISA). 196 dog sera samples were examined with FAVNT, RFFIT methods and the ELISA test. Sera with low and sufficiently high titre of antibodies had a similar result in determining by all methods. A critical level of rabies antibodies close to the required protection level (0.5 IU/cm^3) was seen in sera of 18 dogs (9.18%); these were the sera obtained after primary vaccination of dogs. At this level, even small differences can cause a change in the assessment of the patient's serum seronegativity or seropositivity. Therefore, it is important to choose the appropriate method that has sufficiently strict criteria while having a good reproducibility.

Key words: Rabies, antibodies, dog, comparison, RFFIT, FAVN, ELISA

Introduction

Prophylactic vaccination is irreplacable and very important when it comes to the prevention of rabies in animals and humans. Detection and quantification of antibodies is used primarily for the control of the status of humoral immunity of the animal after antirabic vaccination and also for the characterization of antigens activity in rabies vaccines. Antibodies are the results of the humoral immune response of the organism to the antigens; it is a process that is driven, and influenced by many factors: amounts of antigens, way of application, and the participation of the main histocompatibility complex (MHC) genes and, especially the status of the animal (Moore and Hanlon 2010).

WHO Expert Committee on Rabies determined that the level of the antibodies of at least 0.5 IU/cm^3 means the adequate protection by vaccine (Smith 1991, Meslin et al. 1996). This value of 0.5 IU antibody titre was first mentioned by the Expert Committee on Rabies in the 8th WHO Report in 1992 and is recognized throughout the world. For the detection and quantification of antibodies multiple methodologies have been developed, standardized and recommended by supranational institutions. Performance

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of serological tests aimed at the detection of rabies antibodies resulted in that many countries free of rabies accepted new measures for quarantine and introduced the terms, which requires rabies vaccination and subsequent serological testing of animals (Aubert 1992, Fooks et al. 2003, Mansfield et al. 2004). The European Directive requires the detection of antibodies as evidence of protection, allowing the free movement of pet animals within the European Union, European countries and also between third countries not listed in Schedule C of Regulation 998/2003.

For the detection of specific antibodies, two neutralizing tests are being used (RFFIT and FAVN tests), which are not only time-consuming and costly, but also require highly skilled technique and specific laboratory equipment (OIE 2004, 2008). RFFIT and FAVN tests are the current reference methods, which are recognized and intended by WHO and OIE. In recent years, several ELISA tests have been developed, which have been used to determine rabies antibodies in sera from vaccinated carnivores in combination with the neutralization test (Wasniewski and Cliquet 2012).

Materials and Methods

For comparison of rabies antibodies detection means of individual serological methods we used 173 sera obtained from dogs vaccinated with commercial rabies vaccines as well as from 23 unvaccinated dogs. The blood of vaccinated dogs was collected on the 30th day after vaccination. Sera obtained from vaccinated and unvaccinated dogs were heated before the testing (30 minutes at 56°C) and stored at -20°C. The sera from dogs were tested by three different serological methods: RFFIT (Smith et al. 1996), FAVNT (Cliquet et al. 1998) and ELISA test (Suliovh et al. 1997). For statistical evaluation of the results obtained by three diagnostic methods we used the method of repeated measures ANOVA ($P<0.0001$) and Tukey's Multiple Comparison Test.

Results

The results of the titration of rabies antibodies in the serum of dogs obtained by RFFIT; FAVNT and ELISA tests are shown in Fig. 1. When testing samples that came from unvaccinated dogs (23 sera) in six samples we saw low levels of antibodies (0.22 IU/cm^3 in RFFIT; 0.11 and 0.17 IU/cm^3 FAVN and 0.101, 0.254, 0.356 UE/cm^3 in ELISA test); these were probably due to the persistence of maternal antibodies, i.e. serum came from dogs, whose mothers were vaccinated closely before mating. In 155 sera (89.60%) coming from vaccinated dogs required levels of antibodies were detected, antibody titers were greater than 0.5 IU/cm^3 in RFFIT and FAVN test. The titer of rabies antibody less than the demanded value was detected by RFFIT and FAVN tests in 18 sera, which represents 10.40%. The titers of rabies antibody obtained by ELISA test are in correlation with the results obtained with RFFIT and FAVN tests. From 173 sera originating from vaccinated dogs, titer was higher than 1.0 UE/cm^3 in 165 sera (95.38%) tested sera. In the 9 investigated sera (5.20%) lower antibody titer than determined protective value 1.0 UE/cm^3 was determined. Statistical evaluation of the results of three diagnostic methods has shown statistically significant difference ($P<0.05$) between the average values obtained from different methods.

