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CORRECTION OF THE SEXUAL FUNCTION IN DOMESTIC ANIMALS BY MEGESTROL ACETATE

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Summary. Correction of sexual function in animals is a topical issue today, despite a large amount of scientific data on this problem. The study aimed to scientifically substantiate the effectiveness of hormonal veterinary drugs in cats and dogs of different breeds and genders. Veterinary drugs with the active substance megestrol acetate were used in the experiments. The studies were performed on clinically healthy cats and dogs of both sexes, different breeds, and ages vaccinated against infectious diseases and treated with antiparasitic drugs. The study was conducted according to the current regulatory documentation. It has been found that the use of hormonal drugs to interrupt/delay estrus in female cats at a dose of 5 mg of megestrol acetate for 8 days leads to a significant decrease in follicle-stimulating hormone, and the content of luteinizing hormone in the blood of cats in the experimental groups reliably decreased throughout the experiment. The use of contraceptives based on megestrol acetate in doses of 2.5 mg for 14 days for cats leads to a reliable decrease in the concentration of testosterone in the serum. It was found that the use of experimental hormonal drugs for female dogs in doses of 5 mg of megestrol acetate per 2.5 kg of body weight in the first 3 days, and half the daily dose from the 4th to the 10th day, leads to a reliable decrease in concentrations of follicle-stimulating and luteinizing hormones in serum compared to the control. Oral administration of experimental hormonal drugs to male dogs in doses of 5 mg of megestrol acetate per 2.5 kg of body weight for 8 days, led to a reliable decrease in the concentration of testosterone in the serum of males. It was found that the estrous cycle in female cats and dogs of the experimental groups after the cessation of hormonal drugs was completely restored, which indicates the safety of drugs with an active ingredient megestrol acetate

Keywords: dogs, cats, hormonal drugs

Introduction. The issue of preserving the reproductive potential of animals is a very important issue in veterinary science and practice (Shakhova et al., 2021). In this regard, veterinary hormonal contraceptives, which are used to regulate the sexual cycle and correct behavior in cats and dogs, are especially popular (Wildt, Brown and Swanson, 1998; Romagnoli, 2015).

Conservative method of contraception is the use of synthetic hormonal highly active drugs (Ginel et al., 2012; Attalah et al., 2016). The use of contraceptives often provides rapid suppression of undesirable behavior of the female during estrus and creates an alternative to surgery (Vasetska, 2020; Paliy et al., 2021).

The effects of exogenous sex hormones on the hormonal status of female cats and dogs during the sexual cycle and changes in progesterone levels in animal blood remain poorly understood (Akison and Robker, 2012).

Therefore, there is a need for research in the field of suppression of sexual function in females of small domestic animals (dos, cats) for more effective and safe use of hormonal contraceptives and the development of new, alternative, non-surgical, safe schemes and methods of drug suppression of sexual arousal with minimal risk of pathology of the reproductive system, breast and adverse effects on the body as a whole (Sarasola et al., 2002; Asa, 2018; Urfer and Kaeberlein, 2019). The estrous cycle in cats and dogs is manifested by morphological changes that are cyclically repeated in the reproductive system of females, which are associated with the maturation of gametes and their release into the abdominal cavity during ovulation. Hormones of the hypothalamic-adenohypophyseal system and ovary are involved in the regulation of the estrous or sexual cycle (Burke, 1976; Aspinall, 2011). The estrus corresponds to the period of sexual activity of females and coincides in time with the maturation of follicles in the ovaries (follicular phase of the sexual cycle). It is also called one of the stages of the vaginal cycle of animals (corresponds to the end of the sexual cycle) (Concannon, 2011).

Manifestations of normal physiological activity of the reproductive system in cats and dogs are accompanied by hypersexual, aggressive and antisocial behavior, which brings significant inconvenience to owners. For a long time, scientists have been developing non-surgical, safe for animals, easy to use, most effective, with regulated period of effect on the body drugs for the suppression of sexual function of animals (Diederichs et al., 1991; Junttila, Huohvanainen and Tiira, 2021).

Drugs that regulate the sexual cycle in female cats are also widely used because of the specific behavior that a cat exhibits during estrus (Greenberg et al., 2013; Driancourt and Briggs, 2020).

An important requirement for hormonal contraceptives used in veterinary practice is long-term prevention (suppression of estrus), short-term delay of estrus or interruption of estrus at different stages of the sexual cycle (Refsdal, 2000).

Scientists have found that progestin megestrol acetate is the most commonly used. Megestrol acetate is a synthetic progestogen, affects the hypothalamic-pituitary system of the animal, blocks the secretion of gonadotropic hormones by the adenohypophysis (follicle-stimulating and luteinizing). Decreased levels of these hormones in the blood of animals lead to impaired folliculogenesis in females, which provides antiestrogenic and antiovulatory effects on the gonads of animals, resulting in delayed, suppressed, and interrupted estrus and suppression of sexual desire (Luvoni, 2000; Han et al., 2014; Jang et al., 2014).

Sexual desire in cats and dogs after receiving the full course dose is renewed in 3–4 months. Megestrol acetate is well absorbed in the gastrointestinal tract and excreted with the urine within 1–2 days after administration to the animal's body (Mertens, 2006; Wiebe and Howard, 2009).

Megestrol acetate binds to cytoplasmic progesterone receptors. After transfer of progesterone receptor complexes from megestrol acetate to the cell nucleus, RNA synthesis is stopped, which in turn inhibits protein synthesis. This reduces the number of cytoplasmic estrogen receptors, that is estrogen cannot reach the target molecule and cause DNA damage in the cell nucleus.

Megestrol acetate posseses a high affinity for the progesterone receptors, and significant ability to bind to androgen and glucocorticoid receptors. Megestrol acetate reduces the release of follicle-stimulating hormone in the pituitary gland and, as a consequence, slows down the synthesis of estrogen in the ovaries relative to the synthesis of androgen in the testes. Megestrol acetate counteracts the stimulating effect of estrogen on the growth of hormone-receptor of positive cell lines, reduces the secretion of luteinizing hormone in the pituitary gland. According to animal studies, megestrol acetate reduces the secretion of prolactin in the pituitary gland. According to studies in animals, megestrol acetate reduces the secretion of adrenocorticotropic hormone in the pituitary gland (Colon et al., 1993; Kutzler and Wood, 2006).

Megestrol acetate is almost completely absorbed. After oral administration of one dose of megestrol acetate, the maximum concentration in blood plasma is reached within 2–3 h. The level of concentration in blood plasma depends on the dose, but is not directly proportional to it (Chainey, McCoubrey and Evans, 1970). The half-life is 15–20 h. At steady state, which is reached on the 3rd day of oral administration of the drug, the peak concentration in plasma is 90%. Megestrol acetate is metabolized in the liver. According to the results of the analysis of urine, which was collected within 7 days, megestrol acetate is excreted from the body in the urine by 56–78%, and in the feces for the same period — by 8–30%.

Along with the use of hormonal drugs, the issue of preserving the functions of germ cells for further reproduction of animals both naturally and through biotechnological methods remains relevant (Smorag et al., 2008; Shakhova et al., 2020).

The aim of the study was to scientifically substantiate the effectiveness of hormonal veterinary drugs in cats and dogs of different breeds and genders.

Materials and methods. Veterinary drugs were used in the experiments:

— composition of the drug No. 1 (1 tablet (0.25 g)): active substance: megestrol acetate (5 mg); excipients: lactose, calcium stearate;

— composition of the drug No. 2 (1 tablet (0.30 g)): active substance: megestrol acetate (5 mg); excipients: powdered sugar, calcium carbonate, carboxymethylcellulose sodium salt, polyvinylpyrrolidone, flavoring, magnesium stearate, sucram.

Studies of the effectiveness of veterinary drugs were conducted in the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv) and in the animal shelter (Balaklia, Kharkiv Region).

The experiments were performed on clinically healthy cats and dogs of both sexes, different breeds and ages, vaccinated against infectious diseases and treated with antiparasitic drugs.

Cats and dogs were kept in cages on a standard diet with free access to water.

Prior to the experiment, the animals underwent clinical studies, including examination, palpation, thermometry, studies of respiratory rate, heart rate. Animal body weight, fatness, condition of skin, auricles, teeth, oral mucosa were recorded. The hormonal status of cats and dogs was studied, and the phases of the sexual cycle (follicular and luteal) were determined. During the quarantine period, cats and female cats of both experimental groups showed signs of sexual hunting. The obtained data were entered into individual registration cards of animals. Prior to the experiment, the animals of the experimental groups were treated against ectoparasites and helminths, after that they were quarantined for one month (Kotsiumbas, 2013).

To conduct research, control and research groups were formed on the principle of analogues, taking into account body weight, age and type of animal constitution.

At the first stage of the study, experiments were conducted to establish the effectiveness of veterinary drugs in female cats and cats. For this purpose, one control and two experimental groups of female cats with five animals in each were formed. The animals had a

normal estrous cycle. Female cats of the experimental group I on the 1st day of sexual hunting were given oral drug No. 1 for interruption of heat (1 tablet daily for 8 days from the beginning of heat).

Female cats of the second experimental group were given drug No. 2 following the similar scheme.

The cats in the control group were not given hormonal contraceptives. The animals received dry food and pure drinking water. Prior to administration, on the 1st, 3rd, 5th, and 8th days of the experiment, blood samples were taken from cats for endocrinological studies. Quantitative determination of progesterone, follicle-stimulating hormone, and luteinizing hormone was performed.

The next stage of research was to conduct an experiment on cats. At the appearance of signs of sexual arousal, hormonal drugs were given to cats 1–2 tablets per day for 14 days for sedation. Cats in the control group were not given hormonal contraceptives. On 1st, 3rd, 7th, and 14th days of the experiment, blood samples were taken from cats to quantify testosterone.

At the second stage of the study, a series of experiments were conducted to establish the effectiveness of hormonal veterinary drugs on female and male dogs. Females of the first experimental group were orally administered the drug No. 1 according to the scheme for interruption of the heat (1 tablet per 2.5 kg of body weight in the first 3 days, and half the daily dose from the 4th to the 10th day). Females of the second experimental group were given drug No. 2 following a similar scheme.

Female dogs of the control group were not given hormonal contraceptives. The animals received dry food and pure drinking water. On 1st, 3rd, 4th, 7th, and 10th days of the experiment, blood samples were taken from female dogs to determine progesterone, follicle-stimulating hormone, and luteinizing hormone.

In the second experiment, hormonal drugs were given to female dogs following the scheme to delay heat (1 tablet per 10 kg of body weight for 7 days before the start of heat for 32 days). On 1st, 7th, 14th, and 32nd days of the experiment, blood samples were taken from bitches to quantify progesterone, follicle-stimulating hormone, and luteinizing hormone.

In an experiment on male dogs, when signs of sexual arousal, the drugs were given to calm down following the scheme: 1 tablet per 2.5 kg of body weight for 8 days, then 1–2 tablets per 2.5 kg for the next 8 days. On 1st, 5th, 7th, 10th, and 16th days of the experiment, blood samples were taken from males to quantify testosterone.

The blood of cats and dogs for testing was obtained from the forearm lateral subcutaneous vein (*v. cephalica*). Blood sampling was performed following the rules of asepsis and antiseptics. Serum was obtained by settling for 15 min in test tubes in a thermostat. The serum was separated from the clot with the help of a stainless steel rod. Centrifugation was performed at 3,000 rpm for 20 min. Serum was collected using a dosing pipette into sterile Eppendorf tubes.

The concentration of sex hormones, namely progesterone, follicle-stimulating hormone, and luteinizing hormone was determined in the serum of female cats and dogs. Testosterone levels were determined in the serum of male cats and dogs. Fresh serum was used for the studies.

Enzyme-linked immunosorbent assays of blood to determine the concentration of progesterone, follicle-stimulating hormone, and luteinizing hormone in the serum of female cats and dogs were performed using 'Granum' test systems (Ukraine).

The principle of competitive enzyme-linked immunosorbent assay was used to study progesterone. The test sample and conjugate (peroxidase-labeled progesterone) were added to the well with the immobilized antigen (specific anti-progesterone antibodies). The progesterone in the sample competes with the conjugate for binding to the antigenic surface of the well. After washing, the activity of the enzyme bound on the surface of the well was shown by the addition of substrate and measured at a wavelength of 450 nm. The intensity of the color reaction was inversely proportional to the amount of progesterone in the sample.

The principle of two-site enzyme-linked immunosorbent assay (sandwich method) was used to study luteinizing hormone. The test sample and conjugate (second peroxidase-labeled anti-luteinizing hormone antibodies) were added to the well with the immobilized antigen (specific luteinizing hormone antibodies). Luteinizing hormone from the sample binds to the antigen on the surface of the well and the conjugate.

Unbound material was removed by washing. After washing, the activity of the enzyme bound on the surface of the well was shown by adding the substrate and measured at a wavelength of 450 nm. The intensity of the color reaction was directly proportional to the amount of luteinizing hormone in the sample.

The principle of two-site enzyme-linked immunosorbent assay (sandwich method) was used to study follicle-stimulating hormone. The test sample and conjugate (second peroxidase-labeled anti-follicle-stimulating hormone antibodies) were added to the well with the immobilized antigen (specific anti-follicle-stimulating hormone antibodies). Follicle-stimulating hormone from the sample binds to the antigen on the surface of the well and the conjugate. Unbound material was removed by washing. After washing, the activity of the enzyme bound on the surface of the well was shown by the addition of substrate and is measured at a wavelength of 450 nm. The intensity of the color reaction is directly proportional to the amount of follicle-stimulating hormone in the sample.

The principle of competitive enzyme-linked immunosorbent assay was used to study testosterone. The test sample and conjugate (peroxidase-labeled testosterone) were added to the well with the immobilized antigen (specific anti-testosterone antibodies). The testosterone from the sample competes

with the conjugate for binding to the antigen on the surface of the well. After washing, the activity of the enzyme bound on the surface of the well was shown by the addition of substrate and measured at a wavelength of 450 nm. The intensity of the color reaction is inversely proportional to the amount of testosterone in the sample.

Statistical processing of the results was performed by Student's *t*-test with STATISTICA v. 10.0 for Windows (Rebrova, 2006).

Experiments on animals were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and in accordance with Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). The research program was reviewed and approved by the Bioethics Commission of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' in the current order.

Results and discussions. To achieve this goal, a series of experiments were conducted to determine the effectiveness of hormonal contraceptive veterinary drugs in female cats, cats, female and male dogs.

When studying the effectiveness of veterinary drugs in female cats and cats, we conducted clinical studies of animals in control and research groups. No deviations from the physiological norm were registered. Animals of the control and experimental groups were active, willingly took food and water, visible mucous membranes were pink, normal respiration and heart rate, average fatness. It was found that throughout the experiment, female cats periodically spontaneously aroused. In animals of the experimental groups, signs of sexual desire were registered before the use of veterinary drugs, as well as after discontinuation of drugs, when the sexual cycle resumed. It was found that the use of hormonal drugs leads to the suppression of signs of sexual desire in female cats of experimental groups.

Observations of the behavior of female cats showed that within 3–5 days of drug application, the sexual activity of animals was significantly reduced compared with the control group. In control group female cats, which did not received hormonal contraceptives during the experiment, showed clinical signs and behavioral changes characteristic of the stage of sexual arousal.

As a result of a comprehensive study of the effectiveness of experimental drugs, we determined the dynamics of the concentration of sex hormones in the serum of female cats and cats (Table 1).

Fig. 1 shows the dynamics of progesterone content in the serum of female cats receiving hormonal drugs to delay heat (M ± m, n = 5).

Fig. 1 shows that oral administration of hormonal drugs to delay heat at a dose of 5 mg of megestrol acetate every two weeks led to a reliable decrease in the concentration of progesterone in the serum, compared with control.

The data obtained (Table 1) indicate that the use of drugs for interruption of heat in female cats at a dose of 5 mg of megestrol acetate for 8 days led to a reliable decrease in follicle-stimulating hormone levels in animals of experimental groups I and II, namely: on the 3rd day — by 65.34% and 66.48% relative to control, on the 5th day — 48.18% and 49.27% respectively, on the 8th day — 73.10% and 69.66% respectively.

It was found that the content of luteinizing hormone in the blood of female cats of experimental groups I and II reliably decreased during the entire experimental period: on the 1st day — by 15.51% and 20.32% relative to control, on the 3rd day — 10.17% and 6.78% respectively, on the 7th day — 42.98% and 45.71% respectively, on the 14th day — 46.38% and 44.60% respectively. It should be noted that when using the drugs in females of the experimental groups we did not register changes in the general clinical state, animals willingly consumed food and water.

To study the effectiveness of hormonal drugs to delay heat in female cats, the concentration of sex hormones was determined (Table 2).

From Table 2 it is seen that oral administration of experimental hormonal drugs to female cats at a dose of 5 mg of megestrol acetate every 2 weeks, leads to a reliable decrease in follicle-stimulating hormone levels in animals of experimental groups I and II, namely: on the 3rd day — 51.98% and 57.43% compared to control, on the 5th day — 45.66% and 49.13% respectively, on the 8th day — 65.31% and 63.78% respectively.

It has been found that the content of luteinizing hormone in the blood of female cats of experimental groups I and II reliably decreased throughout the experiment period: on the 1st day — 18.75% and 10.68% compared to control, on the 3rd day — 27.04% and 30.53% respectively, on the 7th day — 50.65% and 45.29% respectively, on the 14th day — 34.69% and 31.23% respectively.

Experiments have shown that the use of contraceptives based on megestrol acetate in doses of 2.5 mg for 14 days in cats leads to a reliable decrease in serum testosterone (Table 3).

Table 3 shows that application of hormonal drugs to cats of experimental groups I and II at a dose of 2.5 mg of megestrol acetate for two weeks causes a reliable decrease in the concentration of testosterone in the serum: on the 3rd day — 56.85% and 52.15% relative to control, on the 7th day — 67.16% and 66.42% respectively, on the 14th day — 55.20% and 52.65% respectively.

According to the results of observations, it was found that the use of hormonal drugs in cats reduces the clinical manifestations of sexual arousal.

