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STUDY ON INFECTION WITH RABIC VIRUS IN WILD AND DOMESTIC ANIMALS

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Key words: *rabies virus, rabies, brain fragments, wild and domestic animals*

INTRODUCTION

Rabies are nowadays a totally incurable disease after they have settled in the body. It is a disease of the rapidly evolving nervous system and invariably leads to death.

The rabies virus, heavily circulating in the fox population, is transmitted to wildly animals (roebuck, badger, ferret, polecat, marten, bat, rat) and domestic animals. The increased number and diversity of the affected animals denotes the dangerous extent of the epizootic process in rabies and suggests the need to step up measures to combat rabies.

A recently published European report shows that Romania has a real record of cases of rabies in wild and domestic animals, and the risk of transmitting rabies to humans is very high. Foxes have proven to be the most important reservoir of virus.

Rabies is produced by a virus with neurotrophic properties. The rabies virus, the etiological agent of rabies in animals and humans, is framed by Libermann (1972) in the family *Rhabdoviridae*, the genus *Liyssavirus*, the viruses characterized by their "bullet" shape. The *Rhabdoviridae* family comprises approximately 75 viruses that can infect vertebrates, invertebrates and plants.

The rabies virus is not stable, it can easily change under the influence of environmental conditions, due to the fact that different strains of virus, such as virulence and pathogenicity, are encountered in different geographical areas.

MATERIALS AND METHODS

During the period 01.01.2014 - 30.04.2017, in DSVSA Galati laboratories, 147 cases of rabies were recorded, of which 105 foxes, 15 dogs, 9 cattle, 5 domestic cats, 3 wolves, 3 deer, 4 sheep, 2 wild cats, a boar and a ferret.

The study material consisted of fragments of brain harvested from suspect animals - foxes and dogs - from the following brain areas: cerebellum, bark and cerebral trunk.

For the virological and histological diagnosis of rabies the following working methods were used:

a. Method of identifying the viral antigen by direct immunofluorescence (IFD).

b. Intracerebral inoculation test on mice.

c. The Mann staining method for the Babeş-Negri corpuscles.

a. Method of identifying the viral antigen by direct immunofluorescence:

The purpose of this method is to highlight by microscopic examination in the U.V. of cells infected with rabies virus.

Immunoflorescence is routinely practiced, being a specific and rapid test, providing a diagnosis of certainty up to 98-100% of cases, within 24 hours, and can be used as a unique diagnostic method.

The principle of the method. Detection of rabies virus antigen in infected tissues results from specific binding of the rabies virus by a fluorocromus conjugated antiserum and visualization in U.V. light. of the bound fluorochrome. Based on this principle, direct detection of rabies antigen on the brain imprint or infected cell cultures can be performed.

The assay follows detection by microscopy examination U.V. specific fluorescence corpuscles, which are formed from a mixture with the homogeneous structure of viral antigen and cytoplasmic material of the previously infected cell (fragments of cerebellum, bark and cerebral trunk).

b. Intracerebral inoculation test on mice

The test is applied to samples of nervous tissue harvested from animals that have experienced nervous symptoms. Cultivate rabies virus on mice brain after their intracerebral inoculation. Suspension encephalous triturations in physiological saline suspension was inoculated intracerebrally, 0.015 ml and 0.03 ml respectively, in 10 3-4 week old mice.

The principle of the method. Intracerebral inoculation of the 3-4 week old mice that are susceptible to the rabies virus causes their death from the seventh day after inoculation.

Brain (brain) fragments of rabies suspected of having rabies and a batch of 10 mice 3-4 weeks old in perfect health were used to prepare the brain suspension (inoculum). Preparation of the inoculum was done in a sterile dish. Brain suspension was 1/5 (one brain part + 4 parts diluent); 10 x sterile

phosphate buffer was used as the diluent. The inoculation operation was performed in the biobase inoculation chamber. Mice are observed on a daily basis for a period of 28 days.

c. Mann staining histopathological method

This method can be used to highlight the rabies-specific Babeş-Negri inclusions specific to the rabies and the structural and pathological structural elements of the central nervous system.

The techniques of serial fragments of brain fragments have been tilted. The brain being a soft tissue was completely harvested and immersed in fixative fluid, after 3 to 4 hours, necessary fragments were taken. Sections were made at the CUT 4050 microtome at a size of about 3-5 m. For staining, the permanent histological sections displayed on the blades through the semiautomatic protocol were placed in the special stands of the MEDITE TST 33 coloring machine according to IL-M-21.

