THE PROGRAM FOR ORAL RABIES VACCINATION USED IN ROMANIA AND THE METHODS FOR MONITORING ITS EFFECTIVENESS

PROGRAMUL UTILIZAT ÎN ROMÂNIA PENTRU VACCINAREA ORALĂ A RABIEI ȘI METODELE DE MONITORIZARE A EFICACITĂȚII SALE

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ABSTRACT

Rabies, a fatal zoonotic disease, produced by a Lyssavirus, causes more than 70,000 human deaths every year. In Romania, foxes are the main reservoir. Oral rabies vaccination (ORV) of this species is the most effective method to control and eradicate rabies, through many countries in Western, Central and Northern European have become free of rabies.

Since 2011, a supported by co-financing program between Romania and European Union is performed by two successive ORV campaigns every year, spring and autumn. The vaccine distribution was performed on the entire Romanian territory, approx. 237.500 km^2 by air distribution of baits using 8 aircraft, an approx. 25 baits/km^2, with a distance between flight lines of 500 meters and 150 meters altitude, by avoiding the territories of localities, water surfaces, highways, etc. The estimated surface suitable for aerial vaccination is approximated at 213.375 km^2. Around localities and areas difficult to reach by plane, it was done a manual distribution, almost 25 baits/km^2. In order to assess the efficiency of vaccination, at 45 days following vaccination campaign, the hunting of foxes shall be performed; for this purpose, there shall be shot 4 foxes/year/100 km^2. Samples of tooth/bone are tested by microscope examination, UV light, in order to detect tetracycline biomarker. Immune response is assessed using the enzyme-linked immunosorbent *assay (ELISA)* method.

Keywords: Oral Rabies Vaccination Program, bait, tetracycline, ELISA

REZUMAT

Rabia, o zoonoză fatală produsă de un lyssavirus, induce anual peste 70.000 de victime în rândul populației umane. In România rezervorul principal este reprezentat de vulpi. Vacinarea orală a acestei specii s-a dovedit a fi cea mai eficientă metodă de control și eradicare a bolii, prin care multe țări din Vestul, Centrul și Nordul Europei au devenit indemne.

Din 2011 în țara noastră se desfășoara un program cofinanțat de Uniunea Europeană de vaccinare orală a vulpilor împărțit în două campanii pe an, primăvara și toamna. Distribuția vaccinului s-a făcut pe tot teritoriul României, cca 237.500 km^2, prin distribuție aeriană a momelilor, utilizând 8 aeroporturi, cu o densitate de 25 momeli/km^2 cu o distanță între liniile de zbor de 500 m și o altitudine de 150 m evitându-se localităţile, apele, autostrăzile. Suprafața estimată pentru distribuția aeriană este de cca 213.375 km^2. In jurul localităților și a zonelor dificile pentru zbor, distribuția de momeli vaccinale s-a făcut manual, aproximativ 25 momeli/km^2. In vederea monitorizării eficienței vaccinării, la 45 de zile de la distribuția vaccinului are loc împușcarea vulpilor, 4 vulpi/an/100 km^2. Secțiunile de dinte/os se examinează la microscop în lumină UV în vederea detecției biomarkerului tetraciclină, iar răspunsul imun este evaluat folosind testul imunoenzimatic ELISA.

Cuvinte cheie: Program de vaccinare orală a rabiei, momeală, tetraciclină, ELISA

Rabies is a Central Nervous System zoonotic disease, with the causative agent Rabies virus, the negative-sense single stranded RNA viruses of the Lyssavirus genus within the family *Rhabdoviridae*, distributed worldwide and found in terrestrial mammals, causing between 37,000 and 87,000 human deaths annually (7, 9). In Europe, the major reservoir of rabies is wild animals, especially red fox (*Vulpes vulpes*) (2). Extensive oral vaccination programs (ORV) with baits for red foxes have reduced the incidence of rabies in many Western European countries (5, 8).

A co-financed by the EU and the Romanian state budget oral vaccination trial of foxes has been conducted in 16 counties from the western part of Roma-

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nia in spring and autumn 2011. From 2012 the OV programs were implemented throughout the Romanian territory. The objective of this study is to present the ORV program used in Romania and the methods for monitoring its effectiveness.

ORAL RABIES VACCINATION PROGRAM

Study aria.

The vaccination area involved of the entire Romanian territory (237.500 km^2). Romania is situated in the East of Europe and it has borders with Moldova to the Northeast, Ukraine to the North and East, Hungary and Serbia to the West and Bulgaria to the South. The Black Sea forms the Eastern border of the country (6).

Since 2018 two buffer zones will be included, in Moldavia and Ukraine, 50 km and 100 km respectively, from Romanian border.

Vaccine.

Lysvulpen vaccine (Bioveta®, Czech Republic) was used for ORV program. According to the vaccine data sheet, it contains SAD Bern modified attenuated strain cells (min 1.8×107 TCID50-max1.8×108 TCID50/bait). In one bait, there is one vaccination virus dose (1.8 ml) closed in aluminum-plastic blister. Round, dark brown bait is made of feed mixture attractive for foxes and other target animal species (Fig.1 A).

Each bait contains 150 mg of tetracycline HCl, which is intended as an indicator of ingestion by target animal species (Lysvulpen data sheet). Baits were stored in freezers at -20°C prior to use and during the entire vaccination campaign as well.

Baits distribution.

