

VETERINARY PHARMACOLOGY


UDC: 615.326:552.52

DOI: 10.18413/2500-235X-2017-3-1-97-104

Bukhanov V.D.¹
Vezentsev A.I.¹
Filippova O.V.²
Nadezhdin S.V.¹
Pankova O.N.³
Firsova T.I.⁴
Mikhailyukova V.O.¹
Tishin A.N.¹

**THE INFLUENCE OF THE CONCENTRATION
 OF MONTMORILLONITE CONTAINING SORBENT
 AND pH OF THE CULTURE MEDIUM
 ON THE ANTIBIOTIC SENSITIVITY OF ESCHERICHIA COLI,
 AS WELL AS THE EFFECT OF GROUND ON GROWTH
 OF ESCHERICHIA**

¹Belgorod State University, 85 Pobedy St., Belgorod, 308015, Russia

²Sechenov First Moscow State Medical University, 8-2 Trubetskaya Sst., Moscow 119991, Russia

³FSBSI Kovalenko All-Russian Research Institute for Experimental Veterinary, 4, Kurskaya St., Belgorod, 308002, Russia

⁴Regional State Funded Healthcare Facility Regional Clinical hospital, 8/9 Nekrasova St., Belgorod, 308000, Russia
 e-mail: valabu55@bk.ru

Abstract.

Diseases of a digestive tract in frequency and mass manifestation of young farm animals represent one of challenges for modern veterinary medicine. Giving chelators specific properties by immobilization on the surface of the medicinal substances in the form of active ligands is a promising direction to reduce side effects and increase treatment efficacy. The aim of this work was the comparative sensitivity test of E. coli to enrofloxacin and doxycycline and bactericidal action of montmorillonite containing sorbent and its combination with enrofloxacin, as well as the influence of the concentration of the enriched montmorillonite containing sorbent and pH of the culture medium on the antibiotic sensitivity of Escherichia coli. The sensitivity test of Escherichia coli to enrofloxacin, and the combination of enrofloxacin with sorbent was performed by the method of double serial dilutions of drugs in a liquid culture medium. The influence of the concentration of the enriched montmorillonite containing sorbent and pH of the culture medium on the sensitivity of E. coli to enrofloxacin and doxycycline was performed by diffusion method on Mueller-Hinton agar (MHA). Value of the bactericidal action of montmorillonite containing sorbent and its combination with enrofloxacin and expressing the sorption activity of the sorbent in relation to E. coli, were performed by confocal laser scanning microscopy (CLSM) using the fluorescent probe 5-carboxyfluorescein diacetate. The effect of ground on growth rate and reproduction of E. coli was performed in a grounded Petri dishes. Based on the undertaken studies it was found that the montmorillonite- based sorbent has no antibacterial and growth-boosting action against E. coli, while the sorbent effectively accumulates on the surface of live bacteria E. coli; in a combination of the sorbent with enrofloxacin, a bactericidal effectiveness of the latter against the E. coli decreased; antibacterial activity of enrofloxacin and its combinations with enriched montmorillonite containing sorbent to E. coli; the combination of enrofloxacin with the sorbent at the respective concentrations of 2, 1, 0.5 µg/ml and 4, 4, 2 mg/ml has bacteriostatic effect; enrofloxacin spectrum of a growth inhibition of E. coli increases with increasing the culture medium pH (from 6 to 8), but for doxycycline it reduces; a loss of size of growth inhibition zones of Escherichia coli due to enrofloxacin in acidic culture medium (pH 6) was at concentrations of sorbent from 2000 to 31,25 µg/ml in comparison with a control, such results were observed in neutral (pH 7) and alkaline culture medium (pH 8), but with the concentration of the sorbent from 2000 to 500 µg/ml; in 1.2 times more E. coli grows in grounded Petri dishes on a meat-and-peptone agar (MPA) than in ungrounded dishes.

Key words: colibacteriosis, sensitivity test, Escherichia coli, the culture medium, enrofloxacin, doxycycline, montmorillonite containing sorbent, composite drugs, confocal laser scanning microscopy method.

Introduction.

A transition of the Russian Federation in the new market relations demands a fundamental revision of the veterinary service of animal industry with the goal of more efficient use of labor, material resources and money. Activities aimed at the implementation of these requirements needs to be not only feasible and effective but also economically efficient, financially viable and profitable. A main purpose of modern transformations is to achieve high performance of veterinary work and the growth of economic efficiency of veterinary measures to ensure the welfare of animal industry, the loss of economic enhancement associated with loss of productivity of sick animals.

