

**Приватна наукова установа
«Науково-дослідний інститут єдиного здоров'я»**

**Державний науково-дослідний інститут
з лабораторної діагностики та ветеринарно-санітарної експертизи**

ГО «Інститут Єдиного Здоров'я»

**Private Scientific Institution
“One Health Scientific and Research Institute”**

**State Scientific and Research Institute for Laboratory Diagnostics and
Veterinary and Sanitary Expertize**

One Health Institute, NGO

**ISSN (print): 2786-7420
ISSN (online): 2786-7439**

ONE HEALTH JOURNAL

**Healthy community, Healthy livestock,
Healthy wildlife, Health environment
One World – One Health**

Том / Vol. 2

Номер / N 3

DOI: 10.31073/onehealthjournal2024-III

Київ / Kyiv – 2024

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Журнал заснований 25 жовтня 2022 р. Державним науково-дослідним інститутом з лабораторної діагностики та ветеринарно-санітарної експертизи та Громадською організацією "Інститут Єдиного Здоров'я". Видання зареєстроване Міністерством юстиції України, Свідоцтво КВ № 25382-15322Р від 10.01.2023 р. Включено до Переліку наукових фахових видань України (Категорія «Б») для ветеринарних наук (наказ МОН України N 768 від 20 червня 2023 р.) та біологічних наук (наказ МОН України № 1309 від 25 жовтня 2023 р.). Матеріали номеру схвалені до друку Редакційною колегією 1.07.2024 р.



Dear colleagues,

“One Health” is a multidisciplinary approach implemented at local, national, and global levels, aimed at achieving optimal outcomes in the realm of health and well-being via interdisciplinary collaboration of human health, animal health, plant health, and environmental safety domains.

Our multisectoral team, led by the State Scientific and Research Institute for Laboratory diagnostics and Veterinary and Sanitary Expertize (SSRILDVSE) – leading veterinary science and reference activities establishment of the State Food Safety and Consumer Protection Service of Ukraine and Non-Governmental Organization One Health Institute (NGO OHI), created the One Health Journal in

2022. Our multidisciplinary editorial team and respected society of contributing authors and reviewers demonstrated proficiency of our issue, that allowed to be included in the State register of professional journals for veterinary, biological and medical sciences since 2023-2024.

Our Editorial team hopes, that our multidisciplinary profile will be interesting for wide auditorium of scientists and practical specialists in human medicine, veterinary medicine, biology, biotechnology and biosafety. We invite new authors for fruitful collaboration and joint development of the One Health multidisciplinary approach implementation.

This issue is dedicated to different medical, veterinary and ecological problems in areas of infectious diseases, biological and food, and ecological safety.

The editorial board is grateful to German Biosecurity Program and GIZ GmbH for of English translation of papers for this issue.

Sincerely yours,

Prof. Dr Anton Gerilovych,
Editor-in-Chief



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**NON-STEROIDAL ANTI-INFLAMMATORY
DRUGS "CELEXIB" AND "DIBUTALIASTIN" EFFICACY IN INDUCED
INFLAMMATION IN RATS**

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Abstract. *Non-steroid Anti-Inflammatory Drugs (NSAIDs) are the most widely used analgesics in veterinary medicine. Induced inflammation in laboratory animals is one of the most common methods to determine the anti-inflammatory and analgesic properties of new drugs. We studied the anti-inflammatory properties of the drugs "Celecoxib", with the celecoxib (100 mg in 1 ml) active pharmaceutical ingredient and "Dibutalastin" ointment (1 g of the drug contains 61.0 mg of methyl salicylate). The studies resulted in the following, after supplantation of 1% carrageenan solution into laboratory rats, animals of the control and experimental groups developed an acute inflammatory process (swelling, increased volume of the paw, hyperemia, and tenderness). The most significant changes were observed in rats of all groups three hours after carrageenan administration. It was found that rats of the control group that did not receive anti-inflammatory drugs had the volume of the paw increased on the 4th and 6th hours of the experiment. The animals of experimental E₁ and E₂ groups, after 4 hours showed decreased intensity of the inflammatory process under the influence of "Celecoxib" and "Diclofenac" anti-inflammation drugs. The volume of the paw was 25.6, 8.2, and 14.3% less in the E₁, and E₂ groups of animals, compared to the period before induction of inflammation. By the 6th hour of the study, the animals of group E₁ had virtually no visible clinical signs of the inflammatory process caused by carrageenan, which confirms the anti-inflammation effectiveness of the "Celecoxib" drug. It was found that inflammation induced by allyl isothiocyanate and formalin in rats, the investigated drug "Dibutalastin" ointment also showed peculiar analgesic properties. It was observed that its analgesic and anti-inflammatory effects were like the reference drug "Dolaren-gel".*

Keywords: Celecoxib, Dibutalastin, carrageenan-induced inflammation, white rats, analgetic and anti-inflammatory effect

Increasing the effectiveness of treatment of diseases in animals that occur with inflammation is one of the challenges in veterinary medicine.

Inflammation is connected with a range of biochemical and immunological processes, their control is conditioned by a large number of humoral mediators, among which a prominent place belongs to low-molecular-weight proteins called cytokines.

Pain, as an unpleasant sensation accompanying the inflammation process, often performs an alarming function, warning the body of danger and protecting it from possible damage. Special neural structures – nociceptors are responsible for the perception and analysis of the pain signals in animal bodies, they are mainly sensitive to pathological marks or to marks of the pathologies after prolonged exposure. The pain syndromes are associated with the activation of nociceptive receptors in trauma, inflammation, or damage to the structures

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of the central or peripheral nervous systems involved in the transportation of pain signals (Bennet and Villa, 2000; Laporte et al., 2004).

Non-steroid Anti-Inflammatory Drugs (NSAIDs) are the most widely used analgesics in veterinary medicine. After the discovery of preferential and selective cyclooxygenase – 1, 2 inhibitors (COX-1, -2), these inhibitors became even more extended due to their anti-inflammatory, analgesic, and antipyretic effects (Brune et al., 2015; Blanca-Lopez et al., 2019).

More than 1000 types of drugs containing NSAIDs, or their active ingredients are used in humane and veterinary medicine. They have been primary analgesics in pain therapy in animals' musculoskeletal disorders for many years. Typically, NSAIDs exert their analgesic effect by inhibiting the cyclooxygenase (COX) enzyme, which is involved in the synthesis of prostaglandins from arachidonic acid in animal bodies. The discovery of selective COX-2 inhibitors was caused by the adverse effects (gastrointestinal and renal dysfunction) of COX-1 (KuKanich et al., 2012; Salazar Alcala et al., 2019). Modern drugs of this group include meloxicam, nimesulide, diclofenac, ibuprofen, ketoprofen, etc. Recently, newer NSAIDs under the 'coxib' generic name (celecoxib, cimicoxib, deracoxib, firocoxib, mavacoxib, robenacoxib, etc.) have been approved and appeared on the pharmaceutical market (Reinoso et al., 2001). It is believed that the new anti-inflammatory drugs should be focused not so much on improving efficacy but on enhancing their safety, since in Ukraine NSAIDs are the cause of 50% of all complications from pharmacotherapy.

The adverse effects of anti-inflammatory drugs also depend on the dose and the delivery. Topical NSAIDs have almost the same analgesic effect as oral administration, but their systemic effect on the body and adverse effects are less.

In human medicine, NSAIDs with various active ingredients are often used as topical therapies. They include soft drugs with methyl salicylate as the active ingredient. Such as Bom-Benge ointment, Capsin, Saliniment, and others. Methyl salicylate is a derivative of salicylic acid and has a local irritating effect. Methyl salicylate irritates the skin receptors, causing the formation and release of many active substances into the blood that regulate pain sensitivity. This releases a substance from the neurons. Reduced accumulation of this substance in the nerve endings leads to a decrease in pain sensitivity. Besides, methyl salicylate, which is a non-steroidal anti-inflammatory ingredient (NSAID), inhibits prostaglandin synthesis by inhibiting cyclooxygenases (COX-1, 2), which reduces swelling and infiltration of inflammatory tissues (Li et al., 2016; Yeoh and Goh, 2022).

The common non-steroidal anti-inflammatory drugs are widely used in veterinary medicine. However, the problem is there are few drugs approved or licensed specifically for the treatment of animals, which can create certain therapeutic and legal issues. Therefore, in our opinion, the development of new safe, and effective NSAIDs for veterinary medicine is urgent and requires prompt resolution.

