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Immunogenicity and efficacy of an in-house developed cell-culture derived veterinarian rabies vaccine

Héla Kallel [a,*], Mohamed Fethi Diouani [b], Houssem Loukil [a], Khaled Trabelsi [a], Mohamed Ali Snoussi [b], Samy Majoul [a], Samia Rourou [a], Koussay Dellagi [c]

[a] *Unité de Développement de Vaccins Virologiques, Institut Pasteur, 13 Place Pasteur, BP 74, 1002 Belvédère, Tunis, Tunisia*
[b] *Laboratoire d'Epidémiologie et d'Ecologie Parasitaire, Institut Pasteur, Tunis, Tunisia*
[c] *Laboratoire d'Immunopathologie, de Vaccinologie et de Génétique Moléculaire, Institut Pasteur, Tunis, Tunisia*

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Abstract

The efficiency of an inactivated tissue culture rabies vaccine produced on BHK-21 cells, according to an in-house developed process, was evaluated and compared to a commercial cell-tissue culture vaccine (Rabisin). Fifteen experimental dogs from local common breed were duly conditioned during a quarantine period, then vaccinated via the subcutaneous route with 1 ml of either the tissue culture vaccine developed in-house or the commercial vaccine Rabisin. The immune response of each dog was monitored for 162 days. Serum-neutralizing antibodies titers to rabies virus were determined by the rapid fluorescent focus inhibition test (RFFIT) which confirmed the strong response of dogs to both vaccines except one dog in the Rabisin group. The dogs were then challenged in the masseter muscle with a rabies street virus of canine origin. All vaccinated dogs except the single dog in the Rabisin group that failed to respond to the vaccine, survived the challenge. In contrast, 80% of animals in the control non-vaccinated group, developed rabies and died.

A field vaccine trial was also conducted: 1000 local dogs living in field conditions received one subcutaneous dose of the locally developed vaccine. Serum neutralizing antibody titers to rabies virus was determined by RFFIT at days 0, 60 and 360. Mean rabies neutralizing antibody titers were equal to 0.786, 3.73 and 1.55 IU/ml, respectively.

The percentage of dogs with a neutralizing rabies antibody titer higher than the 0.5 IU/ml mandated WHO threshold, was 30%, 91.4% and 87.5% at day 0, 2 months and 1 year post-vaccination, respectively.

These data demonstrate the efficiency of the in-house developed vaccine produced on BHK-21 cells in both experimental and field conditions and support its use in dog mass vaccination campaigns.

Keywords: Veterinary rabies vaccine; BHK-21 cells; Field and laboratory trials

1. Introduction

Rabies is a zoonotic disease that causes 40,000–70,000 annual deaths worldwide [1]. More than 99% of all human rabies deaths occur in the developing world, mainly in Africa and Asia. Today, 10 millions post-exposure treatments are reported yearly among humans [2,3].

The domestic dog is the main reservoir and vector for rabies virus and accounts for more than 99% of all human rabies cases. Although canine rabies was successfully eliminated from most developed countries, it is still widespread in over 80 developing countries [4].

Vaccination of dogs against rabies effectively controls this zoonosis and prevents its transmission to humans [4,5]. Programmes of mass vaccination of dogs via the parenteral route remain the most efficient control measure of endemic canine rabies [4,6] since dog culling alone is not effective [4].

* Corresponding author. Tel.: +216 71 783 022; fax: +216 71 791 833.
E-mail address: hela.kallel@pasteur.rns.tn (H. Kallel).

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Nowadays, it is estimated that at least 50 millions dogs are vaccinated each year against rabies either in private practice or during national mass campaigns [7].

In Tunisia, canine rabies is still considered as a serious public health concern, although tremendous efforts were spent to control this zoonosis. A national control program was launched in 1982, based on epidemiological surveillance of animal rabies and on dog vaccination during mass campaigns, initially conducted every 2 years then on a yearly basis since 1992. As a result, incidence of animal rabies was significantly reduced to about 170 cases per year and human cases strikingly decreased leading to the effective reduction of human rabies. Zero to two human cases are currently reported each year compared to 16 cases per year prior to 1982 [8,9].

