

THE SPATIAL DISTRIBUTION OF RABIES ANTIBODIES IN WILD BOARS IN ROMANIA AND THEIR SIGNIFICANCE IN THE FRAME OF ORAL RABIES VACCINATION

DISTRIBUȚIA SPAȚIALĂ A ANTICORPILOR ANTIRABICI LA MISTREȚII DIN ROMÂNIA ȘI SEMNIFICAȚIA ACESTORA ÎN CADRUL PROGRAMULUI DE VACCINARE ORALĂ

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ABSTRACT | REZUMAT

Rabies is a fatal zoonotic viral disease produced by a Lyssavirus and is causing more than 70,000 human deaths each year. In Romania, like in Europe, foxes are the main reservoir. Oral rabies (ORV) vaccination of this specie is the most effective method to control and eventually eradicate rabies. However, the vaccine used for the oral vaccination of foxes could be taken up by other wild species as well. The prevalence of rabies antibodies in wild boar population in Romania, which may represent competitor for foxes, has not yet been studied. Therefore, in the present study, we evaluated samples from the peripheral blood of wild boar (*Sus scrofa*) (n=625) from 14 counties of Romania by serological assays for the presence of specific antibodies against rabies. Rabies virus-specific antibodies were detected in 28% (n=175 sera) in wild boars, using a commercial enzyme-linked immunosorbent assay (ELISA). Further analysis of 63 out of the 625 samples using the fluorescent antibody virus neutralisation (FAVN) test showed the seroconversion in 50 (94%) samples. This is the first research reporting that antibodies against rabies virus following oral vaccination of foxes are present in other wild animals than targeted species in Romania.

Keywords: rabies, serology, vaccination FAVN, wild boar

Rabia este o zoonoză fatală și cauzează anual peste 70.000 de victime în rândul populației umane.

În România ca și în Europa, vulpea este principalul rezervor. Vaccinarea orală a acestei specii este cea mai eficientă metodă de control și eventual de eradicare a rabiei. Totuși, vaccinul folosit pentru vaccinarea orală a vulpilor poate fi folosit și la alte specii de animale sălbatice. Prevalența anticorpilor antirabici în populația de mistreți, care poate reprezenta un competitor pentru vulpi, nu a fost încă studiată.

De aceea, în lucrarea de față am testat probe de sânge de la mistreți (*Sus scrofa*) (n=625) din 14 județe din România, prin teste serologice pentru detecția anticorpilor antirabici specifici. Aceștia au fost detectați în 28% (n=175) din cazuri, folosind un kit imunoenzimatic comercial (ELISA).

În continuare, 53 de probe din 625 testate prin metoda de seroneutralizare folosind anticorpi fluorescenți (FAVN) au arătat seroconversie pentru 50 de probe. Acesta este primul raport științific care demonstrează că anticorpii antirabici apăruți ca urmare a programului de vaccinare orală a vulpilor, sunt prezenți și la alte animale sălbatice în afară de speciile țintă din România.

Cuvinte cheie: rabie, serologie, vaccinare FAVN, mistreț

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*, causing between 37,000 and 87,000 human deaths every year (6, 10).

In Europe, the major reservoir of rabies is represented by wild animals, especially red fox (*Vulpes vulpes*) (1). Extensive oral vaccination programs (ORV) with baits for red foxes have reduced the incidence of

rabies in many Western European countries (4, 5).

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 Romania in spring and autumn 2011. From 2012, the OV programs were implemented throughout the Romanian territory (8, 9).

Other wild or domestic animals could eat baits used for the oral vaccination of foxes (7).

Thus, characterization of rabies immune response in the other wild animals' species than foxes should greatly facilitate the design of eradication programs with potentially high social and human healthy impact.

Prevalence of rabies antibodies in the wild boar

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population in Romania, to the authors' knowledge, has not been addressed yet.

Therefore, the aim of this study was to investigate the Rabies seroconversion in this species after ORV from Romania, which may represent competitor for foxes, and, in this respect, could influence the efficacy of the eradication program. For this purpose, animals were studied for the presence of viral-specific antibodies by ELISA and FAVN test.