The purpose of the study is to substantiate and develop a formulation of new NSAIDs for veterinary medicine, and to study their adverse effects and efficacy in the treatment of musculoskeletal diseases in animals.

Materials and methods. Inducing an inflammatory process in laboratory animals is one of the most common methods to determine the analgesic and anti-inflammatory properties in developing new drugs or synthesizing substances. The study on the effect of non-steroidal anti-inflammatory drugs (NSAIDs) "Celexib" and "Dibutalastin" was conducted in the vivarium and laboratory of the Department of Pharmacology and Toxicology of Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Ukraine, on laboratory rats of 3-4 months old, with body weight 260-290 g. The laboratory animals were selected on the analogue principle by way of randomization. The housing and feeding of laboratory rats met the established requirements. The air temperature in the room was 20-24 °C, the humidity – 45-65%, and the lighting regime was comfortable for the animals. The animals were kept in

cages (3 animals each), with regular ventilation and disinfection with a UVC germicidal lamp. The access to food and water was constant. The laboratory rats were fed with granular meal.

Before the research procedures, the laboratory animals had a preparation and acclimation period. The animals were kept in a separate room, had daily examinations and behavior assessments, their body temperature was measured, and their functional characteristics, reflex activity, etc. were monitored for a week.

The studies were conducted in compliance with the rules and requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Strasbourg, 1986).

For the study, we substantiated and developed the formulation, technological aspects of preparation, and preliminarily studied acute and chronic forms in laboratory rats, analyzed the toxicity and cumulation of two new NSAIDs, "Celexib", a solution for injections with celecoxib (100mg in 1ml) active ingredient, and "Dibutalastin" ointment for external use (1g of the drug contains 61.0 mg of methyl salicylate).

The study was conducted in several stages. First, we studied the anti-inflammatory effect of the new celecoxib-containing drug "Celexib" in three groups of white rats (six animals each): The Control (C) and two Experimental (E₁ and E₂). Animals of all groups were clipped and shaved on the thigh area of the right hind paw. The rats of the Control group were administered 0.15 ml of 0.9% sodium chloride solution intramuscularly, the rats of the Experimental groups had: first group – 0.15ml of "Celexib" and the second group – 0.15ml of diclofenac. After 1 hour, all rats involved in the study were induced with an inflammatory process by subplantar (under the plantar aponeurosis of the right hind paw) injection of 1% carrageenan solution (with 0.9% sodium chloride solution) (Morris, 2003). The anti-inflammatory pharmacological effect was assessed by measuring the paw thickness (at the widest point) using an electronic caliper (before and after 1, 2, 3, 4, 6-hour time after carrageenan administration).

In the second stage, like the previous procedure, the anti-inflammatory effect of another NSAID the topical drug "Dibutalastin", was investigated. White rats of the control group (C₂) were treated with petroleum ointment rubbed on the shaved thigh area of the right hind paw, the III experimental group (E₃) with "Dibutalastin" ointment and the IV experimental group (D₄) with the reference drug "Dolaren-gel". In 30 min, animals of all groups were subplantar administered with 0.15ml of allyl isothiocyanate (AITC) solution in 1.2-propylene glycol. Inflammation signs were assessed visually (redness, swelling, higher body temperature) and changes of the paw volume before and in 1, 3, 6, 12, and 24 hours after induction of inflammation in laboratory animals by subplantar administration of allyl mustard oil. During each measurement of the paw thickness, it was additionally lubricated with petroleum ointment and the ointment and gel under study.

The analgesic effect of experiment ointment was also studied in rats when the inflammation was induced with formalin. Petroleum ointment, "Dibutalastin" ointment, and "Dolaren-gel" were applied to the prepared thigh area in the Control and 2 Experimental groups, respectively. In 30 min animals of all three groups were subplantar administered with 0.15ml of 2% formalin solution (in 0.9% sodium chloride), to induce the inflammatory process. The effect of the experimental drugs was assessed by the time animals spent licking the paws with inflammation.

To determine whether "Dibutalastin" ointment has analgesic activity, white rats of the control (C₄) and experimental groups (E₇, E₈) were shaved the thigh area of the right hind paw, a "hot plate" test was performed. With that, the animals of Experimental groups E₇ and E₈ were pre-applied with "Dibutalastin" ointment and "Dolaren-gel", and the rats of the Control group were rubbed with petroleum ointment. In 30 min the rats were alternately placed on a metal plate heated to 50-55 °C. The analgesic activity of "Dibutalastin" and "Dolaren-gel" was

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assessed by their ability to change the threshold of pain sensitivity, namely by assessing the period, or the time until the animal would try to lick the paw.

After 24 hours, the animals were decapitated under light chloroform anesthesia, and their blood was taken for morphological studies. The study was performed on a Dymind DF51 hematology analyzer.

Results. It was found that after subplantation of 1% carrageenan solution into laboratory rats, animals of Control and Experimental groups E₁ and E₂ developed an acute local inflammatory process with signs in the thigh area of the hind right paw. The thigh swelled, increased in volume and thickness, and had significant redness. The rats felt pain while being palpated, and this area was hotter when touched.

In the time range of the study, it was noted that during the continuous 6 hours of observation in the control group of rats, the pattern of inflammation hadn't changed significantly. As for the rats of Experimental groups E₁ and E₂, which before the administration of carrageenan were injected intramuscularly with the experimental anti-inflammatory drugs "Celexib" and the reference drug "Diclofenac", it was found that the pain in the area of experimentally induced inflammation, swelling and temperature were slightly lower than in the rats of the control group.

Fig. 1 shows the results of measuring the thickness of the paws with inflammation of the control and experimental groups rats.

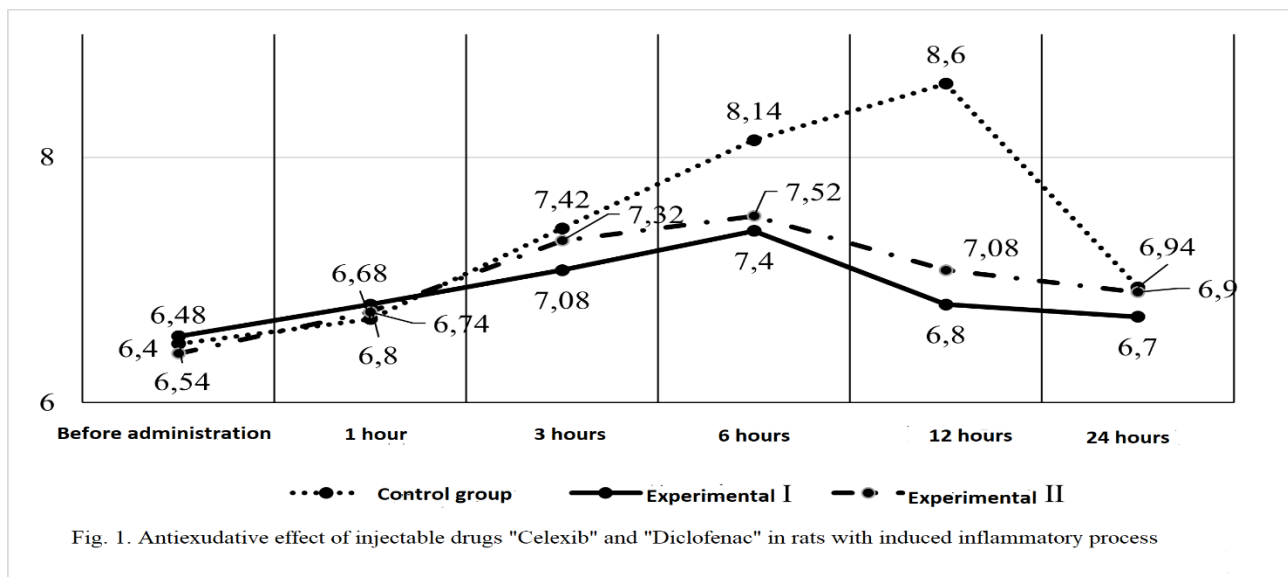


Fig. 1. Antiexudative effect of injectable drugs "Celexib" and "Diclofenac" in rats with induced inflammatory process

We found that the most peculiar changes in the paw's thickness were observed in rats of all groups three hours after carrageenan administration. The thickness of the paws, at this time of the procedure, in the rats of groups C, E₁, and E₂ was more than the values obtained before the drug administration by 25.6, 8.2, and 14.3%, respectively. It was found that rats of the control group that did not receive anti-inflammatory drugs had the volume of the paw increased on the 4th and 6th hours of the experiment. Regarding the experimental groups, we noted that after 4 hours the intensity of the inflammatory process decreased.

