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MONITORING BAIT UPTAKE THROUGH TETRACYCLINE PRESENCE AND AGE STRUCTURE OF FOXES IN ORAL VACCINATION AGAINST RABIES CAMPAIGNS IN R. MACEDONIA

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ABSTRACT

Key objectives of this paper were to present the prevalence of tetracycline presence in fox's teeth and jaws and their age structure after two performed campaigns for oral rabies vaccination in 2011 in Macedonia and to evaluate the successfulness of the bait uptake. The method used for detection of tetracycline was based on fluorescent microscopy of fine sections of teeth and mandibular bones of foxes in ultra-violet light. Age determination in the canine sections was based on presence/absence and number of annual growing lines in the cementum. The total number of examined samples was 141 (n=141) out of which 86 (60,9%) were tetracycline positive. The Southeastern region had the most positive samples [26 (68,4 %)]. Ninetyeight samples (69,5 %) originated from foxes older than three years, although there was high prevalence of tetracycline positive samples in all age classes. The presented results showed relatively high percentage of baits uptake by foxes for 2011 in Macedonia, but more thorough sampling on national level is needed, especially in Northeastern, Polog, Skopje and Vardar region.

Key words: rabies, monitoring of vaccination, tetracycline, fox, age determination

INTRODUCTION

Rabies as a zoonotic viral disease is of a great concern in the European countries. The main reasons are the ability of the virus for easy transmission through direct contact with saliva from the infected animals and the fatal results for human and other animals after the development of the disease's symptoms. Although rabies is widely known as a disease of dogs and cats, the analyses show that the wild animals are the main reservoirs. From the mid of the twentieth century the disease virtually disappeared from dogs, established itself in the fox population in Eastern Europe and unavoidably spread on south and west. Following the high co-adaptation of the current rabies virus strain to the fox, and due to the fox's ecology, no other species play a significant role in maintaining the disease in the infected areas. Therefore red foxes (*Vulpes vulpes*) are considered as the main reservoir as well as the main vector of the virus [1].

Countries across Europe have created vaccination strategies of foxes in order to monitor and control this disease. As a result of implemented oral rabies vaccination campaigns there was a great improvement of rabies situation across the Europe, especially in western European countries [1]. Conducted researches concluded that oral application of inactivated rabies vaccines cannot confirm satisfactory level of immunization in red foxes [2]. Therefore, in vaccine strategies for foxes attenuated vaccines are used.

Fox vaccination monitoring is crucial for the successfulness of implemented vaccination strategy. The ability of tetracycline molecule to incorporate into bones and teeth and to serve as a long term post-mortem

tissue marker is widely used in oral rabies vaccines for monitoring the bait uptake by wildlife. Therefore, WHO expert committee on Rabies suggested that the testing for the occurrence of a biomarker (tetracycline) – incorporated into the bait is one of the three methods that could be used for the evaluation of vaccination [3]. Tetracycline, as a marker of bait uptake, provides a life-long marking of bones and teeth that is easily detected on post-mortem. At the same time, during the tetracycline determination, the age of the animal can be determined, which provides additional data of the conducted vaccination strategy on one region.

At the Balkan region, including Macedonia, the main vector and reservoir of the disease, also, is the red fox (*Vulpes vulpes*), which constitutes 88% of the rabies cases [4]. Therefore, part of the European Instrument for Pre-Accession Assistance (IPA) project "Capacity building of the veterinary services for implementation of EU Acquis" has a component for control and eradication of rabies in Macedonia. The project has started on 16 August 2010 and in order to create a multiannual strategy for control (oral vaccination of foxes), improve passive surveillance and reporting, as well as to enhance public awareness for the risks of this disease and ways of its prevention.

In 2011, as part of this project, two oral vaccination campaigns were completed. The first one was initiated in spring 2011 (May, 19 – June, 9) and the second campaign was undertaken in autumn (October, 11 – 31). The aerial distribution of vaccine Fuchsoral® (SAD B19 strain) was performed using an automatic dropping system. A total of 500 000 baits per campaign were dropped, covering the whole territory of the country excluding water

surfaces and dense urban settlements. The vaccine bait density was 21.6 baits/km², the distance between dropping lines was 500 – 600 meters [4].

The main objectives of this paper were to present the presence of tetracycline in fox's teeth after two performed campaigns for oral vaccination in 2011 in Macedonia on national and regional level; to determine the age structure profile of collected samples of foxes on national and regional level; to demonstrate the predominant age category of foxes in bait uptake and to evaluate the successfulness of the bait uptake campaign for oral vaccination of foxes for 2011.

