Identification of causes of death of Baikal seal (*Pusa sibirica* Gmelin, 1788)

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Abstract: The Baikal seal is the only endemic mammal of Lake Baikal. In late October 2017, 132 dead Baikal seals were found on the shore of the lake. To identify causes of death, postmortem necropsy, virological, chemical toxicological, microbiological, and parasitological studies were carried out. Several hypotheses for explaining the causes of the deaths were put forward. The most probable causes are heart failure and asphyxia due to echo sounders (sonar). The causes were confirmed by the results of histological examination of the hearts and lungs of a large number of dead seals found on the southern shore of Lake Baikal (a center for the fishing industry).

Key words: Baikal seal, organs, necropsy, causes of death

The Baikal seal (*Pusa sibirica* Gmelin, 1788 [syn. *Phoca sibirica]*) is one of the dwellers of Lake Baikal. It is the only endemic mammal of the lake.

The first reported mass death of Baikal seals occurred in the autumn of 1987 due to a distemper outbreak, as a result of which more than 6000 animals died (Petrov, 2009; Pronin and Kabanov, 1992). This was the first report of epizooty in Baikal seals. The disease was accompanied by symptoms typical of morbilliviral infections (Bame et al., 1988; Goodman, 2016; Mamaev et al., 1995).

The second mass death of Baikal seals was reported in the late 1990s and early 2000s; it was due to emissions of sulfur components from the Baikal Pulp and Paper Plant. Water samples contained chloride of lime.

In late October 2017, 132 dead seals were found on the Baikal coast: 38 bodies along the Circum-Baikal railway between Port Baikal and Kultuk (in the Irkutsk region), and 94 in the Republic of Buryatia near Novy Enkhaluk, Murino settlement.

Identification of the cause of mass deaths of the Baikal seal in 2017 is extremely important for preserving the endemic Baikal seal population. In 2016, there were 128,700 Baikal seals (Goodman, 2016; Tkachev et al., 2016); however, increased anthropogenic loading, pollution of Lake Baikal, and poaching have had negative effects on the endemic Baikal population.

For our research, we examined dead seals and their organs (n = 20).
Specific bands of the amplified cDNA (cDNA of the carnivorous plague 270 polynucleotides, CTP CDV 670 bp) shown by the electrophoregram were recorded.

The RNA of the carnivorous plague and rabies viruses in the Baikal seal brain samples was determined by the RT-PCR method using primers specific for morbilliviruses and rhabdoviruses.

For chemical, toxicological, and parasitic testing, parts of the esophagus and stomach, a segment of the thick intestine (up to 10 cm in length), a part of the liver (a solid organ) with the gallbladder, one kidney, urine, and skeletal musculature in the amount of 500 mg were used. Samples were placed in sterile test tubes and bags. For each sample, the log included information on the type of material and date of sampling. Parasitic studies were carried out according to the guidelines for the diagnosis of animal helminth infections approved by the General Directorate of Veterinary Medicine (1980; http://docs.cntd.ru/document/1200106918).

The bacteriological diagnosis was performed in accordance with the methodological guidelines for the bacteriological diagnosis of mixed intestinal infection of young animals caused by pathogenic enterobacteria (MU 13-7-2/1759, 1999; http://docs.cntd.ru/document/550855605).

The necropsy material was sampled in accordance with sanitary and epidemiological regulations (SanPiN 2.3.2.560-96: “Hygienic requirements for quality and safety of food raw materials and food products”).

For histological testing, organ tissues were cut from standard segments and fixed in 10% neutral buffered formalin (ErgoProduct, St. Petersburg, Russia). After fixation, the material was paraffin-embedded. Sections 2–7 µm in thickness were cut using an MPS-2 microtome (TOCMEDPRIBOR, Kharkov, Ukraine). Structural components of the organs were identified by staining tissue sections with hematoxylin and eosin produced by Biovitrum-Siberia Company (Novosibirsk, Russia). To identify changes in the myocardial microstructure, tissues were stained with hematoxylin–Rego (Biovitrum-Siberia Company, Novosibirsk, Russia), which helped identify initial changes. Anatomical structures were classified according to the International Veterinary Anatomical Nomenclature (2017) and International Histological Nomenclature (2017; published on the website of the World Association of Veterinary Anatomists www.wava-amav.org).

The necropsy of the bodies revealed that all of the Baikal seals under study were female specimens 20–30 years of age. External examination revealed good nutritional status, no postmortem changes, and natural (wounds, fractures, tumors, etc.) and anthropogenic (marks of fishing nets, bullet wounds, etc.) injuries. Internal examination revealed no visible damage. The organs were located anatomically in the thoracic and abdominal cavities. However, the examination identified edema and emphysema in the lungs, and nematodes *Parafilaroides krascheninnikovi* in the parenchyma (Examination Report No. GD 11-01-173 of 2 November 2017, Irkutsk Interregional Veterinary Laboratory). Indurations with crater-shaped indentations in the center were revealed on the gastric mucosa, where thin white *Contracaecum osculatum baikalensis* nematodes were found (Examination Report No. GD 11-01-174 of 2 November 2017, Irkutsk Interregional Veterinary Laboratory).

