

# Applied autecology on the model of cationic proteins of neutrophil granulocytes of carnivores by adverse environmental effects

*Nikolay Pimenov, Christina Permyakova, Regina Ivannikova\**, and *Saida Marzanova*

Federal State Budgetary Educational Institution of Higher Education «Moscow State Academy of Veterinary Medicine and Biotechnology - MVA named after K.I. Skryabin», 23, Akademicheskaya St., Moscow, 109472, Russia

**Abstract.** The article presents studies related to the evaluation of the reaction of cationic proteins of neutrophil granulocytes as markers of environmental impact on the animal body on the example of carnivores (dogs). As is known, neutrophils are one of the first lines of defense of the macroorganism against many impacts, such as environmental pollution by various products of anthropogenic activity, bacterial and viral infections, etc. Therefore, taking into account the reaction of neutrophil cationic proteins to adverse environmental effects may be of interest for the early detection of immunological changes. We found that the level of neutrophil cationic proteins in stray dogs was significantly higher than in domestic dogs. This fact may indicate a longer adaptation process of the body of homeless animals in response to changes in the environmental situation and requires further more detailed study.

## 1 Introduction

Ecological consciousness determines the living conditions of individuals, as well as the value orientations by which representatives of various social groups perceive environmental factors. Autecology, which studies the interaction of the organism with the environment, examines the processes of adaptive physiological and pathogenetic reactions of the organism to the effects of environmental factors, including adverse environmental factors that are a reflection of the environmental situation. In connection with the above, applied autecology is inextricably linked with the problems of preserving the species biodiversity of organisms. Autecology allows, according to a number of reactions of internal metabolism and morphogenesis, to assess the state of environmental impact and develop ways to stabilize the ecological situation and preserve the biodiversity of species by methods of predicting the degree of impact and leveling the negative effect on the endoecology of the organism of external factors.

Orientation to the formation of factors of the ecologization process in close connection with the physiological markers of autecology is of current interest, especially in the field of anthropogenic transformation of the environment, biogeocenoses, natural landscapes and

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\* Corresponding author: [regiotf@yandex.ru](mailto:regiotf@yandex.ru)

gene drift [1, 2]. The issues of the environmentally safe development of civilization require more and more attention, as well as the ecology of the inner world of a person is connected with autecological knowledge and philosophical culture of thinking. Ecological culture as a form of theoretical return of a person to unity with nature is based on the issues of assessment and indication of factors of response to ecotransformation.

Indicators of health and adaptive reactions are presented in the body at different levels – from the molecular to the system-organ level. The oldest innate marker unit of immunoreactivity at the cellular level are cationic proteins of neutrophil granulocytes. Neutrophils of animals do not have quantitative characteristics of such an important criterion of functional activity as granularity of the cytoplasm.

Cationic proteins carry a significant positive charge on the surface of the protein micelle and provide increased permeability of vascular walls, nonspecific bactericidal activity, stimuli for leukocyte migration and phagocytosis [3, 4].

In inflammatory and toxic processes, cationic proteins are able to be released into the extracellular environment and participate in the resorption of immune complexes and the neutralization of extracellularly located bacteria and viruses. This leads to an intracellular decrease in the content of cationic proteins, which affects the shape and color intensity of cytoplasmic granularity [5, 6]. In research practice, the assessment of cationic protein levels can be useful as markers of systemic activation of neutrophils when monitoring reactions to adverse external influences.

However, there is currently no data on how much the number of granules and their sizes change during these processes, what part of the cell is occupied by granular and intergranular cytoplasm.

In this regard, the purpose of this work was set: to study cationic proteins as markers of individual and population health of the ecosystem.

## **2 Research methodology**

The methodology of the research was based on a system for assessing the environmental impact on the body by morphotinctorial changes in intracellular cationic proteins in response to adverse environmental factors on the example of carnivores (dogs). With the natural variability of immunological parameters in practically healthy animals, it is necessary to take into account the fact that many of the identified deviations do not reflect a violation of the functioning of the system, but adaptive changes in response to an irritating factor. Because of adaptive restructuring, the imbalance of individual parts of the immune system is eliminated and an adequate immune response to the antigen is formed. The orientation of adaptive reactions ultimately determines the type of immune response.

The following research methods were used: system analysis and synthesis, environmental monitoring, determination of cationic proteins according to Pigarevsky in the modification of Nesterova I.V. (1992) with microscopy and staining with diachromic lemon dye green durable.

## **3 Research results and discussion**

The idea was based on a comparison of changes in the reactions of cationic proteins to environmental factors. For this purpose, stray dogs were used – natural synanthropes of an anthropogenic ecological niche with «former pets», who later ended up in the same kennel.