According to the results of the studies, the application of experimental hormonal drugs to female dogs in doses of 5 mg of megestrol acetate per 2.5 kg of body weight in the first 3 days, and half the daily dose from the 4th to the 10th day, leads to a reliable decrease in concentrations of follicle-stimulating and luteinizing hormones in blood serum, compared with control indicators (Table 4).

Table 1 — The concentration of hormones in the blood serum of female cats that received hormonal drugs for the interruption of heat (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	1st	3rd	5th	8th
Follicle-stimulating hormone, ng/ml					
Control	0.49 ± 0.01	0.56 ± 0.04	1.76 ± 0.04	2.74 ± 0.03	1.45 ± 0.01
Experimental I	0.58 ± 0.03	0.55 ± 0.06	0.61 ± 0.05**	1.42 ± 0.02**	0.39 ± 0.04**
Experimental II	0.56 ± 0.01	0.57 ± 0.03	0.59 ± 0.02**	1.39 ± 0.04**	0.44 ± 0.02**
Luteinizing hormone, nmol/l					
Control	3.26 ± 0.04	3.74 ± 0.14	6.49 ± 0.04	14.33 ± 0.15	20.18 ± 0.13
Experimental I	3.20 ± 0.06	3.16 ± 0.07**	5.83 ± 0.01**	8.17 ± 0.01**	10.82 ± 0.07***
Experimental II	3.32 ± 0.01	2.98 ± 0.02**	6.05 ± 0.02**	7.78 ± 0.03**	11.18 ± 0.11***

Notes:** — $p < 0.01$,*** — $p < 0.001$ according to the indicator in the control.

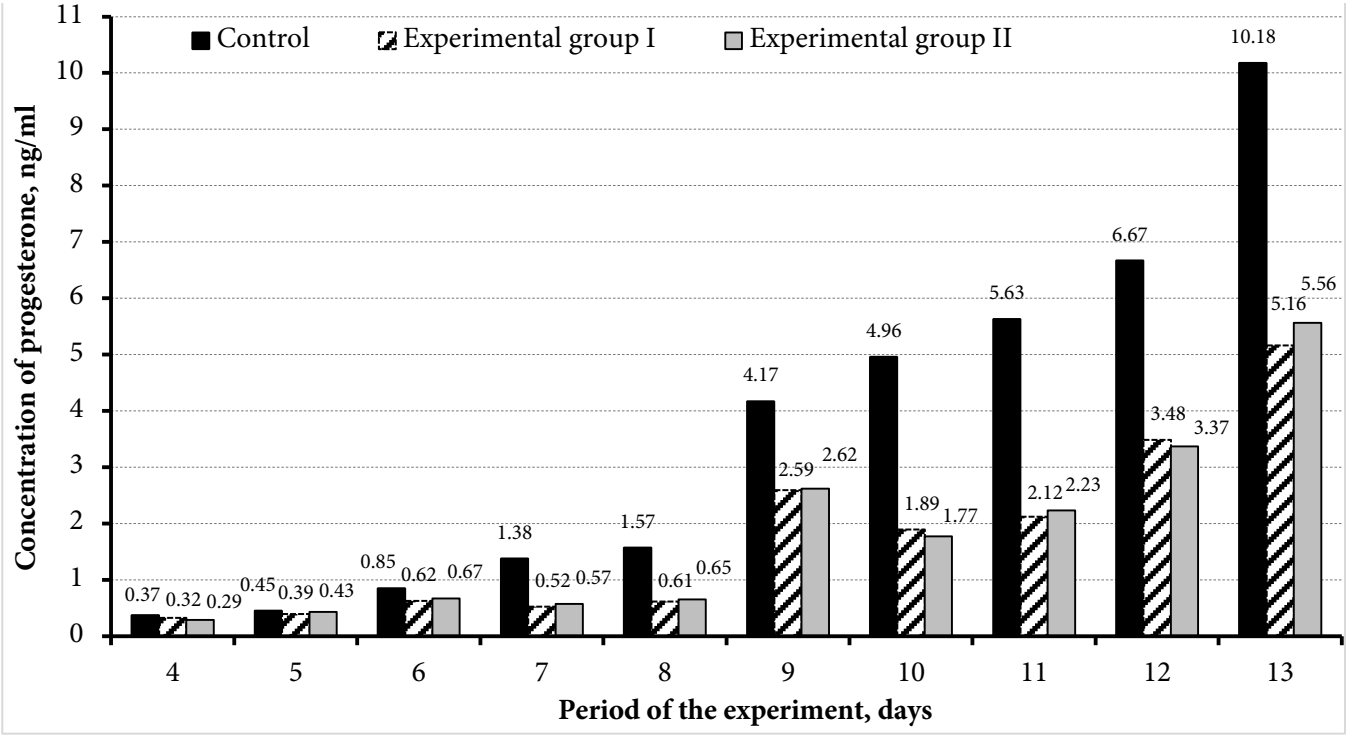

Figure 1. Dynamics of progesterone levels in the serum of female cats receiving hormonal drugs to delay heat (M ± m, n = 5)

Table 2 — The concentration of hormones in the serum of female cats receiving hormonal drugs to delay heat (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	1st	3rd	7th	14th
Follicle-stimulating hormone, ng/ml					
Control	0.59 ± 0.02	0.64 ± 0.05	2.02 ± 0.06	1.73 ± 0.02	1.96 ± 0.04
Experimental I	0.61 ± 0.05	0.60 ± 0.02	0.97 ± 0.03*	0.94 ± 0.15*	0.68 ± 0.07**
Experimental II	0.57 ± 0.02	0.58 ± 0.07	0.86 ± 0.05*	0.88 ± 0.10**	0.71 ± 0.03**
Luteinizing hormone, nmol/l					
Control	4.16 ± 0.09	3.84 ± 0.11	8.32 ± 0.10	14.55 ± 0.21	19.31 ± 0.52
Experimental I	3.58 ± 0.07	3.12 ± 0.09*	6.07 ± 0.08**	7.18 ± 0.12**	12.61 ± 0.10**
Experimental II	4.27 ± 0.04	3.43 ± 0.05*	5.78 ± 0.11**	7.96 ± 0.15**	13.28 ± 0.19**

Notes:* — $p < 0.05$,** — $p < 0.01$ according to the indicator in the control.

Table 3 — The concentration of testosterone (nmol/l) in the serum of cats, which received hormonal drugs to calm down when signs of sexual arousal (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	1st	3rd	7th	14th
Control	4.26 ± 0.01	4.29 ± 0.07	4.89 ± 0.07	5.45 ± 0.12	4.71 ± 0.04
Experimental I	4.15 ± 0.03	3.78 ± 0.04	2.11 ± 0.02*	1.79 ± 0.04*	2.11 ± 0.01*
Experimental II	3.96 ± 0.02	4.26 ± 0.03	2.34 ± 0.05*	1.83 ± 0.02*	2.23 ± 0.05*

Note.* — $p < 0.05$ according to the indicator in the control.

Table 4 — The concentration of hormones in the serum of female dogs, which received hormonal drugs (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	1st	3rd	5th	8th
Follicle-stimulating hormone, ng/ml					
Control	0.65 ± 0.01	0.73 ± 0.04	2.31 ± 0.01	2.97 ± 0.16	1.67 ± 0.19
Experimental I	0.56 ± 0.03	0.52 ± 0.06*	1.16 ± 0.03*	0.82 ± 0.01**	0.51 ± 0.05**
Experimental II	0.69 ± 0.05	0.68 ± 0.03*	1.57 ± 0.02*	0.94 ± 0.02**	0.46 ± 0.01**
Luteinizing hormone, nmol/l					
Control	3.21 ± 0.10	4.52 ± 0.15	8.12 ± 0.24	14.27 ± 0.15	19.57 ± 0.32
Experimental I	2.79 ± 0.12	4.18 ± 0.12	3.15 ± 0.17*	2.98 ± 0.12**	4.19 ± 0.25**
Experimental II	3.30 ± 0.14	4.26 ± 0.31	3.47 ± 0.29*	3.12 ± 0.11**	4.83 ± 0.42**

Notes:* — $p < 0.05$,** — $p < 0.01$ according to the indicator in the control.

Table 4 shows that oral administration of experimental drugs to female dogs at a dose of 5 mg of megestrol acetate per 2.5 kg of body weight in the first 3 days, and half the daily dose from the 4th to the 10th day, leads to delayed heat and a reliable decrease in follicle-stimulating hormone in animals of experimental groups I and II, namely: on the 1st day — 28.77% and 6.85% compared to control, on the 3rd day — 49.78% and 32.03% respectively, on the 5th day — 72.39% and 68.35% respectively, on the 8th day — 69.46% and 72.46% respectively.

It was found that the content of luteinizing hormone in the blood of female dogs of experimental groups I and II reliably decreased during the entire experiments: on the 3rd day — 61.21% and 57.16% respectively, on the 5th day — 79.11% and 78.14% respectively, on the 8th day — 78.59% and 75.32% respectively. It should be noted that during the application of drugs in female dogs of the experimental groups we did not register changes in the general clinical condition, animals willingly consumed food and water, the behavior did not differ from the animals of the control group.

Experimental drugs were used to delay heat (Table 5).

Table 5 shows that oral administration of drugs to female dogs in doses of 5 mg of megestrol acetate per 10 kg of body weight a week before the heat leads to a reliable decrease in the concentration of follicle-stimulating hormone in the blood of female dogs of the groups I and II on the 1st day — 31.33% and 24.09% compared to control, on the 3rd day — 69.70% and 67.15% respectively, on the 5th day — 83.41% and 81.52% respectively, on the 8th day — 82.39% and 77.84% respectively.

During the experiment, the concentration of luteinizing hormone was reliably lower than the control level, in particular: on the 3rd day — 53.25% and 53.92% respectively, on the 5th day — 79.03% and 79.62% respectively, on the 8th day — 87.15% and 86.21% respectively. In female dogs of the control group, the concentration of hormones was within the physiological norm, according to the specific phase of the sexual cycle.

During the clinical examination of female dogs of experimental groups I and II, no signs of heat in female dogs were registered.

According to the results of experimental studies, it was found that hormonal drugs affect the level of testosterone in the serum of male dogs (Table 6). The results of the experiment showed that oral administration of experimental hormonal drugs to male dogs in doses of 5 mg of megestrol acetate per 2.5 kg of body weight for 8 days, led to a reliable decrease in testosterone concentration in serum of male dogs of both experimental groups on the 3rd day by 68.99% and 68.84% respectively, on the 7th day — 26.85% and 21.75% respectively, on the 14th day — 53.98% and 51.99% respectively. The concentration of testosterone in the blood of male dogs of the control group was within the physiological norm. It was found that the estrous cycle in female cats and dogs of the experimental groups after the cessation of hormonal drugs was completely restored, which indicates the safety of drugs with the active ingredient megestrol acetate.

Table 5 — The concentration of hormones in the serum of female dogs, which received hormonal drugs to delay heat (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	1st	3rd	5th	8th
Follicle-stimulating hormone, ng/ml					
Control	0.78 ± 0.04	0.83 ± 0.03	2.74 ± 0.09	2.11 ± 0.07	1.76 ± 0.04
Experimental I	0.71 ± 0.02	0.57 ± 0.03*	0.83 ± 0.06**	0.35 ± 0.01**	0.31 ± 0.02**
Experimental II	0.68 ± 0.08	0.63 ± 0.04**	0.90 ± 0.02**	0.39 ± 0.06**	0.39 ± 0.05***
Luteinizing hormone, nmol/l					
Control	2.84 ± 0.03	4.05 ± 0.05	7.53 ± 1.02	15.21 ± 1.13	19.22 ± 2.11
Experimental I	2.79 ± 0.07	3.99 ± 0.11	3.52 ± 0.04*	3.19 ± 0.12*	2.47 ± 0.18**
Experimental II	2.80 ± 0.12	4.16 ± 0.12	3.47 ± 0.08**	3.10 ± 0.15**	2.65 ± 0.23***

Notes:* — $p < 0.05$,** — $p < 0.01$,*** — $p < 0.001$ according to the indicator in the control.

Table 6 — The concentration of testosterone (nmol/l) in the serum of male dogs, which received hormonal drugs at the appearance of signs of sexual arousal for sedation (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	1st	3rd	7th	14th
Control	5.87 ± 0.32	6.67 ± 0.54	6.58 ± 0.09	2.16 ± 0.13	4.52 ± 0.23
Experimental I	5.26 ± 0.16	3.11 ± 0.24	2.04 ± 0.13*	1.58 ± 0.10**	2.08 ± 0.15***
Experimental II	4.97 ± 0.12	3.34 ± 0.19	2.05 ± 0.18**	1.69 ± 0.18**	2.17 ± 0.19***

Notes:* — $p < 0.05$,** — $p < 0.01$,*** — $p < 0.001$ according to the indicator in the control.

It should be noted that the pituitary gland secretes gonadotropins — luteinizing hormone and follicle-stimulating hormone (Scanes et al., 2005). Follicle-stimulating hormone stimulates ovarian estrogen production and testicular testosterone, egg follicle maturation, and spermatogenesis (Smitz et al., 2016). Luteinizing hormone is responsible for ovulation and corpus luteum formation in the ovaries and testosterone synthesis by Leydig cells in the testes (Rama Raju et al., 2013).

The active substance of the studied drugs is megestrol acetate, a synthetic progestogen that affects the hypothalamic-pituitary system of the animal and blocks the release of gonadotropic hormones (follicle-stimulating and luteinizing) (Pirzada, 2002). The systemic effect of megestrol acetate is higher than administration in nanocrystalline form (Jang et al., 2014).

Therefore, our results are consistent with the results of other researchers on the high effectiveness of hormonal drugs with the active substance megestrol acetate for sexual function suppression in animals.

Along with the use of hormonal veterinary drugs, a scientifically sound approach to the use of drugs for the destruction of pathogens of infectious and parasitic animal diseases remains relevant (Mateus et al., 2011; Paliy et al., 2019, 2020).

Conclusions. According to the results of experimental studies of hormonal drugs with the active substance megestrol acetate, it was found that they are well tolerated by domestic animals and do not cause side effects and changes in their clinical condition.

It has been scientifically proven that hormonal drugs have anti-estrogenic and anti-ovulatory effects. It has been found that the tested hormonal drugs are effective for interruption and suppression of heat in female cats and dogs. They also inhibit sexual activity and regulate the behavior of male cats and dogs.

The prospect of further research is to develop a scientifically sound scheme for the use of veterinary drugs for domestic animals, depending on their physiological condition, general epizootic situation and housing conditions.

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USE OF GIS TECHNOLOGIES TO ANALYZE THE SPREAD OF MAREK'S DISEASE VIRUS IN UKRAINE

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Summary. The article presents data on the use of GIS technologies to visualize the spread of Marek's disease virus in Ukraine since 2011. The ArcGis v. 10.4.1 was used as a geographic information system. Three vector maps were designed, with different data on Marek's disease virus outbreaks, and a map showing the density of the poultry population in Ukraine, to better understand the possible risks associated with the spread of the virus and to predict the epizootic situation regarding Marek's disease

Keywords: ArcGis, vector maps, risk assessment

Introduction. Today, the Marek's disease virus (MDV) is a major threat to the poultry industry in Ukraine. Marek's disease is a highly contagious herpesvirus neoblastic disease of poultry and wild birds, which is common in countries around the world.

Vaccination of day-old young birds with live vaccines is the basic strategy for the prevention and control of Marek's disease (Stegniy, 2010; Sostin, Stegniy B. and Stegniy M., 2017). Effective vaccination prevents clinical manifestations of MD, such as tumors from latently infected T lymphocytes, but although vaccination reduces infection and its spread, it is not able to completely prevent it (Gerilovych et al., 2010). Annually in countries with developed poultry losses from MD are estimated at 1–2 billion dollars (Morrow and Fehler, 2004). Marek's disease virus belongs to the genus *Mardivirus*, subfamily Alphaherpesvirinae, family Herpesviridae. There are three serotypes: 1) Gallid herpesvirus 2 (GaHV-2), 2) Gallid herpesvirus 3 (GaHV-3), 3) Meleagrid herpesvirus 1 or herpesvirus of turkeys (HVT). However, only representatives of the first serotype are oncogenic (Murata et al., 2007; Kozdruń et al., 2020). This infectious disease of birds (mainly chickens) is caused by the oncogenic herpesvirus of group B and manifested by paresis, paralysis of the extremities, change in the color of the iris, deformation of the pupil, and tumors in internal organs, skeletal muscles and skin. Under natural conditions, chickens are susceptible to the disease.

Turkeys, guinea fowl, quail and pheasants may be susceptible to infection. Young birds are most susceptible to infection in the first days of life. Marek's disease virus is characterized by pronounced tropism in T lymphocytes, in which it persists for a long time. In the body of a sick bird it is contained in the blood, feces, tumors, pathologically altered organs, as well as in the epithelial cells of feather skin follicles, lymphoreticular cells (legs, comb, wattles). The source of infection is sick birds, as well as clinically healthy birds-virus carriers. The pathogen can be transmitted from the surface of the shell of an infected egg. The incubation period is from 14 days to 6 months. Virus excretion begins 7–20 days after infection and can continue throughout life. The pathogen spreads to the environment with the epithelium of feather follicles, through the gastrointestinal tract, as well as through the respiratory organs. The main route of infection is aerogenic. Marek's disease can take the form of an epizootic outbreak or be sporadic. If Marek's disease is suspected, measures are taken to clarify the diagnosis. For this purpose, carcasses or sick birds in the amount from 5 to 10 individuals are examined.

During the first week after infection, the virus is found in the thymus, spleen and fabric bursa, from the 23rd day and even earlier — in the epithelium of feather follicles. 5–7 days after inoculation of the pathogen, round cells of various shapes and sizes appear in the blood, then multinucleated giant cells with eosinophilic cytoplasmic granulation and perinuclear inclusions are detected, and by the 10th day foci of star-shaped cells appear. In the stage of nerve damage, circulatory disorders are observed.

Lymphocytes infiltrate the stroma of the nerve that germinates in the connective tissue, as a result, the nerve thickens, changes its normal structure. This leads to disruption of nerve trophism and, as a consequence, disorders of the entire physiological system of metabolic processes, mechanisms of regulation and adaptation, depletion and death (Stegniy B., Stegniy M. and Sostin, 2014).

