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Use of filter paper blood samples for rabies antibody detection in foxes and raccoon dogs

Marine Wasniewski[a], Jacques Barrat[a], Benoit Combes[b], Anne Laure Guiot[c], Florence Cliquet[a,*]

[a] French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Nancy Laboratory for Rabies and Wildlife, European Union Reference Laboratory for Rabies, European Union Reference Laboratory for Rabies Serology, WHO Collaborating Centre for Research and Management in Zoonoses Control, OIE Reference Laboratory for Rabies, Technopôle agricole et vétérinaire de Pixérécourt, CS 40009, 54220 Malzéville, France
[b] ELIZ – Entente Interdépartementale de Lutte contre les Zoonoses, Technopôle agricole et vétérinaire de Pixérécourt, CS 40009, 54220 Malzéville, France
[c] Conseils en Pharmacie et Biologie, 2 place des Quatre Vierges, 69110 Sainte Foy les Lyon, France

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A B S T R A C T

The effectiveness of oral rabies vaccination in wildlife is usually evaluated by the detection of rabies antibodies. However, the assessment of rabies antibodies has several technical difficulties in the field, such as the collection, storage, transport and titration of blood samples, often of poor quality.

The objective of this study was to assess the feasibility of collecting blood on a filter paper (FP) coupled with enzyme-linked immunosorbent assay (ELISA) titration of rabies antibodies in raccoon dogs and red foxes.

The FP blood sampling method was found highly specific and repeatable in both species. Overall, results obtained with the FP sampling method were highly concordant with the conventional (venipuncture) sampling methods. Blood eluates from FP samples from foxes and raccoon dogs tested using ELISA showed concordance values of 92% and 95%, respectively, with serum samples tested using the seroneutralisation test and values of 95% and 91%, respectively, when the ELISA was used on both types of sample.

The use of FP blood sampling coupled with the titration of rabies antibodies by ELISA provides a reliable alternative to conventional blood sampling and serum testing by seroneutralisation. This simple procedure is particularly attractive and cost-effective for assessing the effectiveness of oral rabies vaccination in field conditions.

1. Introduction

Rabies has been controlled in several European countries through oral vaccination (OV) programmes targeting wildlife (Aubert et al., 2004; Brochier et al., 1996; Bugnon et al., 2004; Capello et al., 2010; Cliquet et al., 2012). The effectiveness of OV campaigns in wildlife is usually assessed by the quantification of rabies antibodies and the detection of a biomarker (tetracycline) incorporated into the vaccine bait in the teeth of sampled target species.

Samples from red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) are usually taken by hunters in rabies vaccinated areas (Cliquet et al., 2012; WHO, 2013). The assessment of rabies antibodies in animals killed and sampled in the field has technical difficulties, leading to inadequate monitoring of the effectiveness of OV. The sampling method must be simple, practical, reliable and usable under any field conditions and samples need to be transported easily. The collection of blood on filter paper (FP) is a promising sampling method that can satisfy these conditions. In addition, the serological testing should be suitable for samples of poor quality (insufficient volume, haemolysis, bacterial contamination) collected from animals killed or found dead (Cliquet et al., 2000).

The specificity, sensitivity and reliability of an enzyme-linked immunosorbent assay (ELISA) kit (BioPro ELISA, BioPro, Prague, Czech Republic) used to detect rabies antibodies in blood samples collected in the field from foxes and raccoon dogs were previously demonstrated (Wasniewski et al., 2013). There is high concordance (95%) between the BioPro ELISA results and those of the fluorescent antibody virus neutralisation test (FAVN test), one of the two reference methods recommended by the World Health Organisation (WHO) and the World Organisation for Animal Health (OIE) to quantify neutralising rabies antibodies (OIE, 2012; WHO, 2013). ELISAs are more suitable than neutralisation tests for quick and

* Corresponding author. 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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easy detection of rabies antibodies in large-scale screening programmes. Furthermore, neutralisation tests 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 (Wasniewski et al., 2013).

A reliable technique for field sampling of fox and raccoon dog blood to improve the monitoring of OV in Europe (WHO, 2013) is needed. As part of the national programme for active rabies surveillance in bats, the ANSES laboratory validated a method of FP blood sampling for the detection of rabies antibodies in bats (Picard-Meyer et al., 2011). In the present study, the FP method was tested and evaluated for the collection and transport of blood samples from foxes and raccoon dogs collected either in experimental conditions or from wild cadavers. After an elution step, FP blood samples were titrated with the BioPro ELISA kit.

The objective of the study was to assess the feasibility of the FP collection method coupled with the BioPro ELISA kit for the titration of rabies antibodies in blood samples from raccoon dogs and red foxes.

