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COMPARATIVE STUDIES OF TETRACYCLINE DETECTION IN FOX TEETH IN THE REGIONS WITH ORAL VACCINATION AGAINST RABIES DURING 2016-2017 IN BULGARIA

Darinka Ilieva, Reneta Petrova, Valentina Marasheva

National Diagnostic& Research Veterinary Medical Institute (NDRVMI), Sofia, Bulgaria
E-mail: emd@mail.orbitel.bg

ABSTRACT

During the period 2016–2017 in connection with control the effectiveness of oral vaccination, laboratory studies of tetracycline detection of teeth fox (*Vulpes vulpes*) were carried out in Bulgaria. From the regions with rabies vaccination during last two years total 1834 samples for presence of biomarker tetracycline were examined and determined age categories of target animals. The teeth samples were collected from a total 578 settlements in 16 regions. The data indicated, that the rate of prevalence tetracycline in fox teeth varied between 65–73%.

Key words: tetracycline, oral vaccination, rabies, fox.

Introduction

With regard to the complicated epidemic situation in Bulgaria since 2009 at this moment rabies eradication program is approved and co-financed by the EU in the frame of veterinary fund and for 2016–2018 it was approved as multiannual program. In our country for oral vaccination of foxes was implemented vaccine Lysvulpen (Bioveta, Czech Republic), containing attenuated vaccine strain SAD Bern and oral marker tetracycline.

Tetracycline is widely used as biomarker for rabies vaccine bait uptake evaluation. After its consumption the antibiotic is incorporated into bones and teeth in vaccinated fox populations. There is no currently available effective marker, which could be used instead of tetracycline, as the best long term post-mortem tissue marker. Age estimation is a second criterion regularly analysed along with tetracycline detection to assess vaccination effectiveness across age class (Robardet and Cliquet, 2015).

The scope of this investigations is to compare the laboratory data to detect the tetracycline in red fox teeth and to determine age categories of target animals during 2016–2017, because the both techniques are crucial factor in the evaluation of oral vaccination effectiveness.

Materials and methods

For oral vaccination of foxes 2016-2017 were included total 16 regions. In North Bulgaria were included 9 regions: Vidin, Montana, Vratza, Silistra, Dobrich together with part of district regions – Pleven, Veliko Tarnovo, Rouse, Razgrad and 20 km belt on the north border with Romania (the river Danube). Whole territory of west and part of south bordering regions were included 7 regions: Sofia town, Sofia-district, Pernik, Kyustendil, Pazardjik, Blagoevgrad and Smolyan (BG eradication program, 2016–2018, BFSA).

Biomarker tetracycline was detected by examination of transverse sections of teeth canines with size 150 μm (microtome Leica SP-1600, Germany), Low Speed Saw. The teeht sections were stored at T-20 ° C until being microscoped. The samples were investigated with fluorescence microscopy Carl Zeiss, Germany magnify 10x/0,25 or magnify 40x/0,75 and filter DAPI with excitation wavelength at 390 nm and emission wavelength at 560 nm.

To determine the age of foxes, the same section was analyzed. Age is determined by categorizing the target animals to junior (J) < 1 year and adult (A) ≥ 1 year. Age estimation based on dental eruption and morphology observation (Harris, 1978). To determination the age was used light microscope (Carl Zeiss, Germany). The technique consists in counting the number of cementum lines and observing dentine, which increases with age (centrifugal growth). Large pulp cavity is consequently observed in young animals. Cementum is deposited over the dental root annuli (centripetal growth) (Morris, 1972).

Detection of tetracycline and age categories determination wildlife were perfomed according to operating procedures in BG NRL of Rabies and Control the Effectiveness of the Vaccination (NRL of RCEV), which were harmonization in accordance with tehniques in European Union Reference Laboratory for Rabies (EURL, ANSES, France, 2012).

Results and discussion

In 2016-2017 the brain samples of the target animals were previously investigated for detection of rabies virus by Fluoreccent antibody test (FAT) (Dean et al., 1996).

For the detection of tetracycline, transverse sections of canine teeth of foxes with mandibular bone were prepared (EC and ANSES, Demerson and Andrieu, 2013).