Clinical picture. Marek's disease manifests itself at the age of 6 weeks, but more often from 12 to 24 weeks. The incubation period lasts from 3–4 weeks to several months. Previously, the course of MD according to the features of clinical signs and lesions of tissues and organs in sick birds was divided into three forms: neural, ocular and visceral. At present, the course of MD by the form of manifestation is classified into classical and acute. In the classical form, a small percentage of the herd is affected (up to 10%), and in the acute form — 20–30%. Death often begins at 8 weeks of age in laying hens, but its peak usually occurs at 5 months of age (Berezhna, Ivashchenko and Polishchuk, 2015; Morrow and Fehler, 2004).

In the case of artificial infection of day-old chickens that do not have maternal antibodies, death can occur in 3 weeks and even in 10–17 days after depression and stunted growth. The classical form is subacute and chronic. The incubation period of the disease lasts from 2–3 to 6 months. A sick bird dies at the age of 3–5 months.

Acute form of Marek's disease is characterized by a short incubation period — from several weeks to 2–3 months. The virus with blood leukocytes penetrates the internal organs and spreads in the cells of the lymphoid tissue of the fabric bursa, thymus, spleen, in places of lymphoid infiltration of organs and nerve trunks. Due to the death of lymphocytes, the normal functioning of the immune system is disrupted, which contributes to the generalization of infection and the formation of tumors in many organs. Clinical symptoms of the disease in experimentally infected chickens are manifested by cachexia, paresis and paralysis of the limbs, neck, crop, wings, lesions of the digestive tract, eyes. Their appearance is caused by lesions of internal organs, tumors that lead to a general violation of the bird body. In some cases, outbreaks are dominated by skin lesions. This form occurs suddenly, is rapid, and is characterized by high morbidity and mortality of up to 80% of chickens aged from 1 to 5 months.

Summarizing the above, it should be noted that among infectious diseases of birds, Marek's disease continues to be widespread throughout the world. In this regard, due attention should be paid to monitoring, specific prevention and control measures.

Cartographic methods are widely used for monitoring studies of animal and human diseases, which allow to study the patterns of spatial location of objects and certain aspects of the development of disease epizootics in a particular area by compiling and using nosological maps.

A new stage of technological development is characterized by the emergence of geographic information systems (GIS). Geoinformation system — computer technology that allows you to combine a model image of the territory with tabular information (statistics, lists, economic indicators, epidemiological data, *etc.*) (Kaliuzhnyi and Ushkalov, 2013; Mengistu and Haile, 2017).

The work **aimed** to visualize the spread of Marek's disease virus in Ukraine using GIS technologies.

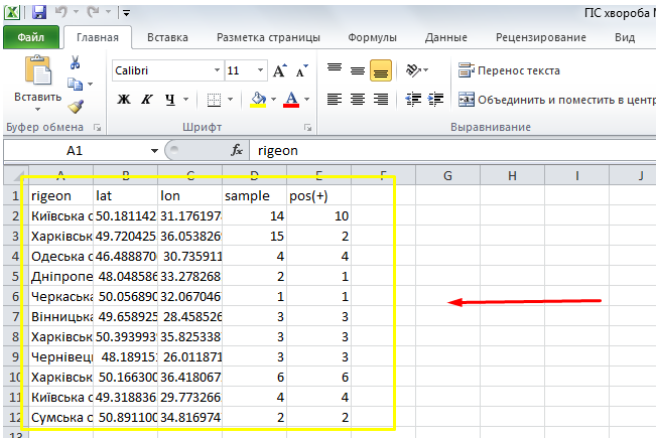
Materials and methods. The research was conducted based on analysis of the epizootic situation in Ukraine and worldwide, own monitoring research and patent-license search in PubMed databases, and with the use of geographic information system (GIS technologies). Preliminary MD was diagnosed based on epizootic data, symptoms of the disease (lesions of the peripheral and central nervous system in the classical form of MD, almost any nerve can be affected, and the symptoms can be varied: lameness, paresis, ataxia, paralysis of one or two limbs, wings, neck, and tail. The clinical signs of the acute form of the disease are often nonspecific: lethargy, anemia, sometimes shortness of breath and cough, indigestion, exhaustion, refusal to feed, dehydration, *etc.*), and characteristic pathological and anatomical changes. At the autopsy of the dead bird, in the case of the classical form, diffuse-focal thickening of the nerve trunks of the lumbar and humeral plexuses is found, which acquire a dull gray color.

Tumor-like growths are observed in the lungs, kidneys, heart, and gonads. In the carcasses of birds that died of acute Marek's disease, there are single (in the initial stage) and later — numerous tumors in the internal organs, skin, muscles, rarely — changes in the nerves. Tumors are usually found in the ovaries, heart, glandular stomach, lungs, skeletal muscles, less often in the cloacal sac, kidneys, liver and spleen. At the same time, the liver and spleen increase in sizes several times, and the wall of a glandular stomach thickens 2–5 times.

For final diagnosis, from 5 to 10 clinically sick chickens are sent to the laboratory, where blood is taken, and pathological material during the autopsy: pieces of affected organs, nerves, skin. On the other hand, from the outer surface of the thigh of each bird 10–15 feathers with the presence of tissue (epithelium of feather follicles) are taken. Pathological material is used for virological (not later than 2–3 h after collection) and molecular-genetic (PCR) (Murata et al., 2007) or pathomorphological studies.

This work used data for the period 2011–2021. 57 samples of biological material from dead birds were examined for the presence of MDV, studies were performed by molecular-genetic and virological methods, the results of these studies revealed a total of 39 positive samples. Experimental studies on animals have been conducted following the basic principles of bioethics. Euthanasia of animals was performed by inhalation of chloroform anesthesia.

We used ArcGis v. 10.4.1 as a GIS. Then, with the help of Microsoft Excel, a table in CSV format was created (Fig. 1), in which data on cases of MDV detection were entered.



	A	B	C	D	E	F	G	H	I	J
1	rigeon	lat	lon	sample	pos(+)					
2	Київська с	50.181142	31.176197	14	10					
3	Харківськ	49.720425	36.053826	15	2					
4	Одеська с	46.488870	30.735911	4	4					
5	Дніпропе	48.048586	33.278268	2	1					
6	Черкаськ	50.056896	32.067046	1	1					
7	Вінницьк	49.658925	28.458526	3	3					
8	Харківськ	50.393993	35.825338	3	3					
9	Чернівец	48.18915	26.011871	3	3					
10	Харківськ	50.166300	36.418067	6	6					
11	Київська с	49.318836	29.773266	4	4					
12	Сумська с	50.891100	34.816974	2	2					

Figure 1. Tabular data in CSV format

Fig. 1 shows the location of MDV outbreaks, the number of positive and negative samples.

After that the data was transferred to ArcGis program, which was later converted into an attribute table. Then all the data with the help of the 'data display' function (Fig. 2), the coordinates of the MDV pathogens outbreaks were plotted on the map.

Results. According to the monitoring results, two maps were created, one with the marked outbreaks of MDV, which have been detected since 2011, and with the marked poultry farms of Ukraine, the data of which were taken from open sources (Fig. 3).

A map was also created with the same data, but with additional data on the number of positive and negative samples, which were plotted on the map in the form of pie charts (Fig. 4).

Subsequently, we created a map of Ukraine with marked data on poultry population. The statistics on the number of poultry of all species in farms of all categories were taken from official site of the State Statistics Service of Ukraine (http://www.ukrstat.gov.ua/druk/publicat/kat_u/2020/zb/05/zb_tvaryny_2019.pdf).

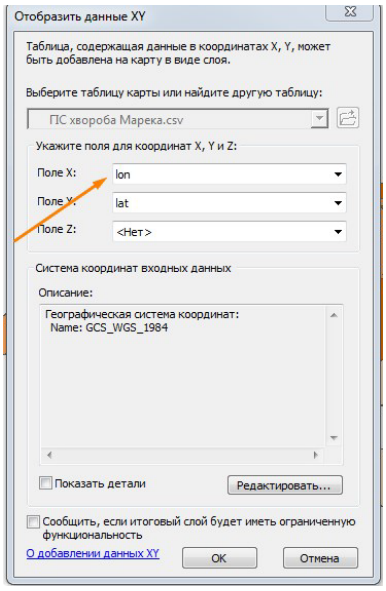


Figure 2. Data display function

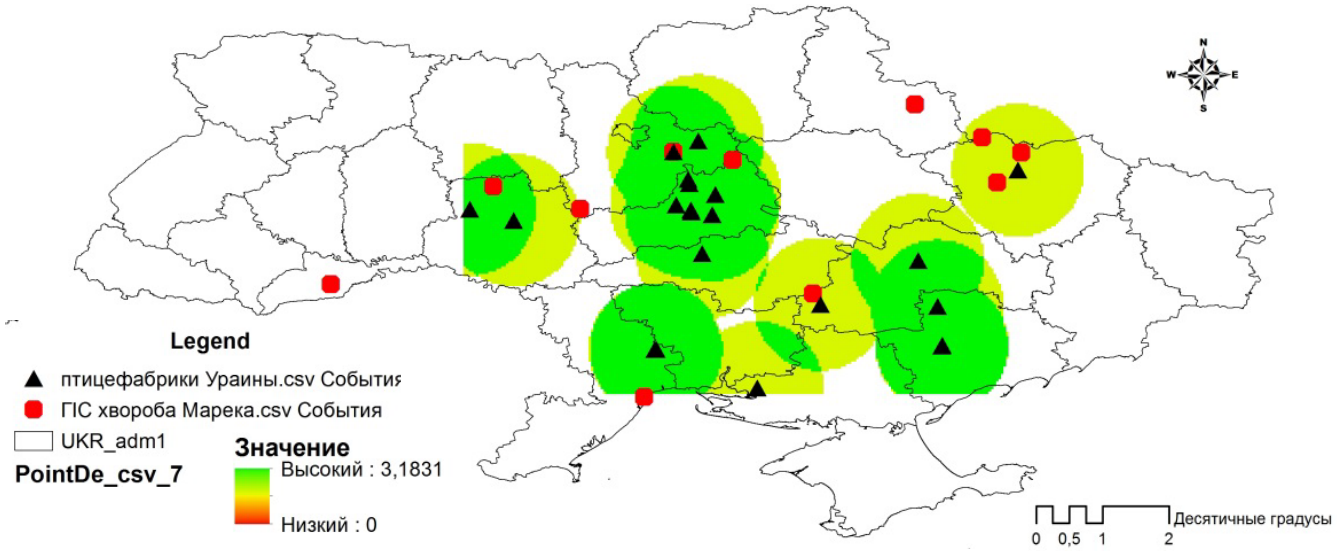


Figure 3. Map of Ukraine with data on MDV outbreaks and poultry farms

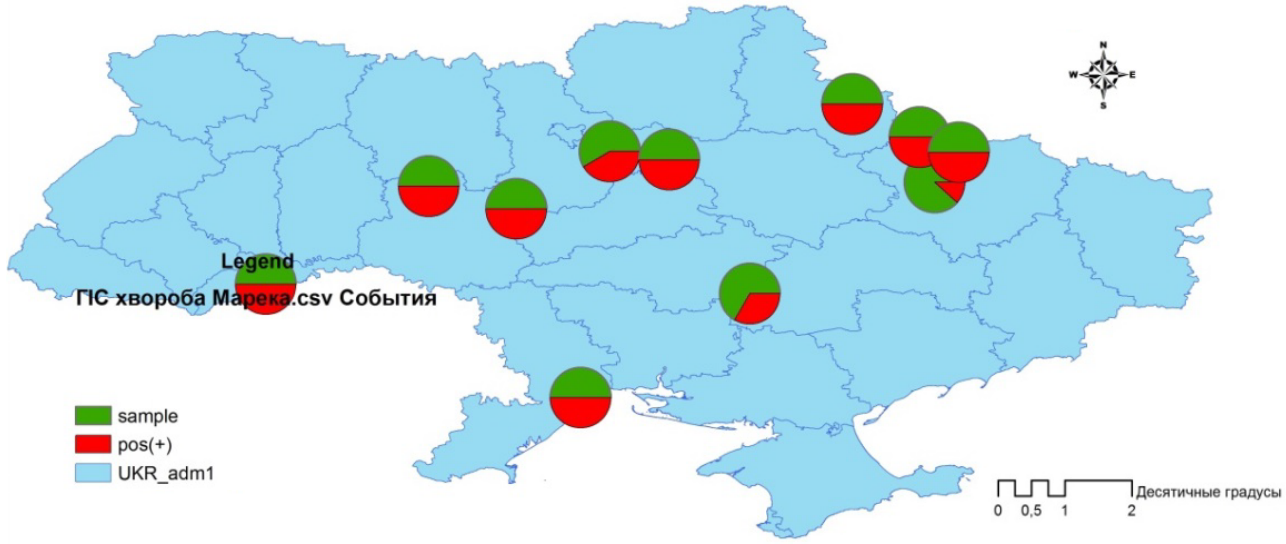


Figure 4. Map of Ukraine with data on MDV outbreaks in the form of pie charts

Two additional columns were created in the attribute table, which included data on the number of poultry in each region, and data on the area of these regions (Fig. 5).

Then a map was created, which visually displayed the poultry population in the form of points (Fig. 6).

1	NAME_1	TYPE_1	ENGTYPE_1	NL_NAME_1	VARNAME_1	poultry	area
4	Crimea	Autonomous Republic	Autonomous Republic		Crimée|Criméia|Krim|Krymskaya Respublika|Respublika Krym	0	0
11	Kiev City	Independent City	Independent City		Kyiv	0	0
20	Sevastopol'	Autonomous Republic	Autonomous Republic		Sebastopol	0	0
1	Cherkasy	Oblast'	Region		Cherkas'ka Oblast'|Cherkasskaya Oblast'|Cherkassy	25261	20916
2	Chernihiv	Oblast'	Region		Chernigov|Tschernigow	3548	31903
3	Chernivtsi	Oblast'	Region		Chernivets'ka Oblast'|Chernovitskaya Oblast'|Chernovtsy|Czernowitz|Tschernowzy|Tchernovtsy	3726	8096
5	Dnipropetrovs'k	Oblast'	Region		Dnipropetrovsk|Dniepropietrovsk|Dnjepropetrowsk	17763	31923
6	Donets'k	Oblast'	Region		Donetsk|Donetskaya Oblast'|Donezk|Stalino	6104	26517
7	Ivano-Frankivs'k	Oblast'	Region		Ivano-Frankovsk|Ivano-Frankovskaya Oblast'|Stanislav	4908	13927
8	Kharkiv	Oblast'	Region		Charkow|Jarkov|Karkov|Khar'kov	8151	31418
9	Kherson	Oblast'	Region		Cherson|Khersons'ka Oblast'	5703	28461
10	Khmel'nyts'kyy	Oblast'	Region		Khmelnitsky|Khmelnytskyi|Chmelnizkij|Hmelnicki|Kamenets-Podol'skaya Oblast'|Khmel'nyts'ka Oblast'	8157	20629
12	Kiev	Oblast'	Region		Kiev Oblast|Kiew|Kijew|Kiiv|Kijev|Kiyev|Kyiv|Kyjiv|Kyyiv|Kyyivs'ka Oblast'	31387	28121
13	Kirovohrad	Oblast'	Region		Kirovograd|Kirovogradskaya Oblast'	5481	24588
14	L'viv	Oblast'	Region		Lemberg|Livov|L'vov|Lwow|L'vivs'ka Oblast'	9914	21831
15	Luhans'k	Oblast'	Region		Luhansk|Luganskl|Luhans'ka Oblast'|Voroshilovgrad	931	26684
16	Mykolayiv	Oblast'	Region		Mykolaiv|Nikolajew|Nikolayev	2453	24585
17	Odessa	Oblast'	Region		Odesa|Odes'ka Oblast'|Odesskaya Oblast'	2624	33314
18	Poltava	Oblast'	Region			5388	28750
19	Rivne	Oblast'	Region		Rovno|Rivnens'ka Oblast'|Rovenskaya Oblast'	7266	20051
21	Sumy	Oblast'	Region			5310	23832
22	Ternopil'	Oblast'	Region		Ternopol|Ternopol'	5197	13824
23	Transcarpathia	Oblast'	Region		Transcarpathian|Zakarpattia|Ruthenia|Zakarpats'ka Oblast'|Zakarpatskaya Oblast'	3572	12753
24	Vinnytsya	Oblast'	Region		Vinnytsia|Vinnitskaya Oblast'|Vinnyts'ka Oblast|Winniza	37550	26492
25	Volyn	Oblast'	Region		Volhynia|Volyns'ka Oblast'|Volynskaya Oblast'|Wolynien	7759	20144
26	Zaporizhzhya	Oblast'	Region		Saporoshje|Zaporizhia|Zaporiz'ka Oblast'|Zaporoje|Zaporozhskaya Oblast'|Zaporozh'ye|Zaporožje	4952	27183
27	Zhytomyr	Oblast'	Region		Zhitomir|Jitomir|Shitomir|Zhitomirskaya Oblast'	7416	29827

attribute table

Figure 5. Table of attributes with the marked data on the number of poultry in the country

Figure 6. Map of Ukraine showing poultry population

Conclusions. Thus, according to the results of our research, three maps were created, two of which showed MDV outbreaks, in two different ways, one map showed disease outbreaks with additional application of poultry farms in Ukraine, the other the same data, but in the form of pie charts showing positive and negative samples, for more detailed visualization.

The map of Ukraine showing the poultry population showed the distribution of poultry in Ukraine in the form of points. This map was created for a more detailed understanding of the areas of animal accumulation, linking this factor with the possible further spread of Marek's disease outbreaks in areas of poultry concentration.