RESULTS AND DISCUSSIONS

Rabies is a disease that causes infection with a virus as a result of contact with saliva of infected animals.

It is transmissible to humans and is characterized by nerve disorders: hyperexcitability, aggression, paralysis, and even death. There is no specific treatment, evolution is fatal, vaccination being the only way to prevent the disease

Throughout the development of human society through the demographic explosion, through urbanization and industrialization, the natural environment of life has changed profoundly and so has changed the body-environment relationship.

A neglect of environment related interrelations would pose a danger to human and animal health, therefore future epidemiological studies will address this problem in the field of zoonoses that can be transmitted from wild, domestic animals to humans.

During the study period, laboratory tests were performed on a number of 366 samples by three working methods, namely: direct fingerprint immunofluorescence (IFD) test, bioprobe of white laboratory mice and histological examination.

147 cases of wild rabbits were confirmed in wild and domestic animals, with the highest number of cases being recorded: 106 foxes, 14 dogs, 9 cattle, 5 cats, 3 wolves, 3 deer, 3 sheep, 2 wild cats, one wild boar and one ferret.

According to Table 1, the incidence of rabies in Galaţi is increasing until 2016, then decreases in 2017. During the research period 2014-2017, the number of wild rabies affected was higher (62 %) than that of domestic animals (Table 1, Figure 1).

Of the domesticated animals investigated, the high percentage of infected animals is represented by the dogs (50%), being the guards of the houses that first contact with the diseased wild animals.

A significant percentage of diseased animals are also cattle (26%), animals which by their nature can not defend themselves from the attack of potentially diseased wild animals (Figure 2). Among the wild animals (Figure 3) a high percentage is the foxes (96%), many present near the large human settlements because of the abundant food resources to which they have access due to the increasingly significant disappearance of the forest. In most cases, diseased foxes have penetrated into localities and even in people's yards, increasing the risk of spreading rabies in domestic animals and humans by biting, scratching, etc. Bioprobe on white mice (Figures 4, 5) and histopathological examination of the brain by direct fingerprint immunofluorscence test revealed virus-infected cells (Figures 6, 7) and the presence of Babes-Negri intracytoplasmic corpuscles occurring in soma and cell expanses nerve (figures 8, 9).

The localities where most of the cases of ravages have been confirmed are those near the forests, as by destroying the forest environment the wild animals have left their habitat and have fled to the neighboring localities in search of food and shelter spreading and transmitting in this way rabies.

Table 1. Situation of animals investigated for rabies during 2014-2017

Year	Animal category	Total investigated animals	Number of positive cases	%
2014	Wild animals Domestic animals	6 20	3 5	50% 25%
2015	Wild animals Domestic animals	9 26	12 6	77,7% 23,07%
2016	Wild animals Domestic animals	89 38	52 13	58,4% 34,21%
2017	Wild animals Domestic animals	122 56	48 8	39,14% 14,8%
TOTAL	Wild animals	226	115	50,88%
TOTAL	Domestic animals	140	32	22,85%