According to the evaluation of the Anti-Exudative Effect Index (AEEI), which is the factor of the thickness of the paw during the relevant study period and the size before the drug that induced the inflammation administration, we found that the experimental non-steroidal anti-inflammatory drugs "Celexib" and "Diclofenac" had a certain anti-inflammatory effect (Table 1). From the 4th hour of observations, the AEEI decreased and by the 6th hour, it was 0.22 and 0.30, respectively. During this period of observation, there were practically no visible clinical signs of the inflammation induced by carrageenan, the rats of the experimental groups

reluctantly allowed palpation of the thigh area of the hind right paw, pain and hyperemia were already not significant.

The efficacy of the drug "Celecoxib" developed by us for veterinary medicine, in terms of anti-inflammatory effect, was comparable to the anti-inflammatory and pain-relieving effect of the non-steroidal anti-inflammatory drug "Diclofenac", which has been widely used in medicine for a long time.

We have also studied the morphological composition of the blood of control and experimental groups' laboratory rats with induced inflammation. (Table 2).

Table 1

Anti-Exudative Effect Index (AEEI) of "Celecoxib" and "Diclofenac" in rats with carrageenan-induced inflammation, $M \pm m$, n=6

#	Group of rats	NSAID	Anti-Exudative Effect Index after carrageenan administration (hours)				
			1	2	3	4	6
1	Experimental I (E ₁)	Celecoxib	0.4±0.08	0.36±0.08	0.57±0.04	0.49±0.08	0.22±0.06
2	Experimental II (E ₂)	Diclofenac	0.47±0.6	0.50±0.06	0.67±0.04	0.53±0.02	0.30±0.08

Table 2

The effect of "Celecoxib" and "Diclofenac" drugs on the morphological composition of the blood of laboratory rats with induced inflammations, $M \pm m$, n=6

#	Values	Units of measurement	Groups of rats		
			C	E ₁	E ₂
1	WBC	10 ⁹ /l	13.0±0.6	8.40±0.22**	8.2±0.32**
2	RBC	10 ¹² /l	6.88±0.22	6.77±0.36	6.9±0.26
3	Hb	g/l	140.1±2.4	137.0±12.4	146.0±7.8
4	HCT	%	37.0±0.9	35.2±0.52	33.2±0.88
5	Thrombocytes	10 ⁹ /l	560±40.2	573±28.6	592±40.2
6	MCV	fl	53.7±1.3	52.0±2.4	54.4±3.3
7	MCH	pg	20.4±0.7	20.2±0.64	20.8±0.82
8	MCHC	g/l	380.0±12.4	388.0±16.6	398.6±24.2

Note in this Table: ** – P<0,01

It was found that in the blood of the rats of the Control group during the first 6 hours of the study, the leukocyte counts significantly increased, which is a strong confirmation of the development of an inflammatory process. The number of WBCs in rats that did not receive "Celecoxib" or "Diclofenac" (control group) was 68 and 74% higher (P<0.01) than in the blood of rats of the first and second groups. It is important to note that there were no peculiar changes in the qualitative composition of WBC during this period of the experiment (6 hours) (Table 2). However, in 24 hours after carrageenan-induced inflammation, it was found that the percentage of eosinophils in the blood of the control group rats increased from 3 to 8%, the level of segmented neutrophils was 17.8% higher compared to the blood of the rats that did not receive NSAIDs, and the number of lymphocytes decreased by 13.6%.

Blood leukogram of rats with carrageenan-induced inflammation, $M \pm m$, $n=6$

#	Values	Units of measurement	C	E ₁	E ₂
1	Eosinophils	%	8±0.01	3±0.01	3±0.01
2	Neutrophils with a drum-sticks shaped nucleus	%	4±0.01	3±0.01	2±0.01
3	Segmented neutrophils	%	53±2.6	37±4.0	40±3.4
4	Lymphocytes	%	29±2.2	51±4.8	47±6.0
5	Monocytes	%	6±0.8	7±0.4	5±0.2

The analysis of the parameters showed that in the rats of the experimental groups that had non-steroidal anti-inflammatory drugs "Celecoxib" and "Diclofenac", no significant changes in the blood were observed.

Thus, based on the results of the experiment, it can be stated that "Celecoxib" has anti-inflammatory and analgesic properties. The mechanism of this effect is not simple. Here, it is necessary to assume that the carrageenan-induced inflammation was characterized not only by local manifestations but also had a generalized character because the content of leukocytes in the blood of the experimental rats increased. The infiltration of tissues by inflammatory cells (neutrophils, monocytes, lymphocytes) at the area of administration is the critical feature of any inflammation. Such inflammatory cells release many enzymes (proteases, elastases, collagenases, acid hydrolases, phosphatases, lipases) and chemical mediators (eicosanoids, cytokines, chemokines) and induce tissue damage and the development of inflammation.

We had an experimentally induced inflammation and it resulted in tissue damage. Toll-like receptors (TLRs), NOD-like receptors (NLRs), and receptors for advanced glycation end products (RAGE) responded to tissue injury. These receptors, as J. White et al. (2010) L. Moilanen think when binding to pathogenically active molecules, stimulate and, through signal transduction, accelerate transcription factors, in particular nuclear factor – κ B (NF- κ B) and activate protein – 1 (AP-1), where there is expression of an anti-inflammatory gene. Nuclear factor- κ B (NF- κ B), according to the authors, regulates the expression of genes for anti-inflammatory cytokines, chemokines, inflammatory enzymes, adhesion molecules, receptors, and microRNAs (John et al., 2010; Lauri et al., 2012).

We have investigated the anti-inflammation effect of another drug developed by us – "Dibutalastin" ointment in white rats with evoked inflammation by allyl isothiocyanate (AITC) injection. Methyl salicylate is the active ingredient of this ointment, international classification says that it is a non-steroidal anti-inflammatory drug. We found out that after subdermal injection of AITC in the laboratory rats of the control and experimental groups E₃ and E₄, an acute inflammatory process developed in the thigh area of the right hind paw. Like in a carrageenan-induced case, it had the clinical signs of inflammation from the 1st hour of observation and was characterized by an inflammatory reaction and swelling, increased paw volume, hyperemia, higher body temperature, and significant pain. However, the dynamics of inflammation in the control group and experimental E₃ and E₄ rats were different (Fig. 2).

