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Evaluation of an ELISA to detect rabies antibodies in orally vaccinated foxes and raccoon dogs sampled in the field

M. Wasniewski[a], A.L. Guiot[b], J.L. Schereffer[a], L. Tribout[a], K. Mähar[c], F. Cliquet[a,*]

[a] ANSES, Nancy Laboratory for Rabies and Wildlife, European Union Reference Laboratory for Rabies (EURL), WHO Collaborating Centre for Research and Management in Zoonoses Control, OIE Reference Laboratory for Rabies, European Union Reference Institute for Rabies Serology, Technopôle agricole et vétérinaire de Pixérécourt, B.P. 40009, F-54220 Malzéville, France

[b] CPB – Conseils en Pharmacie et Biologie, 69110 Sainte Foy les Lyon, France

[c] Estonian Veterinary and Food Laboratory, Virology-Serology Department, Kreutzwaldi 30, 51006 Tartu, Estonia

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A B S T R A C T

The assessment of the efficacy of oral vaccination in wildlife is based on detection in the teeth of a biomarker (tetracycline) which is incorporated in the vaccine bait, and the quantification of rabies antibodies.

A blocking ELISA was evaluated and compared with the FAVN test and a validated in-house ELISA, using sera from foxes and raccoon dogs collected following oral vaccination campaigns in France and Estonia.

Specificity reached 100% in sera from naïve animals. A high concordance (95%) was observed between the BioPro ELISA and the FAVN test, which was similar in sera from red foxes and raccoon dogs. Concordance between the BioPro ELISA and the in-house ELISA reached 96.5% for sera from red foxes.

The agreement with tetracycline results was excellent in the fox for both the BioPro ELISA (95.9%) and the FAVN test (91.8%). Concordance was slightly lower in the raccoon dog, with a value of 82.8% for the BioPro ELISA and 78.4% for the FAVN test.

Rabies antibodies were detected with the BioPro ELISA in animals vaccinated with different types of vaccines and in highly haemolysed sera.

The BioPro ELISA is a valuable test to assess the efficacy of oral vaccination in foxes and raccoon dogs.

1. Introduction

The oral vaccination of wildlife against rabies was shown to be effective for eliminating rabies in several European countries (Aubert et al., 2004; Brochier et al., 1996; Bugnon et al., 2004; Capello et al., 2010; Cliquet et al., 2012). The assessment of oral vaccination efficacy in wildlife is based on two methods: the detection of a biomarker (tetracycline) incorporated in the vaccine bait and revealed in the teeth of sampled target species, and the quantification of rabies antibodies. The first method demonstrates bait uptake, and the second, effective immunisation (Anonymous, 2005).

Neutralising antibodies are recognised as the most reliable parameter for assessing the efficacy of vaccination because they are closely correlated with protection against rabies infection (Aubert, 1992, 1993). The fluorescent antibody virus neutralisation test (FAVN) (Cliquet et al., 1998a) and the rapid fluorescent focus inhibition test (RFFIT) (Smith et al., 1973) are the reference methods recommended by WHO and OIE to quantify neutralising rabies antibodies (Anonymous, 2005; Cliquet and Barrat, 2008). However, they are time-consuming, expensive, require highly trained technicians, the maintenance of cell cultures, laboratories with a high containment level and vaccinated technicians to handle live rabies virus. In addition, since they are based on cell cultures, they are sensitive to any cytotoxic products and contaminating agents present in samples (Barton and Campbell, 1988; Cliquet et al., 2003; Servat et al., 2006). This is an important issue for field samples, which are body fluids collected by puncturing the heart or thoracic cavity of animals killed or found dead. Thus, these samples are often of poor quality (haemolysis, bacterial contamination) (Cliquet et al., 2000).

Other techniques have been developed, including an indirect immunofluorescence test which was shown to be suitable for detecting rabies antibodies in body fluids from foxes (Hostnik and Grom, 1997). This test correlates well with the RFFIT and is not sensitive to the cytotoxic activity of some sera. It is a quick test, but requires skilled personnel to read the fluorescence results and handle the rabies virus when preparing infected slides.

* Corresponding author at: ANSES, Nancy Laboratory for Rabies and Wildlife, Technopôle agricole et vétérinaire, BP 40009, 54220 Malzéville Cedex, France. Tel.: +33 3 83 29 89 50; fax: +33 3 83 29 89 58.
E-mail address: florence.cliquet@anses.fr (F. Cliquet).

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Enzyme-linked immunosorbent assays (ELISA) are quick, easy to perform, do not require live rabies virus or cell culture, are suited to large-scale screening and can be automated. In addition, they can be performed in any laboratory. These tests appear particularly suitable for assessing the effectiveness of oral vaccination in field samples.

Few ELISAs have been evaluated using sera from wild animals (Barton and Campbell, 1988; Cliquet et al., 2000; Knoop et al., 2010; Servat et al., 2007, 2008; Vengust et al., 2011).

The BioPro company's blocking ELISA kit, designed to detect rabies antibodies, showed a good correlation with the FAVN test and the Biorad Platelia Rabies II ELISA in sera from dogs and foxes (Mojzis et al., 2008). Recently, specificity, sensitivity and reliability of the BioPro Rabies ELISA Ab kit were assessed in sera from dogs and cats received in the framework of international trade. The assay was shown to be highly specific and had 86.2% concordance with the FAVN test (Wasniewski and Cliquet, 2012).

The objective of this study was to evaluate the BioPro Rabies ELISA Ab kit for assessing the efficacy of oral vaccination in sera from raccoon dogs and red foxes collected in the field.