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Part 2. Biotechnology

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DEVELOPMENT OF DIFFERENTIATION METHOD FOR BOVINE HERPESVIRUS SEROTYPES (BHV-1, BHV-4, BHV-5) USING POLYMERASE CHAIN REACTION

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Summary. Infectious pneumoenteritis of cattle is etiologically caused by viruses of different families and species. Bovine herpesvirus type 1 — infectious rhinotracheitis virus — is the main and the most dangerous pathogen transmitted by cattle semen. At the same time, recently, according to European scientists' data, in addition to this pathogen, other herpesviruses have been circulating in cattle groups, in particular, bovine herpesvirused of types 4 and 5. Studies have been conducted using molecular-genetic and bioinformatic methods. Based on the analysis of the genomes of bovine herpesvirus of types 1 (IBR virus), 4 and 5 we developed primers BoHV-1 F/R, which flanks the DNA fragment of the IBR virus with the length of 204 bp, BoHV-4 F/R, which flanks the DNA fragment of bovine herpesvirus type 4 with the length of 615 bp, and BoHV-5 F/R for bovine herpesvirus type 5 DNA amplification with the formation of specific fragments 158 bp in length. The tests demonstrated that primers specific for bovine herpesvirus of types 1, 4 and 5 can be used in multiplex amplification format and hybridized only with specific DNA matrices of bovine herpesviruses. A standard operating procedure 'Indication of DNA of infectious bovine rhinotracheitis virus and bovine herpesviruses of types 4 and 5 by polymerase chain reaction' has been developed

Keywords: infectious bovine rhinotracheitis, infectious pneumoenteritis, cattle, primers

Introduction. The vertically transmitted infectious diseases of cattle are of great economic importance, they significantly hinder the development of the livestock industry, causing significant damage by reducing fertility, viability of young animals, productivity of the parent herd and the cost of veterinary measures. On the other hand, the detection of a number of infectious diseases of cattle is important for the development of international trade in animals and animal products.

One of the main factors in the transmission of infections and their spread among susceptible cattle is the semen of breeding bulls. There is a lot of information in the literature on the transmission of viral and chlamydial infections in cattle by semen (Muylkens et al, 2007; Sarangi et al, 2021; Roberts et al, 1981).

As the main object of international trade, the semen of breeding bulls needs close monitoring and comprehensive quality research, which is regulated by several European Union directives and the OIE Code (Edwards, 2007). From this point of view, the control of breeding semen is an important task of the veterinary service, as timely implementation of product screening for virus and chlamydia contamination can prevent economic losses, loss of productivity and ensure the efficiency of the industry, as well as high quality sperm and normal reproduction of farm animals.

The semen is considered suitable for artificial insemination if the following conditions are met: firstly, if the semen does not contain pathogenic and opportunistic microorganisms; secondly, if it is obtained from clinically healthy donor bulls that have negative results of laboratory tests performed in accordance with current veterinary regulations, for a number of infectious diseases included in the OIE list (infectious rhinotracheitis, chlamydia, viral diarrhea, *etc.*).

When screening of breeding animal semen for the presence of pathogens, high efficiency and reliability are demonstrated by molecular-genetic methods based on PCR. It allows the rapid direct detection of genetic material of a particular infectious agent. PCR, which is recommended by the OIE for the detection of viral contamination and in some countries for the detection of chlamydia, has significant advantages in semen testing compared to traditional virological tools due to, firstly, significantly higher sensitivity and specificity, and secondly, the toxicity of sperm to cell cultures and its possible contamination by microflora, which leads to a high probability of incorrect test results (Givens, 2018).

The main and most dangerous pathogen transmitted by cattle semen is bovine herpesvirus type 1 (BHV-1) — infectious rhinotracheitis virus (IBR virus). At the same time, recently, according to European scientists, in addition to this pathogen, other herpesviruses are circulating in cattle groups, in particular, bovine herpesvirus of types 4 (BHV-4) and 5 (BHV-5). They cause IBR-like clinic in newborn calves and serologically similar to IBR virus (Nefedchenko et al., 2019; Rapaliute et al., 2021).

The only effective mechanism for the differentiation of these viruses is molecular genetic testing. It is suitable for targeted identification of herpesviruses, which contributes to the accurate diagnosis and selection of effective treatment regimens and prevention of viral pneumoenteritis.

The **aim of our work** was to develop a method of PCR detection of bovine herpesviruses of different epizootically significant types.

Materials and methods. In order to perform computer analysis of the genes of IBR virus and other bovine herpeviruses from the databases DDBJ (https://www.ddbj.nig.ac.jp/ddbj) and GenBank (https://www.ncbi.nlm.nih.gov/genbank), the corresponding sequences of different lengths were obtained.

The resulting sequences were aligned using the ClustalX module to search for conserved sites in the DNA sequences of herpesvirus pathogens in cattle.

After conservative sites were identified, primer pairs were searched using AmplifX software.

The calculated primer sequences were synthesized, after which the amplification mode and parameters of the reaction mixture were selected using standard base kits: GenePack IsoGen (Denmark), Thermo Scientific Maxima Hot Start Green PCR Master Mix (2X) (USA), and Fermentas MasterMix (Lithuania).

Matrix for annealing primers in the development of methods for the indication of BHV DNA were: DNA of IBR virus, strain Cooper (Friedrich Loeffler Institute, Germany) and strain Moldavsky (National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine (NSC 'IECVM'), Ukraine), DNA of BHV-4 and BHV-5 (İstanbul University — Cerrahpaşa, Turkey), Marek's disease virus, strain SB-1 and Aujeszky's disease virus, strain UNDIEV 18-B (NSC 'IECVM', Ukraine)

Mathematical analysis of the obtained data was performed using the Excel XP spreadsheet editor.

For the development and validation of methods we used the approaches previously developed in the NSC 'IECVM' (Stegniy and Gerilovych, 2014).

Results. At the first stage of research on the development of methods for differentiation of BHV-1, BHV-4, BHV-5 serotypes by PCR we conducted research on the selection of specific primers for amplification of DNA of pathogens.

At the beginning of this work, we analyzed the DNA sequences of herpesviruses in order to select target genes.

In order to study the polymorphism of nucleotide sequences of the *gC* gene, 12 sequences of BHV-1 isolates of different lengths obtained online from the GenBank database were studied.

The analysis showed that the nucleotide sequence of the studied gene has a length of 1,786 bp, and the mass is 1.09 MDa, G+C content is 72.32%, and A+T — 27.68%. The last parameter indicates the possibility of designing primer systems with a high melting point.

After analyzing the result of multiple alignments of *gC* gene sequences, we found that it contains 5 conservative nucleotide alignments with a length of 23–47 bp, the average entropy (Hx) of these segments was 0.000–0.001, which allowed them to be used to search for probable primer pairs. Subsequent bioinformatics analysis of the sequences of other types of herpesviruses showed that serotype-specific sites suitable for primer design, this gene does not contain.

After analyzing the sequences of *gC* and *gB* genes of different types of herpesviruses available in the GenBank database, we concluded that when creating primer systems to differentiate all three types of bovine herpesviruses that have epizootic significance, it is necessary to use DNA matrices of different genes as targets.

Thus, based on the analysis of multiple alignments obtained with BioEdit and the calculation of probable primer sequences, we identified oligonucleotides with AmplifX to detect different types of bovine herpesviruses (Table 1).

Table 1 — Oligonucleotide sequences for DNA detection of BHV-1, BHV-4, BHV-5

Agent	Primer system	Sequences 5'–3'	Annealing temperature, °C	Product size, bp
Bovine herpesvirus type 1 (IBR virus)	BoHV-1_F	GCGGGCCTGGTTGCGTACTAC	58	204
	BoHV-1_R	AGCAGATCTTCCGCGTTGATC		
	BoHV-1 gE_F	GCCAGCATCGACTGGTACTT	57	325
	BoHV-1 gE_R	GCACAAAGACGTAAAGCCCG		
Bovine herpesvirus type 4	BoHV-4_F	CCCTTCTTTACCACCACCTACA	58–61	615
	BoHV-4_R	TGCCATAGCAGAGAAACAATGA		
Bovine herpesvirus type 5	BoHV-5_F	CGGACGAGACGCCCTTGG	58–61	158
	BoHV-5_R	AGTGCACGTACAGCGGCTCG		

To facilitate the procedure of future amplification, all selected primer pairs were selected taking into account the divergence of the flanked areas to facilitate visual differentiation and with close 57–61°C hybridization temperatures to the matrix.

Thus, to detect IBR virus, we created BoHV-1 F/R primers suitable for amplification of a 204 bp *gB* region of the virus and BoHV-1 gE_F/R, complementary to the DNA sequence of the *gE* gene with a length of 325 bp. Both oligonucleotide systems had a theoretical annealing temperature of 57–58°C.

BoHV-4 primer set for the detection of BHV-4 DNA limited the region of the *gB* gene to 615 bp and were characterized by theoretical annealing temperature 58–61°C. Oligonucleotides BoHV-5 F/R flanked a region of the *gB* gene of BHV-5 with a length of 158 bp and were characterized by a similar theoretical annealing temperature.

Theoretical verification of the developed oligonucleotides showed their satisfactory qualities (absence of palindromes, GC content, proximity of melting temperatures), which was recorded at the limit of 87–94% of the maximum.

These primer sequences were synthesized and used for further research on PCR protocols.

Development of amplification reaction regulations was performed using strain Cooper of BHV-1 with a titer of 6.5 lg TCD_{50}/ml and strain Moldavsky as a positive control reaction. DNA of BHV-4 and BHV-5 from broods with virus titers of 6.7 lg TCD_{50}/ml and 5.5 lg TCD_{50}/ml, respectively.

Standard PCR parameters were tested for a total reaction volume of 30 µl containing 0.5 U of Taq polymerase, 2.0 mm Mg^{2+} ions, with the introduction of 5 µl of viral DNA solution isolated by sorbent method and from the obtained reference materials.

The first step in optimizing the reaction was to select the appropriate temperature regime. PCR was performed on three consecutive five-fold dilutions of standard DNA solution using 50 pM primers BoHV-1 F/R, BoHV-1 gE_F/R, BoHV-4 F/R and BoHV-5 F/R at temperatures of 50°C, 52°C, 54°C, 56°C, 58°C, and 60°C.

The results obtained during amplification showed the possibility of detecting the system BoHV-1 gE_F/R in five consecutive dilutions of viral DNA at 50°C and 56°C, however, together with a specific fragment in the tracks (325 bp), we observed bands of different lengths (approx. 150–200 bp and 700–800 bp), which made it impossible to properly account for the reaction, as specific, at the specified temperature.

The result of amplification at 60°C indicated an excessively high annealing temperature, as evidenced by the absence of specific bands in the third dilution.

Therefore, this technique, showing satisfactory sensitivity in five replicates at 58°C amplification, can be recommended for the detection and identification of viral isolates of IBR in titers 3–6 lg TCD_{50}/ml, which is a satisfactory analytical threshold for screening for virus from semen samples and other clinical materials, as well as in the study of samples of viral isolates.

Similar results were obtained using oligonucleotides BoHV-1 F/R. The 204 bp length DNA fragment amplified in all dilutions for use at a temperature of 58°C.

Subsequent studies of the primers of the BoHV-4 F/R system according to the scheme described above showed that the effective annealing temperature for these oligonucleotides is 56–58°C. Bands of amplicons of specific calculated length (615 bp) were observed in all tracks containing reaction products with specific DNA, which corresponded to the sensitivity threshold for the minimally detected virus titer of 2.0–2.5 lg TCD_{50}/ml. Due to the similarity of the lengths of the fragments amplified by BoHV-1 F/R and BoHV-4 F/R primers, it was decided to use the BoHV-1 primer system gE_F/R for the detection of BHV-1 DNA.

Optimization of the annealing regime of BoHV-5 F/R primers for the excretion of BHV-5 DNA also showed that annealing at temperatures of 58–60°C is optimal, and the detection threshold is 1.5–2.0 lg TCD_{50}/ml. Specific fragments of 158 bp in length were formed in the tracks of samples containing BHV-5 DNA.

At the next stage of the work, the influence of different concentrations of magnesium ions on the nature of amplicon formation and their contrast was tested. When checking the results of the reaction with concentrations of magnesium ions from 1.0 mm/µl to 4.0 mm/µl in steps of 1 mm/µl and then 0.5 mm/µl, it was found that the formation of a specific amplicon of maximum contrast was observed in the case adding 2.0–2.5 mm/µl of the reaction mixture of magnesium ions in the form of magnesium sulphate. At the same time, minimal comet tail and unspecific product formation was noted (visually detected only in 2 from 48 samples).

Analysis of the absence of cross-reactions with the genetic material of other alpha-herpesviruses (Marek's disease virus, strain SB-1; Aujeszky's disease virus, strain UNDIEV 18-B) and different types of bovine herpesviruses showed intraspecific specificity of primers of the developed systems. Thus, BoHV-1 primers gE_F/R amplified only IBR virus DNA, BoHV-4 F/R — only BHV-4 DNA, and BoHV-5 F/R — only BHV-5 DNA (Figs. 1–3).

To test the sensitivity and reproducibility of the developed protocol of IBR virus DNA amplification, the possibility of detecting different amounts of specific DNA (3–8 lg TCD_{50}/ml) was studied. IF-negative and IF-positive sperm samples were examined, as well as virus culture broods with different titers.

It was found that the proposed method is able to detect IBR virus DNA with a titers from 3 TCD_{50}/ml to 8 TCD_{50}/ml, so the sensitivity exceeds the sensitivity of IF in the twice amount. The studies were conducted three times, with repeated results reproduced (Fig. 4).

Thus, IBR virus DNA detection protocol based on amplification of 245 bp region of *gB* gene and 325 bp area of *gE* gene. In terms of sensitivity, the second method was more effective, which allowed to choose it as the basis for creating a diagnostic technique.

The testing of primers of BoHV-4 F/R and BoHV-5 F/R systems at the specified composition of the reaction mixture for annealing at a temperature of 58°C demonstrated the synthesis of the specific fragments with 615 bp and 158 bp length, respectively, that were formed in specific samples corresponding to the content of viral DNA viral titers of BoHV-4 broods — from 2 lg TCD_{50}/ml, and BoHV-5 — 2.5 lg TCD_{50}/ml.

Therefore, the developed amplification regulations were quite sensitive (analytical sensitivity 2–3 lg TCD_{50}/ml) and specific for cross-reactions with heterologous herpesviruses and animal DNA. This allowed us to move to the creation of methods for detecting and differentiating of bovine herpesviruses' different subtypes.

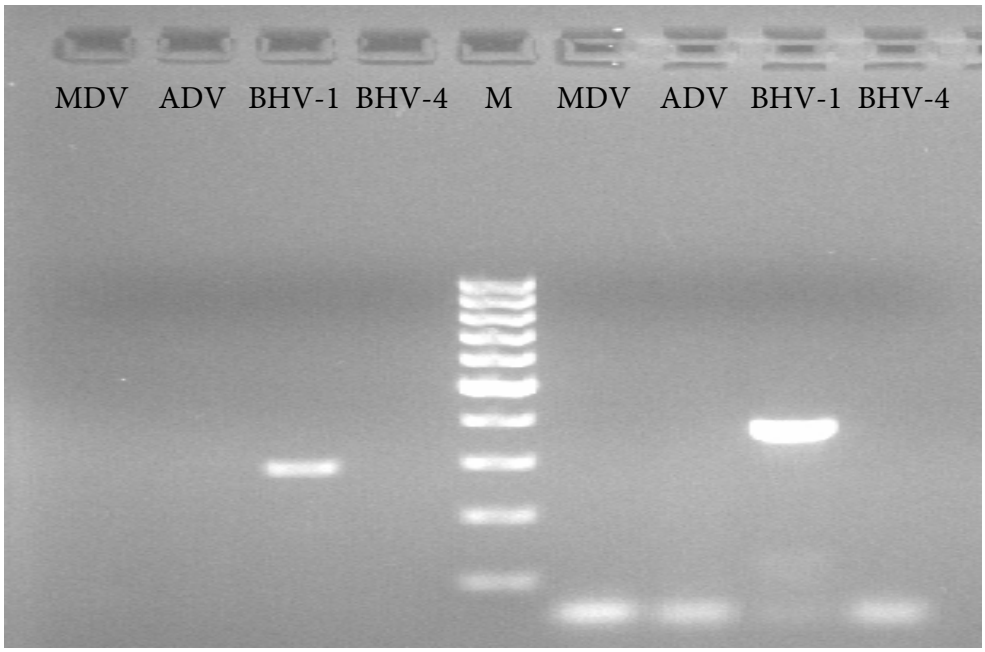

Figure 1. Amplification results for DNA of IBR virus and other herpesviruses with primers of BoHV-1 F/R (first 4 tracks) and BoHV-1 gE_F/R (4 tracks on the right, after the mass ladder) systems: M — ladder (100–1,000 bp); MDV — DNA of Marek's disease virus, strain SB-1; ADV — DNA of Aujeszky's disease virus; BHV-1 — DNA of IBR virus, Moldavsky strain, BHV-4 — DNA of BHV-4

To develop a diagnostic test system we optimized the amplification temperature and the number of cycles and selected the appropriate reaction mixture.

We optimized amplification cycles to detect small amounts of specific IBR virus DNA more effectively. PCR was performed using the components of the reaction mixture GenePack IsoGen, Thermo Scientific Maxima Hot Start Green PCR Master Mix (2X), and Fermentas MasterMix.