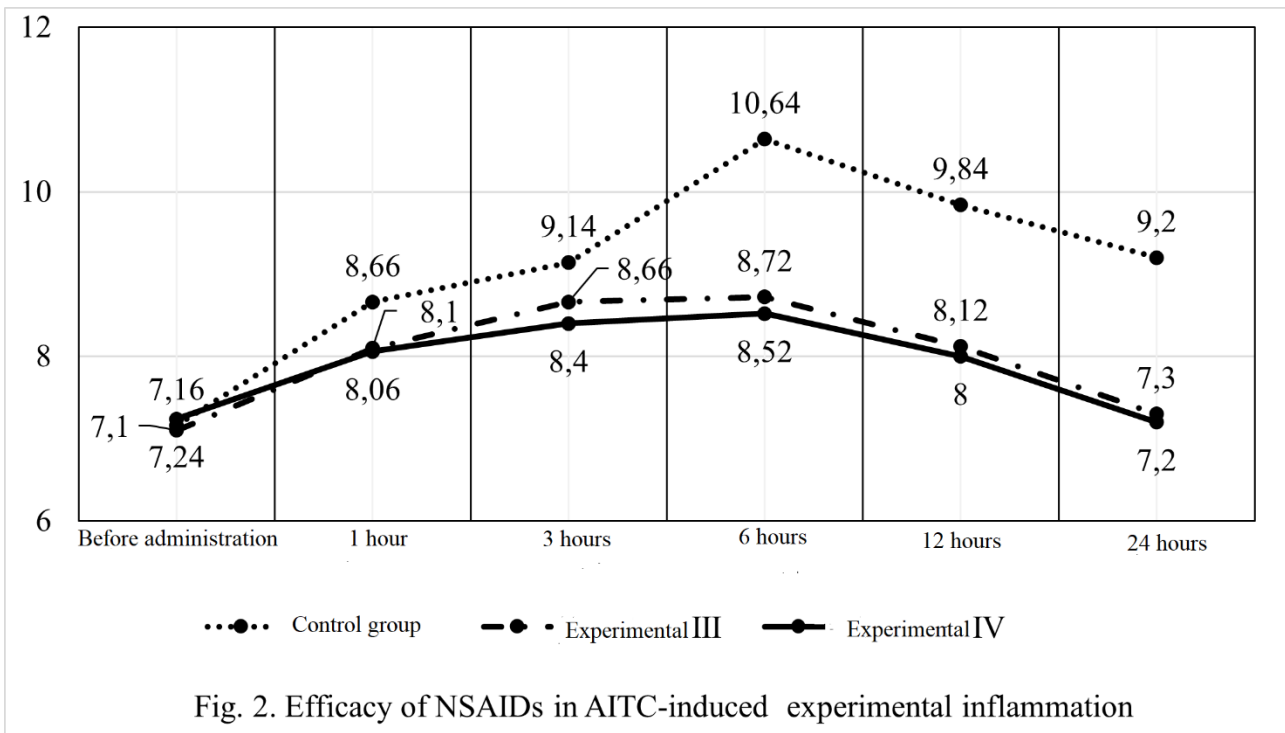


Fig. 2. Efficacy of NSAIDs in AITC-induced experimental inflammation

The diagram shows that the thickness of the paws with signs of inflammation in the Control group was the largest at the 6th hour of the study and was more than the normal (before AITC administration) by 40.6%. It can be assumed that the intensity of the inflammation process tended to decrease, although it remained quite high (after 24 hours, the thickness of the paws was 20.9% more).

With the effect of anti-inflammatory drugs "Dibutalastin" ointment and "Dolaren-gel", a significant decrease in the intensity of the inflammatory process was observed. As Table 4 shows the anti-exudative effect of the investigated drugs can be assessed by evaluating their indices.

Table 4

Comparative efficacy of non-steroidal anti-inflammatory drugs according to the anti-exudative effect index in rats, $M \pm m$, $n=6$

#	Groups of rats	AEEI after AICT administration (hours)				
		1	3	6	12	24
1	E ₃	0.67±0.08	0.78±0.02	0.65±0.02	0.41±0.03	0.27±0.06
2	E ₄	0.48±0.04	0.59±0.08	0.52±0.08	0.36±0.04	0.11±0.08

Thus, "Dibutalastin" ointment and "Dolaren-gel" can be asserted that these NSAIDs, when applied to the inflamed area, AEEI started decreasing from the 6th hour of observation, and 24 hours after it was the lowest. It can be stated as a conclusion that the investigated soft drug showed a high anti-inflammation effect, apparently due to the same active ingredient – methyl salicylate.

The analgesic effect of "Dibutalastin" and "Dolaren-gel" was also investigated in a rat model of formalin-induced inflammation (Table 5).

It was found that the time spent by the animals of the control group on licking the paw with signs of inflammation in the first phase, when the pain was apparently because of the formalin irritating effect on sensitive receptors, was 68 seconds. In the later period (20-30 min)

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after formalin administration, i.e. when we could observe the development of inflammation in the tissues, the time was 120 seconds. In the rats of groups E₅ and E₆, which were treated with "Dibutalastin" ointment and "Dolaren-gel" before formalin administration, the time spent on licking the paw with inflammation was less compared to the control group: in the first phases 24 and 38 seconds less and 54 and 64 seconds less in the second phase of the investigation. The results verify the analgesic effect of the investigated drugs.

Table 5

Analgesic effect of a new methyl salicylate "Dibutalastin" ointment and "Dolaren-gel" in formalin-induced inflammation in rats, $M \pm m$, n=6

#	Groups of rats	Evaluation by the time of response (sec.)	
		I phase 0-5 min.	II phase 20 -30 min.
1	Control (C ₃)	68	120
2	Experimental V (E ₅)	44	66
3	Experimental VI (E ₆)	30	56

The "hot plate" test also showed that soft drugs containing methyl salicylate "Dibutalastin" and "Dolaren-gel" have analgesic properties (Table 6)

Table 6

Investigation of analgesic effect of non-steroidal anti-inflammatory drugs in the "hot plate" test, $M \pm m$, n=6

#	Investigated drug	Reaction time (sec.)
1	Control (C ₄)	20±4.0
2	Experimental VII (E ₇)	42±2.8
3	Experimental VIII (E ₈)	46±3.6

It was found that the response time of rats before the onset of the defense reflex, after putting them on the "hot plate", ranged from 20±4.0 to 46±3.6 seconds. It was observed that when using the investigated soft drugs with analgesic effect, the response time was 22 and 26 seconds more, respectively, compared to the control group.

Conclusions. 1. Celecoxib, when administered intramuscularly in white rats with carrageenan-induced inflammation, has anti-inflammatory effects (reduces swelling, pain, and hyperemia in the inflammation area). Its effectiveness is comparable to the Diclofenac reference drug.

2. The anti-inflammatory and analgesic effect of "Dibutalastin" ointment is similar in effectiveness to the "Dolaren-gel" reference drug.

The next stage of our scientific experiments will be the study of the effectiveness of "Celexib" and "Dibutalastin" in pets with musculoskeletal disorders.

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ЕФЕКТИВНІСТЬ НЕСТЕРОЇДНИХ ПРОТИЗАПАЛЬНИХ ПРЕПАРАТІВ “ЦЕЛЕКСИБ” І “ДІБУТАЛЯСТИН” ЗА ІНДУКОВАНОГО ЗАПАЛЕННЯ В ЩУРІВ

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Резюме. Нестероїдні протизапальні засоби є найбільш широко використовуваними анагетиками в практиці ветеринарної медицини. Одним з поширених методів з'ясування протизапальних і анагетичних властивостей нових препаратів є індукування в лабораторних тварин запального процесу. Нами в умовах експерименту було вивчено протизапальні властивості препаратів “Целексиб”,

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діючою речовиною якого є целекоксиб (в 1 мл 100 мг) і мазі “Дібуталестин” (в 1 г препарату міститься 61,0 мг метилсаліцилату). В результаті проведених досліджень з'ясовано, що за субплантарного введення лабораторним щурам 1% розчину каррагінану у тварин контрольної і дослідних груп розвивався гострий запальний процес (набряк, збільшення об'єму і товщини кінцівки, гіперемія, болючість). Найбільш характерними були зміни в щурів усіх груп через три години після введення каррагінану. Відзначено, що у щурів контрольної групи, які не отримували протизапальні засоби об'єм лапки збільшувався і на, 4-ту та 6-ту години дослідю. У тварин дослідних груп Д₁ і Д₂ вже починаючи з 4-ої години, на тлі дії протизапальних засобів “Целексиб” і “Диклофенак”, інтенсивність розвитку запального процесу зменшувалась. Товщина лапки, при цьому, була, порівняно з періодом до індукування запалення, у тварин К, Д₁ і Д₂ меншою на 25,6, 8,2 і 14,3%, відповідно. На 6-ту годину досліджень у тварин групи Д₁ видимі клінічні ознаки запального процесу, викликаного каррагінаном, були практично відсутні, що підтверджує протизапальну ефективність досліджуваного препарату “Целексиб”. Встановлено, що в щурів за умови індукування запального процесу алізізотіоціанатом і формаліном досліджуваний засіб у формі мазі “Дібуталестин” також проявляв характерні аналгетичні властивості. Відзначено, що протизапальна та знеболювальна його дія була подібною за ефективністю до препарату-порівняння “Доларен-гель”.

Ключові слова: “Целексиб”, “Дібуталестин”, каррагінан-запалення, щури білі, протизапальна, аналгетична дія

DOI: 10.31073/onehealthjournal2024-III-01

DOI: 10.31073/onehealthjournal2024-III-02

UDK: 619:615.015.35-285:615.454.15

**EXPERIMENTAL ASSESSMENT OF THE ACUTE TOXICITY OF "KUBAZOL" –
SOLUTION FOR EXTERNAL APPLICATION BASED ON BIRCH TAR**

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Abstract. *Laboratory studies were conducted to determine the acute toxicity of the veterinary drug "Kubazol" on white rats.*

The drug "Kubazol" - a veterinary drug (spray for external use, solution) contains birch tar in its composition. Intended for the treatment and prevention of lesions of the skin (wounds, dermatitis, eczema), claws (injuries, superficial panaritium), hooves and hooves (foot rot, etc.) in dogs, wild pigs, wild birds and sports horses.