Currently, especially in the farms with the continuous flow system of livestock reproduction, there are established conditions with managed ecosystems, which aggravate the relations between the organism and opportunistic microflora. Permanent presence in anthropogenic environment of extremely dangerous in epidemic relation zoonoses of reemergent pathogens of colibacillosis, dysentery, and pneumonia is one of important problem of veterinary epizootology. Mechanisms of contraction and advance of reemergence zoonoses, an activation of cycles of its agents, traffic pathogens with vector from natural zoonotic pools to artificially created biocoenosis, are contributed by some kind of transformation in the system "pathogen + host + environment" due to various circumstances, including the main phenomena of natural and/or anthropogenic nature.

In this regard, diseases of the digestive tract in frequency and mass manifestation of young farm animals represent one of challenges for modern veterinary medicine. While in the nosological structure of alimentary infections, colibacteriosis is one of the leading places.

Variant multifactorial nature of this disease makes it difficult to control, doing huge damage to the animal industry from disease and mortality in pre- and postnatal periods of development [1, 2, 3, 4, 5].

The main directions in the colibacteriosis control are chemotherapy and antibiotic therapy, and also a specific prophylaxis. Large variability of *Escherichia coli* strains and a high degree of variations make difficult specific prophylaxis and treatment of animals suffering from colibacteriosis.

Used antimicrobial drugs (antibiotics, sulfonamides, nitrofurans) and other therapeutic agent in the most are inefficient and environmentally hazardous in connection with the formation of resistant forms of enteropathogenic *E. coli* and decrease the total organism reactivity of the animal. Along with this it is a cause of allergy and often leads to the development of dysbiosis [6, 7, 8, 9].

Therapeutic interventions are usually implemented in two complementary ways: by the introduction into the body of necessary and useful and the excretion of unwanted and harmful. In most cases the first dominates but gradually there is maturing understanding of the importance and necessity of the second, as evidenced by the successful development of efferent methods in medicine. Due to the fact that the overall ecological condition of the environment worsens: there is the increase the impact of industrial and domestic pollutants, pesticides, herbicides, nitrates, nitrites, growth stimulants, antibiotics, other biochemically foreign substances, electromagnetic fields, etc., humans and animals are constantly exposed to various xenobiotics. Therefore, the additional administration of another drug can lead to negative consequences, instead of the expected positive results. Methods of efferent veterinary medicine, which allow the correction of the state of the internal environment and reduce the toxic load on the body [10, 11] are useful in this situation.

The substances, which can adsorb on its surface pathogenic microorganisms and their metabolic products, are enterosorbents. It facilitates the search and development by a modern science of drugs, having high effective in the treatment and prevention of colibacteriosis and influencing on pathogenic microorganisms, irrespective of their antigenic display [12, 13].

Currently, there is a wealth of scientific-based information on the practical use of enterosorption. In addition, the creation of composite drugs using different groups of the sorbents, expanded opportunities of enterosorption in complex treatment of acute intestinal infections, because enterosorbents are the drugs with multifaceted efficacy, defined not only by their symptomatic (antidiarrheal) and pathogenetic (desintoxication, etc.), but also the etiotropic action against pathogenic bacteria and viruses [14].

However, many of the enterosorbents haven't yet wide use due to various reasons:

1) information gap of doctors about the role of enterosorbents in the treatment of sick animals with infectious and non-infectious pathology of the gastrointestinal tract;

2) ignorance of the advantages and disadvantages of various sorbents in concrete disease and skeptical stance of doctors on enterosorption.

The role of electrons in biology has been important for a long time. Overriding concern in the study of the "electronic" aspects of life belongs to the Nobel laureate Albert Szent-Györgyi, who created a scientific field called electronic biology. Opening a revitalizing action of "ground" by Clinton Ober, Stephen Sinatra and Martin Zucker suggests that ground may influence on the intensity of growth and reproduction of *Escherichia coli*, since the cultivation of microorganisms in

laboratory conditions almost break its contact with the free electrons of the Earth's surface [15].

The aim of our work was to study:

- sensitivity of *Escherichia coli* to antibacterial drugs and its combinations with the montmorillonite containing sorbent in different concentrations and different pH values of the culture medium due to the fact that in the Russian Federation there are huge deposits of montmorillonite containing clay;
- ground effect on growth and reproduction rate of *Escherichia coli*.

Materials and methods

The sensitivity test of *Escherichia coli* to enrofloxacin, and the combination of enrofloxacin with sorbent was performed by a conventional method of double serial dilutions of drugs in a liquid culture medium. Dilution of drugs was performed in beef-extract broth (BEB). Each series of dilutions consisted of 9 test tubes containing 5 ml of BEB. In the first series of tubes the concentration of enrofloxacin in the primary tube was 4 µg/ml in the last it was 0.016 µg/ml.