MATERIALS AND METHODS

The method used for detection of tetracycline in canine and premolar teeth and mandibular bone of red foxes is based upon the protocol of ANSES, Nancy Laboratory for rabies and wildlife [5]. The detection method uses the fluorescence of tetracycline in ultra – violet light. First, sections of teeth and jaw were made, according to the protocol for "section of teeth and bone of foxes to monitor age and bait uptake" by ANSES, Nancy Laboratory for rabies and wildlife [6]. The canine is fixed by its point and a transversal section is done. The cut is done between 2 and 3 mm from the end of the root. For very young animals, the mandible is sectioned at right angle at the beginning of the first premolar. The result is acceptable when the section is 0.2 to 0.6 mm thick and when different elements may be identified such as the end of the root, pulp cavity, dentine, cementum and some pieces of bone [6]. The created section is placed on the microscope slide on a drop of buffered glycerol. Tetracycline lines will appear as more or less intense yellow lines on the bluish background. These lines could be found at the premolar teeth (characteristic for the young foxes), dentine and cementum of the canine, as well as into the mandibular bone. Depending of the number of uptake baits, the number of campaigns that animals were subjected and the age of the animal, the tetracycline lines will vary in thickness, number and location, respectively (Figure 1).

The transversal fine section of fox's canine was submerged into the glycerol. Using classical transmitted light microscope the annual growing lines were searched in the tooth cementum. The main parameters for the age determination are: the size (diameter) of pulp cavity, presence/absence of cementum and the number of dark lines in cementum that correspond to the number of winter passed by the animal. These parameters are summarized and expressed as fox's age through the following age classes: 0-1 year; 1-2 years; 2-3 years and over 3 years.

All of the results (positive/negative samples and age of foxes) were processed statistically and geographically analyzed using the LABIS® (Laboratory Information System) software and MS Excel 2010.

RESULTS

In 2011 the total number of examined samples for detection of tetracycline was 141 (n=141) of which 86 (60,99%) samples were tetracycline positive. Predominantly tetracycline was found in mandibular bone, canine dentine, canine cementum and in the dentine of the first premolar respectively. The Southeastern region had the most positive samples on tetracycline presence, 26 (68,42 %) out of 38 collected samples, while at the Northeastern region there was no positive sample with the lowest rate of collected samples (Table 1).

CONCLUSIONS

The presented results showed relatively high percentage of baits uptake by foxes for 2011 in Macedonia. In the regions with high number of collected samples the percentage of positive samples was high, suggesting successfulness of the implemented vaccination campaigns. However, the number of collected samples from Northeastern, Polog, Skopje and Vardar region is very low, so the level of bait uptake could not be determined. Therefore, more frequent sampling from the mentioned regions is necessary.

Regarding the age structure of collected samples, most of them were older than 3 years. Although there is lower percentage of occurrence of other age classes,

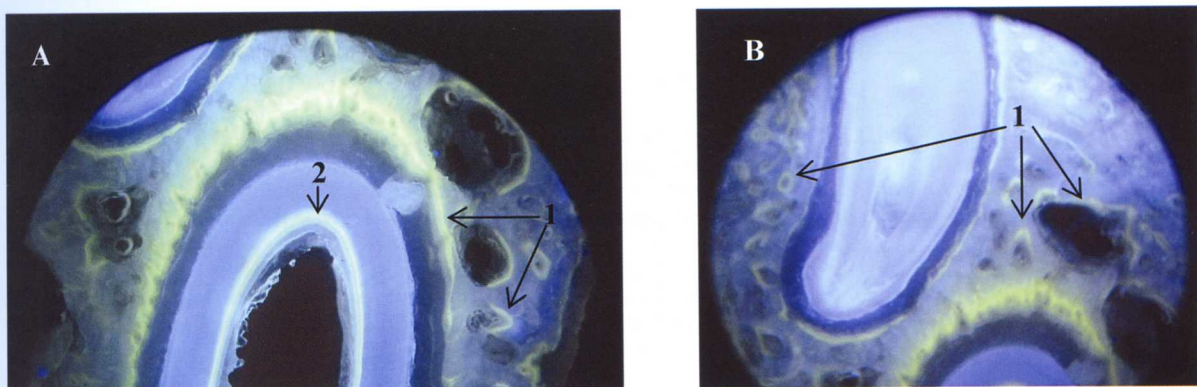


Figure 1. Fluorescence of tetracycline in positive tetracycline samples seen from the transversal fine section of fox's (A) canine and (B) premolar. 1 - Tetracycline presence in mandibular bone; 2 - Tetracycline lines presence in the dentin of the canine tooth.

Regarding the age determination of foxes, the procedure "Age determination on sections of teeth" by ANSES, Nancy Laboratory for rabies and wildlife was used [7]. Buffered glycerol was dropped on the micro-

a high affinity of bait uptake was noted in all age classes. This led us to a conclusion that the implemented vaccination campaigns are equally influencing all age categories of foxes.

