

Kastriot KORRO*, Kristaq BERXHOLI*, Vasilika DINI*, Bejo BIZHGA*,
Fejzo SELAMI*, Luigj TURMALAJ*, Ali LILO**

THE IDENTIFICATION OF RANAVIRUSES IN RANA TEMPORARIA AT SHKODRA LAKE

Përmbledhje: Ranavirusët janë një grup virusësh, që prekin disa specie, por veçanërisht dëmtime të mëdha shkakton në amfibët, duke reduktuar ndjeshëm popullatën e tyre. Në kuadrin e projektit FP 7 me titull „WildTech”, Shoqata Shqiptare e Sëmundjeve të Kafshëve të Egra në bashkëpunim me Fakultetin e Mjekësisë Veterinare, gjatë perjudhës Tetor 2009 – Maj 2010 ka grumbulluar disa kampione nga 15 bretkosa të ngordhura dhe 13 bretkosa të gjalla rreth Liqenit të Shkodrës. Të 28 kampionet janë grumbulluar në zonat përqark Liqenit të Shkodrës. Midis studimeve të bëra, për shkakun e ngordhjes të bretkosave, ranovirusët janë konsideruar një shkak i mundshëm, pasi virusi është identifikuar në disa vende të Europës, por nuk ka qënë i identifikuar më parë në Shqipëri. Vendosja në dispozicionin e shoqatës, e një kiti special për ranovirusët, ka bërë të mundur kryerjen e një testi eksperimental për praninë apo jopraninë e këtij virusi. Nga eksperimenti janë vërtetuar 3 raste pozitive për ranovirus, të cilat mund të kenë shaktuar edhe ngordhjen. Rastet pozitive të identifikuara me kitet speciale, do të dërgohen në Universitetin „Aristotle” të Selanikut për t’iu nënshtruar metodës PCR. Ky studim do të verifikojë përfundimisht praninë e ranovirusëve në popullatën e amfibëve në Shqipëri.

Fjalët kyçe: *ranavirus, bretkosë, sëmundje, kite, Liqeni i Shkodrës*

Abstract: Ranaviruses are a group of viruses that affect some species, but particularly in amphibians they cause dangerous deceases and that can cause severe injuries in the population of amphibians as well as reducing their elasticity. In the framework of the FP 7-KB-BE-2007-2 A „WildTech” project, as well as a monitoring project of the Albanian Association of Wildlife Diseases and Faculty of Veterinary Medicine of Tirana, during the month of October 2009 – May 2010 were collected some samples from 15 dead frogs and 13 live frogs in the lake of Shkodra. The 28 samples belonged to the common frogs that live in the suburbs of the Shkodra Lake. Among the studies made for the causes of death, ranaviruses were considered to be a probable cause, a virus identified in some European countries, but not de-

* Kastriot Korro, Kristaq Berxholi, Vasilika Dini, Bejo Bizhga, Fejzo Selami, Luigj Turmalaj, Agricultural University of Tirana, Faculty of Veterinary Medicine Tirana Albania, Albanian Association of Wild Animals Diseases

** Ali Lilo, Institute for Food Searching and Veterinary Tirana Albania

terminated before in Albania. Taking advantage from the special kit in disposal of our association, the samples were submitted to the experimental test to prove whether this virus is present or not. The presence of this special virus was proven in 3 of them, which might have caused their death. The positive samples proven by the means of special kits method will be sent to the „Aristotle” University in Thessalonica to perform the PCR test. This study will show the presence of ranavirus in the amphibian community.

Key words: *ranavirus, frog, diseases, kite, Shkodra Lake*

INTRODUCTION

Ranaviruses (family *Iridoviridae*) are emerging pathogens of farmed and wild amphibians and cause high mortality rates in these animals. These viruses are associated with massive population decreases of some species, outbreaks have been reported in the United States, Asia, Micronesia, and Europe. At the general meeting held by the International Epizootic Office in May 2008, iridoviruses of amphibians were added to the list of pathogens of wildlife that should be monitored (Wolf *et al.*, 1968). Clinical signs in frogs were depression; lethargy; palpebral, hyperemia; abdominal edema, petechiae, and erythema on the ventral surface; skin ulcers; limb and tail necrosis; and emaciation. Pathologic changes were similar in all larvae. At necropsy, subcutaneous edema, body cavity effusions, and swollen and friable livers were observed. Ranoviruses, as dangerous agents of the population of amphibians and frogs in general, can damage the populations of fish as well (Daszak *et al.*, 1999). Considering the fact that this virosis, can be transmitted from amphibians to fish and vice versa, the importance of the research takes another importance (Docherty *et al.*, 2003). The dangerousness of the illness, is evaluated from OIE (International Organization of Epizootic), by classifying it in the illnesses with high dangerousness of the wild world. This illness is not known in Albania at all. It is often confused with bacterial infections, or the parasite ones and the first mentioning of this illness is made in the text of veterinary virology (Berxholi, 2002). Building of small plants for growing of the frogs, as well as their trading for consume in luxurious restaurants, in some districts of the country, but from the farmers of the villages surrounding the Shkodra Lake, became a reason for starting of this monitoring from Albanian Wildlife Diseases Association with the staff of pedagogue of Faculty of veterinary Medicine in cooperation with European Wildlife Diseases Association in the framework of the project FP 7-WildTech, in which the Department of Veterinary Public Health is an associated partner. The manifestation of the signs of this illness in some handicraft micro plants built in some villages surrounding the lake triggered our staff to restart a monitoring in the common frogs in the ponds surrounding this lake (Maeda & Matsui, 1989). Considering the fact that the illness causes serious damage of the species, but on the other hand serves as another potential danger for the population of fish, the study takes a special veterinary and ecological importance, as our study aims at the conservation of the population of *Rana temporaria*, as well as the protection of the whole ecosystem of underwater fauna of the lake.