The second series of dilutions of enrofloxacin 1:1 with the sorbent contained the same concentrations of each ingredient. In the third series of dilutions of enrofloxacin with sorbent concentration of enrofloxacin was the same as in the previous series, and for sorbent in 2nd and 3rd tubes it was 4 mg/ml and then 2 mg/ml.

In the control there were used two series of tubes. The first series of dilutions contain the sorbent only at the concentrations from 4 to 0.016 µg/ml. In the second control series there was enrofloxacin with a sorbent at the concentrations similar to the second experimental series. In three experimental and the first control series tenth test tubes contained only the BEB.

After dilutions each tube (except the second control series) was applied by 0.1 ml of the day old culture of *Escherichia coli* (500000 microbial cells), that accounted for 100,000 microbial cells in 1 ml of BEB. In the second control series of dilutions culture of *E. coli* haven't been applied, to test the sterility of the prepared dilutions in the BEB.

The next experiment studied the influence of the concentration of the enriched montmorillonite containing sorbent and pH of the culture medium on the sensitivity of the *E. coli* to enrofloxacin and doxycycline and was performed by diffusion method on Mueller-Hinton agar (MHA). The study was performed in one-use Petri dishes with a diameter of 5.5 cm, three series, eleven dishes in each series. Previously Petri dishes were added: the first (20 mg) and the second (10 mg) dishes of each series by sterile sample of enriched montmorillonite containing sorbent and 1 ml of sterile isotonic sodium chloride solution, the next dishes (from 3 to 10) by 1 ml of dilutions of sterile sorbent at concentrations from 500

to 3.91 µg/ml in isotonic sodium chloride solution. For the experiment there were produced three vials of 110 ml of the MHA. After autoclaving and cooling of MHA to 45-42°C, we produced fixing a pH level of the culture medium: in the first vial to 6, in the second to 7 and in the third to 8. Further, these the culture medium was added by the day old culture of *Escherichia coli*, at the rate of 1×10^7 CFU (colony forming units) in 1 ml of the MHA. Then in each dish there was added 9 ml of the MHA and carefully suspended with a sorbent. After compaction of the MHA on its surface there were placed a discs containing enrofloxacin (5 µg of the Bayer) and doxycycline (10 µg, Federal State Institution of Science, Pasteur Research Institute of Epidemiology and Microbiology, St.-Petersburg). The control dishes (eleventh) of each series were added only 10 ml of the MHA, infected by *E. coli*.

The tubes and dishes with the tested dilutions were cultured for 16-18 hours in a thermostat at 37°C, after there was performed the analysis of findings. Aiming to obtain consistent results, the experiments were repeated three times.

Assessment of the bactericidal action of montmorillonite containing sorbent and its combination with enrofloxacin and the sorption activity of the sorbent in relation to *E. coli*, was performed by confocal laser scanning microscopy [16] using the fluorescent probe 5-carboxyfluorescein diacetate (5-CARBOXYFLUORESCHEIN DIACETATE, C4916 SIGMA-ALDRICH) [17]. For the study, in sterile conditions, in five test tubes there were prepared samples of the following composition (in three replications): sample I – 9 ml of isotonic sodium chloride + 10,0 µg of enrofloxacin, sample II – 9 ml of isotonic sodium chloride solution + 20 mg of sorbent, sample III – 9 ml of isotonic sodium chloride + 10,0 µg of enrofloxacin + 20 mg of sorbent, sample IV – 9 ml of isotonic sodium chloride solution, sample V – 9 ml of the BEB. Then each tube was added by 1 ml of the day old culture of *Escherichia coli* whose concentration was 9×10^8 CFU in 1 ml. The content of the tube was thoroughly suspended. After that, all five samples were subjected to 24-hour exposure in thermostat at 37°C.

Stock solutions of the fluorescent probe were prepared according to the manufacturer's instructions. From stock solutions there was received a working solution. For this, 2 µmol of 5-carboxyfluorescein diacetate was dissolved in 1.5 ml of sodium chloride solution. 100 µl of test sample in the Petri dish of 35 mm diameter with a central hole and a cover glass (SPL Lifesciences), was added 10 µl of the fluorescent probe and incubated at 37°C for 30 min. After incubation the Petri dish was placed on the stage of the CLSM Nikon DIGITAL ECLIPSE C1 plus, the scanning of the samples was carried out at a wavelength of 488 nm. The

results of the CLSM we determined the number of living cells in a field of the microscope.