According to the results of determining the parameters of the acute toxicity of the drug "Kubazol" (spray for external use, solution), in the case of a single intragastric administration, the LD₅₀ for male rats is 7328.87±878.80 mg/kg of body weight, which allows us to classify it as toxic up to V class - practically non-toxic substances (LD₅₀ 5001-15000 mg/kg), and according to the degree of danger - up to IV class - low-hazard substances (LD₅₀ > 5000 mg/kg).

According to the results of toxicological studies of the veterinary drug "Kubazol" (spray for external use, solution), the LD₅₀ indicator could not be calculated, since the death of laboratory animals was not detected within 14 days after application to the skin of rats. At the same time, the maximum dose of the drug "Kubazol (spray for external use, solution) applied to the skin of rats (based on the absolute weight of the drug) was 15,000.0 mg/kg of body weight, which allows the drug to be classified according to the degree of danger to the IV class - low-hazardous substances (LD₅₀Cut>2500.0 mg/kg of body weight).

Further studies will be the next stage of pre-registration tests aimed at studying the chronic toxicity of "Kubazol", which is mandatory material in the section "Studies on safety and residues" of the dossier for this drug.

Key words: "Kubazol", rats, acute toxicity, dose, lethality, toxicity.

Effective treatment and prevention of lesions of the skin (wounds, dermatitis, eczema), claws and hooves (injuries, superficial panaritium) in Ukraine are possible only with the use of highly effective and affordable medicines. Therefore, the development of drugs with significant effectiveness and environmental safety does not lose its relevance today. Thus, "DEVIE" LLC was offered a new veterinary drug - "Kubazol" (spray for external use, solution). One milliliter of the drug contains: birch tar – 45 mg, auxiliary substances: ethyl acetate, polybutyl methacrylate – up to one milliliter. Birch tar has strong antiseptic, local irritant, anti-inflammatory, insecticidal, antiparasitic and disinfectant effects, improves blood circulation, stimulates the regeneration of the epidermis of damaged tissues, enhances the process of keratinization, dries wounds and accelerates their healing. In small concentrations (3-5%) it activates the growth of granulation, in large ones (above 10%) it sharply weakens it (Kaliuzhna & Bardova, 2011; Orlovetzka & Lukianchuk, 2018; Kychan & al., 2024).

The drug "Kubazol" (spray for external use, solution) is used for the treatment of hoof and claw diseases in cattle, horses, sheep, goats, pigs, European fallow deer, deer, wild pigs, dogs

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and poultry, in which the use of tar is recommended (rotting of the horn arrow); postoperative intervention on hooves and claws; for the treatment of superficial scratches and skin and claw defects; for nail care after cosmetic procedures; to stabilize bandages on hooves; treatment of dermatomycoses of domestic animals, especially in the initial stage or during recovery, when the drying effect of tar is manifested; for the treatment of wounds caused by cannibalism of poultry and pigs.

Therefore, the purpose of the research was to provide a toxicological (preclinical) assessment of the veterinary medicinal product "Kubazol" (spray for external use, solution) by determining its acute toxicity on white rats.

The purpose of the study is to carry out a toxicological assessment of the veterinary drug "Kubazol" (spray for external use, solution), under the conditions of an acute toxicological experiment on a model of white rats.

Materials and methods. An experiment to determine the parameters of the acute toxicity of the drug "Kubazol" (spray for external use, solution), with a single intragastric administration to laboratory animals, was conducted on 58 male non-linear white rats (3-4) months old and weighing (180-200) g, which were kept under optimal vivarium conditions (Zapadniuk, 1983; Kotsiumbas & al., 2006; Karkyshchenko & Hrachev, 2010): the temperature in the room was (18±2)°C, the relative humidity was (60-70)%, the day-night lighting cycle, during the experiment, was (10-14) hour, and the air volume in the vivarium room was changed 10 times per hour.

Rats were fed complete rodent feed. Animals had free access to water and feed.

Each animal was weighed before the start of the research. The administered doses were calculated individually according to the weight of each rat, while the volume of the drug administered intragastrically at one time did not exceed 2,5 cm³. Determination of the dose range for the main experiment was carried out in a previous experiment.

For this purpose, a control and 3 experimental groups of 4 animals each (n=4) were formed in the previous experiment based on the principle of analogues. The drug was administered in doses of 2000,0; 6000,0 and 12000,0 mg/kg of body weight, based on the absolute weight of the drug, once orally using an esophageal-gastric probe. Animals of the control group were injected with distilled water.

After taking into account the results of the previous experiment, 6 experimental groups were formed in the main experiment, whose rats were administered the drug in doses of 2000,0; 4000,0; 6000,0; 8000,0; 10,000,0 and 12,000,0 mg/kg of body weight, as well as a control group, the animals of which were injected with distilled water in a volume of 2,0 cm³ according to a similar protocol. There were 6 animals in each group (n=6).

It should be noted that the manipulations on rats were carried out in accordance with the existing regulatory documents, which regulate the organization of work using experimental animals and compliance with the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

The clinical condition of the experimental animals was observed for 14 days, noting the appearance and development of clinical signs of poisoning, the time of death or recovery to the physiological norm. During the clinical examination of rats, attention was paid to behavior, reaction to external stimuli, presence of appetite, skin condition, color of mucous membranes, frequency of breathing and defecation, changes in color and consistency of feces, etc (Zapadniuk, 1983; Kotsiumbas & al., 2006; Karkyshchenko & Hrachev, 2010).

After the death of the animals, a pathological autopsy was performed. The macroscopic method of research was used to establish patho-anatomical changes (Zharov & al., 2003). Pathological autopsy was performed according to the following scheme:

- at the first stage, an external examination was performed, noting the condition of the wool coat and mucous membranes;

- on the second - an autopsy and examination of body cavities and internal organs, such as: pharynx, trachea, larynx, heart, lungs, liver, spleen, kidneys, stomach, intestines, was performed, noting changes in color, consistency, pattern and shape of organs.

Based on the results of death, LD₁₀, LD₁₆, LD₅₀, LD₈₄, LD₉₀, LD₁₀₀ and the error of LD₅₀ were calculated using the method of probit analysis in the modification of V. B. Prozorovsky.

The parameters of acute dermal toxicity of the drug "Kubazol" (spray for external use, solution) were studied on 36 white male rats, aged (4-5) months, weighing (250-260) g. The animals were kept in standard vivarium conditions at a temperature of (18- 21)^oC, humidity (55-65)%, on a standard diet that meets the standards.

Experiments on animals were carried out in compliance with the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes".

One control and 5 experimental groups, 6 rats in each, were formed for the research. A day before the beginning of the experiment, wool was removed at the intended place of application, and it was carefully cut with scissors.

Observation of experimental animals lasted 14 days, taking into account the general condition of animals, the nature of skin lesions at the application site, as well as the time of death or recovery of animals. The application of the drug "Kubazol" (spray for external use, solution) was carried out in the morning before feeding the animals.

The drug "Kubazol" (spray for external use, solution) was evenly applied to a 4×4 cm area of the skin of rats.

The animals of the experimental groups were given the drug "Kubazol" (spray for external use, solution) on the skin in doses (by absolute weight): 5000.0; 7500.0; 10000,0; 12500,0; 15000,0 mg/kg of body weight, respectively. Animals of the control group, under similar conditions, were given distilled water.

The experimental animals were observed for 14 days, taking into account the general condition of the experimental rats, the amount of feed and water consumed, the depth and nature of the skin lesions at the application site, as well as the time of death or recovery of the animals.

The obtained results were processed by methods of variational statistics using the StatPlus 7.6.5.0 software package. Data were presented as mean values with standard deviation at the 95% confidence level.