Discussion

Specific antibodies play a central role in the prevention of many infectious diseases. The gold standard for detection of rabies virus neutralizing antibodies is the rapid fluorescent focus inhibition test (RFFIT). Instead of using neutralization, it is possible to use a method based on blocking the binding activity of antibodies to the specific antigen (ELISA), as well as other available methods. In any infectious disease, but in particular in rabies, the aspect of choosing the appropriate diagnostic test for the detection and determination of antibody titer, its validation and interpretation is crucial (Moore and Hanlon 2010).

WHO Expert Committee on Rabies has determined that the level of rabies antibodies of least 0.5 IU/cm^3 indicates adequate protection (WHO 2005). Evidence of the level of 0.5 IU/cm^3 antirabies antibodies in serum set in a single test determines whether the animals entering the country being free of rabies should be quarantined or not. Ultimately, small variations in the test have a significant economic impact for animal owners (Briggs et al. 1998).

In some countries, vaccination policy, certification and detection of rabies antibodies have been introduced to reduce the time dogs and cats would have to spend in quarantine after transport; it was the rabies-free countries that have tried to review the lengthy quarantine system (Briggs and Schweitzer 2001).

Detection of rabies antibodies proves that, in dogs and cats, the probability of survival after the vaccination is high regardless of the used dose and the strain of the virus. The level of antibodies titer in serum of animals vaccinated against rabies, reported in the pet passport, guarantees vaccination and sufficient

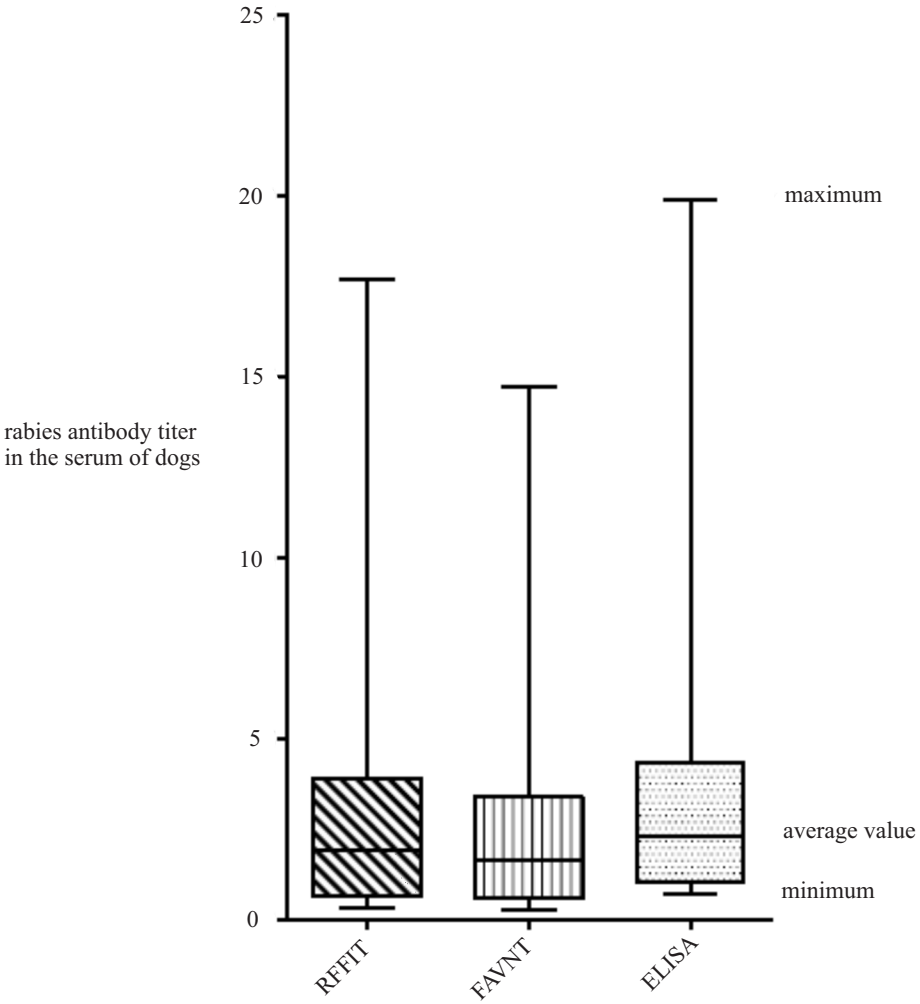

Fig. 1. Comparison of the performances of the RFFIT, FAVN and ELISA test (n = 173, SD: RFFIT 2.988; FAVNT 2.477; ELISA 3.326; P<0.05)

immunity of the animal during transport between countries (Aubert 1992).