The study of the effect of ground on growth rate and reproduction of *Escherichia coli* was performed in the grounded Petri dishes. To ground a diameter of experimental dishes bottom was placed a strip of copper foil (1cm of width), wrapping the edges to the walls of the dish. One of the foil edges was soldered on copper wire, which was connected with the grounding device. Control Petri dishes were not grounded. In the experimental and control dishes were poured 15 ml of meat-and-peptone agar (MPA). After autoclaving the MPA was cooled to 42° and added by the day old culture of *Escherichia coli*, in the rate of 1×10^7 CFU per 1 ml of the culture medium. Then studied in the dishes *Escherichia coli* inoculation was cultured for 16-18 hours in a thermostat at 37°C. Next, from the surface of the MPA of every dish there were made wipe samples of *Escherichia coli* 8 ml of the sterile isotonic sodium chloride solution. Then we determined the number of CFU *E. coli* in 1 ml of wipe sample.

Determination of the concentration of *E. coli* was carried out with the help of the device for measuring the turbidity of bacterial suspension Densi-La-Meter, the principle of operation is based on the optical density of the suspension with the results of the measurement in McFarland units.

The final results were obtained using computer programs of variation statistics using the nonparametric student's t-test.

Research results

On the basis of the performed researches there was established the antibacterial activity of enrofloxacin and its combinations with enriched

montmorillonite containing sorbent in relation to *E. coli* (table. 1).

Presented in the table 1 data indicate a high sensitivity of *E. coli* to complex drug, combines 1:1 enrofloxacin with sorbent where the concentration of each drug is 0.063 µg/ml of the BEB. Minimum inhibitory concentration of enrofloxacin, against *E. coli* was lower (0.125 µg/ml) in comparison with the above composition. This combination, as the experiment showed, in addition to well-known detoxification function of the enterosorbent (active absorption of toxins reduces both local and general toxicity, metabolic load on the organs of detoxification: liver, kidneys, immune system) leads to a synergistic enhancement of the bacteriostatic effect. Also, we should pay attention to the gradual increase in the number of microbial cells in the liquid culture medium (from 7 to 9 tubes) in which the content of enrofloxacin decreased by double dilutions. This effect was not evident in the combination of enrofloxacin with a sorbent. Using the combination of enrofloxacin with sorbent led to more rapidly growing of the number of microbial cells (8-9 tubes), this suggests that enrofloxacin partially bound with the sorbent.

Guided by the study results, one may talk of the combination of enrofloxacin with the sorbent at the respective concentrations of 2, 1, 0.5 µg/ml and 4, 4, 2 mg/ml has a bacteriostatic effect. Mechanism of antimicrobial action of the studied combinations looked different, if the level of the sorbent increased to 4-2 mg/ml of the culture medium. Constant values of densilameter as before as after cultivation of the studied strain coupled with the above drug combinations support a conclusion drawn.

Table 1

The sensitivity of *Escherichia coli* to enrofloxacin and its combination with the montmorillonite-containing sorbent

№	Drug	Sorbent concentration, µg/ml.									Control	
		4	2	1	0.5	0.25	0.125	0.063	0.031	0.016		
Optical density in McFarland units, mg/ml												
1	Enrofloxacin	– 0	– 0	– 0	– 0	– 0	– 0	– 0	+	+	+	+5.0
2	Enrofloxacin + Sorbent	– 0	– 0	– 0	– 0	– 0	– 0	– 0	+	+	+	+ 5.2
3	Enrofloxacin + Sorbent	– 4	– 4	– 4	+	+	+	+	+	+	+ 5.1	
		Value of an optical opacity before cultivation										
		9.8	9.8	7.0	7.0	7.0	7.0	7.0	7.0	7.0		
		Value of an optical opacity after cultivation										
9.8	9.8	7.0	10.0	11.0	11.0	11.0	11.0	11.0	11.0			
Control												
1	Sorbent	+	+	+	+	+	+	+	+	+	+	+ 4.7
2	Enrofloxacin + Sorbent	– 0	– 0	– 0	– 0	– 0	– 0	– 0	– 0	– 0	– 0	

Comment: + a presence of the bacterial growth; – an absent of the bacterial growth.

In later combinations with lower concentrations of enrofloxacin from 0.016 to 0.25 mcg/ml, but with a consistent level of sorbent (2 mg/ml), there was a growth of *Escherichia coli* with McFarland value of the optical density by 1-2 units lower than in control. It suggests that the sorbent at high concentrations does not bind fully enrofloxacin. Also it may be assumed unstable immobilization of enrofloxacin by sorbent by forming on its surface specific ligands. In this case, the formed ligands rather easily strip from the surface of the sorbent, and enrofloxacin gets in a liquid culture medium. In such an embodiment, the system sorbent + immobilized fluoroquinolone has a certain buffer capacity, i.e., working as a warehouse, from which as needed a certain amount of an antibacterial drug can be allocated.