2. Materials and methods

2.1. Samples

2.1.1. Naïve sera used for specificity tests

A total of 71 red fox sera were obtained from night shootings of naïve foxes (animals not vaccinated against rabies and collected in a rabies-free country) performed in 2010–2011 in the French department of Meurthe-et-Moselle. Serum samples were also taken from 20 unvaccinated caged foxes used for breeding and 117 unvaccinated caged raccoon dogs used in experimental laboratory protocols.

2.1.2. Field sera collected after oral vaccination campaigns

Red fox sera ($n = 359$) were collected during night shootings from 2000 to 2006 to assess the efficacy of oral vaccination campaigns in France in the following departments: Meurthe-et-Moselle, Meuse, Moselle, Bas-Rhin, Haut-Rhin, and Vosges. The vaccines used for oral vaccination were Vaccinia Glycoprotein Recombinant (VRG, Merial Limited, Athens, USA) and Street Alabama Gif (SAG2, Virbac Laboratories, Carros, France) baits. The last vaccination campaign took place in autumn 2005. For most sera collected in France, the extent of haemolysis was reported according to the following scale: A = no haemolysis, B = little haemolysis, C = haemolysis, and D = substantial haemolysis.

Raccoon dog ($n = 274$) and red fox sera ($n = 49$) were also collected in Estonia from September 2010 to March 2011 in areas vaccinated with SAG2 baits. In addition, tetracycline (150 mg per bait)—used as a biological marker for bait uptake—was sought in the lower jaw of the Estonian foxes and raccoon dogs.

2.1.3. Sera collected from foxes and raccoon dogs vaccinated experimentally

Foxes and raccoon dogs were housed outside in individual cages and appropriately sheltered to conform with instructions relating to animal experimentation as given by the European Community's Directive (Anonymous, 1986).

A total of 50 serum samples came from an experiment using foxes vaccinated orally either with VRG rabies vaccine baits containing $10^{8.8}$ $TCID_{50}$/dose ($n = 25$) or SAG2 rabies vaccine baits containing $10^{7.9}$ $TCID_{50}$/dose ($n = 25$), each animal consuming a single bait. All the foxes were sampled at the same time after bait uptake.

Serum samples were also obtained from raccoon dogs vaccinated orally with SAG2 ($n = 81$) or with VRG ($n = 53$) in separate experiments designed to evaluate the immunogenicity of the vaccine baits.

2.2. Titration methods

All sera were heat inactivated (30 min at $56 \pm 2\,^{\circ}C$) and stored at $-20\,^{\circ}C$ until use.

2.2.1. Standard reference serological method: the FAVN test

Rabies-neutralising antibodies were determined by the FAVN test as described by Cliquet et al. (1998a). The OIE serum or a positive reference serum of dog origin adjusted to 0.5 IU/mL was used as a positive control. Briefly, each serum sample together with the positive and negative controls were placed in four consecutive wells and then serially diluted. The challenge rabies virus (CVS-11, ATCC VR 959) containing a 50% tissue culture infective dose (TCID50) of 100 in 50 μL was then added to each well. After 60 min of incubation, a volume of 50 μl of 4×10^5 BHK-21 cells/mL suspension was added to each well and the microplates were incubated for 48 h at $36 \pm 2\,^{\circ}C$ in a humidified incubator with 5% CO_2. The microplates were stained by adding 50 μl of an appropriate dilution of a fluorescein isothiocyanate (FITC) anti-rabies monoclonal globulin (Fujirebio Diagnostics, Malvern, USA) to each well. Plates were qualitatively read according to an "all or nothing" scoring method. The titres of serum samples were expressed in international units per millilitre (IU/mL) by comparing results obtained with those of the positive standard. According to previous results, the threshold of positivity was 0.24 IU/mL (Cliquet et al., 1998a, 2000).

2.2.2. Rabies ELISA Ab kit developed by BioPro

The BioPro Rabies ELISA Ab kit (BioPro ELISA) and its reagents were purchased from BioPro (Prague, Czech Republic). The calibrated positive controls were those supplied by the manufacturer (CS1, CS2 and CS3). Sera were titrated according to the manufacturer's instructions. This test was a blocking ELISA that detected rabies virus antibodies. Microplate wells were coated with antigens derived from rabies virus containing virus glycoproteins and it was likely to find residues of other rabies virus proteins. Briefly, each serum sample was diluted to 1:2 then 100 μL of this dilution was distributed in the microplates. And 100 μl of the diluted positive and negative controls was distributed in duplicate. The microplates were incubated overnight at 2–8 °C with gentle shaking then washed six times to remove unbound antibodies and other proteins in the samples. Then 100 μL of the biotinylated anti-rabies antibodies were distributed in each well. The microplates were incubated for 30 min at 37 °C with gentle shaking then washed four times to remove unbound biotinylated anti-rabies antibodies. Next, 100 μL of the streptavidin peroxidase conjugate were placed in each well. The microplates were incubated for 30 min at 37 °C with gentle shaking then washed four times to remove unbound conjugate. The presence of antigen-biotinylated antibody complexes was revealed by adding 100 μL of TMB chromogen solution to each well. The microplates were incubated in the dark for 15–20 min at room temperature with gentle shaking. The enzymatic reaction was stopped by adding a solution of 0.5 M H_2SO_4. The microplates were read at 450 nm. The conditions of validation described by the manufacturer were implemented to interpret the results obtained for the different samples. The percentage of blocking (%PB) was calculated for each sample according to the manufacturer's specifications (i.e. $\%PB = [(ODNC - OD_{sample})/(ODNC - ODPC)] \times 100$) where ODNC was the optical density of the negative control, ODPC the optical density of the positive control and "OD sample" the optical density of the sample. The positivity threshold recommended by the manufacturer is 40% for assessing the effectiveness of oral vaccination campaigns.