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**STUDY OF RABIES VIRUS IN AZERBAIJAN 2016-2017****Chichak Vali Aliyeva**

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*Due to the fact that rabies causes 100% of deaths among humans and animals, it is still considered a particularly dangerous disease. Rabies is caused by an RNA-containing virus that affects nerve cells. In order to contract rabies, a person must be bitten by a sick animal. World Organization for Animal Health (WOAH-OIE) has mandated that member countries report on rabies. Hence, Azerbaijan submits rabies statistics to the OIE every year.*

*According to OIE data, rabies kills about 70,000 people worldwide every year. According to statistical data, rabies disease is registered in the country every year. The virus is widespread mainly among pets and stray dogs.*

*Azerbaijan is located in the South Caucasus, a region of geopolitical importance located at the gateway between Europe and Asia. This geographical location makes the region of central importance to epidemiological study and the control of transboundary infectious diseases such as rabies.*

*The purpose of the conducted research is to study the regions where the rabies virus spread in 2016-2017, the type of infected animals and laboratory diagnostics. As a result, in 2016-2017, the zone where the virus spread the most in the country was Zagatala-Sheki and Baku. The main advantage in laboratory diagnostics is given to the polymerase chain reaction. This method is used for early detection and is considered confirmatory.*

**Keywords:** Rabies, Azerbaijan, foci, PCR (polymerasa chain reaction), virus

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**INTRODUCTION**

Rabies is present on all continents and this one is a vaccine-preventable, zoonotic, viral disease [1, 2]. Once clinical symptoms appear, rabies is virtually 100% fatal. In up to 99% of cases, domestic dogs are responsible for rabies virus transmission to humans [3]. Yet, rabies can affect both domestic and wild animals. It is spread to people and animals through bites or scratches, usually via saliva [5]. World Animal Health Organization (WOAH-OIE) estimates that rabies kills approximately 70,000 people each year. In European countries, red foxes (*Vulpes vulpes*) are the main reservoir and vector of rabies, while dogs (*canis*) are the most common cause of rabies cases in countries such as Ukraine, Belarus, and the Russian Federation, as well as Africa, Asia, and the Middle East. Dogs and foxes are both thought to act as reservoirs of rabies in Turkey, according to recent statistics.

The causative agent of rabies is a neurotropic RNA-containing virus belonging to the Lyssavirus genus of the Rhabdoviridae family. It is shaped like a bullet, and its size is 90-170 nm, and 110-120 nm. Their reproduction (in vitro and in vivo) leads to the formation of Babesh-Negri bodies, special derivatives of various shapes (big, oval, thread) in the cytoplasm of neurons, 0.5-2.5 nm in size, stained red with acid dyes. Two variants of the virus are known: a) street or "wild" variant - which circulates among animals under natural conditions, b) Fixed variant-obtained by infecting rabbits by L. Pasteur. Since fixed viruses are not excreted in saliva, they cannot be transmitted during a bite. Neuronal proliferation is not accompanied by the formation of Babesh-Negri bodies. Fi-



xed viruses are used to obtain an antirabies vaccine and their administration forms permanent immunity against the street variant virus. This indicates that both variants have the same antigenic structure. Rabies viruses have two - S and V antigens. The S antigen is the same as for lyssaviruses and induces the formation of complement-binding and precipitating antibodies. V-antigen (surface antigen) causes the synthesis of neutralizing antibodies and participates in the formation of immunity. Wild viruses are pathogenic to humans, warm-blooded animals and birds. High sensitivity to the virus is noted in foxes and Siberian mountain mice.

Azerbaijan is located in the South Caucasus, a region of geopolitical importance located at the gateway between Europe and Asia. This geographical location makes the region of central importance to epidemiological study and the control of transboundary infectious diseases such as rabies. Throughout Azerbaijan, rabies is a reportable disease. There is little known about the characteristics (density, growth dynamics) of the dog population, which is thought to be the main source of rabies. Therefore, there is a need to conduct scientific and epidemiological studies in this regard. There is a large population of stray dogs in Azerbaijan. Current management of these animals consists of neutering and vaccination programs and culling is prohibited.

According to reports from the Azerbaijan State Veterinary Control Service rabies cases are observed among animals and fatal cases occur sporadically among people in Azerbaijan annually [4, 6, 7]. However, despite all the research conducted there is still a lack of information regarding the circulation of rabies in Azerbaijan. Therefore, the goal of this study is the continuing study of rabies positive samples for further identification of the circulation of the virus in Azerbaijan in order to plan a vaccination program.