MATERIAL AND METHODS

Study area

In our study, 625 samples were collected from 14 counties covering central and eastern part of Romania. The geographical information of the sites where the sampling from wild boars was performed is shown in Figure 1.

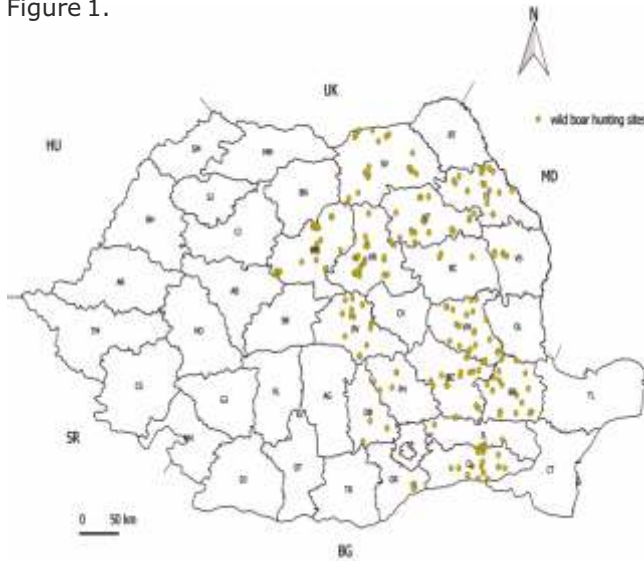


Fig. 1. The sites where the wild boar's samples were collected

Serum samples

During the hunting seasons from 2012-2015, a total of 625 blood samples from 625 animals from different sites, belonging to 14 counties of Romania were collected (Fig. 1, Table 1): 387 were males and 238 females; 287 were under 1 year of age (183 males and 104 females). Blood was collected separately from individual wild boars, immediately transferred to sterile tubes by the veterinary physicians, and transferred refrigerated at $+4^{\circ}\text{C}$ in 30-60 min. to the County Sanitary Veterinary Laboratories.

In the laboratories, the blood was centrifuged to obtain serum, which was stored at -20°C until transfer to our laboratory.

Table 1

Results of evidence of rabies antibodies in wild boars

County	ELISA
Brăila	14/47 (31)
Braşov	10/42 (24)
Buzau	10/36 (27)
Călăraşi	13/55 (25)
Dâmboviţa	8/28 (28)
Giurgiu	14/39 (39)
Harghita	13/51 (26)
Iaşi	24/57 (42)
Ialomiţa	6/33 (19)
Mureş	10/47 (22)
Neamţ	15/48 (31)
Prahova	10/39 (25)
Suceava	17/59 (28)
Vrancea	11/44 (25)
Total number of samples	175/625 (28)

Results are presented as: positive samples / total number of samples tested (positive sample percentage).

Enzyme-linked immunosorbent assay (ELISA)

Detection of specific Rabies antibodies for all 625 sera was performed using a commercially available ELISA (Platelia, Bio-rad, France). The test was carried out according to the manufacturer's instructions.

Fluorescent antibody virus neutralisation (FAVN)

The gold standard to measure level of Rabies antibodies, according to OIE, is fluorescent antibody virus neutralisation. The test was performed based methods established by OIE manual and Meslin et al (2, 3).

RESULTS AND DISCUSSIONS

Out of 625 wild boar sera assayed by ELISA, 175 (28%) had antibody levels against rabies virus equal to or higher than 0.5 IU/ml. The O.D. values were calculated to IU/ml based on the calibration curve. An additional FAVN test revealed 50 (94%)/53 positive samples. All ($n=10$) samples that were negative in the ELISA reacted negative in the FAVN test (Fig. 2).

Reactivity to Rabies virus was detected in 85 (22%) of 387 sera from male wild boars, while 90 (38%) of 238 serum samples from females showed virus-specific antibodies (Table 2).

Juvenile wild boars (45%) showed no significantly difference of specific antibodies (20%) compared with adult animals (32%; Table 2).