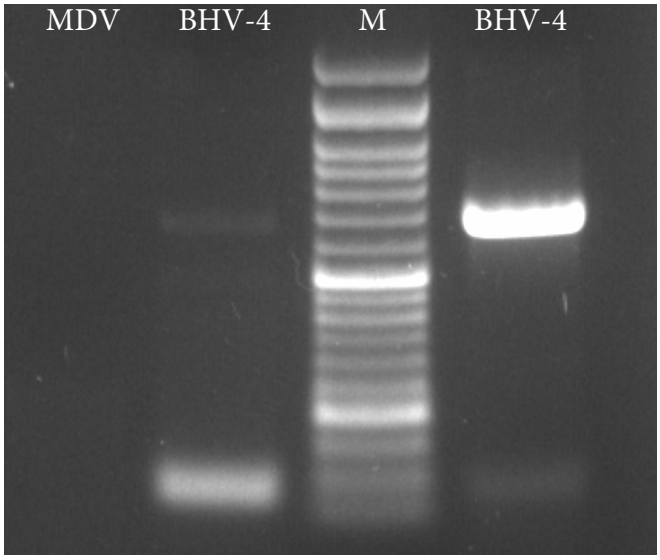

Figure 2. Amplification results for DNA of IBR virus and other herpesviruses (MDV) with primers of BoHV-4 F/R system: M — mass ladder (100-10,000 bp); MDV — DNA of Marek's disease virus, strain SB-1; BHV-4 — DNA of BHV-4 (2 lg TCD_{50}/ml and 5 lg TCD_{50}/ml)

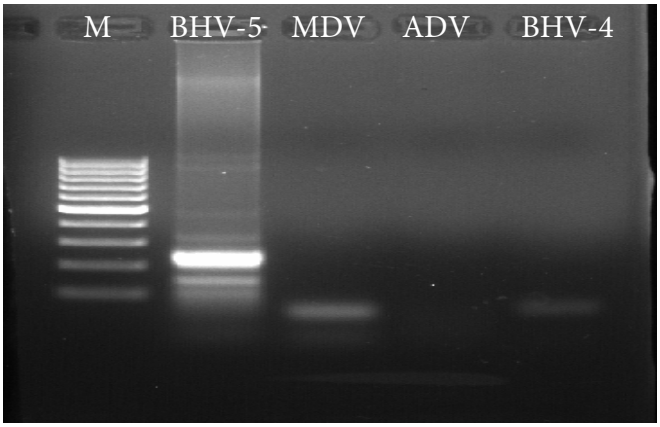

Figure 3. Amplification results for DNA of IBR virus and other herpesviruses with primers of BoHV-5 F/R system: M — mass ladder (50–1,000 bp); MDV — DNA of Marek's disease virus, strain SB-1; ADV — DNA of Aujeszky's disease virus; BHV-4 — DNA of BHV-4; BHV-5 — DNA of BHV-5

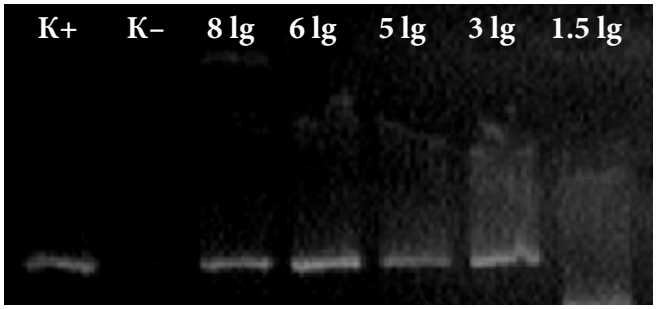

Figure 4. Sensitivity determination for the detection of 325 bp fragment of *gE* gene of IBR virus

According to the results of the experiments, it was found that the most contrasting strips of amplicons were formed using the reaction mixtures Thermo Scientific Maxima Hot Start Green PCR Master Mix (2X) and GenePack IsoGen, but the latter option required increasing the annealing temperature of primers by 2–3°C, which led to a false negative result in the maximum dilutions of the virus (4 lg TCD_{50}/ml) and 3 lg TCD_{50}/ml) (Table 2).

Table 2 — The effectiveness of different reaction mixtures in the detection of IBR virus DNA

Master mix	Viral seed titer, lg TCD_{50}/ml							
	1.5	2.5	3.0	4.0	5.3	6.4	7.2	8.7
Thermo Scientific Maxima Hot Start Green PCR Master Mix (2X)	–	+	++	++	++	++	++	++
GenePack IsoGen	–	±	+	++	++	++	++	++
Fermentas MasterMix	–	–	±	++	++	++	++	++

Thus, the optimal mixtures for amplification were recognized as basic sets of Thermo Scientific Maxima Hot Start Green PCR Master Mix (2X), which became the basis of the amplification part of the method for detection of IBR virus DNA.

When using these mixtures, amplification should be performed using the 45-cycle protocol presented in Table 3.

Table 3 — Temperature cycles for the detection of IBR virus DNA by 325 bp fragment of the *gE* gene by PCR

No.	Temperature, °C	Time, min	Number of cycles
1	94	4	1
2	94	1	45
	58	1	
	72	1	
3	72	10	1
4	4	600	1

Similar results were obtained using BoHV-4 F/R and BoHV-5 F/R primer systems.

Therefore, Thermo Scientific Maxima Hot Start Green PCR Master Mix (2X) reagents using specific primers at a concentration of 20 pM/μl proved to be the most effective as basic reagents for amplification of BHV-1, BHV-4, and BHV-5 DNA.

In order to verify the sensitivity, reproducibility of the results and the specificity of the methodology, laboratory tests were conducted.

To test the specificity and sensitivity, we used total DNA samples from virus-containing material from 10 IBR-infected animals, as well as dilution of BHV-4 and BHV-5 DNA, and 5 samples of sperm contaminated with IBR virus.

To check the taxonomic specificity of the test system were used broods of heterologous herpesviruses:

— Aujeszky's disease virus,

— Marek's disease virus,

— Infectious laryngotracheitis virus,

as well as eukaryotic DNA:

— from intact cattle (4 samples: lungs, kidneys, liver, blood and semen);

— from cell cultures of PT, TrT, PO-2.

In order to determine the reproducibility of the results obtained using PCR techniques, the study was performed three times.

Tests demonstrated, that primers specific for BHV-1, BHV-4, and BHV-5 hybridized only with specific matrices: BoHV-1 gE_F/R amplified only IBR virus DNA; BoHV-4 F/R — annealed only with samples containing BHV-4 DNA; BoHV-5 F/R — only with BHV-5 DNA; and does not hybridize to bovine and other herpesvirus DNA. The results were reproduced at repetitions (n = 3).

Based on the conducted study the standard operative procedure 'Indication of DNA of infectious bovine rhinotracheitis virus and bovine herpesviruses of types 4 and 5 by polymerase chain reaction' has been developed.

Conclusions. Based on the analysis of the genomes of bovine herpesviruses of types 1 (IBR virus), 4 and 5 developed primers BoHV-1 F/R, which flanks the DNA fragment of IBR virus with length of 204 bp, BoHV-4 F/R, which limits the DNA fragment of bovine herpesvirus type 4 with a length of 615 bp, and BoHV-5 F/R for excretion of bovine herpesvirus type 5 DNA with the formation of specific fragments of 158 bp.

It was shown that primers, specific for herpesviruses of types 1, 4 and 5 can be used in multiplex amplification format and hybridized only with specific matrices: BoHV-1 gE_F/R amplified only IBR virus DNA; BoHV-4 F/R — annealed only with samples containing BHV-4 DNA; BoHV-5 F/R — only with BHV-5 DNA; and does not hybridize to bovine and other herpesvirus DNA.

The standard operating procedure 'Indication of DNA of infectious bovine rhinotracheitis virus and bovine herpesviruses of types 4 and 5 by polymerase chain reaction' has been developed.

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Part 3. Biosafety

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STUDY OF THE SAFETY AND HARMLESSNESS OF A DISINFECTANT IN LABORATORY ANIMALS

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Summary. The work aimed to investigate the effect of the disinfectant 'Diolaid' based on sodium chlorite and sodium chloride on acute toxicity indicators, as well as on blood parameters of laboratory animals. The experiments were carried out on 6-month-old clinically healthy male rats (5 groups, 6 animals in each group, n = 30) and female rats (5 groups, 6 animals in each group, n = 30) weighing 200–220 g. The drug was administered to animals intragastrically (by probe) and aerosol treatment of cells with animals was carried out. Separately we studied the skin-irritating and sensitizing action of the disinfectant 'Diolaid' on the groups of clinically healthy guinea pigs and rats weighing 250–300 g by a daily application on their back and sides of different concentrations of the drug for 30 days for 30 min periods. In addition, we tested the effect of 'Diolaid' on nonspecific immune response indicators of these animal species (bactericidal activity of blood serum, level of circulating immune complexes, T and B cells, *etc.*). The work used modern humane methods of care and use of laboratory animals. It was found that after intragastric administration of 'Diolaid', the average lethal dose (LD_{50}) for male rats was 182 mg/kg of body weight, and for female rats it was 170 mg/kg. It has been proven that the drug has a temporary irritating and sensitizing effect and does not adversely affect the parameters of hematopoiesis and non-specific immune response in the form of a 0.06% solution. The research results indicate the low toxicity of the 'Diolaid' drug for laboratory animals and the possibility of its use in low concentrations both for treating cages in the presence of animals and for treating the animals themselves. For disinfection of water during its storage in containers, we used the concentration of the 'Diolaid' drug (by chlorine dioxide) of 0.5–2 mg/l (0.0002–0.0008%), depending on the degree of purity of the water to be treated. Such concentrations ensure compliance of the chlorite residual concentrations with hygienic standards

Keywords: rats, guinea pigs, acute toxicity, irritating and sensitizing effects, immune response

Introduction. *Relevance of the topic*. In the system of veterinary and sanitary measures, disinfection occupies an important place. It is carried out in farms for the prevention and elimination of dangerous infectious diseases of animals and birds, as well as the destruction of the disease-causing pathogens together with opportunistic pathogens in the external environment (Kovalenko and Nedosekov, 2011).

Water purification and disinfection of the water supply system are of great importance in livestock farms, as microorganisms can enter the animals' bodies with drinking water, along with vitamins and other additives (Gebel et al., 2013).

Certainly strict observance of veterinary and sanitary measures requires the use of highly effective, safe, easy to use disinfectants with a wide spectrum of bactericidal action, which are non-toxic, non-carcinogenic, do not cause habituation of the microflora and provide a prolonged effect in the presence of poultry.

Analysis of recent research and publications. Recently, manufacturers offer a wide range of low-toxic disinfectants based on quaternary ammonium and peroxide compounds, aldehydes and dialdehydes, low-molecular organic acids, guanidines and surface-active substances. However, not all disinfectants fully meet expectations. The quality of disinfectants can be affected by a decrease in activity during storage when working solutions come into contact with chemicals, which leads to resistance of microorganisms and carcinogenicity (Addie et al., 2015; Kovalenko et al., 2018; Lineback et al., 2018).

Taking into account the scientific studies and the analysis of active substances for disinfectants, the next development of the authors was a new, environmentally safe, highly effective disinfectant 'Diolaid', which includes sodium chlorite, sodium chloride. These components make it possible to effectively carry out not only comprehensive sanitation of livestock and poultry facilities in the presence of animals and poultry, but also sanitation of water supply (Ge et al., 2021; Ma et al., 2017; Ngwenya, Ncube and Parsons, 2013).

In the conditions of a subchronic experiment on white rats, scientists found that chlorine dioxide in a concentration of 1.35 mg/dm^3 in water does not cause significant changes in blood parameters, lipid peroxidation, as well as structural and functional changes in internal organs in adult animals and in the offspring of females which consumed water containing chlorine

dioxide at the same concentration, except for the stimulation of spermatogenesis and nitric oxide synthase activity in the cellular elements of the spleen (Mokienko et al., 2008).

According to the WHO, the recommended concentration of chlorine dioxide in drinking water has not been established due to its rapid decomposition. The temporarily recommended value for chlorites (0.2 mg/l) provides sufficient protection against the potential toxicity of chlorine dioxide: it has been established that the threshold concentration of chlorine dioxide for the effect on the smell of water is 0.45–0.40 mg/l. An aftertaste with an intensity of 1-2 points has been detected at higher concentrations of this compound in water. The results of chronic experiments on laboratory animals have shown that chlorine dioxide, even in high concentrations — 0.5 mg/kg and 5 mg/kg (or 10 mg/l and 100 mg/l) does not have a pronounced toxic resorptive effect on the body (Mokienko, 2021).

In Ukraine, the maximum permissible concentration of chlorite anions is 0.2 mg/l, chlorate anions — 20 mg/l (MHU, 2007, 2010; Gosstandart, 1976; MHUSSR, 1991).

An analysis of the market of disinfectants and literary sources indicates that the use of chlorine dioxide in veterinary medicine is almost not used, compared to its spread in human medicine, as well as in related fields (Kovalenko et al., 2018; Mokienko and Petrenko, 2008; Mokienko, Petrenko and Gozhenko, 2006).

The **purpose of the work** and the task of the experiments was to investigate the acute toxicity, skin-irritating and sensitizing effects, as well as the effect on the immunological parameters of the blood of laboratory animals when using the disinfectant 'Diolaid' based on sodium chlorite, sodium chloride.

Materials and methods. The research was conducted in the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE) (Kyiv, Ukraine).

The experimental part of the work was carried out taking into account 'Council Directive 2010/63/EU on the Protection of Animals Used for Scientific Purpose' (CEC, 2010), 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (CE, 1986) , and in accordance with Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006). Modern humane methods of care and use of laboratory animals were used in the experiments (Buckmaster, 2012).

Disinfectant 'Diolaid', developed by employees of the SSRILDVSE, consists of Components 1 and 2, which are white powders. pH of a 1% solution is 4.0–6.0. Components 1 and 2 of the 'Diolaid' disinfectant dissolve very well in water without sediment, the working solution of the drug is from transparent to yellowish in color, with a moderate specific smell of chlorine.

Main active substances: component 1 – sodium chlorite (42%), sodium chloride (47%); component 2 — citric acid (95%), adipic acid (3%).

Mass fraction of chlorine dioxide — not less than 1%. To use the 'Diolaid' disinfectant, it is necessary to prepare starting solutions from Components 1 and 2, which are prepared using special equipment for automatic dosing of powdered Components 1 and 2, preliminary dilution and metered introduction of the drug solution with a given concentration of chlorine dioxide into water.

The content of chlorine dioxide in the starting solution is 0.0025% (25 mg/l). The starting solution of the product is a transparent to slightly yellowish liquid with a mild chlorine smell, after diluting it in drinking water, the water remains transparent. Working solutions of the drug for wet and aerosol disinfection of objects are prepared by mixing Components 1 and 2 with drinking water. A mixture of Components 1 and 2 in a ratio of 1:1, 10% of the mixture of components + 90% of the filler (sodium chloride or zeolite or bentonite), respectively, is used for the use of chlorine dioxide aerosol for air disinfection in a small volume of air.

Acute toxicity was studied on clinically healthy male rats (5 groups, 6 animals in each group, n = 30) and female rats (5 groups, 6 animals in each group, n = 30) aged 6 months with a body weight of 190–210 g. The drug 'Diolaid' in different doses: 30 mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg, and 300 mg/kg was administered to animals intragastrically using a probe and inhaled, by treating cages. The average lethal dose (LD_{50}) and indicators of acute toxicity were determined following the method of Kerber. In the course of the experiment, animals that died during the experiment and those that survived and were killed, were subjected to a pathological autopsy in order to identify changes in organs and tissues. The classification of substances by toxicity was carried out in accordance with SOU 85.2-37-736:2011 (MAPFU, 2011), and toxicological studies were carried out following the monograph 'Preclinical studies of veterinary drugs' (Kotsiumbas, 2006).

Experiments to determine the skin-irritating effect upon skin application of the disinfectant 'Diolaid' were conducted in compliance with the general rules adopted in sanitary toxicology. For the experiment, healthy animals were selected — guinea pigs weighing 250–300 g. The drug (suspension 1:3) was applied to a pre-shaved site of the animal's skin with an area of 4×4 cm in a dose of 2,500 mg/kg in an open manner, at an ambient temperature of 12–18°C . Laboratory animals were placed in individual fixing cabins and kept for 4 h. After the end of the exposure, the animals were removed from the cabins and the applied drug sample was washed off with warm water and soap. The total duration of animal observation was 2 weeks. On the 1st day after applying the drug sample to the skin, the animals were under the constant observation.

The general state of the experimental animals, their behavioral characteristics, the intensity and nature of motor activity, coordination of movements, reaction to tactile, light and sound stimuli, the condition of the hair coat and mucous membrane, as well as the number of

dead animals in the group were recorded daily. The skin reaction at the application site was evaluated 1 h and 16 h after the end of the exposure.

The functional state of the skin was assessed by the presence of erythema, edema, and visual manifestations of the disease (ulcers, cracks, *etc.*). The degree of expression of signs of irritation was evaluated in points following the requirements of Methodological Guidelines No. 2102-79 'Assessment of the Impact of Harmful Chemical Compounds on the Skin and Substantiation of the Maximum Permissible Levels of Skin Contamination' (MHUSSR, 1980).

Sensitizing properties of the drug were studied on clinically healthy rats of one group (n = 10). On the right side, for 30 days, a single application of a 0.3% solution of the drug was carried out daily, at the place where the hair was shaved. The skin on the left side served as a control. A single application on it was made with 0.9% physiological solution (Kovalenko and Nedosekov, 2011). Additionally, animal tails were exposed in a flask with disinfectant in the same concentrations for 30 min.

Immunological studies were carried out by the indicators of the opsonophagocytic reaction: phagocytic activity, phagocytic index and macrophage transformation index were carried out following the modified method of V. Yu. Chumachenko.

In addition, we analyzed the indicators of blood serum bactericidal activity, level of circulating immune complex, and also studied the quantitative content of immunocompetent cells in the peripheral blood of rats — T and B cells.

Statistical processing of the results obtained in the course of studies on non-specific immune response was carried out using the software Epitools — Epidemiological Calculators, which is freely available at https://epitools.ausvet.com.au. It was used to estimate confidence limits for the means (Cl) at a confidence level (P = 0.95), and P-Value (a measure of the probability that an observed) was carried out. The estimated confidence limits for the means were calculated taking into account the means value, standard deviations, and sample size. P-Value Definition was conducted with Desired significance level of 0.05.

Results and discussions. The results of the clinical condition after oral administration of a lethal dose of 'Diolaid' on the functional indicators of rats are presented in the Table 1. As can be seen from Table 1, 8 h after the administration of a lethal dose of the drug 'Diolaid' in the stomach, the animals demonstrated a shaky gait, apathy, depression of the central nervous system, immobility, at the same time, the experimental rats showed a reduced reaction to touch, pain interference, grip strength, and decreased respiratory rate. After 24 h, anemia was recorded, the eye slits were narrowed, and the animals tightened their stomachs. The rats died within 3 days.

The pathological autopsy of the dead animals showed that the walls of the abdominal cavity were unchanged, smooth, and moistened; the liver is smooth and shiny, slightly hyperemic on the right side; the lung tissue is pink, hyperemic, unchanged, of uniform consistency; the heart is unchanged.