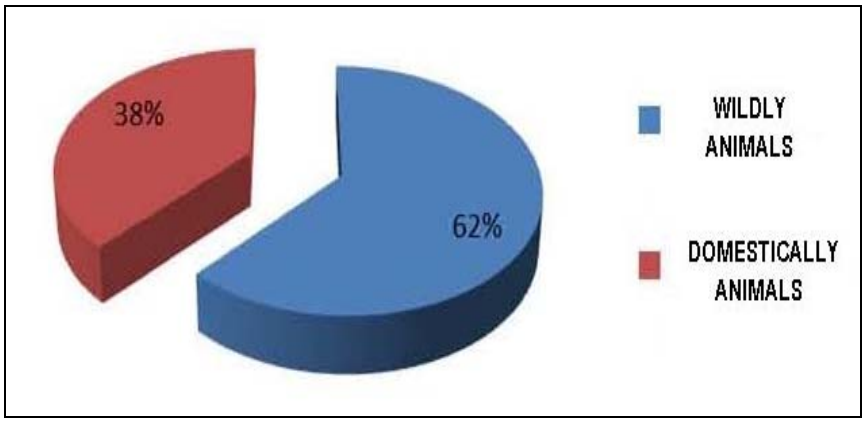

Fig. 1. Percentage distribution of domestic and wild diseased animals

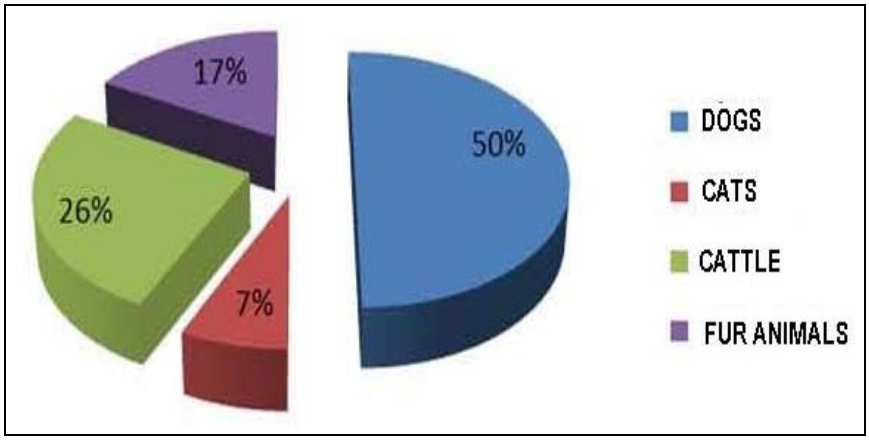

Fig. 2. Percentage distribution of domestic animals analyzed

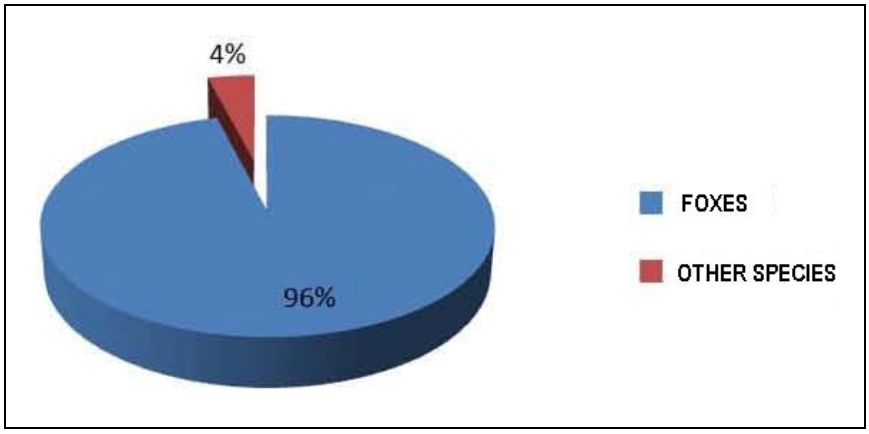

Fig. 3. Percentage distribution of wild animals analyzed

Fig. 4. White mice prepared for inoculation

Fig. 5. Inoculation of brain suspension in mice

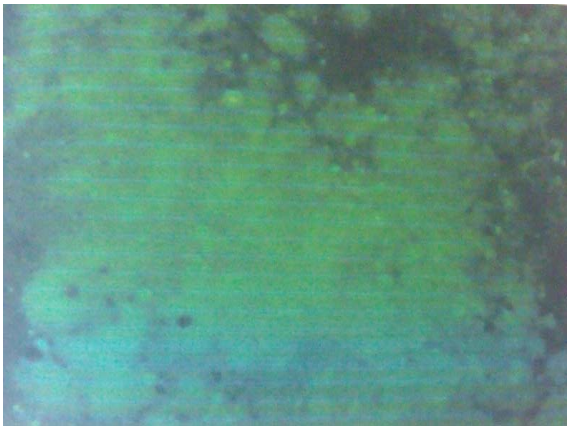

Fig. 6 Cells infected with rabies virus in foxes

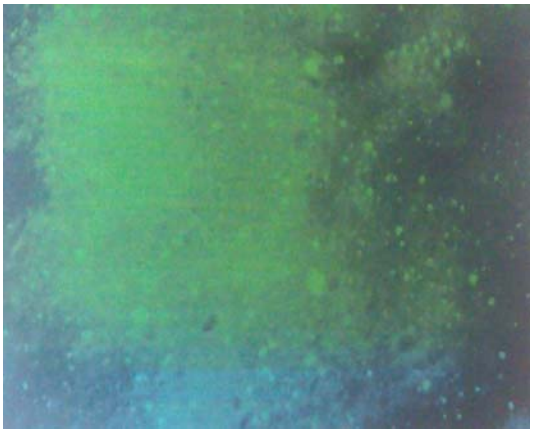

Fig. 7. Cells infected with rabies virus in the dog

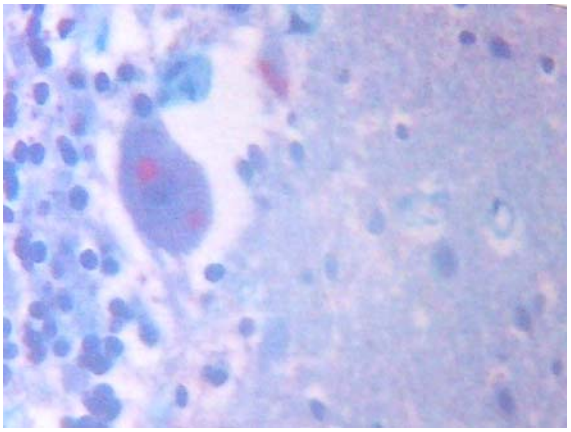

Fig. 8. Babeş-Negri corrpuscles to foxes: red purple nucleoli, blue chromatin, dark blue cytoplasm, pink erythrocytes

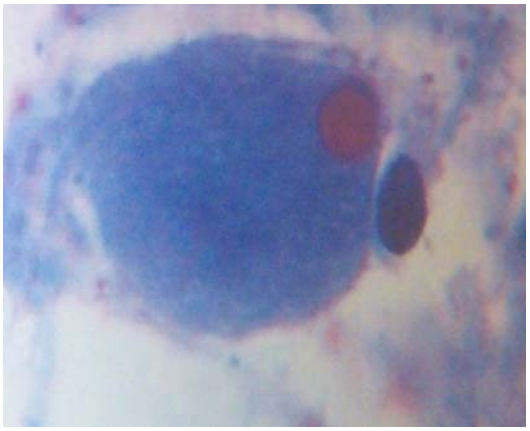

Fig. 9. Corpuscles Babeş-Negri in the dog

CONCLUSIONS

Rabies must be a priority for the Romanian authorities, both because of the high number of cases recorded in recent years and the danger the disease poses as the oldest human-known zoonosis.

Continue to implement the program of oral vaccination of foxes without interruptions, as well as

changing and adapting the vaccination strategy according to the evolution of epidemiological situation in previous years.

Start recording data on the age and sex of foxes diagnosed positively by rabies as well as those shot for evaluation of oral rabies vaccination programs. This aspect is particularly important serving to accurately assess the effectiveness of vaccination.

Considering that there are generally more foxes near the large human settlements due to the abundant food resources they have access to, we consider it necessary to increase the number of vaccinated baits administered using the Bavarian distribution model.