In addition, the immobilization of the antibacterial complexes on the surface of enterosorbents allows creating a range of new complex drugs that combine sorption and antimicrobial properties. A rational solution of a

problem in this way optimizes and minimizes a consumption of antibacterial agents and, in some cases, increases its specific activity due to the transition from bulk to surface concentration.

In the broth of the control tubes, free of the studied drugs there were observed active growth and reproduction of *Escherichia*, the concentration of microorganisms according to the densilameter, ranged up to 4.7 from 5.2 units. Identical concentrations of *E. coli* were recorded in the control test tubes with different amounts of the sorbent. The absence of the microorganisms growth in the second control series of test tubes, containing double dilutions of composite drug, is indicative to sterile conditions of the experiments.

The results of the experiments to identify the effect of concentration of enriched montmorillonite containing sorbent and pH of the culture medium on the sensitivity of *E. coli* to antimicrobial drugs distinguished with reliable results (table 2).

Table 2

The sensitivity of *Escherichia coli* to enrofloxacin and its combination with the montmorillonite-containing sorbent in different pH and sorbent concentration in MHA

№	Sorbent concentration, µg/ml	Inhibition zone, mm					
		pH of culture medium is 6		pH of culture medium is 7		pH of culture medium is 8	
		Test	Control	Test	Control	Test	Control
		Enrofloxacin Doxycycline	Enrofloxacin Doxycycline	Enrofloxacin Doxycycline	Enrofloxacin Doxycycline	Enrofloxacin Doxycycline	Enrofloxacin Doxycycline
1	2000	8.2±0.04		11.2±0.21		13.1±0.08	
		8.4±0.04		8.0±0.04		–	
2	1000	9.1±0.08		12.5±0.08		14.1±0.08	
		10.1±0.13		8.6±0.04		–	
3	500	10.1±0.08		13.1±0.04		15.0±0.13	
		12.2±0.04		9.2±0.17		–	
4	250	10.1±0.08		16.0±0.13		16.1±0.04	
		9.7±0.21		9.2±0.04		–	
5	125	10.1±0.08		16.0±0.13		16.1±0.04	
		11.3±0.13		10.0±0.00		–	
6	62.50	12.6±0.08	16.2±0.17	16.1±0.04			
		12.0±0.04	10.0±0.17	–			
7	31.25	12.2±0.13	16.1±0.04	16.2±0.13			
		12.3±0.08	10.3±0.17	–			
8	15.63	12.8±0.21	16.6±0.13	16.2±0.13			
		13.1±0.04	10.0±0.00	–			
9	7.81	13.0±0.42	17.0±0.42	16.5±0.13			
		11.4±0.33	10.0±0.17	–			
10	3.91	13.1±0.21	17.0±0.84	16.8±0.25			
		12.0±0.84	10.1±0.04	–			
		12.8±0.004	16.0±0.13		16.1±0.08		
		10.0±0.004	10.2±0.004		8.0±0.04		

Comment: numerator – enrofloxacin; denominator – doxycycline; – an absence of the inhibition zone.

The information contained in the table, fully illustrate the mechanism of the inhibitory and synergistic effect of the studied substances. The integration of antibacterial drugs and enriched montmorillonite-containing sorbent into a single

system led to emergent effect. Consequently, the significantly different sizes of the inhibition zones of *E. coli* in the control and test Petri dishes are the basis of the established fact.

Judging by the size of the inhibition zones on MHA of the control dishes as result of bacteriostatic action of enrofloxacin and doxycycline, a fact that pH of the culture medium without sorbent deserves a special attention. Based on a quantitative material of the experiment, it must be concluded that the range of inhibiting the growth of *Escherichia coli* by enrofloxacin increases with increasing pH (from 6 to 8) of the culture medium, and for doxycycline it is reduces.

A similar mechanism of action of these drugs was observed on the test samples of MHA with double serial dilutions of the sorbent. As it follows from the attached table, the process of bacteriostasis of *Escherichia coli* by enrofloxacin, diffusing from the disk in MHA depends not only on the pH of the culture medium, but also on the concentration of the sorbent in it. A decrease in the sizes of the inhibition zones of *Escherichia coli* by enrofloxacin in acidic culture medium (pH 6) in comparison with the control was at the concentrations of the sorbent from 2000 to 31.25 µg/ml. Such results were in neutral (pH 7) and alkaline culture medium (pH 8), but at the concentrations of the sorbent from 2000 to 500 µg/ml.