The first test to detect the virus neutralizing antibodies was *in vivo* mouse neutralization test (MNT) (Bourhy and Sureau 1991). Rabies antibody levels in serum in RFFIT are measured by their ability to inhibit viral replication in a cell culture. Any non-specific factors in the sample that inhibit the growth of virus can also be measured as neutralizing antibodies RFFIT. Due to time and technical reasons, but mainly with respect to ethical principles, RFFIT is more preferable than the MNT method (Smith et al. 1996). The great advantage of FAVN test in comparison with the RFFIT is the elimination of errors using fourfold examination of the same sample as well as simple subtraction of the result all-or-nothing reading of the fluorescence (Cliquet et al. 1998).

In determining titre of rabies antibodies, RFFIT and FAVN tests detected in 155 of examined sera (89.60%) the required level of antibodies, i.e. antibody titer greater than 0.5 IU/cm^3.

The titer of rabies antibodies less than the desired value was detected by RFFIT and FAVN test in 18 sera, which represents 10.40%. In all cases, it was serum that originated from the young dogs after primary vaccination. In older dogs the titre determined was greater than 0.5 IU/cm^3; most of the older dogs were vaccinated more than once against rabies.

Jakel et al. (2008) in their work indicate that young dogs are at high risk of rabies if having titre lower than 0.5 IU/cm^3. This risk can be minimized by using the second vaccination, or performing duplicate primary vaccination against rabies within a short interval of 7-10 days, and applying a booster dose one year latter (Zanoni et al. 2010). The results of detection of rabies antibodies by FAVN test were comparable with the results published by Briggs et al. (1998); when the final assessment prefers FAVNT rather than the evaluation by RFFIT which is time and labor consuming.

In recent years, multiple ELISA techniques have been described for quantification of rabies antibodies (Cliquet et al. 2003). Even though they are quick and

simple, their disadvantage is that they detect almost all IgG antibodies that bind to the antigen-coated plates, and not only the actual anti-rabies neutralizing antibodies. Unless highly purified anti-rabies antigen is used, other antibodies can be detected than those to be quantified giving distorted and incorrect results (Elmgren and Wandeler 1996). It is also one of the reasons why the level of antibodies is expressed in equivalent units (UE) and not in international units (IU). In order to detect all antibodies and not only the neutralizing ones, the WHO (1996) recommended raising the threshold levels of rabies antibodies by ELISA test at 1.0 UE/cm^3.

In our study, rabies antibody titers obtained by ELISA correlated with the results obtained by the above mentioned methods. Out of 173 sera originating from vaccinated dogs the titer was determined to be greater than 1.0 UE/cm^3 in 95.38% of the testested samples. In nine cases, the serum antibody titer was detected only a few thousandths lower than the threshold value 1.0 UE/cm^3. The levels of rabies antibody titers by ELISA are also generally higher when compared with the results of other tests (Otachel-Hawranek 2003). Sera derived from unvaccinated dogs were determined by ELISA as negative. Results of detection of rabies antibodies obtained by different methods mentioned above are directly correlated. All investigated sera derived from unvaccinated dogs were negative, in six sera low levels of antibodies were determined, presumably the persistent maternal antibodies. In 89.60% of tested sera derived from vaccinated dogs, the required protection level of antibodies was detected, in 10.40% of sera the antibody titer was lower than the desired value (0.5 IU/cm^3). The results of detection of rabies antibodies show that all three used *in vitro* methods are reliable and time-saving. The advantage of FAVNT is its greater ability to capture under-immunized animals with some threshold level of rabies antibodies around 0.5 IU/cm^3. ELISA method due to easier implementation and lower time complexity is suitable for mass screening of large numbers of animals (Wasniewski and Cliquet 2012).

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References

Aubert MF (**1992**) Practical significance of rabies antibodies in cats and dogs. Rev Sci Tech 11: 735-760.