Table 1. Tetracycline presence distribution in the examined samples on national and regional level

Statistical Region	No. Examined	No. Positive	No. Negative	Positive samples %
Eastern	26	18	8	69,23
Northeastern	1	0	1	0,00
Pelagonia	34	21	13	61,76
Polog	4	2	2	50,00
Skopje	5	2	3	40,00
Southeastern	38	26	12	68,42
Southwestern	26	15	11	57,69
Vardar	7	2	5	28,57
TOTAL:	141	86	55	60,99

Ninetyeight samples (69,5 %) originated from foxes older than three years, and the rest 43 samples (30.49%) were within the other age classes (2-3, 1-2 and 0-1). The distribution of positive tetracycline samples according the age class showed that there was high percentage of positive samples in all age classes, especially in age classes 0-1 and over 3 years. (Figure 2).

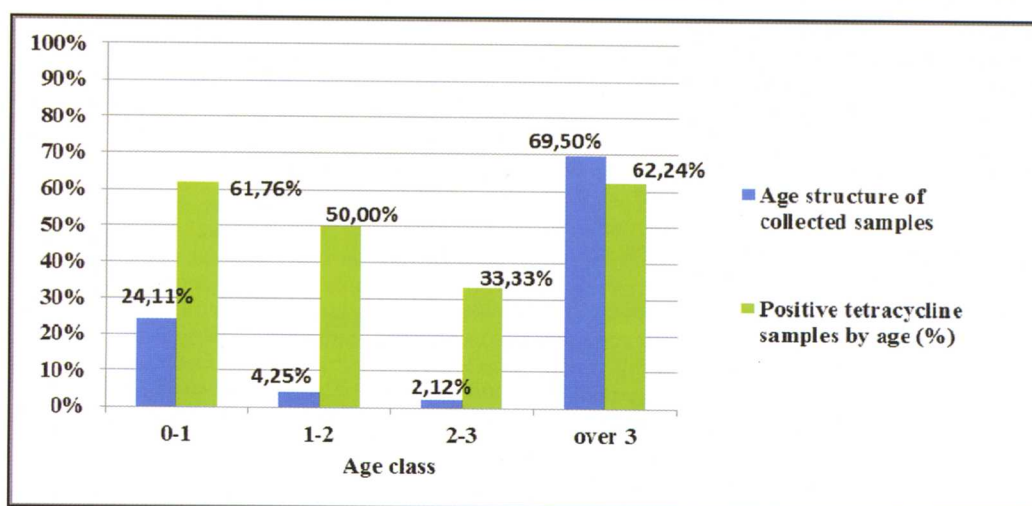


Figure 2. Age structure and distribution of positive tetracycline samples. The age structure is presented as a percentage from the total number of samples. The category "positive tetracycline samples" is shown as a percentage of positive samples in the appropriate age class.

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МОНИТОРИНГ НА ВНЕСОТ НА ВАКЦИНАЛНИ МАМКИ ПРЕКУ ДЕТЕКЦИЈА НА ТЕТРАЦИКЛИН И ВОЗРАСНА СТРУКТУРА НА ЛИСИЦИ ВО КАМПАЊИТЕ ЗА ОРАЛНА ВАКЦИНАЦИЈА ПРОТИВ БЕСНИЛО ВО Р. МАКЕДОНИЈА

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АПСТРАКТ

Главни цели на овој труд беа да се прикажат преваленцата на присуството на тетрациклин во забите и вилиците од лисици, нивната возрастна структура по две спроведени кампањи за орална вакцинација против беснило во 2011 година и евалуација на успешноста на внесот на вакциналните мамки. Детекцијата на тетрациклин базираше на флуоресцентна микроскопија на фини секции од забите и долните вилицы на ултра-виолетова светлина. Возрасната структура во секциите од забите беше одредена на база на присуството/отсуството и бројот на годишни линии на раст во цементот. Вкупно беа испитани 141 (n=141) вилица, од кои 86 (60,9%) беа тетрациклин позитивни. Најмногу позитивни примероци [26 (68,4 %)] имаше во Југоисточниот регион. Деведесет и осум (69,5 %) испитани примероци потекнуваа од лисици постари од три години и имаше висока преваленца на тетрациклин позитивни примероци кај сите возрастни класи. Резултатите покажаа висок процент на внес на вакцинални мамки кај лисиците во текот на двете кампањи во 2011 година, иако на национално ниво, потребно е потемелно мострирање особено во Североисточниот, Полошкиот, Скопскиот и Вардарскиот регион.

Клучни зборови: беснило, мониторинг на вакцинација, тетрациклин, лисица, одредување на возраст