As a consequence of rising rabies cases in European bats, we consider it appropriate to create a bats surveillance network in Romania for lyssaviruses in Romania.

Along with veterinary services, the Ministry of Health and the Ministry of Education have an important role to play in eliminating human rabies of animal origin, and they must work together to actively contribute to the ultimate goal of eradicating rabies as a zoonosis. We believe that this can be achieved by raising awareness and educating the population about rabies and their implications, as well as highlighting the vaccination program and, in particular, its benefits.

Considering that a country's "free of rabies" status also depends on the epidemiological situation of neighboring countries, we recommend intensive collaboration with the veterinary authorities of neighboring countries to synchronize vaccination campaigns to maximize their effectiveness and shorten the period of getting the desired status.

ABSTRACT

During the 2014-2017 study, 226 wild animals and 140 domestic animals suspected of rabies were investigated. Laboratory analyzes were performed on a number of 366 samples by three working methods, namely the direct fingerprint immunofluorescence (IFD) test, the bioprobe of white laboratory mice and the histological examination.

147 cases of wild rabbits were confirmed in wild and domestic animals, with the highest number of cases being recorded in foxes: 105 foxes, 14 dogs, 9 cattle, 5 cats, 4 sheep, 3 wolves, 3 deer, 2 wild cats, a boar and a ferret.

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