Go by the obtained data there was revealed that the sorption activity of enriched montmorillonite containing clay in relation to organic compounds, in an acidic medium is higher than in alkaline.

Also the pH of the culture medium and the concentration of the sorbent have an impact on the decrease in bacteriostatic action of doxycycline. However, a revealed in table dependence fall a long way short of previous, because a decrease of the pH of culture medium to the acid side in the presence of sorbent at the concentration of 2000 µg/ml, blunts the effectiveness of doxycycline. In the neutral culture medium at the concentration of the sorbent from 2000 to 250 µg/ml the bacteriostasis of *Escherichia coli* by doxycycline was not significantly achieved its control value. Lower concentrations of sorbent (from 125 to 3.91 µg/ml) in most cases only approached the inhibitory activity of doxycycline to the control value. In alkaline culture medium (pH 8) sorbent in

the used concentrations completely inhibited the antimicrobial effect of doxycycline.

We must assume that the prerequisites for the manifestation of the inhibition of antibacterial activity of enrofloxacin and doxycycline there was increasing a dissociation of aluminum ions, iron, magnesium, calcium, sodium from the montmorillonite-containing sorbent in the culture medium, which have formed an inactive chelate complex with the drugs [18].

Only at a very low concentrations of the sorbent 7.81-3.91 (the pH of the culture medium 6); 62.50-3.91 (the pH of the culture medium 7) and 31.25 to 3.91 µg/ml (the pH of the culture medium 8) there was observed a potentiating effect of enrofloxacin. Identical result was observed for doxycycline if in an acidic culture medium the concentration of the sorbent was not higher than 1000 µg/ml.

Explanation of high potentiating efficacy of the drugs, apparently, is the process of immobilization on its surface of the active ligands.

The analysis of fluorescent images was established that the largest number of live bacteria (196.67 ± 8.16) was in the sample V, and the complete absence of live *E. coli* detected in the sample I. There was revealed non-significant difference of the content of viable cells of *E. coli* in samples IV (31.0 ± 15.34) and II (35.83 ± 12.45), which indicated an absence of antibacterial and growth promoting properties of enriched montmorillonite-containing sorbent. Along with it false differences observed between the sample IV (31.0 ± 15.34) and sample III (22.17 ± 4.49) that indicated the decrease in bactericidal effectiveness of combination of enrofloxacin with the sorbent. A significant difference ($p < 0.05$) between samples II and III confirms the possibility of partial binding of enrofloxacin by montmorillonite-containing sorbent.

Fluorescence microscopy of samples containing sorbent, confirms the adsorption of *Escherichia coli* by containing montmorillonite sorbent. In addition, none of *E. coli*, fluorescent light green glow was separately from the sorbent particles (figure 1-2).

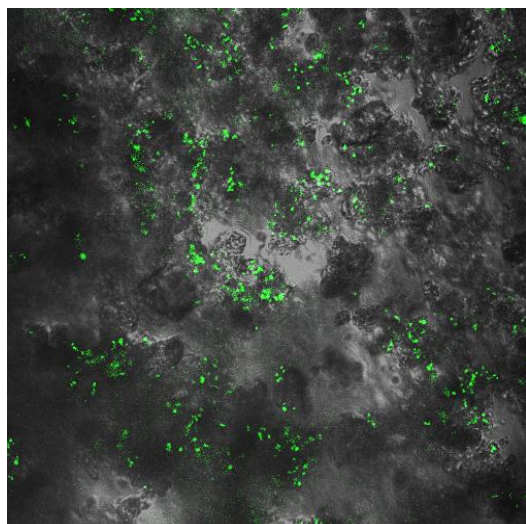


Figure 1. Fluorescent glow of live *E. coli* on the surface of sorbent particles (sample II)

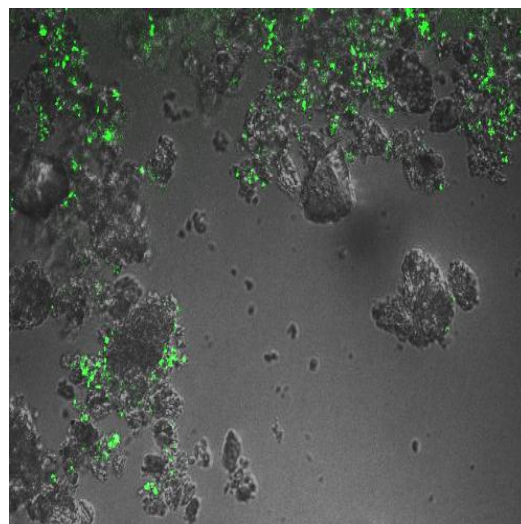


Figure 2. Fluorescent glow of live *E. coli* on the surface of sorbent particles (sample III)

Thus, the results of confocal laser scanning microscopy found that the sorbent based on montmorillonite containing clay has no antibacterial and growth promoting properties against *Escherichia coli*, wherein the sorbent effectively accumulates on the surface of live *E. coli*. However, in the combination of the sorbent with enrofloxacin, a bactericidal effectiveness of last against *E. coli* decreases.