Results. In the experiment, the drug Kubazol (spray for external use, solution) was administered to rats in doses of 2000,0; 6000,0 and 12000,0 mg/kg body weight. Clinical observations showed that the intragastric administration of the drug in a dose of 2000,0 mg/kg of body weight to rats of the 1st experimental group after (5-10) minutes caused slight depression and impaired coordination of movements, which disappeared within 2 days after administration. At the same time, a decrease in appetite and thirst was registered. Starting from the 3rd day after administration, the animals were active, responded adequately to external stimuli, consumed food and water. Until the end of the experiment, the parameters of the physiological state and behavior of the rats did not differ from those of the animals of the control group. Death of rats in this group was not observed.

The pattern of poisoning by the drug was more pronounced in rats of the II experimental group (dose 6000,0 mg/kg of body weight). After 5-10 minutes, after the introduction of the drug, pronounced depression, impaired coordination of movements and heavy breathing, refusal of feed and water were observed. On the 2nd and 3rd day, the rats did not move much, they breathed hard. A decrease in feed intake and thirst was also observed. Starting from the 4th day of the experiment, the clinical condition of the 2 rats gradually recovered and on the 10-11th day of the experiment did not differ from that of the control, along with this, a comatose state and death were observed in the 2 animals on the 3rd day of the experiment.

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In the rats of the III experimental group (dose 12000,0 mg/kg of body weight), 5-10 minutes after administration, pronounced increasing depression was observed. Along with this, impaired coordination of movements and heavy breathing were recorded. After 3-4 hours, after the administration of the drug, the rats sat in one place, some lay on their stomachs with their pelvic limbs stretched back and practically did not react to external stimuli, their breathing was shallow and heavy. The death of all animals occurred within the first day after administration of the drug (Table 1).

Table 1

The dynamics of the death of rats in the previous experiment, to determine the acute toxicity of the drug "Kubazol" (n=16)

Terms of death rats, through	Groups of rats and doses, mg/kg body weight			
	Control	Experiment		
		I (2000,0)	II (6000,0)	III (12000,0)
1	–	–	–	4
2	–	–	–	–
3	–	–	2	–
4 – 14 days	–	–	–	–
All died	–	–	2	4

In the main experiment, the drug Kubazol (spray for external use, solution) was administered to rats in doses of 2000,0; 4000,0; 6000,0; 8000,0; 10000,0 and 12000,0 mg/kg body weight. Intragastric administration of the drug in a dose of 2000.0 mg/kg of body weight to rats of the 1st experimental group, 5-10 minutes after administration, caused slight depression and impaired coordination of movements, which disappeared within 2 days after administration. At the same time, a decrease in appetite and thirst was registered. Starting from the 3rd day after administration, the animals were active, responded adequately to external stimuli, consumed food and water. Until the end of the experiment, the parameters of the physiological state and behavior of the rats did not differ from those of the animals of the control group. The deaths of rats in this group were not observed (Table 2).

Table 2

The dynamics of the death of rats in the main experiment, to determine the acute toxicity of the drug Kubazol (n=42)

Groups of rats and doses, mg/kg body weight		Terms of death rats, through				
		1 day	2 day	3 day	4 – 14 day	Everything died
Control		–	–	–	–	–
Experiment	I (2000,0)	–	–	–	–	–
	II (4000,0)	–	–	1	–	1
	III (6000,0)	–	–	2	–	2
	IV (8000,0)	–	2	1	–	3
	V (10000,0)	4	1	–	–	5
	VI (12000,0)	6	–	–	–	6

The pattern of poisoning by the drug in rats of II-IV experimental groups (doses of 4000,0-8000,0 mg/kg of body weight) was more pronounced and dose-dependent. After 5-10 minutes, after the introduction of the drug, pronounced depression, impaired coordination of movements and heavy breathing, refusal of feed and water were observed. On the 2nd and 3rd day, the rats did not move much, they breathed hard. A decrease in feed intake and thirst was also observed. Starting from the 4th day of the experiment, the clinical condition of 5 rats from the II, 4 from the III, and 3 from the IV experimental groups gradually recovered and on the 10-14th day of the experiment did not differ from that of the control, along with this in one rat from the II, 2 from the III and 3 from the IV experimental groups was observed to be in a comatose state and died on the 2nd-3rd day of the experiment (Table 2).

A severe course of poisoning was observed in rats of V-VI experimental groups (doses of 10000,0-12000,0 mg/kg of body weight). After 5-10 minutes after the introduction, pronounced increasing depression was observed. Along with this, impaired coordination of movements and heavy breathing were recorded. After 3-4 hours, after the administration of the drug, the rats sat in one place, some lay on their stomachs with their pelvic limbs stretched back and practically did not react to external stimuli, their breathing was shallow and heavy. The death of all animals from the VI experimental group occurred within the first day after the administration of the drug, and in the V experimental group, 5 animals died within 2 days after the administration. The animal from V experimental group, which remained alive, recovered by the end of the experiment.

After the death of the rats, a pathological autopsy was performed. During the external examination, discharge from the nasal and oral cavity was noted, the wool was disheveled and dirty-white in color. The specific smell of the drug came from the rats that died on the first day. Paleness of visible mucous membranes was also observed.

At the autopsy, the animals were registered:

- blood filling of the heart, an increase in the volume of the atria, the blood was not coagulated;
- the lungs and spleen were slightly congested without visible changes;
- the stomach is very swollen with the remains of the drug;
- liver - light brown in color, not increased in volume, flabby consistency;
- kidneys slightly increased in volume, light brown in color, elastic consistency;
- in the small and large intestines, swelling and inflammation of varying degrees of severity, from serous to catarrhal-hemorrhagic, were detected.

The next stage of studying the toxicological characteristics of the drug "Kubazol" (spray for external use, solution) was the determination of the average lethal dose and its standard error (LD₅₀, LD₁₀, LD₁₆, LD₈₄, LD₉₀, LD₁₀₀).

The average lethal dose LD₅₀ was calculated using the method of probit analysis according to V. B. Prozorovsky. The toxicometric parameters of the drug were calculated using the method of least squares, for probit analysis - lethality curves. The percentage of lethality, probits (Y), weighting coefficients of probits (Z) are established.

To construct the graph, the values of drug doses (mg/kg) were plotted on the abscissa axis, and the effect values (%) were plotted on the ordinate axis.

A graphic representation of the curve showing the dose-effect relationship for rats is presented in Fig. 1.

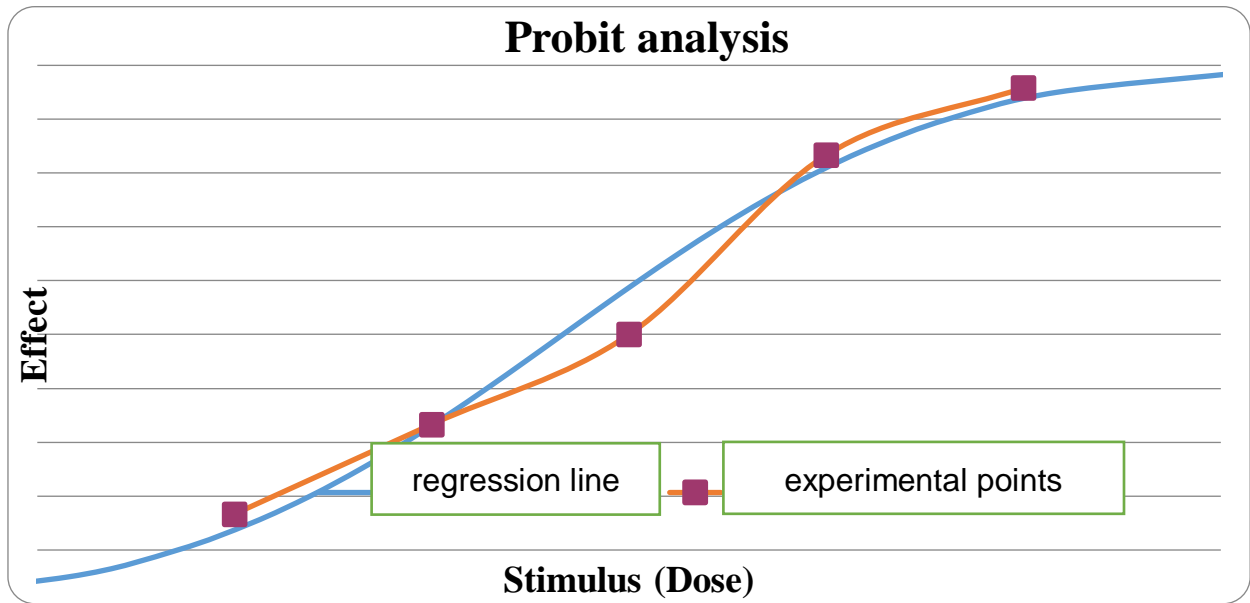


Fig. 1. The curve of lethality of male rats, under the conditions of a single oral administration of the drug "Kubazol"

The results of calculating the average lethal dose of the drug for rats, under the conditions of oral administration, are given in table. 3.