Bourhy H, Sureau P (**1991**) Laboratory methods for rabies diagnosis. Commission des Laboratoires de Référence et d'Expertise de l'Institut Pasteur, Institut Pasteur, Paris, France, p 197.

Briggs DJ, Smith JS, Mueller FL, Schwenke J, Davis RD, Gordon CR, Schweitzer K, Orciari LA, Yager PA, Rupprecht CE (**1998**) A comparison of two serological methods for detecting the immune response after rabies vaccination in dogs and cats being exported to rabies-free areas. Biologicals 26: 347-355.

Briggs DJ, Schweitzer K (**2001**) Importation of dogs and cats to rabies-free areas of the world. Vet Clin North Am Small Anim Pract 31: 573-583.

Cliquet F, Aubert M, Sagné L (**1998**) Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantification of rabies-neutralising antibody. J Immunol Methods 212: 79-87.

Cliquet F, Müller T, Mutinelli F, Geronutti S, Brochier B, Selhorst T, Schereffer JL, Krafft N, Burow J, Schameitat A, Schlüter H, Aubert M (**2003**) Standardisation and establishment of a rabies ELISA test in European laboratories for assessing the efficacy of oral fox vaccination campaigns. Vaccine 21: 2986-2993.

Elmgren LD, Wandeler AI (**1996**) Competitive ELISA for the detection of rabies virus-neutralizing antibodies. In: Meslin FX, Kaplan MM, Koprowski H (eds) Laboratory Techniques in Rabies. 4th ed., World Health Organization, Geneva, pp 200-208.

Fooks AR, McElhinney LM, Brookers SM, Johnson N, Keene V, Parsons G, Soldan A (**2002**) Rabies antibody testing and the UK Pet Travel Scheme. Vet Rec 150: 428-430.

Jakel V, König M, Cussler K, Hanschmann K, Thiel HJ (**2008**) Factors influencing the antibody response to vaccination against rabies. Dev Biol (Basel) 131: 431-437.

Mansfield KL, Burr PD, Snodgrass DR, Sayers R, Fooks AR (**2004**) Factors affecting the serological response of dogs and cats to rabies vaccination. Vet Rec 154: 423-426.

Meslin FX, Kaplan MM, Koprowski H (**1996**) Laboratory Techniques in Rabies. 4th ed., World Health Organization, Geneva, p 476.

Moore SM, Hanlon CA (**2010**) Rabies-Specific Antibodies: Measuring Surrogates of Protection against a Fatal Disease. PLoS Negl Trop Dis 4: e595.

OIE (**2004**) Manual of Standards for Diagnostic Tests and Vaccines. Rabies. 4th ed., Office International des Epizooties, OIE, Paris, pp 276-291.

OIE (**2008**) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds, and Bees). 6th ed., Office International des Epizooties, OIE, Paris, pp 304-322.

Otachel-Hawranek J (**2003**) Comparison of RFFIT and ELISA in detecting post-vaccine anti-rabies antibodies in dog and fox sera. Med Weter 59: 1099-1102.

Smith JS (**1991**) Rabies serology. In: Baer GM (ed) The natural history of rabies. 2nd ed., CRC Press, Boca Raton, pp 235-252.

Smith JS, Yager PA, Baer GM (**1996**) A rapid fluorescent focus inhibition test (RFFIT) for determining rabies virus-neutralizing antibody. In: Meslin FX, Kaplan MM, Koprowski H (eds), Laboratory Techniques in Rabies. 4th ed., World Health Organization, Geneva, pp 181-192.

Süliová J, Benišek Z, Švrček Š, Ďurove A, Ondrejka R (**1997**) The effectiveness of inactivated, purified and concentrated experimental rabies vaccine for veterinary use: immunogenic activity. Vet Med (Praha) 42: 51-56.

Wasniewski M, Cliquet F (**2012**) Evaluation of ELISA for detection of rabies antibodies in domestic carnivores. J Virol Methods 179: 166-175.

WHO (**2005**) World Health Organization Expert Consultation on Rabies. Second report. World Health Organ Tech Rep Ser, Geneva, pp 1-139.

Zanoni RG, Bugnon P, Deranleau E, Nguyen TM, Brugger D (**2010**) Walking the dog and moving the cat: rabies serology in the context of international pet travel schemes. Schweiz Arch Tierheilkd 152: 561-568.