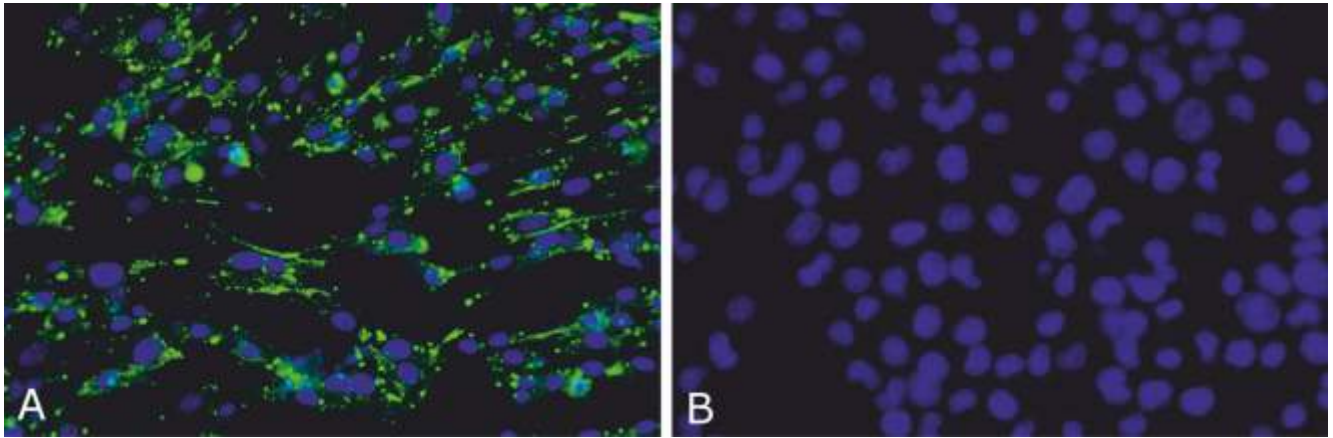


Fig. 2. A-wild boar FAVN negative (Rabies antibodies present); B-wild boar FAVN positive (no Rabies antibodies)

Age class, sex and positive results of animals serologically tested

Age class	Sex	ELISA
Juveniles	male	30
	female	30
Adults	male	60
	female	55
Total		175

Table 2

The spatial distribution of the ELISA positive results of our subsequent serological analysis of rabies are summarized in Figure 3.

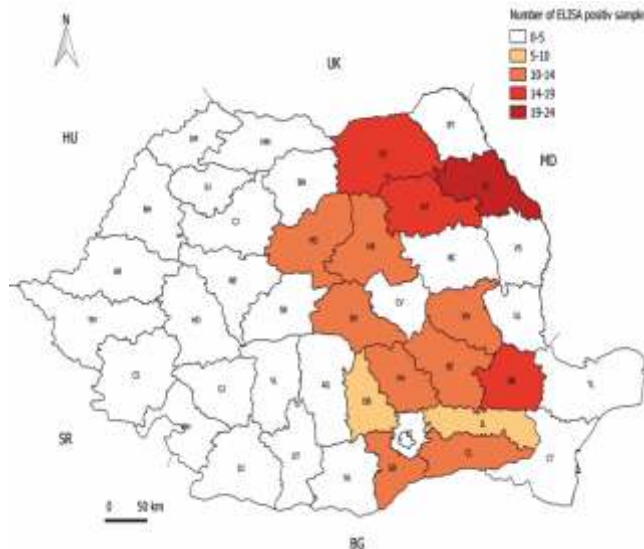


Fig. 3. The spatial distribution of the ELISA positive results

The baits are also exposed to other wild animal species. In Romania, these species could be jackals (*Canis aureus*), wild cats (*Felis silvestris*), wolves (*Canis lupus*) and based on our results, wild boars as well.

The results of our investigation have confirmed the hypothesis that wild boars have considerable levels of antibodies against rabies.

The density of wild boars makes this animal species a real competitor for baits. However, there is no correlation between level of antibodies in wild boars and seroconversion in foxes in studied counties (data no shown). Foxes showed a significantly higher prevalence of specific antibodies compared with wild boars in all counties (Fig. 4). Therefore, we can say that wild boars don't influence the efficacy of ORV in Romania.

Further studies are needed to confirm that the consumption of baits by other wild animal species mentioned above could influence the efficiency of the Oral Rabies Vaccination programs of foxes.