The stomachs were empty but filled with gases, with no chyme in the intestines. The left horn of the two-horned uterus was enlarged in all bodies under study. One uterus contained a fetus 18 ± 2.3 cm in length and 400 ± 56.4 g in weight.

The histological testing of various organs stained with hematoxylin and eosin revealed no pathological changes in most tissues. The analysis revealed hyperinflated alveoli, swollen peribronchial connective tissues, thickened collagen fibers in the lungs, and a transudate with air bubbles in the bronchi. Induration areas on the gastric mucosa were vast necrotic zones with focal lymphoplasmacytic infiltrates. Changes in the heart structure were observed as well. However, staining with hematoxylin and eosin did not allow us to determine their nature and size (see Figure 1).

Staining with hematoxylin–Rego helped identify focal necrosis of muscle fibers in the heart, as shown in Figures 2 and 3. Necrotic areas were evenly distributed in the myocardium. They were rarely located subendocardially or in groups. Contraction bands without signs of fiber disintegration were found. Sometimes 2 contraction bands were located side by side, forming 1 double contraction band.

Virology testing did not reveal distemper, rabies, or viral enteritis antigens (Examinations Report No GD 10-25-170...

Virology testing revealed parvoviral enteritis antigens (Examinations Reports No GD 11-02-181/1 of 3 November 2017, No GD 11-02-181/2 of 3 November 2017, No GD 11-02-180/1 of 3 November 2017, No GD 11-02-180/4 of 3 November 2017, No GD 11-01-173 of 2 November 2017, No GD 11-01-174 of 2 November 2017, No GD 11-01-175 of 8 November 2017, Irkutsk Interregional Veterinary Laboratory) and viral hepatitis antigens (Examinations Reports No GD 10-30-172/1 of 1 November 2017, No GD 10-30-172/2 of 1 November 2017, No GD 10-30-172/3 of 1 November 2017, No GD 11-01-172 of 8 November 2017, Irkutsk Interregional Veterinary Laboratory). Parvoviral enteritis and viral hepatitis antigens indicate that the Baikal seals were infected with these viruses, but necropsy did not reveal typical features of these diseases.

A causative agent of Aeromonas (Examination Report No. GD 11-01-175 of 8 November 2017, No. GD 11-01-172 of 8 November 2017, Irkutsk Interregional Veterinary Laboratory), which is not a danger to carnivores, was found. Tsybikzhapov et al. (2006) have argued that the agent is representative of the Lake Baikal microflora.

Chemical and toxicological testing revealed no critical chemical elements (Examination Report No. GD 11-01-173 of 8 November 2017, No. GD 11-01-174 of 8 November 2017, Irkutsk Interregional Veterinary Laboratory). Thus, poisoning with sulfur components is unlikely. Testing results did not confirm poisoning with cyanotoxins (a poisonous substance of blue-green algae).

Test results showed that the deaths of the animals under study were caused by asphyxia and heart failure (ventricular fibrillation as contractures and ruptures, which were identified due to a staining method by Rego).

Several hypotheses have been put forward to establish the causes of the deaths of these Baikal seals:

1. Natural deaths caused by overpopulation (however, there were no mass deaths of Baikal seals on the Ushkanii Islands, which are their main rookery);
2. Infections (however, analysis did not reveal distemper, rabies, or viral enteritis antigens, or distemper or rabies RNA);
3. Deaths caused by chemical contamination of the lake (however, laboratory tests did not reveal signs of poisoning or related pathological changes of the organs);
4. Parasites: necropsy results revealed isolated cases of stomach and intestinal parasites (nematodes) which could not have caused the deaths of the mammals;
5. Deaths caused by food deficiency (Petrov [2009] says that the largest number of animals with empty gastrointestinal tracts were recorded during autumn migrations);
6. Heart failure caused by echo sounding (sonar): this hypothesis is most probable as it has been confirmed by necropsy results and the large number of dead seals on the southern coast of Lake Baikal, where there are many fishing boats. Thus, the effect of echo sounding on seals at night would explain the empty stomachs. This hypothesis is also supported by the fact that most of the dead seals were pregnant females. During pregnancy, compensatory physiological mechanisms are in a state of stress, so echo sounders may have provoked cardiac damage, causing death.
To confirm the hypothesis on the effects of echo sounders on the Baikal seal’s heart, it is necessary to conduct in-depth experiments, taking into account the anatomical, biological, and physiological characteristics of the animal. We hope that the hypotheses formulated in the article will be confirmed by subsequent studies.

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References