2. Materials and methods

2.1. Samples

Blood samples from foxes and raccoon dogs were collected in the field and in ANSES-Nancy' experimental station (Atton, Meurthe-et-Moselle *département*, France). The facility has been approved by French Veterinary Services on 19 of April 2011 (approval C-54-431-1). Silver foxes and raccoon dogs used during this experiment originated from Finnish approved fur farms and were housed in similar conditions. Experiments and husbandry were conducted following European Directive, 2010/63/EU (Buzek and Chastel, 2010) and French regulations on ethics in animal experimentation. The national animal welfare group studied environment enrichment conditions.

2.1.1. Samples used for specificity tests

A total of 36 red fox blood samples were obtained from night shooting of naive foxes (unvaccinated animals collected in France, a rabies-free country) from January to March 2013 in the French Meurthe-et-Moselle département. In addition, 116 blood samples were also collected from naive caged foxes ($n = 38$) and 30 from naive caged raccoon dogs ($n = 3$) used in other experimental studies.

2.1.2. Samples used for the repeatability study

Three laboratory foxes and three laboratory raccoon dogs were selected on the basis of their level of neutralising rabies antibodies (negative, low titre, high titre). Blood from each animal was collected from the jugular vein into untreated tubes (four 7.5 mL tubes for each animal).

2.1.3. Samples used for the concordance study

A total of 75 blood samples were obtained from five laboratory foxes and five raccoon dogs that received on day 0 an oral vaccine bait (SAG2 strain vaccine from Virbac Laboratories, Carros, France; SAG2 is a modified live attenuated rabies derived from the SAD Bern strain). These foxes and raccoon dogs were used in previous protocols and some had low residual neutralising antibody titres before day 0. Samples were collected on days −20, −9, −7, −5, −2, 0, 2, 4, 6, 8, 14, 20, 29, 40 and 61.

2.2. Collection and processing of blood samples

2.2.1. Collection of blood samples

FP marketed by BioRad (Mini Trans-Blot Filter Paper, catalogue ref. 170-3932, Hercules, CA, USA) was selected for the collection of blood samples. The surface of the FP (10 cm × 7.5 cm) was saturated with whole blood as described below.

Blood samples from caged animals were taken from the jugular vein into untreated tubes (7.5 mL S-monovette, Sarstedt, Nümbrecht, Germany) to saturate the FP. Blood samples for serology were collected with tubes treated to accelerate clotting (7.5 mL S-monovette Z-gel, Sarstedt), they were centrifuged to obtain serum.

Blood samples obtained in the field during night shooting were collected on FP as soon after death as possible (generally only a few minutes after being shot). The filter paper was applied on the surface of the wound of the shot animal, wherever the wound was located (head, chest, trachea or muscles), to saturate the FP with blood. Then FPs were placed into re-sealable zipper plastic bags for transport to the laboratory. In the event FPs could not be sent within 12 h to the laboratory, FP samples were dried at room temperature to prevent mould growth and the dried FP samples were sent to the laboratory in an envelope.

2.2.2. Processing of FP blood samples

FPs were air-dried in the laboratory at room temperature for at least 4 h under a biocontainment hood, then individually stored at room temperature in a paper envelope labelled with the species, animal number and date of collection.

Our laboratory previously demonstrated that a surface of 0.95 cm^2 saturated with blood and diluted 1:9 in a medium containing antibiotics (elution step) gives reliable results in the FAVN test on bat blood samples (threshold of positivity = 1.67) (Picard-Meyer et al., 2011). For fox and raccoon dog blood samples, a surface of 7.5 cm^2 was considered sufficient for serological testing. To perform the test, a 1 cm × 7.5 cm strip of FP was cut with clean scissors. The FP strip was placed into a haemolysis tube with 890 μL of Dulbecco's modified Eagle medium (DMEM, GIBCO, Life Technologies, France) supplemented with antibiotics for the elution step. The strip was pressed against the bottom of the tube with a plastic rod to ensure full contact with the elution medium. After centrifugation (800 × *g* for 30 min), the supernatant (eluate) was collected and stored at −20 °C until analysis. The FP sample final dilution was calculated based on the quantity of blood absorbed on the FP (7.5 cm^2 are saturated by 750 μL of blood) and the volume of medium used for the elution step. Since the hematocrit is not known, a mean value of 50% was chosen. The FP sample dilution was estimated to be approximately 1:4.5 serum concentration.

2.2.3. Processing of serum samples

Serum samples were heat-inactivated (30 min at 56 °C ± 2 °C) and stored at −20 °C until analysis.

2.3. Titration methods

2.3.1. Standard reference serological method: the FAVN test

Rabies-neutralising antibodies were determined by using the FAVN test as described in Cliquet et al. (1998). Reagents used are described in Wasniewski et al. (2013). The neutralising titres are expressed in international units per millilitre (IU/mL) by comparing results obtained with the test serum to those of the positive standard. According to previous results, the threshold of positivity was 0.24 IU/mL (Cliquet et al., 2000, Wasniewski et al., 2013).

2.3.2. 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). Serum