## **MATERIAL AND METHODS**

### **Research Area**

The State Veterinary Service under the Ministry of Agriculture of the Republic of Azerbaijan carried out passive surveillance for Rabies from 2000 to 2017.

The statistical information regarding rabies cases in animals in 2016-2017 was collected from the Azerbaijan State Veterinary Control Service. The National Reference Laboratory under the Azerbaijan Food Safety Institute (AFSI) tested the collected samples.

This laboratory provided training on rabies diagnosis to the research team participants in 2017. All the samples were tested (from 2000 to 2018) by the National Reference Laboratory and PCR positive samples were sent to the Food Safety Institute (former Veterinary Scientific Research Institute) to assure virus vitality and for sequencing.

### **Laboratory examinations**

All brain samples were stored at -80°C in the BSL-2 laboratory (AFSI). Stored samples were tested by FAT for identification of Babes-Negri bodies. The most widely used test for rabies diagnosis is the FAT, which is recommended by both the WHO and WOAHA.

Due to using different diagnostic tests and comparing the results of these tests, we did PCR in parallel with FAT. Since the samples taken (2016-2017) were not fresh, we considered it appropriate to use PCR together with FAT.

First for identification of the agent, we used the fluorescent antibody test (FAT). PCR assays were conducted using Qiagen RNA mini kit (USA) and GENESIG Standard Kit (manufactured in UK) on the Bio-Rad CFX96 PCR instrument.

## **RESULTS AND DISCUSSION**

In general, this surveillance has determined the extent of the spread of Rabies in the country. The map below shows the level of rabies spread throughout the country during 2000-2017. Mostly, the spread in the Absheron peninsula and northern regions (Gakh, Balakan, Zagatala, etc.) has been more noticeable.



**Fig. 1.** Distribution of rabies in Azerbaijan 2000-2017

In our research, the positive brain samples of 2016 and 2017 were used (Figure 1). Thus, in 2016- 18 domestic and 3 stray dogs, 17 cattle, 1 donkey, 5 cats, and 3 jackals- a total of 48 animals tested positive. The analysis was conducted by the National Reference Laboratory (former Republican Veterinary Laboratory).

In 2017 – 17 domestic and 14 stray dogs, 32 cattle, 2 small ruminants, 1 horse, 1 donkey, 2 cats, 1 wolf, and 3 jackals- a total of 48 animals tested positive. The analysis was conducted by the National Reference Laboratory (former Republican Veterinary Laboratory).

While analyzing the 2016 and 2017 positive samples, we found that there were more cases of rabies among cattle. Thus, in 2016, this number was 17, while in 2017, this number was 32. In addition, rabies was not detected among small ruminants in 2016, but 2 small ruminants tested positive in 2017. Finally, both years of the analysis showed that there were cases of rabies found amongst cats and both domestic and stray dogs.

From 2016 to 2017, 12 randomly selected samples from a total of 121 positive samples were given to the FSI. A total of 12 brain samples from different animal species (5 cows, 3 donkeys, 1 horse, and 3 jackals) were provided by the National Reference Laboratory. No scientific investigation has been carried out since the collection of these 12 brain samples stored in the AFSI, and the samples are simply stored at -80°C in the BSL-2 laboratory. All the samples were tested again for rabies positivity by the NRL. Of the 12 samples, 3 were damaged and were not in good condition, so they were neutralized without being tested. In the other 9 brain samples, firstly the FAT resulted positive. For Agent identification, the gold standard test is the fluorescent antibody test (FAT). The preferred method for routine diagnosis of rabies in fresh or frozen brain tissues is the fluorescent antibody test (FAT). For the 9 samples which were found positive in the FAT a PCR was done and the result was the same as the FAT. Next, molecular epidemiological studies were selected for PCR. Out of the 12 brain samples we observed Babes-Negri bodies in 9 by FAT. The positive results were confirmed by PCR.

The samples that tested positive were sent from different regions of Azerbaijan in 2016-2017 (NRL), including, the Absheron Peninsula, Kurdamir, Barda, Shaki, Zagatala, Ujar, Gakh, Balakan and Agdash (Figure 2).



**Fig. 2.** Randomly selected positive samples 2016-2017

From 12 samples-3 were damaged and were not in good condition. In our opinion, this is due to the replacement of the Institution conditions in a short time (moving the VSRI to another building and giving the samples to the AFSI for the BSL-condition) and the lack of a correct method for storing the samples.