Based on the results of research, it was established that the LD₅₀ of the drug "Kubazol" (spray for external use, solution) under the conditions of its single oral administration to male rats was 7328,87±878,80 mg/kg, LD₁₀ – 3426,94 mg/kg, LD₁₆ – 4284,60 mg/kg, LD₈₄ – 10373,13 mg/kg, LD₉₀ – 11230,79 mg/kg, LD₁₀₀ – 11895,27 mg/kg body weight, respectively.

Table 3

Results of calculation of lethal doses of the drug "Kubazol" under the conditions of a single oral administration to male rats

Stimulus (Dose)	Percentage (%)	N	Probit (Y)	Weighting factor (Z)
2000	4,1667	6	3,2680	1,5359
4000	16,6667	6	4,0326	3,5653
6000	33,3333	6	4,5697	4,5697
8000	50,0000	6	5,0000	5,0000
10000	83,3333	6	5,9674	3,5653
12000	95,8333	6	6,7320	1,5359
Regression statistics				
LD₅₀	7328,87	LD₅₀ standard error		878,80
<i>LD₁₀</i>	3426,94	<i>LD₁₆</i>	4284,60	
<i>LD₈₄</i>	10373,13	<i>LD₉₀</i>	11230,79	
<i>LD₁₀₀</i>	11895,27			

Therefore, the drug "Kubazol" (spray for external use, solution) can be classified in terms of toxicity to class V – practically non-toxic substances (LD₅₀ 5001-15000 mg/kg), and in terms of the degree of danger to class IV – low-hazard substances (LD₅₀ > 5000 mg/kg) (Kotsyumbas, 2006).

It was established that after a single application of the drug "Kubazol" (spray for external use, solution) to the skin of rats in doses (5000,0-15000,0) mg/kg of body weight, no changes in the general condition and appetite of the animals were observed, which indicates absence of toxic effects of the drug. It should also be noted that none of the experimental animals died during the experiment.

Therefore, according to the results of toxicological studies of the drug "Kubazol" (spray for external use, solution), the LD₅₀ indicator could not be calculated, since the death of laboratory animals was not detected within 14 days after application to the skin of rats.

At the same time, the maximum dose applied to the skin of rats (based on the absolute weight of the drug) was 15000,0 mg/kg of body weight, which allows the drug to be classified according to the degree of danger to Class IV – low-hazardous substances (LD₅₀>2500,0 mg/kg of body weight bodies) (Kotsyumbas, 2006).

Conclusions. According to the results of determining the parameters of the acute toxicity of the drug "Kubazol" (spray for external use, solution), in the case of a single intragastric administration, the LD₅₀ for male rats is 7328,87±878,80 mg/kg of body weight, which allows it to be classified as toxic up to V class – practically non-toxic substances (LD₅₀ 5001-15000 mg/kg), and according to the degree of danger – up to IV class – low-hazard substances (LD₅₀ > 5000 mg/kg).

According to the results of toxicological studies of the veterinary drug Kubazol (aerosol), the LD₅₀ indicator could not be calculated, since the death of laboratory animals was not detected within 14 days after application to the skin of rats. At the same time, the maximum dose of the drug Kubazol (aerosol) applied to the skin of rats (based on the absolute weight of the drug) was 15000,0 mg/kg of body weight, which makes it possible to classify the drug according to the degree of danger to class IV – low-hazard substances (LD_{50Cut}>2500,0 mg/kg of body weight).

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ЕКСПЕРИМЕНТАЛЬНА ОЦІНКА ГОСТРОЇ ТОКСИЧНОСТІ “КУБАЗОЛУ” – РОЗЧИНУ ДЛЯ ЗОВНІШНЬОГО ЗАСТОСУВАННЯ НА ОСНОВІ ДЬОГТЮ БЕРЕЗОВОГО

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Резюме. Проведені лабораторні дослідження з визначення гострої токсичності ветеринарного препарату “Кубазол” на білих щурах.

Препарат “Кубазол” – ветеринарний препарат (спрей для зовнішнього застосування, розчин) у своєму складі містить дьоготь березовий. Призначений для лікування та профілактики уражень шкіряного покриву (рани, дерматити, екземи), кігтів (травми, поверхневий панарицій), копит та ратиць (гниття копитної стрілки, тощо) у собак, диких свиней, диких птахів і спортивних коней.

За результатами визначення параметрів гострої токсичності препарату “Кубазол” (спрей для зовнішнього застосування, розчин), у разі одноразового внутрішньошлункового введення LD_{50} для щурів-самців, складає $7328,87 \pm 878,80$ мг/кг маси тіла, що дозволяє за токсичністю віднести його до V класу – практично не токсичні речовин (LD_{50} 5001-15000 мг/кг), а за ступенем небезпечності – до IV класу – малонебезпечних речовин ($LD_{50} > 5000$ мг/кг).

За результатами токсикологічних досліджень ветеринарного препарату “Кубазол” (спрей для зовнішнього застосування, розчин), показник LD_{50} розрахувати не вдалося, оскільки загибелі лабораторних тварин не було виявлено протягом 14-ти діб після нанесення на шкіру щурів. При цьому максимальна, нанесена на шкіру щурів доза препарату “Кубазол (спрей для зовнішнього застосування, розчин) (за абсолютною масою препарату), становила 15000,0 мг/кг маси тіла, що дозволяє віднести препарат за ступенем небезпечності до IV класу – малонебезпечних речовин ($LD_{50cut} > 2500,0$ мг/кг маси тіла).

Подальші дослідження будуть черговим етапом передреєстраційних випробувань, спрямованих на вивчення хронічної токсичності “Кубазол”, що є обов’язковим матеріалом розділу “Дослідження щодо безпеки і залишків” досьє на даний лікарський засіб.

Ключові слова: “Кубазол”, щури, гостра токсичність, доза, летальність, токсичність.

DOI: 10.31073/onehealthjournal2024-III-02

One Health Journal. 2024. Vol. 2. N 3

DOI: 10.31073/onehealthjournal2024-III-03

UDC 616.931-036.23:616.931-053.8-036:579.61

EPIDEMIOLOGICAL INVESTIGATION OF A FATAL CASE OF DIPHTHERIA IN A RESIDENT OF KYIV OBLAST

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Abstract. *Ukraine still has diphtheria. This infection is developed with toxins secreted by pathogens of three Corynebacteria species, such as Corynebacterium diphtheria, Corynebacterium ulcerans, and Corynebacterium pseudotuberculosis.*

In January 2024, for the first time in recent decade, a fatal case of diphtheria in a resident of the Kyiv oblast was registered in Ukraine. The results of laboratory testing showed the toxigenic microorganism Corynebacterium ulcerans. The diagnosis was established based on the results of the pathological and anatomical autopsy and confirmed by laboratory testing during the examination of sectional samples. When a woman sought medical attention with certain clinical signs, medical care personal did not collect biological samples from the patient for testing on the causative agents of respiratory diseases of viral or bacterial origin, and the primary diagnosis did not contain information about the warnings of diphtheria. This indicates a low alertness of doctors regarding the possibility of the occurrence and spread of diphtheria.

The anti-vaccination attitude of the population and migration processes under martial law contribute to a decrease in the level of immunization in the Kyiv oblast and pose a threat to diphtheria outbreaks.

Keywords: diphtheria, epidemiological surveillance, diagnostic studies, immunoprophylaxis

Diphtheria is a contagious disease caused by toxigenic corynebacteria, mainly *Corynebacterium diphtheria* (Atkinson et al., 2007), but recently the disease may be caused by *Corynebacterium ulcerans* (Mattos-Guaraldi et al., 2008) and *Corynebacterium pseudotuberculosis* bacteria.