The bait distribution includes border with Serbia, Hungary, Ukraine, Moldova and Bulgaria. The vaccination is performing every year,in two campaigns, spring and autumn. The vaccination of foxes is carried out by air distribution of baits from 8 aircraft (number of 5.325.200 baits with an approx. 25 baits/km^2), with a distance between flight lines of 500 meters and 150 meters altitude, by avoiding the territories of localities, water surfaces, highways, etc. Estimated surface suitable for aerial vaccination is approximated at 213.375 square kilometers (Fig.1 B, C). It has been done a manual distribution around localities and areas difficult to reach by plane (number of 75400 of baits, almost 25 baits/km^2). The data were recorded on Geographical Identification System (GIS) using Geographical Positioning System (GPS).

Samples collection.

At a 45 days following vaccination campaign, the hunting of foxes is performing in order to assess the efficiency of vaccination. For this purpose, 4 foxes/year/100 km^2 shall be shot. Brain samples were collected as previously described (3). Field blood samples were generally collected from the thoracic cavity of shouted foxes. Samples were stored at -20°C until use for ELISA testing. The lower jaws of foxes were collected from each sample.

ORV program monitoring.

Due to the high incidence of rabies cases in Romania, there has been decided that all foxes shall be tested for the detection of rabies virus antigen on brain samples by fluorescent antibody test (FAT) (4) (Fig. 2 A). Different commercial immunofluorescent conjugates were used in order to perform this assay. FAT negative samples are sent to NRL for further investigations such as tetracycline detection and seroconversion. The 150 µm sections throughout canine tooth with some alveolar bone tissue using a low speed saw have been performed from each lower jaw (Fig.2 B, C). Samples of tooth and surrounding alveolar bone were tested at the NRL for rabies by specific fluorescence to detect tetracycline deposits (Fig.2 D). The age of all the foxes tested was determined on the basis of dental examination (young or adult) (Fig.2 E, F) (1).

Fig 1. A - Bait. Feed mixture surrounding vaccination virus suspension closed in aluminum-plastic blister.
B - The vaccination area involved of the entire Romanian territory.
C -The recorded data by GIS and GPS showing the distance between flight lines and flight altitude.

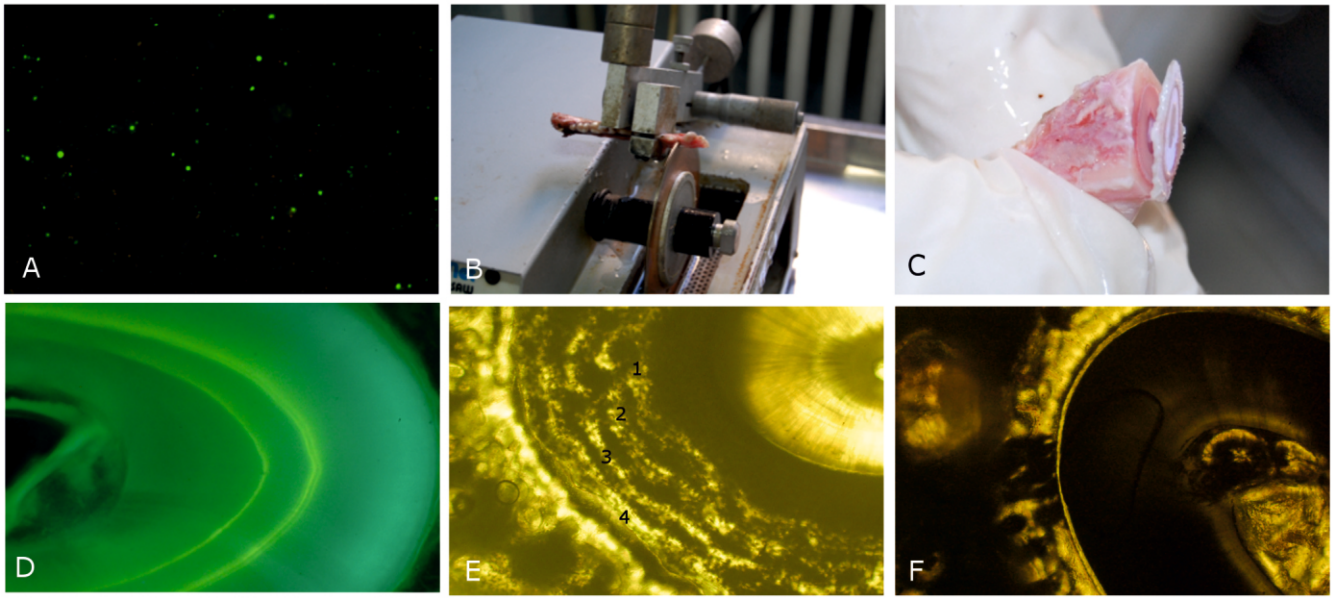

Fig. 2. **A** - FAT positive sample. Different sizes viral particle-apple green brilliance**. B** - Low speed saw. **C** -The 150 µm sections throughout canine tooth. **D** - Detection of tetracycline deposit as specific fluorescent lines. **E** - Dental examination in order to age determination based on annual growing lines in cementum (1,2,3,4)-adult. **F** - No growing lines in cementum-young.

Immune response was assessed using the indirect enzyme-linked immunosorbent *assay (ELISA)*. FAT positive samples are sent to NRL to discriminate between wild and vaccinated strains using molecular biology techniques as previously described (6).

CONCLUSIONS

Romania has implemented a rabies control program since 2011. Sanitary veterinary laboratories from Romania have capability and competence to perform all methods in order to monitor the effectiveness of ORV program. A continuous monitoring and oral vaccination of foxes every year as well as the coordination and cooperation of vaccination programs between neighboring countries are very important.

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