In the coronary vessels, we observed a slight expansion of the venous sinuses and accumulation of blood. In the place where the probe was inserted into the stomach, mechanical stretching of the walls of the stomach and the adjacent part of the small intestine was noted. The colon was unchanged. However, when the drug was used in a subtoxic dose, these symptoms of rat poisoning disappeared after 48–72 h.

Table 1 — The effect of a subtoxic dose of the drug 'Diolaid' under the conditions of oral administration on the general functional indicators of rats

Indicators	Observation period, h		
	8	24	72
Behavioral responses:			
Motor activity	–1	–1	–1
Arousal	–1	–2	–1
Reactivity	–1	–2	–1
Aggressiveness	–1	–1	–1
Neuromuscular reactions:			
Tremor	0	0	0
Convulsions when walking	0	0	0
Reaction to painful stimuli	–1	–1	0
Grip strength	–2	–1	0
Vegetative responses:			
The size of the eye pupil	unchanged		
Breathing rate	slowed down		
Condition of the hair cover	unchanged		
Color of visible mucous membranes	slight cyanosis		
Frequency of defecation	unchanged		
Amount of feces	slight increase		
Fecal consistency	unchanged		
Urinary frequency	unchanged		
Urine color	unchanged		
Heart rate	unchanged		

Notes: 0 — no effect; '–' — inhibition of the effect.

The results of experiments for the determination of the acute toxicity of the disinfectant drug 'Diolaid' are presented in Table 2. It has been established that when the drug 'Diolaid' was administrated into the stomach of male rats, the LD_{50} was 182 mg/kg of body weight, and for female rats it was 170 mg/kg (the difference is approx. 7.0%).

As shown in Table 2, no rats died when the drug was administered to animals (both males and females) at a dose of 30 mg/kg. At the same time, the use of a large dose (300 mg/kg) caused the death of all animals. Administration of the drug in amounts of 50–100 mg/kg caused the death of one to two animals in the group. Taking into account all of the above, according to the classification of SOU 85.2-37-736:2011 (MAPFU, 2011), the drug is considered moderately toxic.

During the 2-hour exposure of rats in cages treated with different concentrations of the drug (0.06%, 0.1%, and 0.16%), death was not observed among them. According to clinical indicators, increased motor activity was noted during the first day. In the future, the behavior of the animals did not differ from those in the control group.

The results of studies of changes in the non-specific immune response in rats after inhalation treatment of cages with disinfectant 'Diolaid' in different concentrations, are presented in Table 3. The results of studies of immunological indicators of blood in laboratory animals after their treatment with 0.06%, 0.1%, and 0.16% solutions of the drug, showed that after 5 h of the first day in rats under the effect of 0.16% solution, a probable insignificant decrease in phagocytic activity was observed in comparison with the parameters of the control group (p-value < 0.001). However, the following 15 and 30 days of research after treatment with a 0.16% solution and lower concentrations did not reveal any suppressive effect on phagocytic activity in animals.

Minor fluctuations determined the indicator of the phagocytic index in rats after treatment of their cages with 0.06%, 0.1%, and 0.16% solutions of the drug for 15 and 30 days in the range from 1.8% to 3.8%, but were within the normal range compared to the control group of animals. The result of studies of the macrophage transformation index on the first day was reliably lower in animals from the group where the cages were treated with 0.16% 'Diolaid' solution (p-value < 0.001). During the following days of the experiment, the indicator was in the same ranges as in the control group of animals. Lower concentrations of the drug did not affect the macrophage transformation index.

It has been established that 0.06%, 0.1%, and 0.16% solutions of the drug 'Diolaid' did not have a suppressive effect on the indicators of the opsonophagocytic reaction in rats, and this is also indicated by the indicators of the content of the circulating immune complex in the blood serum of animals, which characterizes the body's immune response to destruction of toxic products to restore disturbed parameters of hemostasis.

Indicators in experimental groups of rats after their treatment with 0.06%, 0.1% solutions and level of circulating immune complexes in the control group reliably did not differ from the first day of the experiment (p-value < 0.3). Treatment with a 0.16% solution had a negative effect on the animal's body, where it was established that after 5 h of the first day, circulating immune complexes indicators reliably increased by 28.8% compared to similar indicators in control groups of animal (p-value < 0.01). During the next 15 and 30 days of the experiment, the level of circulating immune complexes was within normal limits.

A slight suppressive effect was observed under the effect of 0.1% and 0.16% 'Diolaid' solutions on indicators of bactericidal activity of blood serum and the content of immunocompetent cells in the peripheral blood of rats (T and B lymphocytes).

Table 2 — Study of acute toxicity in rats after intragastric administration of the drug 'Diolaid' by the method of Kerber (n = 6)

Indicators	Drug dose, mg/kg				
	30	50	100	200	300
Males					
Number of animals, indiv.	6	6	6	6	6
of them: survived	6	5	5	3	0
died	0	1	1	3	6
	LD_{50} = 182				
Females					
Number of animals, indiv.	6	6	6	6	6
of them: survived	6	5	4	3	0
died	0	1	2	3	6
	LD_{50} = 170				

Table 3 — Confidence interval (CI) estimate for nonspecific immune response means with a probability (P) of 0.95 (n = 6)

Research period, day	Experimental groups of animals			
	Drug concentration, %			Control
	0.06	0.1	0.16	
Phagocytic activity, %				
1	11.3–12.0	11.5–12.1	10.1–10.5*	11.5–12.2
15	11.7–12.1	11.6–11.9	11.3–11.4	11.3–12.8
30	11.5–12.0	11.8–11.9	11.2–12.0	11.4–12.1
Phagocytic index				
1	4.1–4.4	3.9–4.0	3.7–4.1*	3.8–4.4
15	3.9–4.5	4.1–4.7	3.8–4.4	4.1–4.3
30	3.8–4.5	4.0–4.2	4.0–4.1	4.0–4.2
Macrophage transformation index %				
1	36.1–41.2	35.2–40.5	30.3–35.5*	40.1–43.4
15	38.3–42.1	40.6–42.8	39.8–41.5	41.2–42.2
30	37.5–44.0	41.4–43.4	37.9–42.0	40.4–44.4
Bactericidal activity of blood serum, %				
1	81.0–83.8*	61.3–65.2*	60.1–66.5*	86.2–95.6
15	88.5–91.7	85.7–93.9*	85.1–88.9*	88.8–96.0
30	92.2–98.3	90.4–98.2	98.1–98.1*	94.0–96.5
Circulating immune complexes, CU				
1	10.9–12.1	10.8–12.1	12.5–16.1*	10.5–12.5
15	10.5–12.8	10.7–13.4	12.0–15.0	10.4–12.2
30	10.5–12.2	11.1–12.8	11.3–13.5	10.2–12.8
T lymphocytes (erythrocyte rosette-forming cells)				
1	29.2–32.4*	33.0–34.4*	31.4–32.6*	34.0–36.2
15	33.7–37.9	33.2–38.1	31.6–34.8	34.4–37.5
30	33.3–37.5	34.4–38.2	31.2–34.5	34.5–37.2
B lymphocytes (erythrocyte-antibody-complement rosette-forming cells)				
1	14.4–15.9*	14.8–15.8*	13.3–13.9*	14.2–16.0
15	14.5–15.1	14.9–15.8*	14.5–15.1*	15.1–16.2
30	15.1–15.8	15.1–16.2	13.9–15.0	14.5–16.2

Note. * — the difference between the value of the indicator and the control is probable (p-value is in the range of 0.001–0.05).

On the first day, we noted a suppressive effect on the immune system of rats due to the action of different concentrations of the drug, where a probable decrease in the bactericidal activity of blood serum indicator was established. Especially the 0.16% solution of the drug with a probable decrease of the indicator by 30.3% (p-value < 0.001) compared to the control group. After 30 days of the experiment, indicators of bactericidal activity were within the normal range in all experimental groups.

The study of cellular immunity indicators in rats when using the drug 'Diolaid' showed that after 5 h, a slight inhibition of rosette formation was observed in all experimental groups of animals, regardless of the concentration of the drug, which was characterized by an unprobable decrease in the content of T cells in the peripheral blood. After 15 days, in the group of animals treated with 0.06% and 0.1% solutions of the drug, the relative content of T cells did not differ from the control group and remained so until the end of the experiment.

A study of indicators of the direct immune rosette formation (B cells) showed that the use of a 0.16% concentration of 'Diolaid' reduced the indicators 5 h after inhalation by 6.3%. In the group of animals treated with 0.06% and 0.1% solutions of the drug, the content of B cells did not differ from the control indicators throughout the experiment.

Studying the irritating and sensitizing effects of the drug 'Diolaid' on the skin of laboratory rats for 30 days with 0.06%, 0.1%, and 0.16% solutions, it was established that no skin irritations were detected in the animals at the place of application, which indicates the absence of a negative effect of the drug. Only in the first minutes after application of the concentrate, the animals tried to lick the wetted area of the skin, after that they calmed down and their behavior remained normal. The surface of the skin treated with the disinfectant was unchanged. During a single immersion of the tails of rats in the drug concentrate (exposure time 30 min), increased motor activity was observed in the animals, as a result of the irritating effect. There were no deaths among rats.

The study of the damaging effect on the skin and the development of non-allergic contact dermatitis showed that a single application of 'Diolaid' to the intact skin of the rat backs in the maximum recommended concentration of 1.0% did not cause signs of irritation.

It has been established that 0.06%, 0.1%, and 0.16% concentrations of the drug 'Diolaid' did not cause an irritating and sensitizing effect on rats. At the same time, when the concentrate was applied to the skin of animals, a temporary effect was noted in the form of a change in the behavior of rats.

The research results indicate the low toxicity of the drug 'Diolaid' for laboratory animals and the possibility of its use in low concentrations both for treatment of the cages in the presence of animals and for treatment the animals themselves.

The results of determining the toxicity and establishing the hazard class of the drugs we carried out 5 h after exposure, and then daily for 30 days. During the experiment the death of animals was not observed, the general state was satisfactory, the behavior was without peculiarities, the animals were mobile, the coordination of movements was not disturbed; the animals' skin was smooth and clean. No signs of intoxication were observed; reaction to external stimuli (sound, light, tactile) was normal; the condition of the hair coat and mucous membrane — the fur is neat, dry, the mucous membrane is pale pink, moderately moist.

According to the value of LD_{50} < 2,500 mg/kg, the experimental drug 'Diolaid' when applied once to the skin belongs to the 4th hazard class – low-hazard substances in accordance with SOU 85.2-37-736:2011 'Veterinary Preparations. Determination of Acute Toxicity' (MAPFU, 2011) and GOST 12.1.007-76 'Occupational Safety Standards System. Noxious Substances. Classification and General Safety Requirements' (Gosstandart, 1976).

The results of the study of the skin-irritating effect on guinea pigs after 1 h and 16 h — erythema and edema — 0 points; after 48 h and 72 h — the skin is unchanged, after 96 h and more — the skin of all animals is smooth and clean. According to the results of the study, the skin-irritating effect of the product 'Diolaid' is present, the response assessment — 0 points.

Thus, taking into account the results of the conducted experiments, according to the classification of SOU 85.2-37-736:2011 (MAPFU, 2011), the disinfectant drug 'Diolaid' is considered moderately toxic when administered intragastrically and belongs to the 3rd hazard class; when applied to the skin it belongs to the 4th hazard class — low toxicity.

For disinfection of water when it is stored in containers, drug 'Diolaid' concentrations (by chlorine dioxide) of 0.5–2.0 mg/l (0.0002–0.0008%) are used, depending on the degree of purity of the water to be treated. Such concentrations ensure compliance of the content of residual concentrations of chlorites with hygienic standards. Dosing of the starting solution is carried out using special dosing equipment or manually.

The presented results of our research confirm the information proven in the works of other authors who studied the toxic effect of active substances on the body of laboratory animals (Addie et al., 2015; Kovalenko et al., 2020; Yousef, Abuzreda and Kamel, 2019). Scientists have proven that the acute toxicity of chlorate (LD_{50}), even for two types of short-cycle hydrobionts *D. magna* and *N. spinipes*, is practically identical and amounts to 560 mg/l and 590 mg/l, respectively; chlorate at a concentration of 100–250 mg/l has a probable (P < 0.05) toxic effect on the reproductive parameters of *D. magna* in a chronic experiment.

The indicator of our study of the average lethal dose is 180 mg/kg of the weight of laboratory animals and it corresponds to an effective bactericidal concentration 0.06% (approx. 100 mg/l) of the drug 'Diolaid', which is consistent with the data of the scientists (Daniel et al., 1990), LOAEL (lowest-observed-adverse-effect level) is

25 mg/l (1.9 mg/kg/day), NOAEL (no-observed-adverse-effect level) (Bercz et al., 1982) as a more stringent standard at the level of 30 mg/l (3.5 mg/kg/day). In mice (Moore and Calabrese, 1980), which received drinking water with chlorine dioxide at a dose of 11.7 mg/kg/day (*i.e.* about 100 mg/l) for 30 days, there were no changes in hematological properties.

The same inconsistency was reflected in the regulation of chlorine oxide, chlorites and chlorates in different countries. For example, in the USA the standard for chlorine dioxide and chlorite in bottled water is 0.8 mg/l and 1.0 mg/l, in Germany the maximum limit for chlorite is 0.2 mg/l, in our country the former USSR standard of 20 mg/l remains, and in Italy, which is the leader in changes to chlorine dioxide for water treatment, neither the reagent itself nor its disinfection by-products are standardized at all (Mokiienko et al., 2006).

The main application of sodium chlorite is the generation of chlorine dioxide. Chlorine dioxide, derived from sodium chlorite under certain conditions, is approved by the US Food and Drug Administration (EPA). The EPA has set a maximum drinking water contamination level of 1 mg of chlorite per liter (Haruta and Kanno, 2015; Lin et al. 2018).

Conclusion. According to the results of studies of the effect of the drug 'Diolaid' on the body of laboratory rats, it was established that for intragastric administration of 'Diolaid', the average lethal dose (LD_{50}) for male rats was 182 mg/kg of body weight, and for female rats — 170 mg/kg. The drug exhibits a temporary sensitizing and skin-irritating effect only in the form of a concentrate. During the inhalation of the drug 'Diolaid' in the form of a 0.06% solution, no violations of non-specific resistance indicators were registered in animals.

Based on the results of the analysis, it was established that the disinfectant drug 'Diolaid' according to the classification of SOU 85.2-37-736:2011 is considered moderately toxic and belongs to the 3rd hazard class, when applied to the skin it belongs to the 4th hazard class — low toxicity, which allows it to be used in the presence of animals.

Prospects for further research. To test the drug 'Diolaid' in broiler chickens in production conditions.

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TESTING OF DOMESTIC DISINFECTANTS IN VETERINARY MEDICINE

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Summary. In the system of veterinary and sanitary measures against the background of environmental changes , it remains relevant to search for new high effective means for disinfection to prevent infectious diseases. In a short time, drugs should eliminate pathogens, which requires the special approach to the choice of methods and means of disinfection. To carry out effective disinfection, the availability of appropriate drugs is required, but most of them do not meet one or another requirement, namely: some drugs have a high bactericidal effect, but are toxic, others have a high effect, low toxicity, but have a destructive effect on the treated surfaces. For practical veterinary medicine, drugs that provide a complex effect on viruses, bacteria and fungi are of particular interest. As effective disinfectants, including aerosols and electro aerosols, for many bacterial and viral diseases of animals and birds, preparations from the aldehyde group showed a positive result: a formaldehyde solution with an active substance content of 37%, an alkaline formaldehyde solution prepared from paraformaldehyde and 1% sodium hydroxide. However, despite their advantage, these preparations have a number of disadvantages, namely: high toxicity with a pronounced odor, instability of working solutions, selectivity against pathogenic microorganisms, corrosiveness and carcinogenicity. With the constant use of these agents, the microflora develops resistance. In this regard, it remains relevant to create new environmentally friendly disinfectants, taking into account the achievements of domestic and foreign practice, harmless to humans and animals, environmentally safe and available for consumers

Keywords: pathogens, infectious diseases

Introduction. Disinfectants in the external environment are chemical and physical and used to combat infectious diseases in humans, animals, and plants. They can be in the form of solutions, granules, and tablets. Recently, according to literature data, the process of creating new effective drugs, technologies, and their application has been activated (Firsov et al., 2018; Ivanov et al., 2017).

The most promising developments were in the creation and testing of disinfectants based on peroxide compounds in combination with various stabilizers and surfactants, aerosols, ozone, UV radiation, and ultrasound (Paliy, Palii and Rodionova, 2017). The list of inexpensive traditional disinfectants available to the mass consumer is limited. In addition, in recent years, there has been a trend in world practice to reduce the use of previously widely used disinfectants, such as caustic soda, formaldehyde-containing, and chlorine-active substances, phenols, quaternary ammonium compounds, *etc.* (Simetskiy et al., 2000).

However, they remain relevant in the Ukrainian market. Chlorine-containing products are mainly used in the human medicine — for disinfection of products made of glass, plastic, rubber and other aggressive materials, in veterinary medicine — for disinfecting surfaces and air in the facilities where animals are kept. Drugs from the group of aldehydes are used as effective disinfectants for many bacterial and viral diseases of animals and birds with a positive effect. However, despite their advantages, they also have a number of disadvantages — high toxicity with a pronounced odor, instability of working solutions, selectivity against pathogenic microorganisms, corrosive activity and carcinogenicity. In this regard, it remains relevant to create new environmentally friendly disinfectants, taking into account the achievements of domestic and foreign practice, harmless to humans and animals, environmentally safe and available for consumers.

For more than 50 years, the disinfectant 'Iodine monochloride' (Popov and Udavliev, 2002; Yavnikov, 2020) has been developed and widely tested in Ukraine. It has a high bactericidal activity against bacteria, mycobacteria, viruses, fungi, coccidia, and helminthes. Iodine preparations are widely used for disinfection of premises in cases of many infectious diseases (anthrax, viral hepatitis, foot and mouth disease, tuberculosis, salmonellosis, coccidiosis, ascariasis, *etc.*); for prevention of respiratory diseases; for deodorization and disinfection of air in facilities where farm animals and poultry are kept.