In 2016 in connection with the surveillance of rabies, 835 foxes from 225 settlements were examined. Data show, that in 2016 a high detection rate of tetracycline was found in 9 regions - Vidin, Blagoevgrad, Razgrad, Sofia-town, Kyustendil, Dobrich, Pernik, Pleven and Pazardjik between ≈ 72% and 92%. In others 7 regions - Veliko Tarnovo, Smolyan, Sofia-district, Vratza, Rouse, Silistra and Montana a lower level of tetracycline was reported between 50% and ≈ 68%, (Fig.1). Tetracycline positive samples were total 607 (73%) from which 185 (30%) from junior (J) foxes and 422 (70%) of adult (A) foxes. Tetracycline negative samples were total 228 (27%), respectively 191(84%) for junior (J) foxes and 37 (16 %) to adult (A) one. In 2016 the detection of biomarker tetracycline was ≈ 73%.

In 2017 in NRL 999 tooth samples were examined from 353 settlements where the vaccine baits against rabies was spread. The results of tetracycline detection in the districts of oral vaccination show a high percentage of the oral marker tetracycline in 4 districts - Razgrad, Montana, Kyustendil and Vidin between ≈ 71% and 84%. In 6 districts Sofia-district, Pazardjik, Silistra, Blagoevgrad, Dobrich and Vratza the level of an oral marker varies between 60% and 68%. In others 6 districts - Pleven, Sofia-town, Pernik, Rouse, Veliko Tarnovo and Smolyan a lower level of tetracycline was reported between ≈ 47% and 59%, (Fig.2). Tetracycline positive samples were total 651(≈65,2%) from which 170 (26%) of junior (J) foxes and 481(74%)- adult (A). Tetracycline negative samples were total 348 (34,8%), respectively 314 (≈ 90%) junior (J) foxes and 34 (≈10%) adult (A) one. The level of tetracycline in 2017 reached ≈ 65.2%.

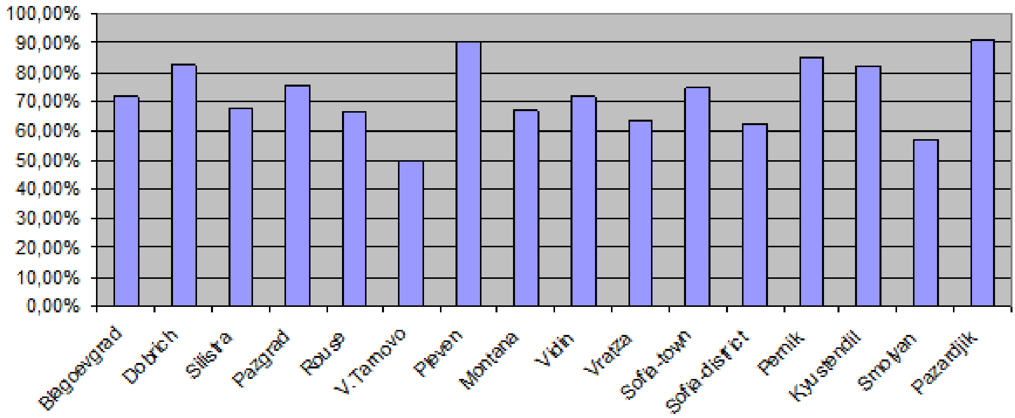

Figure 1: Control of oral vaccination of fox against rabies by tetracycline detection in 2016.

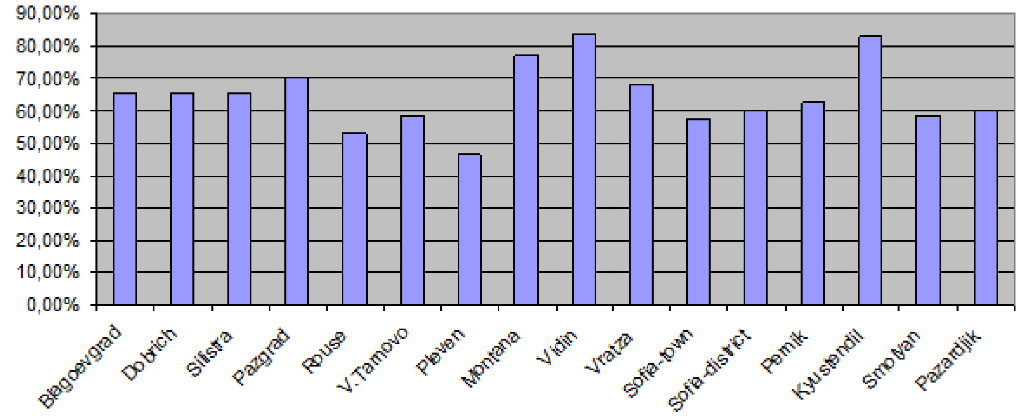

Figure 2: Control of oral vaccination of fox against rabies by tetracycline detection in 2017.