Cationic proteins are a large, heterogeneous group of proteins in their structure and functions, the distinctive physico-chemical property of which is the cationic character of their molecules. Cationic proteins include: defensins, lysozyme, lactoferrin, bactericidal

permeability-increasing protein, cathepsin G, elastase, phospholipase A and myeloperoxidase. Cationic proteins are found only in granulocytes (neutrophils and eosinophils) and are detected at various stages of myelopoiesis, starting with promyelocytes. During the maturation and differentiation of granulocytes, the intracellular content of cationic proteins increases, reaching the maximum level at the stage of mature bone marrow reserve cells. Mature neutrophils of peripheral blood are not capable of synthesis and accumulation of cationic proteins and in the course of performing their functions only consume preformed reserves of these biocidal and regulatory proteins [4].

The material of the study was the whole blood of 20 dogs kept in the conditions of the kennel of the department for the maintenance of neglected animals of the state budgetary institution «Highways» of the Western Administrative District of Moscow: 10 in group 1 (pets trapped on the street) and 10 in group 2 (stray animals). These individuals were selected by sex and age characteristics. Feeding and keeping of dogs was carried out in accordance with zoohygienic indicators.

The selection of groups was carried out according to the principle of analogues – in an equal breed ratio: Central Asian Shepherd Dog, German Shepherd Dog, German Wirehaired Pointer, in the age group of 4-7 years.

Blood from dogs for the study was taken in the morning on an empty stomach with the addition of heparin as an anticoagulant. The level of cationic proteins in neutrophils was calculated using the mean cytochemical coefficient (MCC) according to the modified formula of Astaldi and Vergi, 1957 and expressed in conventional units.

The principle of the method: at pH values of 8.1-8.2, cationic proteins are the only biopolymer with which the molecules of a diachromic lemon dye of strong green durable react to form stable ionic bonds.

- Blood smears are prepared according to the usual rules on cleaned and fat-free slides and dried in air.
- Fixation and incubation are carried out in a buffered alcohol solution of strong green (pH 8.0-8.2) for 15 min. 0.2 M of tris-buffer solution: 24.2 g of dry tris-buffer per 1 liter of distilled water.
- Methanol tris-buffer: 10 ml of tris-buffer and 9 ml of 0.1 N hydrochloric acid and 21 ml of methanol.
- Buffered alcohol solution of durable green: 100 mg of durable green is dissolved in 100 ml of methanol tris-buffer with a pH of 8,0-8,2 (tris-aminomethane (C<sub>4</sub>H<sub>11</sub>O<sub>3</sub>N) is brought to the desired pH).
- After incubation, the smears are rinsed in distilled water.
- Finishing the nucleus with 0.25% azure A solution for 30 seconds.
- Washed with distilled water, dried, microscopied.

The smears were microscopized, neutrophils are counted taking into account the degree of intensity of the color of the granules.

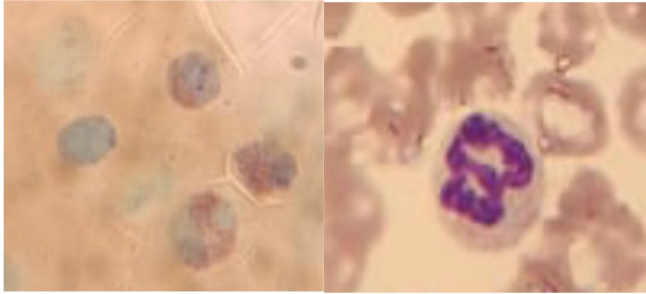
When evaluating the results of the mean cytochemical coefficient (MCC), the data of anamnesis, clinical examination and other clinical diagnostic studies should be taken into account.

**Table 1.** The level of cationic proteins in the blood of dogs.

Animal species	Mean cytochemical coefficient (MCC) (M±m)
Conditionally domestic dogs	0.75±0.1
Stray dogs (kept in a dog shelter)	2.5±0.2

In homeless animals, compared with domestic animals, an increased content of neutrophil cationic proteins was observed. Presumably, this is due to the fact that such animals have a

longer stabilizing adaptation process, which manifests itself in the development of adaptive reactions to the impact of adverse environmental conditions – anthropogenic pressing, insufficient food supply, unbalanced habitat conditions.



**Fig. 1.** Comparative reaction of cationic proteins.

Cationic proteins have a wide spectrum of action. In particular, various studies emphasize their antibacterial effect in the strategy of combating infectious agents, which characterizes the innate ability of the body to resist bacterial infections. Determination of the level of neutrophil cationic proteins can be useful as a marker of systemic activation of neutrophils in the practice of confirming the pathogenetic role of immune-dependent processes in response to adverse environmental impacts [2], when monitoring the course of the body's reactivity to the environmental situation, when assessing the immune status of an animal.

## 4 Conclusion

One of the central tasks of environmental immunology is to identify early changes in the immune system of organisms of biological populations under the influence of adverse environmental factors. In connection with the above, cationic proteins are one of the links in restoring and maintaining both the balance of innate immunity and homeostasis of the organism as a whole, which is important for the survival of an individual in the environment. These studies show the prospects of studying cationic proteins as markers of adverse environmental impacts and a prognostic sign in early prenosological manifestations of environmental consequences. In this regard, there are prospects for using the methodology for assessing cationic proteins in organisms to monitor the environmental situation and develop preventive measures for the conservation of biodiversity.

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