Among foreign-made bactericidal agents, surface-active drugs are more common. They have in their composition highly soluble quaternary ammonium compounds with high bactericidal, fungicidal, virucidal activity and low toxicity. However, they do not affect spores and are ineffective against Mycobacterium tuberculosis. Yet, thanks to these unique properties, the drugs have found application in human and veterinary medicine, cosmetology, the meat and dairy processing industry, and household chemicals.

Unfortunately, in veterinary and medical practice, some chemical disinfectants have a detrimental effect not only on pathogenic, but also beneficial microflora, which are normally always in the air and are less resistant than pathogenic ones. Therefore, substances with a wide spectrum of action are selected for disinfection so that

they have a minimum amount to obtain a positive effect, and they quickly decompose in the environment (Maertens et al., 2018; Jiang et al., 2018; Palii et al., 2019).

To date, more than 200 species of microorganisms have already been identified, which have developed resistance due to the constant and prolonged use of disinfectants. It has been established that the phenomenon of this resistance is a mutation in the bacterial population and the appearance of a resistance gene in certain strains (Chidambaranathan and Balasubramanium, 2019). This once again emphasizes the need for the creation and implementation of new highly effective antiseptics and the study of their bactericidal, toxic-biological properties and methods of application in veterinary medicine, which determined the **aim of our research**.

Materials and methods. The studies used bacteriological and microscopic methods, a colony counter, prototypes of Ag+Bi metal nanoparticles at a concentration of 2.0 mg/cm^3 and 1.55 mg/cm^3 respectively, and the disinfectant 'SDV' we developed.

Results and discussion. To determine the bactericidal properties of disinfectants, test cultures of microorganisms were used: *Bacillus alvei* (strain 5), *Escherichia coli* (strain K 99), *Salmonella* Dublin (strain 41), *Staphylococcus aureus* (strain 209), strain of *Mycobacterium phlei*, and also turbidity standard 500 million bacterial cells, for mycobacteria a suspension of 1 mg/cm^3. The bacterial mass was incubated at a temperature of 37.0 ± 0.5°C on MPB and MPA, mycobacteria — on Pavlovsky's medium.

To study the bactericidal activity of 'SDV', concentrations of 10%, 50%, and 100% were taken with an exposure of 1 h and 3 h on various test objects (glass, plastic, tile, wood, surgical instruments) in comparison with 'Desmol' detergent (Table 1).

Table 1 — Results of determining 'SDV' bactericidal action on test objects

Test objects	Bacterial contamination, CFU/cm^2			
	Before disinfection	Concentration of the 'SDV' solution, %		
		10	50	100
Glass	5.0×10^2	1.3×10^2	1.5×10^2	not found
Plastic	2.5×10^2	1.3×10^2	8.0×10^2	not found
Tile	5.0×10^2	2.0×10^2	not found	not found
Wood	7.8×10^2	1.5×10^2	1.5×10^2	not found
Surgery tools	> 300	not found	not found	not found

According to the results obtained, the bactericidal effect of 100% (concentrated) 'SDV' solution was established on all the studied test objects, as well as on tiles after treatment with 50% 'SDV' (after 3 h), compared with glass, plastic and wood, where from 8% to 15% of colonies of microorganisms were detected, respectively, *i.e.* the activity of the drug was in the range of 85–92%. Lower concentrations of 'SDV' (10%) worked bacteriostatically with an activity of 80–87%.

Regarding the surgery tools, all three 'SDV' concentrations of 10%, 50%, and 100% had a bactericidal effect on microorganisms for 2 h. It has been established that the activity of a 10% 'SDV' solution persists for 10 days (observation period). When test objects were treated with 'Dezmol' detergent, *St. aureus*, *P. vulgaris*, *E. coli* were found in the swabs.

Determination of the bactericidal effect of nanoparticles was carried out by using daily test cultures and their field isolates at a temperature of 26.0°C and 37.0 ± 0.5°C. Daily broth culture served as control. First, the effect of the matrix solution Ag+Bi (2.0 mg/cm^3 and 1.55 mg/cm^3 respectively) on *E. coli* and *St. aureus* was determined. According to the results obtained, the bactericidal effect of the Ag+Bi nanoparticle complex was observed only after 24 h, and when it was diluted 1:2, after 48 h at a temperature of 37.0 ± 0.5°C.

For mycobacteria, the tested complex Ag+Bi was inactive on the *M. phlei* strain (Table 2).

Abundant growth of colonies on the surface of the medium was observed after 3 days at an exposure of 3 h, 5 h, 24 h for 21 days of the experiment. The same results were obtained when the nanoparticles were diluted as Ag (1.0 mg/cm^3) + Bi (0.75 mg/cm^3) and in the control. As for the BCG strain, the nanoparticles at both dilutions did not have either a bactericidal or bacteriostatic effect on bacteria during the entire study period (60 days).

The bactericidal activity of the 'SDV' preparation was studied similarly to nanoparticles. According to the results obtained, it was found that the drug had a 100% effect on pathogenic strains of enterobacteria at concentrations of 0.5% and 1.0% with an exposure of 1 h and 4 h at a temperature of 37.5°C. On *St. aureus*, the tested drug at a concentration of 0.5% and exposure of 1 h acted bacteriostatically. After 4 h, 100% effect of the tested drug concentrations was established.

When using the BCG vaccine at a concentration of 1 mg/cm^3, the activity of 100% (matrix) and 50% (dilution 1:2) 'SDV' solution was tested at an exposure of 3 h, 5 h, and 24 h. During the observation period (60 days), no culture growth was noted; its 100% bactericidal activity was established in comparison with the control (Table 3).

The fungicidal action of the drug 'SDV' was studied on fungi of the genus *Aspergilius*, the cultures of which were grown on Czapek's agar at a temperature 27°C. At aerosol irrigation of the culture with the matrix solution 'SDV', the death of micromycetes was observed after 24 h. The obtained results allowed us to study litter material affected by fungi (Fig. 1).

On the 20th day of observation at a temperature 27°C, growth of fungi appeared in the control and experimental samples, after that the experimental sample was treated with the drug 'SDV' at a concentration of 100% in an amount of 7 ml and placed in a thermostat at a temperature 37 ± 0.5°C, since at a temperature 22–26°C 'SDV' was not active. No growth of fungi was observed in the test sample.

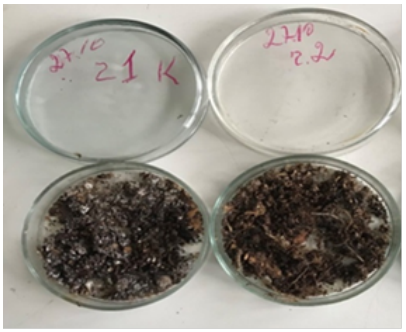
Table 2 — Effect of the Ag+Bi complex on mycobacteria

Drug name	Microorganism species	Concentration	Period of time, h	Growth of colonies after, days						
				3	7	14	21	28	35	45–60
Ag (2.0 mg/cm^3) + Bi (1.55 mg/cm^3)	*M. phlei*	1,0 mg/cm^3 physiological saline	3	+	+	+	+	Utilization of seeding		
			5	+	+	+	+			
			24	+	+	+	+			
Ag (1.0 mg/cm^3) + Bi (0.75 mg/cm^3)			3	+	+	+	+			
			5	+	+	+	+			
			24	+	+	+	+			
Control				+	+	+	+			
Ag (2.0 mg/cm^3) + Bi (1.55 mg/cm^3)	BCG	1,0 mg/cm^3 physiological saline	3	–	–	–	+	+	+	+
			5	–	–	–	–	+	+	+
			24	–	–	–	–	+	+	+
Ag (1.0 mg/cm^3) + Bi (0.75 mg/cm^3)			3	–	–	–	+	+	+	+
			5	–	–	–	+	+	+	+
			24	–	–	–	–	+	+	+
Control				–	–	–	+	+	+	+

Notes: '+' — presence of growth; '–' — lack of growth

Table 3 — Bactericidal effect of the drug 'SDV' on mycobacteria

Drug name	Microorganism species	Concentration	Period of time, h	Growth of colonies after, days							
				3	7	14	21	28	35	45	60
'SDV' 100%	BCG	1,0 mg/cm^3 physiological saline	3	–	–	–	–	–	–	–	Utilization of seeding
			5	–	–	–	–	–	–	–	
			24	–	–	–	–	–	–	–	
'SDV' 50%			3	–	–	–	–	–	–	–	
			5	–	–	–	–	–	–	–	
			24	–	–	–	–	–	–	–	
Control				–	–	–	+	+	+	+	



A B

Figure 1. Effect of 'SDV' on fungi of the genus *Aspergilius* in the litter: A — control sample, B — experimental sample

Conclusion. Based on the reconnaissance laboratory tests of the disinfectant drug 'SDV' developed by us, we can say that the drug has 100% bactericidal and fungicidal properties for pathogenic strains of enterobacteria, mycobacteria, and fungi. The bactericidal effect of a 100% solution of 'SDV' on all studied test objects was established.

Prospects for further research. We believe that it is advisable to continue further research of the disinfectant preparation 'SDV' in order to improve the methods of combating and preventing infectious diseases. In addition, the use of nanotechnologies, first, is a great prospect in the fight against pathogens of infectious diseases of bacterial etiology.

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STATE OF METABOLIC PROCESSES IN CATTLE UNDER THE INFLUENCE OF BIOTIC CONTAMINANTS OF FEED

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Summary. Control of feed contamination by micromycetes and bacteria at all stages of their preparation, storage, and feeding of farm animals is an acute issue of feed safety and one of the principal measures that create an opportunity to prevent their negative impact on animal health. Therefore, the study aimed to investigate the state of metabolic processes in cattle of different physiological groups under the influence of biotic feed contaminants. The material for the research was grain fodder and coarse grinding grain of local production, roughage used on the farm. Veterinary and sanitary condition of grain products was established based on organoleptic, toxico-biological and microbiological studies. To determine the indicators of the state of metabolic processes, 3 groups of cows (n = 5–7) with different physiological conditions were formed: group I — non-pregnant cows, group II — pregnant animals with normal pregnancy, group III — cows after miscarriage. Biochemical parameters (level of total protein, albumin, globulins, vitamins A and E) in blood serum samples were determined spectrophotometrically by conventional methods. The study of the content of inorganic elements in the aggregate samples of bovine sera was performed using an X-ray spectrometer 'Spectroscan MAX'. Laboratory studies have proven the presence of biotic contaminants in the feed base of the experimental farm. Exceedance of maximum permissible levels of feed contamination (max 16.50×10^4 CFU/g when MPL 5.0×10^4 CFU/g) by toxin-forming micromycetes (due to the genera *Fusarium*, *Aspergillus*, *Penicillium*, *Mucor*, and *Rhizopus*; a total of 24 isolates of microscopic fungi were isolated, which showed high toxicity in 11.3% and weak — in 20.1% of samples) and total bacterial contamination (max 18.7×10^5 CFU/g when MPL 5.0×10^5 CFU/g), in the structure of which coliform bacteria and *Salmonella enterica* were isolated. In cattle that consumed feed with an excess of biotic contaminants, disorders of the digestive tract (diarrhea) and reproductive capacity (abortions in the first half of pregnancy) were observed and metabolic disorders were found in cattle: increased Iron (on average 1.5 times) and Bromine (on average 1.6 times) levels, a decrease in the concentration of vitamin A (by 17.4–39.8%), and vitamin E (by 10.0–12.5%), most pronounced in cows after abortion and pregnant cows, respectively, Manganese (on average by 12.5%) and Selenium (by 30.7%)

Keywords: metabolic disorders, microbiological contamination, micromycetes, toxicity

Introduction. High productivity of animals is a genetically determined body's ability to effectively transform feed nutrients, due to the intensive metabolic processes in animals at all levels — from hydrolysis and transport of feed nutrients in the digestive tract and use of energy from their metabolism to biosynthesis of proteins, lipids and other organic substances.

Among them a special place is occupied by minerals — macro- and ultramicroelements. For the manifestation of genetically determined potential properties of animals to synthesize quality products, it is necessary to create ecological conditions for their feeding and maintenance, which provide the optimal course of metabolic processes in the body (Orobchenko, 2012; Doletskyi, 2015).

Feeding poor quality feed to animals with high levels of toxin-forming micromycetes and residues of toxic metabolites of lower fungi — mycotoxins, which are formed under conditions of growth on forage crops in the field, during harvesting and preservation of crops, can lead to weakening of the body's resistance, the emergence of diseases, reduced productivity and deterioration of livestock products (Yaroshenko, 2016; Yaroshenko, Kutsan and Orobchenko, 2018; Kutsan et al., 2020; Kemboi et al., 2020).

According to the Food and Agriculture Organization of the United Nations (FAO), due to the high prevalence of microscopic fungi in almost all biological habitats and their high adaptive properties, 25–40% of the world's food and feed crops are contaminated with mycotoxins annually. This causes annual economic losses of about 20 billion dollars. They produce mycotoxins, which, when entering the body of animals during feeding, can cause dangerous diseases — mycotoxicosis (Gadzalo, 2017; Harčárová, Čonková, Sihelská, 2018).

In the event of action of main mycotoxins on animals, the period of the disease clinical manifestation is determined by a few hours or days. However, this is typical only in acute or subacute mycotoxicosis. Under the action of small doses of mycotoxins a chronic form of toxicosis develops, which is too difficult to diagnose and can appear only after a long time from the beginning of use of the contaminated feed (Gonçalves, Corassin and Oliveira, 2015). Therefore, it is important and relevant to study the assessment of animal welfare, which underlies the functioning of efficient production, especially in the context of the Association Agreement between Ukraine and the European Union (EU) and the WHO strategy 'One Health'(Boqvist, Söderqvist and Vågsholm, 2018; Vandicke et al., 2021; Chiesa et al., 2021).

Due to the fact that the control of contamination of feeds by micromycetes and bacteria at all stages of their preparation, storage and feeding to farm animals is an important issue of feed safety and one of the main measures to prevent their negative impact on animal health (MAPFU, 2012), the study was **aimed** to investigate the state of metabolic processes in cattle of different physiological groups under the influence of biotic feed contaminants.

Materials and methods. The work was performed in the State Enterprise 'Experimental Base 'Dachna' of the Breeding and Genetic Institute — National Center of Seed Science and Variety Study' (SE 'Experimental Base 'Dachna') (Dachna, Odesa District, Odesa Region), laboratories of epizootology, parasitology, monitoring of animal diseases and provision of the Odesa Research Station of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Odesa), laboratories of clinical biochemistry and toxicological monitoring of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (NSC 'IECVM') (Kharkiv).

The material for the research was grain fodder and coarse grinding grain of local production, roughage used on the farm in 2020–2021 (21 samples). The veterinary and sanitary condition of grain products was established on the basis of organoleptic, toxicological and microbiological studies.

Organoleptic analysis was used to determine the appearance, color, odor, and visible signs of fungal infection (Obrazhei, Pohrebniak and Korzunenko, 1998). In order to determine the degree of contamination of feed with microorganisms, mycological and bacteriological studies were carried out by seeding in nutrient media, isolation into a pure culture, according to generally accepted methods (MVDMAUSSR, 1976; MAPFU, 2012).

The species affiliation of microorganism isolates was determined by cultural and morphological characteristics of the isolated mycobiota (features of culture growth on different media, their size, shape, width, construction of the edge and center of colonies, intensity of growth, surface characteristics, color of colonies) (Pidoplichko and Mil'ko, 1971). The main test for determining the toxicity of feed was a skin test on rabbits (Malinin, Khmel'nitskiy and Kutsan, 2002).

To determine the indicators of the state of metabolic processes, 3 groups of cows (n = 5–7) with different physiological conditions were formed: group I — non-pregnant cows, group II — pregnant animals with normal pregnancy, group III — cows after miscarriage.

The presence of possible metabolic disorders in the cattle body was determined by biochemical indicators in blood serum samples, which were examined spectrophotometrically according to generally accepted methods (Stegniy et al., 2007; Vlizlo, 2012) and compared them with the reference levels given in the monograph (Levchenko, 2010).

The determination of the content of inorganic elements in the aggregate blood serum samples of cattle was carried out using the X-ray spectrometer 'Spectroscan MAX' following the X-ray fluorescence methodology developed at the NSC 'IECVM' and approved by the State Committee of Veterinary Medicine of Ukraine (protocol No. 1 of 23–24 December 2009) (Kutsan, Orobchenko and Kocherhin, 2014).

Results. SE 'Experimental Base 'Dachna' is a multi-industry enterprise. Crop production, where the leading industry is seed production, occupies the largest specific weight in the structure of commercial products of the enterprise. However, dairy cattle breeding, which has more than 200 cows in breeding stock and 260 heifers and calves, is also of great significance in the structure. Conditions for feeding and keeping animals comply with sanitary and hygienic norms. Veterinary and sanitary measures are carried out in a timely manner at the farm. All animals are vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea, bovine parainfluenza-3, pasteurellosis and escherichioses.

However, in the autumn period, alimentary abortions were registered among cows in the first third of the pregnancy — the expulsion of a dead fetus (miscarriage) together with the membranes without visible previous clinical signs and disorders of the gastrointestinal tract (diarrhea), the cause of which could be insufficient feeding, protein starvation or, on the contrary, overfeeding with protein substances, mineral starvation (Phosphorus, Calcium, Iron, Potassium, *etc.*), vitamin deficiency (A, E, C, D), feeding with poor-quality feed.

In order to determine the causes of abortions, laboratory tests were conducted for the sanitary quality of the feed and the presence of biotic contaminants (microscopic fungi and coliform bacteria).

According to the results of the sanitary condition of 21 feed samples (roughage: silage, haylage, straw; grain-compound feed, corn, barley) it was established that 47.6% of the feed samples met the sanitary and hygienic requirements and were allowed for feeding, 52.4% did not meet the maximum permissible levels (MPL). Thus, changes in organoleptic indicators were detected in 11 samples of roughage (straw, silage), which had a dark gray color without a shine, a musty mold smell with mycelium and sporulation of mushrooms; in samples of hay we observed a musty smell and a change in color from dark brown to black with the formation of compressed lumps. Such feed is suspicious; a change in color, smell and other characteristics indicates the development of microorganisms.