From Fig. 1 and Fig. 2 it can be seen that the percentage of tetracycline for some regions varies in a high level. The variation in the percentage of oral marker in vaccinated foxes is probably due to the irregularity presented number of samples received in NRL testing from the different districts. On the other side, vaccine baits in separate districts for 2017 were distributed later in November and December. To deliver a larger number of samples to the NRL during the second oral campaign, along with the distribution of the vaccine, Lysvulpen continued the shooting of an extremely large number of target animals. It is a European practice to control the oral vaccination carried out 45 days after bait uptake from the target animals and not parallel to the distribution of the vaccine. These reasons, we believe, are essential for detecting a lower level of tetracycline in 2017.

In our studies we use a mounting medium could the accuracy of the results. Mounting media provide better contrast and allow observation of the entire section in a single plan (Robardet et al., 2012). We used control slides before the test slides observation with microscope (Fig. 3). Tetracycline lines will appear on positive control and test slides as more or less intense yellow on the bluish background (Robardet and Cliquet, 2015).

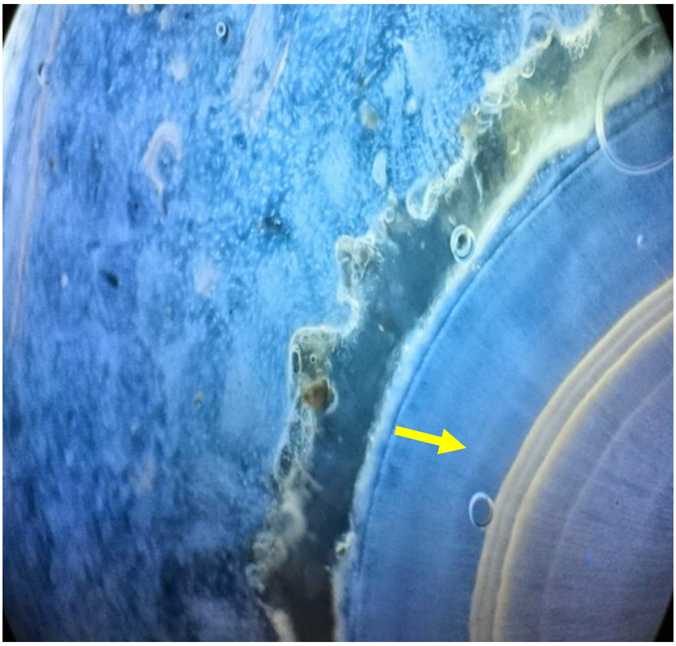

Figure 3: Tetracycline lines localized in dentin of teeth fox deposit in three consecutively lines (arrow) after much more of one bait uptake and light fixing of tetracycline in bone, magnify 10x/0,25.

The proportion of animals identified positive for the presence of tetracycline in teeth (bait uptake level) from the different regions in our country 2016-2017 ranged from 47% to 92%. All the countries except three (Greece, Latvia and Finland) reported a bait uptake that exceeds 70%, which is the minimum recommended vaccination WHO (Robardet and Cliquet, 2017).

Table 1 and Table 2 show data for determining the fox age category 2016–2017. Determination age of the animals allow to determine the vaccination population of adults and junior target animals. The data give on Table 1 and Table 2) show, that the ratio between the junior (J) and adult (A) foxes varies ≈ in the same range, respectively (45% - 48% junior (J) and 52% – 55% adult (A) foxes). In the most cases, in adult foxes baits uptake is better as the level of oral marker found in them is higher. This finding is confirm by the data interpreted above in the report, as well as by our previous observations (Ilieva et al., 2016).

Table 1: Samples, according to age determination 2016 year.

Age categories	Fox J/A	%
Fox J ≤ 1 year	376J	45%
Fox A >12-24 months >24 months	459A	55%
Total tested fox:	835	-

** Junior (J) fox * Adult (A) fox.*

Table 2. Samples, according to age determination 2017 year.

Age categories	Fox J/A	%
Fox J ≤ 1 year	484J	48%
Fox A >12-24 months >24 months	515A	52%
Total tested fox:	999	-

** Junior (J) fox * Adult (A) fox .*

Conclusion

- In 2016-2017 the level of the biomarker tetracycline varies in the range between 65.2% and 73%. The proportion of foxes identified as positive for the presence of tetracycline in teeth from the regions with oral vaccination for the same period in our country ranged from 47% to 92%.
- Adult foxes uptake better vaccine baits compared to junior foxes.

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