In wipe samples from the surface of MPA of the ground dishes the concentration of *Escherichia coli* was 42×10^8 CFU/ml, and unground – 36×10^8 . Studies showed that in ground Petri dishes on the MPA growth of *E. coli* was in 1.2 times more than in ungrounded dishes.

Conclusion

Summarizing above materials it may be noted that the creation of new drugs enhances the use of enterosorption in complex treatment of animals suffering from acute infectious intestinal disease. In general, the development of domestic composite antimicrobial drugs on the basis of enriched montmorillonite containing sorbent allows you to make broader use of effective health-preserving and independent from importing technologies for the prevention and treatment of many pathologies. In addition, the rational use of composite antimicrobial preparations can be used for dosed introduction of drugs in the reverse desorption.

The creation of drugs of the specified group associated with the use of selective enterosorbents with known chemical nature of their surface and pore size and the characteristics of therapeutic action in various parts of a gastrointestinal tract with consideration of pulp pH and the desired concentration of the sorbent.

Giving enterosorbents specific properties by immobilization on its surface of the medicinal substances in the form of active ligands is a perspective direction, allowing to optimize and to minimize the consumption of antibacterial agents and, in some cases, to increase its specific activity due to the transition from bulk concentration to surface. The solution to the current problem in this way reduces or even eliminates the negative impact on the body chemotherapeutic substances. The use of this approach makes it possible, on the basis of already existing drugs quickly create prophylactic and therapeutic complex drugs with high efficiency.

The movement of mineral nutrients and water in the microbial cells is governed by the electricity energy. Ground prevents electrical instability and compensates for a deficiency of electrons constantly pulsating on Earth surface. Ground naturally protects the sensitive bioelectrical system of the body, bacteria from static electrical charges and interference, that greatly affects their growth and development.

References

1. Макаров, В.В. Синантропизация, ветеринарная эпидемиология и зоонозы / В.В. Макаров // *Ветеринарная Патология*. – 2011. – № 4 (38). – С. 7-18. [\[eLIBRARY\]](#) [\[Full text\]](#)
2. Методические рекомендации по применению комплексных антибактериальных препаратов на основе монтмориллонит содержащих глин с энрофлоксацином и тимолом при колибактериозе птиц / В.А. Антипов, А.Н. Трошин, Н.П. Зуев, А.В. Хмыров, В.Д. Буханов, А.И. Везенцев, О. Н. Панькова, П.В. Соколовский, С.Н.Зуев, Р.З. Курбанов. – ФГБНУ КНИВИ. – Краснодар. – 2015. – 48 с. [\[eLIBRARY\]](#)
3. Эффективность композиционного препарата на основе наноструктурных монтмориллонит

содержащих глины при колибактериозе птиц / Зуев Н.П., Буханов В.Д., Хмыров А.В. [и др.] // Сборник: «Актуальные вопросы ветеринарной медицины и технологии животноводства» Материалы научной и учебно-методической конференции профессорско-преподавательского состава, научных сотрудников и аспирантов факультета ветеринарной медицины и технологии животноводства. ФГБОУ ВО "Воронежский государственный аграрный университет имени императора Петра I". – 2016. – С. 11-15. [\[eLIBRARY\]](#)

4. ExPEC-typical virulence-associated genes correlate with successful colonization by intestinal E. coli in a small piglet group / P. Schierack, N. Walk, C. Ewers, H. Wilking, H. Steinrueck, M. Filter, L.H:Wieler // *Environ Microbiol.* – 2008. – Vol.10, №7. – P. 1742-1751. doi:10.1111/j.1462-2920.2008.01595.x. [\[PubMed\]](#) [\[Full text\]](#)

5. Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic Escherichia coli strains with zoonotic potential / C. Ewers, E.M. Antao, I. Diehl, H.C. Philipp, L.H. Wieler // *Appl Environ Microbiol.* – 2009. – Vol. 75, №1. – P. 184-192. doi:10.1128/AEM.01324-08. [\[PubMed\]](#)