As a result of research, it was established that the sanitary condition of grain fodder met the requirements of the MPL. High rates of micromycete contamination were found in rough forages, which amounted to 13.25×10^4 CFU/g in straw, 16.5×10^4 CFU/g in haylage, and 11.25×10^4 CFU/g of forage in silage, which, respectively, in 2.7, 3.3, and 2.3 times exceeded the MPL indicator (Table 1), and 24 isolates of microscopic fungi were isolated.

Table 1 — Results of microbiological studies of feeds

Name of the feed	Microscopic fungi, CFU/g	Total bacterial pollution, CFU/g	The titer of coliform bacteria
Barley	1.65×10^4	3.5×10^5	2
Wheat	not found	2.9×10^5	1
Combined feed	not found	3.5×10^5	2
Wheat bran	0.95×10^4	3.0×10^5	1
Wheat straw	13.25×10^4	9.5×10^5	3
Haylage	16.50×10^4	6.3×10^5	3
Silage	11.25×10^4	18.7×10^5	3
MPL	$\leq 5.0\times10^4$	$\leq 5.0\times10^5$	≤ 3

The maximum indicators of total bacterial contamination were also found in roughage, which 2–3 times exceeded the MPL, and the titer of coliform bacteria was 3.

Mycological studies of two straw samples revealed toxigenic micromycetes of the genus *Fusarium oxysporum*. Samples of peas, combined feed, bran, haylage, silage, and straw were affected by fungi of the genus *Aspergillus* spp., *Mucor* spp., *Penicillium* spp., and *Rhizopus nigrus*, which showed high toxicity in 4 (19.1%) and weak toxicity in 7 (33.3%) samples.

The analysis of research results showed that representatives of the genera *Aspergillus* — 16 isolates (66.7%), *Fusarium* — 1 isolate (4.1%), *Penicillium* — 3 isolates (12.5%), and Mucoraceae family — 4 (16.7%) were the main contaminants of fodder in SE 'Experimental Base 'Dachna' in 2021 (Fig. 1).

A skin test on rabbits revealed weak toxicity of 7 feed samples (33.3%) and toxicity of 4 feed samples (19.1%).

The etiological structure of isolates of microorganisms was represented by cultures of coliform bacteria (69.2%) and *Salmonella enterica* (30.8%).

After the detection of a significant amount of biotic contaminants in feeds, a study of the state of metabolic processes in the body of cattle of critical groups was carried out by means of biochemical studies of blood sera. The results of the research are given in Tables 2–3.

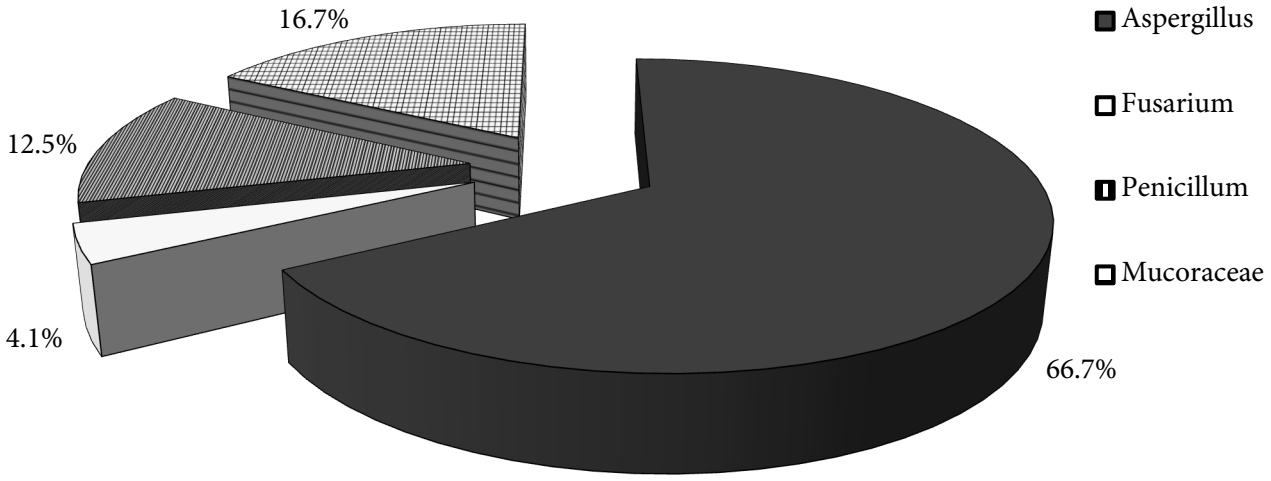

Figure 1. Taxonomic structure of mold fungi isolated from feeds for cattle

Table 2 — Results of biochemical studies of cows' blood sera (M ± m, n = 5–7)

Group	Indicators					
	Total proteins, g/l	Albumin, g/l	Globulins, g/l	Albumin/ globulin ratio	Vitamin A, μg%	Vitamin E, μg/ml
Group I (non-pregnant)	81.7 ± 0.6	36.5 ± 3.2	45.1 ± 2.3	0.80 ± 0.06	20.60 ± 0.30	3.6 ± 0.20
Group II (pregnant)	83.3 ± 2.5	35.2 ± 1.9	48.1 ± 3,7	0.76 ± 0.07	16.50 ± 1.30*	3.5 ± 0.15
Group III (after miscarriage)	80.3 ± 1.8	37.0 ± 1.2	43.3 ± 0.6	0.85 ± 0.01	15.05 ± 1.40**	4.0 ± 0.06
Reference level	72.0–86.0	27.5–39.4	28.9–48.6	0.80–1.00	not less 25.00	4.0–6.0

Notes: * — $P \leq 0.05$, ** — $P \leq 0.001$ relative to the indicators of the group I.

As evidenced by the data in the Table 2, the most pronounced deficiency of vitamin A was observed in cows — a decrease in concentration relative to the lower limit of the reference level was found in animals of all experimental groups: in non-pregnant animals the decrease was 17.4%, in pregnant animals — 34.0%, in cows after miscarriage — 39.8%. When determining the characteristics of the cow body supply in different

physiological groups, it was established that the highest level of vitamin A supply was in non-pregnant animals. Compared to the indicator of the group I, the concentration of vitamin A was significantly lower by 20.9% ($P \leq 0.05$) in pregnant cows, and by 27.1% ($P \leq 0.001$) in cows after miscarriages.

In addition, a slight decrease in the supply of vitamin E in the cows' body was established — in the cows of the group I by an average of 10.0%, in the group II — by 12.5% compared to the lower limit of the reference indicators. In cows of group III, the indicator corresponded to the minimum level of reference values.

The indicators characterizing the state of protein metabolism generally corresponded to the reference values and did not differ significantly in the studied physiological groups of animals, with the exception of the globulin fraction content, which was close to the maximum values of the reference level. This determined the established tendency to decrease (by 5.0%) the albumin/globulin ratio in animals of the group II compared to the minimum reference values and indicators in the cows of the group I.

Studies of the content of inorganic elements in blood serum have established (Table 3) that in the body of animals of the group I (non-pregnant) and group III (after miscarriage) there is a deficiency of Manganese and an excess of Iron and Bromine, and in the group II (pregnant) there is a deficiency of Manganese and Selene and an excess of Iron and Bromine.

Table 3 — The content of inorganic elements in cows' blood sera (M ± m, n = 5–7)

Element	Group I (non-pregnant)	Group II (pregnant)	Group III (after miscarriage)	Reference level
Zinc, μg%	133.30 ± 1.67	133.50 ± 1.22	138.40 ± 1.84	100.00–150.00
Copper, μg%	101.60 ± 1.14	103.90 ± 1.29	110.00 ± 1.42*	80.00–120.00
Iron, μg%	271.40 ± 3.57	330.00 ± 3.76*	321.70 ± 3,84*	90.00–210.00
Manganese, μg%	3.60 ± 0.12	3.80 ± 0.17	3.40 ± 0.15	4.00–6.00
Selenium, μg%	7.90 ± 0.24	5.20 ± 0.13*	9.70 ± 0.27*	7.50–10.00
Lead, μg%	Not found	Not found	Not found	—
Nickel, mg%	5.10 ± 0.18	4.10 ± 0.16*	4.80 ± 0.11	2.80–5.40
Strontium, μg%	Not found	Not found	Not found	—
Cobalt, μg%	4.80 ± 0.14	4.10 ± 0.10*	4.30 ± 0.09*	3.00–5.00
Bromine, mg%	1.71 ± 0.06	2.47 ± 0.04*	2.19 ± 0.02*	0.70–1.30

Note. * — $P \leq 0.05$ relative to the indicators of the group I.

Thus, in the group I Iron and Bromine concentrations exceeded the upper limit of the reference level by 1.3 times, respectively, while the concentration of Manganese was lower than the lower limit of the reference level by 10.0%.

In the group II concentrations of Iron and Bromine exceeded the upper limit of the reference level by 1.6 times and 1.9 times, respectively, while the concentrations of Manganese and Selenium were below the lower limit of the reference level by 10.0% and 30.7%, respectively.

In the group III concentrations of Iron and Bromine exceeded the upper limit of the reference level by 1.5 times and 1.7 times, respectively, while the concentration of Manganese was lower than the lower limit of the reference level by 15.0%. That is, the concentration of Iron in the blood serum of cattle of the experimental groups was higher than the upper limit of the reference level by an average of 1.5 times, Bromine — by an average of 1.6 times, while the concentration of Manganese decreased by an average of 12.5%, and Selenium — by 30.7%.

The analysis of the data in relation to the group I (conditionally control) indicates an increase ($P \leq 0.05$) in the concentration of Iron in the blood serum of cattle of the group II by 17.9%, and Bromine — by 30.8%, while the concentration of Selenium, Nickel and Cobalt was lower ($P \leq 0.05$) of the indicator of the group I by 51.9%, 24.4%, and 17.1%, respectively. In the blood serum of cattle of the experimental group III, the concentration of Iron exceeded the indicator of the conditional control by 15.8%, and Copper, Selenium, and Bromine — by 7.6%, 18.6%, and 21.9%, respectively, while the concentration of Cobalt was lower ($P \leq 0.05$) than the indicator of the group I by 11.61%.

Discussion. Under the influence of fungi and bacteria, the physico-chemical properties of feeds change, various toxins and decomposition products of organic substances accumulate, which leads to animal poisoning. At the same time, the toxicity of feed is caused by the development of not one, but several types of toxic fungi on it. The course of animal poisoning may be in hidden and expressed forms, which depends on the generic and species composition of fungi, their toxicity, the degree of damage to the feed, and the sensitivity of animals. At the same time, the sensitivity of animals to viral and bacterial pathogens also increases, which causes the development of immunodepression, mycotoxicosis, symptoms of various diseases, the risk of ketosis (Marczuk et al., 2012), reduced productivity and reproductive function, abortions and death of animals (Volkov, 2005; Wakelin et al., 2016; Deveau et al., 2018).

Chronic toxicosis is characterized by a decrease in productivity, hemorrhages and necrotic changes in the digestive tract (especially in the large intestine), disorders of the function of hematopoietic organs due to damage

to the liver and kidneys, changes in the cardiovascular system, biochemical indicators of blood, impaired reproductive function, abortions, the birth of a non-viable young animals (pregnant cows abort on the 3rd–17th days under the influence of toxic metabolites of fungi from the genus *Aspergillus*) (Malinin, Khmel'nitskiy and Kutsan, 2002; Adhikari et al., 2017).

According to our data, the above is confirmed both by clinical symptoms in cattle (in particular, such as diarrhea and abortions), and by disorders of metabolic processes in the body. A decrease in the level of vitamin A in the blood serum (A-hypovitaminosis) is observed in the event of a violation of its transformation into vitamin A in chronic inflammation of the intestinal mucosa, a lack of protein in the diet, easily soluble sugars, and the presence of antivitamins (nitrites, chlorides, *etc.*) in the feed. At the same time, the development of endogenous A-hypovitaminosis is caused by liver diseases (hepatodystrophy, purulent hepatitis), since they reduce the secretion of bile, the synthesis and release of bile acids, which participate in the emulsification of retinol. E-hypovitaminosis causes a violation of oxidative processes in organs and tissues, dystrophy and necrosis of hepatocytes, muscular dystrophy, decreased fertility, with a simultaneous lack of Selenium, white muscle disease develops. In addition, the intensive reproduction of mold fungi leads to significant losses of carbohydrates and a decrease in the quality of ensiled fodder due to partial consumption of lactic acid, when animals eat such fodder, A-vitaminosis of animals and fermentation processes in the digestive tract of cattle increase (Parakhyn et al., 2006). Which is reflected in the biochemical profile of the experimental groups of animals — a decrease in the concentration of vitamin A by (15.0-52.0%) and vitamin E in 70.0% of cows by (2.5–25.0%).

It should also be noted that oxidative stress is one of the most important toxic mechanisms of action of waste products of biotic feed contaminants (bacterial and mycotoxins). They are able to generate free radicals, including reactive oxygen species, which induce lipid peroxidation, leading to changes in membrane integrity, cellular redox signaling, and antioxidant status of cells, by excessive use of antioxidant system vitamins (A and E) (Wu et al. 2014). This may be one of the reasons for the established decrease in the body supply of them in the experimental cows.

As is known, the antioxidant system includes several links, one of which belongs to trace elements that are part of antioxidant protection enzymes. In particular, Selenium and Manganese play a leading role in these processes (Aguirre and Culotta, 2012; Fernández-Lázaro et al., 2020). It is possible that the decrease in the content of these elements in the blood serum of experimental cows is associated with the presence of endotoxicosis and excessive use of microelements.

Manganese deficiency can lead to delayed growth and development of animals, impaired reproductive function, and nervous system disorders. Due to a lack of Manganese, carbohydrate, mineral and vitamin exchanges are disturbed, acid capacity decreases, *etc.* Selenium in the body performs the function of an antioxidant, has an immunostimulating effect; affects reproductive function. Selenium deficiency is characterized by a violation of protein, carbohydrate, fat, mineral, vitamin (especially vitamins A and E) exchanges; liver necrosis; disorder of endocrine, cardiovascular, respiratory, digestive and nervous systems. Cow diseases such as metritis, ovarian cystitis, and udder edema are associated with Selenium deficiency (Kutsan, Orobchenko and Kocherhin, 2014).

Today, there is a debate in the world about Iron homeostasis under conditions of inflammation. Some authors indicate that inflammatory reactions in the body lead to anemia (Verma and Cherayil, 2017), while others, especially in the presence of chronic inflammation, claim an increase in the level of the element in the body (Wessling-Resnick, 2010). The last statement is consistent with our data: Iron concentration in cattle increased by an average of 1.5 times relative to the upper limit of the reference level.

Under conditions of excess reception, Iron accumulates in the organs and tissues of animals (especially in the liver) in the form of toxic hemosiderin; with an excess of Iron in the body, the assimilation of Calcium, Manganese, Zinc, vitamins A and E is disturbed, feed consumption and animal productivity decrease (Kutsan, Orobchenko and Kocherhin, 2014), which can also be the cause of a decrease in vitamin A and manganese in blood serum of cows.

Bromine reception in high doses in the body for 6 months causes disorders in carbohydrate and protein metabolism, fatty degeneration of the liver and degenerative-necrotic changes in the myocardium, leads to iodine deficiency, which causes growth retardation and pronounced changes in the endocrine system: a decrease in the content of thyroxine in the tissue of the thyroid gland, an increase in the amount of thyroid-stimulating and adrenocorticotropic hormones in the pituitary gland, a decrease in the content of thyroxine, testosterone and corticosterone in blood serum, an increase in follicle-stimulating hormone and insulin (Kutsan, Orobchenko and Kocherhin, 2014).

Regarding the content of vitamins and microelements in blood serum, their reduced or increased reception with feed and water should not be excluded either, but this is the topic of another work.

The animal's organism is placed in extremely harsh conditions of existence and requires constant monitoring of the state of health, maintenance of homeostasis of various systems and the organism as a whole. An important element of such control is biochemical monitoring, which makes it possible to detect the earliest deviations in health without allowing to go beyond physiological parameters. Changes in biogeocenoses, which occur constantly due to human economic activity and at the same time a natural deficiency of essential trace elements and the presence of a significant number

of contaminants of biotic origin in feed, contribute to the emergence and spread of pathology of mineral metabolism in the body of farm animals, in particular in lactating cows (Doletskyi, 2015; Sachko et al., 2016).

Conclusions. 1. Based on the results of feed safety studies, it was established that 47.6% of the samples met the sanitary and hygienic requirements and were allowed for feeding, 52.4% of the feed did not meet the MPL, of which 33.3% of the samples were mildly toxic and 19.1% of the samples were toxic.

2. As a result of laboratory studies, high levels of biotic contaminants were found in the fodder base of the farm: the maximum total contamination with micromycetes was 16.50×10^4 CFU/g when the MPL was 5.0×10^4 CFU/g (due to toxin-producing representatives of the genera *Fusarium*, *Aspergillus*, *Penicillium*, *Mucor*, and *Rhizopus*, a total of 24 isolates of microscopic fungi were isolated) and the maximum total bacterial contamination was 18.7×10^5 CFU/g when the MPL was 5.0×10^5 CFU/g, in the structure of which coliform bacteria and *Salmonella enterica* were isolated (the titer of coliform bacteria was 3).

3. Clinically, an excess of biotic contaminants in feed was manifested in cattle by the occurrence of disorders of the digestive tract (diarrhea) and reproductive capacity (abortions in the first half of pregnancy).

4. Under the influence of biotic feed contaminants, metabolic disorders in the body of cattle were revealed: an increase in the level of Iron (on average by 1.5 times) and Bromine (on average by 1.6 times), a decrease in the concentration of vitamin A (by 17.4–39.8%), and vitamin E (by 10.0–12.5%), most pronounced in cows after miscarriage and pregnant, respectively, Manganese (by 12.5% on average) and Selenium (by 30.7%).

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Contents

Part 1. Veterinary medicine

Part 2. Biotechnology

Part 3. Biosafety