As a result, the virus suspension and RNA extract from brain samples were stored at under  $-80^{\circ}\text{C}$  degree. The main purpose of this is to allow future work to keep up the RNA as much as possible, that is, the RNA level may be reduced as the brain samples are frozen each time.

## CONCLUSION

According the results in 2016-2017 we evaluated the statistical of positive results. In general, we can say that rabies is actual between agricultural animals, domestic animals and wildlife. Although several studies have been carried out, there is still a lack of information in the areas mentioned below: oral vaccination program and evaluation of statistical data.

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**AZƏRBAYCANDA QUDUZLUQ VİRUSUNUN TƏDQIQI 2016-2017****Ç.V. Əliyeva**

Quduzluq başverdiyi zaman insanlar və heyvanlar arasında ölüm hallarına 100% rastlandığı üçün hələ də bu xəstəlik xüsusilə təhlükəli xəstəlik hesab olunur. Quduzluq sinir hüceyrələrinə təsir edən RNT tərkibli virusdan qaynaqlanır. Quduzluğa yoluxmaq üçün insanı xəstə heyvan dişləməlidir. Ümumdünya Heyvan Sağlamlığı Təşkilatı (WOAH-OIE) üzv ölkələrin quduzluqla bağlı hesabat verməsini tələb edir. Beləliklə, Azərbaycan hər il quduzluq statistikasını OIE-yə təqdim edir.

OIE məlumatlarına görə, quduzluq hər il dünyada təxminən 70.000 insanın ölümünə səbəb olur. Statistik məlumatlara görə, ölkədə hər il quduzluq xəstəliyi qeydə alınır. Virus əsasən ev heyvanları və sahibsiz itlər arasında geniş yayılıb.

Azərbaycan Cənubi Qafqazda, Avropa ilə Asiya arasında keçid qapısında yerləşən geosiyasi əhəmiyyətə malik regionda yerləşir. Bu coğrafi yerləşmə bölgəni epidemioloji tədqiqatlar və quduzluq kimi transsərhəd yoluxucu xəstəliklərə nəzarət üçün mərkəzi əhəmiyyət kəsb edir.

Aparılan tədqiqatın məqsədi 2016-2017-ci illərdə quduzluq virusunun yayıldığı rayonların, xəstəliyə yoluxmuş heyvanların növünün öyrənilməsi və laboratoriya diaqnostikasının aparılmasıdır. Nəticədə 2016-2017-ci illərdə ölkədə virusun ən çox yayıldığı zona Zaqatala-Şəki və Bakı olub. Laboratoriya diaqnostikasında əsas üstünlük polimeraza zəncirvari reaksiyaya verilmişdir (PZR). Bu üsul erkən aşkarlama üçün istifadə olunur və təsdiqedic diaqnostik üsul hesab edilir.

**Açar sözlər:** *Quduzluq, Azərbaycan, ocaqlar, PZR (polimerazisasiya zəncir reaksiyası), virus.*

**ИЗУЧЕНИЕ ВИРУСА БЕШЕНСТВА В АЗЕРБАЙДЖАНЕ 2016-2017 ГГ****Ч.В. Алиева**

В связи с тем, что бешенство является причиной 100% смертей людей и животных, оно до сих пор считается особо опасным заболеванием. Бешенство вызывает РНК-содержащий вирус, поражающий нервные клетки. Чтобы заразиться бешенством, человеку необходимо укубить больное животное. Всемирная организация по охране здоровья животных (ВАОЗ-МЭБ) обязала страны-члены сообщать о бешенстве. Таким образом, Азербайджан ежегодно представляет в МЭБ статистику по бешенству.

По данным МЭБ, ежегодно от бешенства во всем мире умирает около 70 000 человек. По статистическим данным, заболевание бешенством регистрируется в стране ежегодно. Вирус распространен преимущественно среди домашних животных и бездомных собак.

Азербайджан расположен на Южном Кавказе, в регионе геополитического значения, расположенном на стыке Европы и Азии. Такое географическое положение делает этот регион центральным для эпидемиологических исследований и борьбы с трансграничными инфекционными заболеваниями, такими как бешенство.

Цель проведенных исследований - изучение регионов распространения вируса бешенства в 2016-2017 гг., вида зараженных животных и лабораторной диагностики. В результате в 2016-2017 годах зоной наибольшего распространения вируса в стране стали Загатала-Шеки и Баку. Основное преимущество в лабораторной диагностике отдается полимеразной цепной реакции. Этот метод используется для раннего выявления и считается подтверждающим.

**Ключевые слова:** *Бешенство, Азербайджан, очаги, ПЦР (полимеразная цепная реакция), вирус.*