

# Research of animal microflora at the Imperial Kazan University (based on the materials of N.I. Orlov)

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## ABSTRACT

Qualitative and quantitative determination of the intestinal microflora is currently one of the tools for studying the physiological state of both human and animal organisms. Historically, the first observations in this area belong to the turn of the XIX-XX centuries. This article highlights the issues of determining the presence of paratyphoid microbes in the feces of dogs, made at the beginning of the XX century by the Kazan bacteriologist N.I. Orlov. Data on its methodological approaches to culture and identification of the studied group of bacteria are presented, which is of interest from the point of view of the history of development of microbiological and immunological methods.

**Key words:** Microbiocenosis, Intestine, Dogs, Paratyphoid fever, Stool, Nutrient medium.

## Introduction

Currently, the study of intestinal microbiocenoses of both humans and animals is a popular and promising direction in medical and veterinary microbiology. Both for humans (Zakharova, 2006; Zakharova *et al.*, 2009) and for animals (Larionov and Dundukova, 2005; Petrov *et al.*, 2007; Timchenko and Gandraburova, 2012; Gromova, 2017) various pathologies associated with violations of the microbial composition of the gastrointestinal tract, skin, wool, and mucous membranes are described. Active attempts are being made to correct microbial communities using various biologics (Kuryatova, 2005; Ivanovsky *et al.*, 2006; Mazankova *et al.*, 2007; Klimova, 2008). The development of molecular biology methods makes it possible to accurately identify microbes today, based not only on the methods of classical microbiology, but also using genome sequencing and other modern approaches (Zhilenkova *et al.*, 2016; Kardymon and Kudryavtseva, 2016; Dzhaparidze and Suvorov,

2013; Andryushchenko *et al.*, 2018).

Interest in the intestinal microflora began to increase especially towards the end and beginning of the twentieth century. Initially, the attention of researchers was drawn to human microbial communities (Uspensky, 1896; Manual, 1897; Rosenthal, 1912), later attention was paid to animals (Kadio, 1930). The purpose of this work is to highlight the first steps in the study of the intestinal microflora of animals made at Kazan University.

## Work of N.I. Orlov: microbial study of fecal masses of dogs

N.I. Orlov's work begins with highlighting the relevance of the study of a group of paratyphotic bacteria in connection with their ability to cause gastrointestinal disorders. It is noted that Otto von Bollinger and other bacteriologists were the first to work with this group of microbes (Orlov, 1912). Orlov notes that by 1907, the systematics of V. Babes, proposed by him at the Berlin Congress of hygienists, was generally accepted, according to

which all typhoid-like bacteria were proposed to be divided into 3 groups (Orlov, 1912) – I. Typhus, paratyph A, II. Paratyphi b (subgroups A and b), III. Gartner's enteric bacilli. Orlov notes that the task of his research was to detect the microbes of these three groups in the feces of dogs and describe their characteristics. To successfully perform the work, N.I. Orlov mastered the method of cultivating bacteria on various media (Orlov, 1912). Cultures were obtained from the Hygiene Institute and the Institute of infectious diseases in Berlin. N.I. Orlov tested the following media – broth, agar, gelatin, milk, various carbohydrates (Table 1) and other media (total – 13 media).

The author describes the ability of differential diagnostics for the growth of certain microbes (Orlov, 1912) and the transformations that are characteristic of the growth of certain microbes on these media.

N.I. Orlov notes that fecal masses with rare exceptions were collected on the streets of the suburbs of Berlin-Charlottenburg (Orlov, 1912). N.I. Orlov writes about their freshness: "If we take into account the exemplary cleanliness of the streets of Berlin and Charlottenburg, which are swept and washed from early morning to late evening, we can assume with a certain degree of probability that in most cases I had to deal with my experiments with excrement of recent origin" (Orlov, 1912). Fecal matter was collected in sterile Petri dishes, the Central part of the mass was used for analysis, from where a drop of fecal matter was transferred to the saline solution by a loop, and from there – to the nutrient medium (Orlov, 1912). A total of 30 stool samples were col-

lected, 2 of which were liquid, and the rest were solid and semi-solid.

As the author notes, they isolated *B. paratyphoid B.*, which had the form of a rod with mobile flagella, the use of paratyphoid serum to which caused the phenomenon of agglutination. N.I. Orlov describes in detail the changes in nutrient media when this bacterium was cultured in them (Orlov, 1912) but as he notes later - it was impossible to be sure that it was *B. paratyphoid B.*, and not *A. Gartner's enteric Bacillus*. To confirm or disprove the results obtained with the use of nutrient media, serums produced by Merck, manufactured at the Berlin Hygiene Institute, were used. Thanks to the use of three different serums - against paratyphoid A, B and enteric *Bacillus* of A. Gartner (data are presented in three tables, (Orlov, 1912). Orlov found that the bacterium under study is actually *B. paratyphoid* (Orlov, 1912). Then the pathogenicity of the isolated culture was checked in laboratory mice. The bacteria isolated from the liver and spleen of deceased mice were identical to the original *B. paratyphoid* [Orlov, 1912).

The isolated bacterial culture was used for the application of agglutinating serum by immunizing rabbits and its quality was evaluated. Finally, N.I. Orlov asks whether *B. paratyphoid B.* is pathogenic for dogs and immediately assumes that it is not (Orlov, 1912).

Thus, the work of N.I. Orlov was important in terms of the development of veterinary microbiology at Kazan University and, in particular, in terms of improving methodological approaches for identifying bacteria based on their growth characteristics

**Table 1.** Cultivation of various representatives of paratyphotic bacteria on carbohydrate media (according to (Orlov, 1912).

Type of nutrient	<i>Bac. typhus.</i>	<i>Bac. colli</i>	<i>Bac. Paratyph. B.</i>	<i>Bac. Paratyph. A.</i>	<i>Bac. enterit. Gartner</i>
Grape sugar	-	+	+	+	+
Milk sugar	-	+	-	?	-
Cane sugar	-	-	-	-	-
Mannose	-	+	+	+	+
Levulose	-	+	+	+	+
Maltose	-	+	+	+	+
Raffinose	-	-	-	-	-
Dextrin	-	±	±	-	-
Potato starch	-	-	-	-	-
Glycerin	-	-	-	-	-
Adonite	-	-	-	-	-
Dulcet	-	-	+	+	+
Mannitol	-	+	+	+	+

in a variety of nutrient media and in the application of immunology methods.

## Conclusion

Thanks to the analysis of the work carried out by N.I. Orlov, we can conclude that at the beginning of the twentieth century, the identification of intestinal microbes was actively developed. A variety of culture media were used to determine the presence of certain bacteria in the culture medium by the nature of specific changes in them (gas formation, color changes, etc.). A more specific approach to verifying the correctness of identification was the use of serums. The results of N.I. Orlov's experiments contributed to the development of veterinary microbiology at the Imperial Kazan University.

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