CONCLUSIONS

1. The results of our study showed the presence of rabies antibodies in Romanian population of wild boars.
2. There is no correlation between genders and level of antibodies, even ages categories.
3. We found in foxes a higher prevalence of rabies antibodies compared with wild boars.
4. Other wild animal species should be further investigated in order to get a real and complete image of fox competitors.

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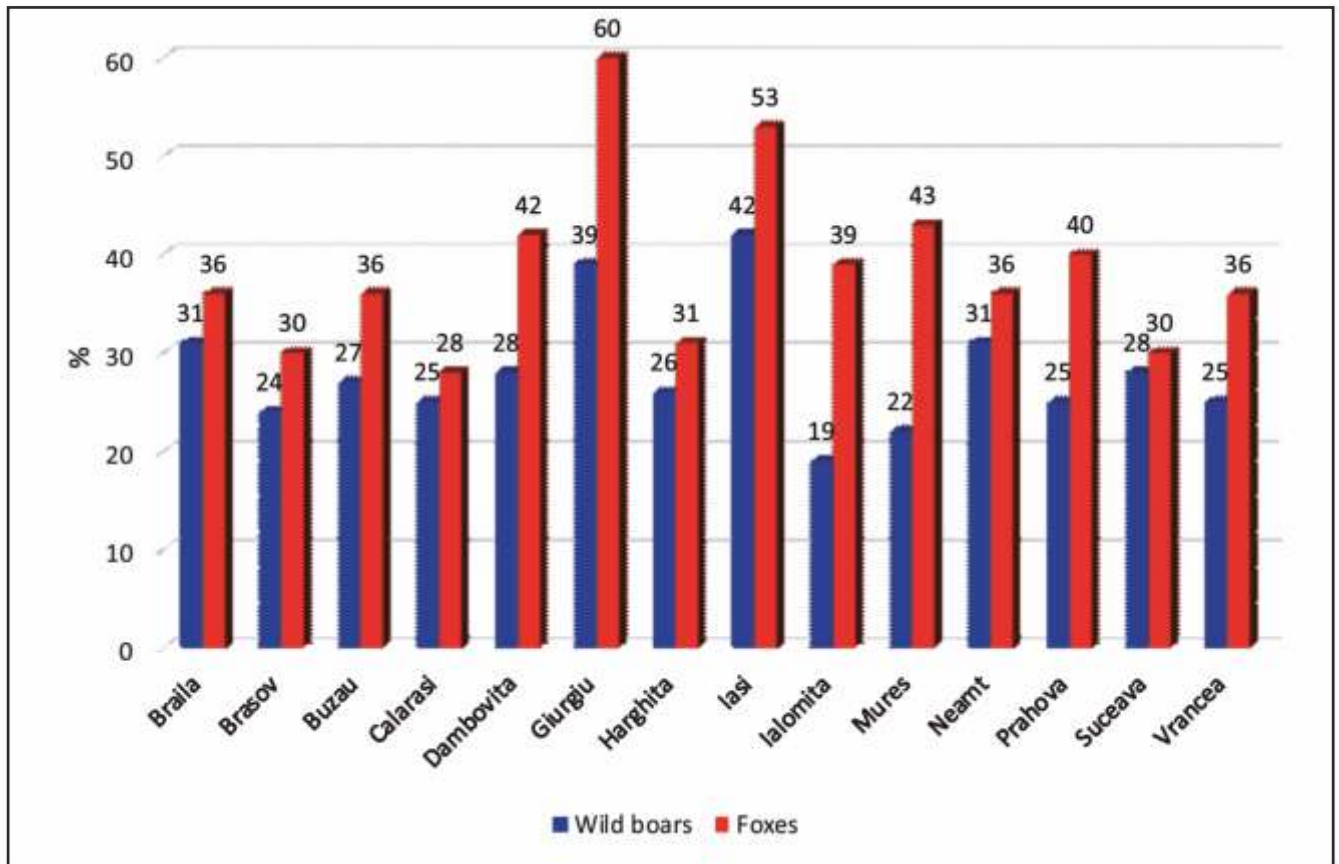


Fig. 4. The correlation between wild boars and foxes antibodies (%) in studied areas

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