6. Егоров, Н.С. Основы учения об антибиотиках: учебное пособие для студентов биологических специальностей университетов. / Н.С. Егоров // М.: Высшая школа. – 1979. – 456 с. [\[Full text\]](#)

7. Тараканов, Б. Г. Механизмы действия пробиотиков на микрофлору пищеварительного тракта и организм животных / Б.Г. Тараканов // *Ветеринария.* – 2000. – №1. – С. 47-54. [\[eLIBRARY\]](#)

8. Пирожков М.К., Ленев С.В., Викторова Е. В., Стрельченко С.А., Тихонов Л.И., Скляр О.Д. Диагностика, специфическая профилактика и лечение при бактериальных болезнях животных // *Ветеринария.* – 2011. – №1. – С. 24-27. [\[eLIBRARY\]](#)

9. Пейсак, З. Болезни свиней / З. Пейсак; пер. с польск. под ред. Д.В. Потапчука, В.В. Петрова. – Брест: ОАО «Брестская типография». – 2008. – 424 с. [\[Full text\]](#)

10. Эфферентная терапия / под ред. А.Л. Костюченко. – СПб: ООО «Издательство Фолиант», 2003. – 432 с. [\[eLIBRARY\]](#) [\[Full text\]](#)

11. Разработка монтмориллонит содержащей матрицы биоактивного сорбирующего раневого покрытия / К.Н. Касанов, В.А. Попов, М.В. Успенская [и др.] // *Научные ведомости БелГУ. Естественные науки.* – 2011. – Вып. 14. – №3 (98). – С. 168-173. [\[eLIBRARY\]](#) [\[Full text\]](#)

12. Применение фитоаскорбоминералосорбента при колибактериозе телят и дизентерии свиней / В.Д. Буханов, А.И. Везенцев, А.А. Шапошников [и др.]

// *Научные ведомости БелГУ. Серия «Естественные науки».* – 2010. – Вып. 11. – №9 (80). – С. 99-103. [\[eLIBRARY\]](#) [\[Full text\]](#)

13. Применение активированной монтмориллонитовой глины в остром эксперименте на цыплятах, зараженных колибактериозом и сальмонеллезом / В.Д. Буханов, А.И. Везенцев, А.А. Антипов [и др.] // *Актуальные вопросы ветеринарной биологии.* – 2011. – № 4 (12). – С. 51-57. [\[eLIBRARY\]](#) [\[Full text\]](#)

14. Учайкин В. Ф., Новокшенов А. А., Соколова Н. В., Бережкова Т. В. Энтеросорбция – роль энтеросорбентов в комплексной терапии острой и хронической гастроэнтерологической патологии // *Пособие для врачей.* М. – 2008. – 24 с. [\[Full text\]](#)

15. Заземление – Самое важное открытие о здоровье? / Клинтон Обер, Синатра Стивен, Зукер Мартин // *Перев с англ.* – М.:ООО Издательство «София», 2012. – 320 с. [\[Full text\]](#)

16. Руководство по конфокальной микроскопии / Штейн Г.И. – СПб: ИНИЦ РАН, 2007. – 77 с. [\[eLIBRARY\]](#) [\[Full text\]](#)

17. A comparison of fluorescent stains for the assessment of viability and metabolic activity of lactic acid bacteria / T. Zotta, A. Guidone, P. Tremonte, E. Parente, A. Ricciardi // *World Journal of Microbiology and Biotechnology March.* – 2012. – Vol. 28. – P. 919-927. [\[PubMed\]](#)

18. Anderson, C.R. Complexities in tetracycline analysis chemistry, matrix extraction, cleanup, and liquid chromatography / C.R. Anderson, H.S. Rupp, W.H. Wu // *J. Chromatogr. A.* – 2005. – Vol. 1075. – P. 23–32. [\[PubMed\]](#)

Bukhanov Vladimir Dmitrievich – Ph.D. in veterinary science, assistant professor of Department of Biomedical Foundations of Physical Culture.

Vezentsev Aleksandr Ivanovich – Doctor of Engineering Sciences, Professor.; Head of the Department of general chemistry.

Filippova Olga Vsevolodovna – MD-PhD, Professor, Pharmaceutical Technology and Pharmacology Department.

Nadezhdin Sergei Victorovich – Ph.D. in biology, assistant professor of Department of biology.

Pankova Olga Nikolaevna – Researcher of campus.

Firsova Tatyana Ivanovna – Gastroenterologist.

Mikhailyukova Variya Olegovna – Postgraduate student of the Department of general chemistry of Medical institute.

Tishin Anton Nikolaevich – Postgraduate student of pharmacological department.