MATERIALS AND METHODS

For the identification of ranavirus was used a special kit provided by European partners of Wildlife Diseases. This kit contains specific chromatographic antibodies for ranaviruses and in collaboration with the antigen, which is found in the provided samples of the frogs, interacts and gives the changing color of the kit from blue in red. The presence of two red lines indicates the presence of ranavirus from the taken samples. In order to control the presence of ranavirus kidneys of turtles were taken and were put into the kit's solution for 20 minutes. Later the extract was filtrated in another test-tube and the kit was put, by letting it for 10 minutes, waiting for reading of the result (Ota, 2000). The samples were provided in September 2009 and May 2010. Thus, the samples were taken in two periods, the autumn and the spring period.

RESULTS AND DISCUSSIONS

From both parties was realized the monitoring for the preserve of ranavirus with the method introduced above. The tests made after the gathering of samples, resulted in the Table 1, 2, 3.

Table 1. The data from the monitoring for ranavirus made in the period of September 2009.

Nr of samples	Time of provision	Positive result for ranavirus	Negative results for ranavirus	Doubt case
3	10.09.2009	1	2	–
6	16.09.2009	–	6	–
5	23.09.2009	–	5	–
Total 14		1	13	–

Altogether were provided 28 samples of the kidneys of frogs, which were taken in some ponds near the Shkodra lake in two periods: (September 2009) and spring period (May 2010). From 28 samples gathered, 15 of them were taken from dead frogs, but that the reason of their death was not known and 13 of them were taken from living frogs found in some of the ponds near the lake.

From the results, are identified three cases with the presence of the ranavirus. Thus this is an initial identification in our country for the presence of this virus (Miller *et al.*, 2007). Even though the number of the samples is relatively low, still our study managed to identify the presence of the virus (Majji *et al.*, 2006). As far as concerning the possibility of a greater number of samples, this was conditioned by the lower number of provided kits (Cunningham *et al.*, 2007).

Table 2. Data taken from the monitoring for ranavirus made in the period of may 2010.

Nr of samples	Time of provision	Positive result for ranavirus	Negative result for ranavirus	Doubt case
5	04. 05. 2010	–	5	–
4	10. 05. 2010	2	2	–
5	23. 09. 2009	–	5	–
Total 14		2	12	–

Table 3. Results from dead and living frogs.

Kind of samples	Number of samples	Positive result for ranavirus	Negative result for ranavirus
Samples from living frogs	13	1	12
Samples taken from dead frogs	15	2	13
Total 14	28	3	25

Our study is focused in this district, but also in some other districts in all the country will continue in all the country, as the identification of the virus, indicates that the infection circulates in our country, as it is confirmed in the neighboring countries (Marsh *et al.*, 2002). We aim that our study will continue even later with the confirming of the virus with the method of PCR, a study which will be done according to our agreements with the partners of the project WildTech in the University „Aristotle”, Thessaloniki, Greece.

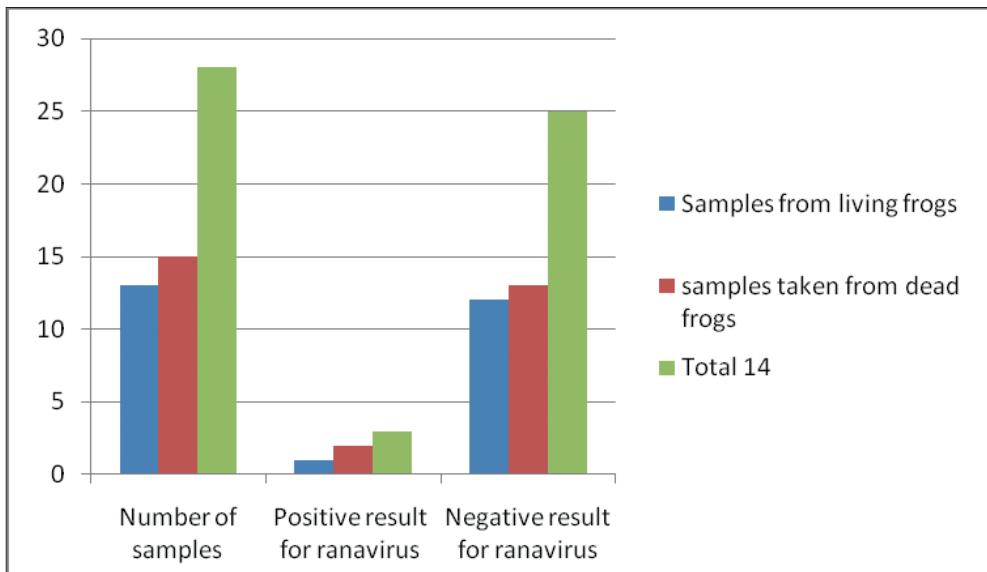


Figure 1. The results according to the seasons

CONCLUSIONS

1. The study will confirm the presence of ranaviruses in frogs, which will allude for the presence of this virus in fish which grow in the Shkodra Lake.

2. The study is the first one in the aspect of the study of the illnesses of the amphibians in Albania, serving as a starting element for deeper and complete studies in the future.

3. The study will serve the identification in genetically level of the strains of the virus which circulates in our country.

4. Our study will serve as a stimulant for the scholars of the country and international scholars to realize such studies in the field of the illnesses which damage the amphibians and reduce the elasticity of this class.

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