Imported vaccine produced by cell-culture techniques (Rabisin, Merial, France) was used in Tunisia from 1982 to 1988 for dog mass vaccination campaigns. Later on it was partially replaced by a vaccine locally produced on lamb brains in order to reduce costs [8,10].

In a previous work, we described a unit process system to produce a cost effective rabies vaccine using BHK-21 cells grown on microcarriers in a 20 L bioreactor [11]. In the present study, we evaluate the efficiency of this vaccine to protect local dogs against street rabies virus challenge and compared it to the commercially available cell-culture vaccine, Rabisin. A field trial including one thousand field dogs was also conducted to evaluate vaccine effectiveness in field conditions.

2. Materials and methods

2.1. Dogs

Fifteen dogs 2–3 months old, issued from the common local breed, were collected from their owners in the field and quarantined at least for 60 days. They were deparasitized, vaccinated against the major canine infectious diseases (Tetradog®, Mérial, France) and properly fed. The animals were then transferred to an experimentation kennel.

2.2. The in-house developed, cell-culture derived rabies vaccine

The vaccine was produced according to the process previously reported [11]. Briefly, the virus (PV/BHK-21 virus strain) was multiplied on BHK-21 cells grown on 4 g/l Cytodex 3 in a 20-l bioreactor. Virus harvests were clarified by filtration through a 8 μm filter and then inactivated by β-propiolactone (final dilution: 1/4000). A stabiliser was added to the inactivated virus harvests and aluminium hydroxide was used as an adjuvant. The vaccine lot was tested according to WHO guidelines [12]. The potency of the prepared vaccine as estimated in vivo according to the NIH test [13], was equal to 2.5 IU/ml.

2.3. Vaccination protocol

At the start of the vaccination protocol, dogs were aged 5–6 months. They were randomly divided into three groups of five dogs each. Animals assigned to group A were vaccinated via the subcutaneous route with 1 ml of a tissue-culture derived rabies vaccine Rabisin (Mérial, France). Dogs of group B were vaccinated via the subcutaneous route with 1 ml of the tissue culture vaccine that was developed by our group at Institute Pasteur of Tunis. Dogs in group C served as non-vaccinated controls.

Dogs assigned to the three groups, were bled at day 0 (pre vaccination) and at days 15, 30, 60 and 162 post-vaccination to determine rabies neutralizing antibody titers.

2.4. Post-vaccination rabies virus challenge

At day 162, all vaccinated dogs and controls were challenged with a rabies street virus isolated from a rabid dog in Tunisia. Each dog was injected via the intramuscular route, 1 ml of the challenge virus (0.5 ml in each masseter muscle) at a concentration of 10^4 DL50/ml. Dogs were then clinically monitored during 5 months. Animals that survived the challenge were further bled at day 150 before euthanasia.

A brain-tissue sample was collected from each experimental dog and was screened for rabies virus antigen presence by the immunofluorescence antibody test (IFAT).

2.5. Field vaccine trial

The study was conducted in the Gouvernerat of Bizerte (North-East of Tunisia) in three districts: Utique, Mateur and Sejnane. In these areas, dogs aged more than 1 year, had been presumably vaccinated with Rabisin vaccine during the previous yearly mass vaccination campaigns. One thousand dogs living in field conditions received by the subcutaneous route 1 ml of our cell-culture derived vaccine. They were not pre treated against parasites. The characteristics of each dog were assessed by qualified veterinarians and recorded in a data record form (coat, colour, sex, age and rearing status: i.e. is the dog restricted to the house, to the field or released). Each dog was clinically evaluated for malnutrition and external diseases and identified by a numeric photograph.

Veterinarians from the Ministry of Agriculture in charge of the Gouvernerat of Bizerte where the study was conducted, were responsible for animal survey after vaccination.

A sample of dogs representative of the global experimental cohort were bled on days 0, 60 and 360 post-vaccination.

2.6. Antibody assays

Sera were evaluated for rabies neutralizing antibody titers by RFFIT [14]. Briefly negative and positive controls and serum samples were diluted in 96-well microplates then a fixed amount of rabies virus was added to each serum dilution well and incubated for 60 min at 37 °C in a humidified incuba-