If diphtheria is caused by *Corynebacterium diphtheria*, it is characterized by an airborne transmission mechanism, accompanied by a peculiar lesion of the oropharynx and respiratory tract, but there are cases of skin and mucous membranes affection. (Atkinson et al., 2007). Infection usually occurs in spring or winter (Lamichhane and Radhakrishnan, 2022).

After the vaccination campaign, the number of cases of diphtheria considerably declined. Before 1920 in the United about 200,000 cases of the disease annually were reported, but after the introduction of the immunization program, the number of reported cases decreased significantly, and today about 1000 cases are reported annually (Lamichhane and Radhakrishnan, 2022). Most cases are observed in people with low socioeconomic status who live in crowded conditions, are not vaccinated, arrive from endemic regions, and have comorbidities. However, in some parts of the world, such as Southeast Asia and Africa the numbers of cases are higher.

In recent years, despite the availability of safe and effective vaccine, the process of vaccination is insufficient and outbreaks of the disease have become more frequent. Nonetheless, widespread immunization has reduced the cases of diphtheria to sporadic ones. Since the 1990s, the incidence

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of diphtheria in Ukraine has become an epidemic that lasted for about 10 years and it was due to numerous violations of immunization procedures, especially in the previous 15-20 years (Prokopiv O.V. et al., 2022).

Corynebacterium ulcerans is a relatively rare species that mostly causes skin diphtheria; however, this species can sometimes cause respiratory signs. The severity of the disease depends on the production of exotoxin. *C. ulcerans* has also been associated with zoonotic transmission to humans and is most seen in agricultural communities breeding livestock (Mattos-Guaraldi et al., 2008).

Infection of humans with *Corynebacterium ulcerans*, including diphtheria, can be fatal and usually occurs in adults in close contact with animals (Wellinghausen et al. 2002, Lartigue et al., 2005). Some cases are not linked to the farming communities or consumption of raw dairy products, suggesting other routes of infection (De Zoysa A. et al. 2005, Lartigue et al., 2005). Patients may have skin lesions that completely resemble diphtheria on the skin or signs of a tracheal-bronchial tree covered with pseudomembranes (Wagner et al., 2001; Dewinter et al. 2005). Infection with *C. ulcerans* can cause clinical syndromes in the lower respiratory tract (Nureki et al. 2007), sometimes associated with signs of systemic inflammatory response syndrome and disseminated intravascular coagulation (Wellinghausen et al., 2005). *C. ulcerans* infection can occur in children who have previously been immunized against diphtheria (Mattos-Guaraldi et al., 2008). Urban older adults may also be at risk of toxic complications due to weakened immunity from remote or incomplete diphtheria immunization (Wellinghausen et al., 2002). In some cases, *C. ulcerans* has been found to cause severe disease in humans, depending on the patient's immunological status rather than the nature of the toxin (Lartigue et al., 2005).

Corynebacterium pseudotuberculosis is found in sick animals, and few cases of diseases caused by this pathogen have been described in humans worldwide. *Corynebacterium pseudotuberculosis* is a well-known pathogen for farm animals, especially sheep and goats. Human contamination is a rare case that can occur in the form of purulent lymphadenitis, or have clinical signs such as severe fever, cough, peripheral blood eosinophilia, and eosinophilic pulmonary infiltrate. *Corynebacterium pseudotuberculosis* was isolated from human transtracheal aspirate and bronchoscopy washings (Keslin et al., 1979; Mills et al., 1997).

C. pseudotuberculosis sometimes causes infection in farm workers who are in close contact with infected animals or raw animal products, causing swelling of the lymph nodes in the neck or groin. This is the third species known to be capable of producing diphtheria toxin. However, toxin-producing *C. pseudotuberculosis* is rarely isolated (Prygel et al., 2022).

Given the above, regardless of the species of corynebacteria, diphtheria is a toxin-mediated infection, the signs of which depend on the anatomical site of infection with the pathogen, the immune status of the host, as well as the production and systemic distribution of the toxin (Atkinson et al., 2007). Pathogens of *Corynebacterium* genus, that can produce diphtheria toxin, *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis* were combined into a group of toxigenic corynebacteria called "*C. diphtheriae* complex" (Riegel et al., 1995).

The purpose of the study was to raise the awareness and increase the alertness of medical doctors and public health workers to diphtheria. Based on the results of the epidemiological investigation, to analyze the fatal case of diphtheria in a resident of Kyiv oblast in 2023.

To achieve this purpose, the **following tasks were set:**

1. Based on the results of the epidemiological investigation, to describe the fatal case of diphtheria in a resident of Kyiv oblast in 2023.
2. To identify the pathogen that caused the fatal disease.
3. To identify the source and routes of transmission of the pathogen.
4. To analyze the state of immunization of the population of Kyiv oblast against diphtheria using combined vaccines according to the National Immunization Schedule.

Materials and methods. A descriptive and evaluative epidemiological analysis was conducted based on the data from the primary accounting documentation form # 058/o "Emergency notification

of an infectious disease, food, acute occupational poisoning, uncommon reaction to vaccination" and information and analytical references, as well as on the results of an epidemic investigation of a case of diphtheria conducted by specialists of the State Institution "Kyiv Regional Center for Disease Control and Prevention of the Ministry of Health of Ukraine" (SI "Kyiv RDCPC of the Ministry of Health of Ukraine").

According to the data provided by the State Institution "Center for Public Health of the Ministry of Health of Ukraine" (SI "CPH of the Ministry of Health of Ukraine"), the incidences of diphtheria in Ukraine in the period 2011-2023 were analyzed. The intensity of diphtheria incidence according to generally accepted methods in terms of 100 thousand people was calculated.

Bacteriological and molecular genetic tests (real-time polymerase chain reaction (PCR)) to detect RNA of viral pathogens were run on sectional samples from a deceased person and clinical samples from contact persons. The study of biological samples was carried out based on the microbiological laboratory of the Department of Biological Factors Research of the SI "Kyiv RDCPC of the Ministry of Health of Ukraine" with further confirmation in the reference laboratory SI "CPH of the Ministry of Health of Ukraine". The real-time examination of samples by the PCR method as part of an epidemiological investigation was carried out in the reference laboratory SI "CPH", for that the authors express their gratitude to the staff.

Results. For 2010-2023, sporadic cases of diphtheria were reported in Ukraine every year, apart from 2017, 2020, and 2021 (Table 1). Overall 63 people were infected during this period, including 13 children (20.64%) under the age of 17 and 50 adults (79.36%) aged 18+.

Table 1

**Cases of diphtheria registered in Ukraine for 2010-2023.
(According to SI "PHC of the Ministry of Health of Ukraine" statistics)**

Year	Number of cases					
	Total		including:			
			children		adults	
abs.	DI*	abs.	DI**	abs.	DI***	
2011	8	0,02	2	0,02	6	0,02
2012	5	0,01	1	0,01	4	0,01
2013	6	0,01	2	0,03	4	0,01
2014	4	0,01	1	0,01	3	0,008
2015	2	0,005	1	0,01	1	0,003
2016	4	0,01	0	0,00	4	0,011
2017	0	0,00	0	0,00	0	0,00
2018	10	0,02	3	0,04	7	0,02
2019	21	0,05	2	0,01	19	0,06
2020	0	0,00	0	0,00	0	0,00
2021	0	0,00	0	0,00	0	0,00
2022	2	0,005	0	0,00	2	0,005
2023	1	0,002	1	0,002	0	0,002
Total:	63	0,011	13	0,01	50	0,012

Note: DI* – diphtheria index for 100 thousand people (population); DI** – diphtheria index for 100 thousand children (from 0 to 17); DI*** – diphtheria index for 100 thousand adults (from 18 years old and older).

From 2010 to 2023, the highest number of diphtheria cases was recorded in Zakarpattia oblast - 16 (25.4%), the city of Kyiv – 7 (11,1 %), Luhansk oblast – 5 (7,9 %). In Zhytomyr,

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Rivne, Volyn, Chernivtsi, Kirovohrad, Mykolaiv, and Kherson oblasts there were no cases reported at all (Fig. 1). There were no fatal cases of diphtheria reported.

In the Kyiv oblast, sporadic cases of diphtheria were recorded during this period in 2012, 2018, and 2023, 2 were reported in children aged 15-17 and 1 in an adult. The information on these cases is shown in Table 2.

with a DI rate of 2.4 per 100 thousand people in this age group (0.3 per 100 thousand children aged 0 to 17).

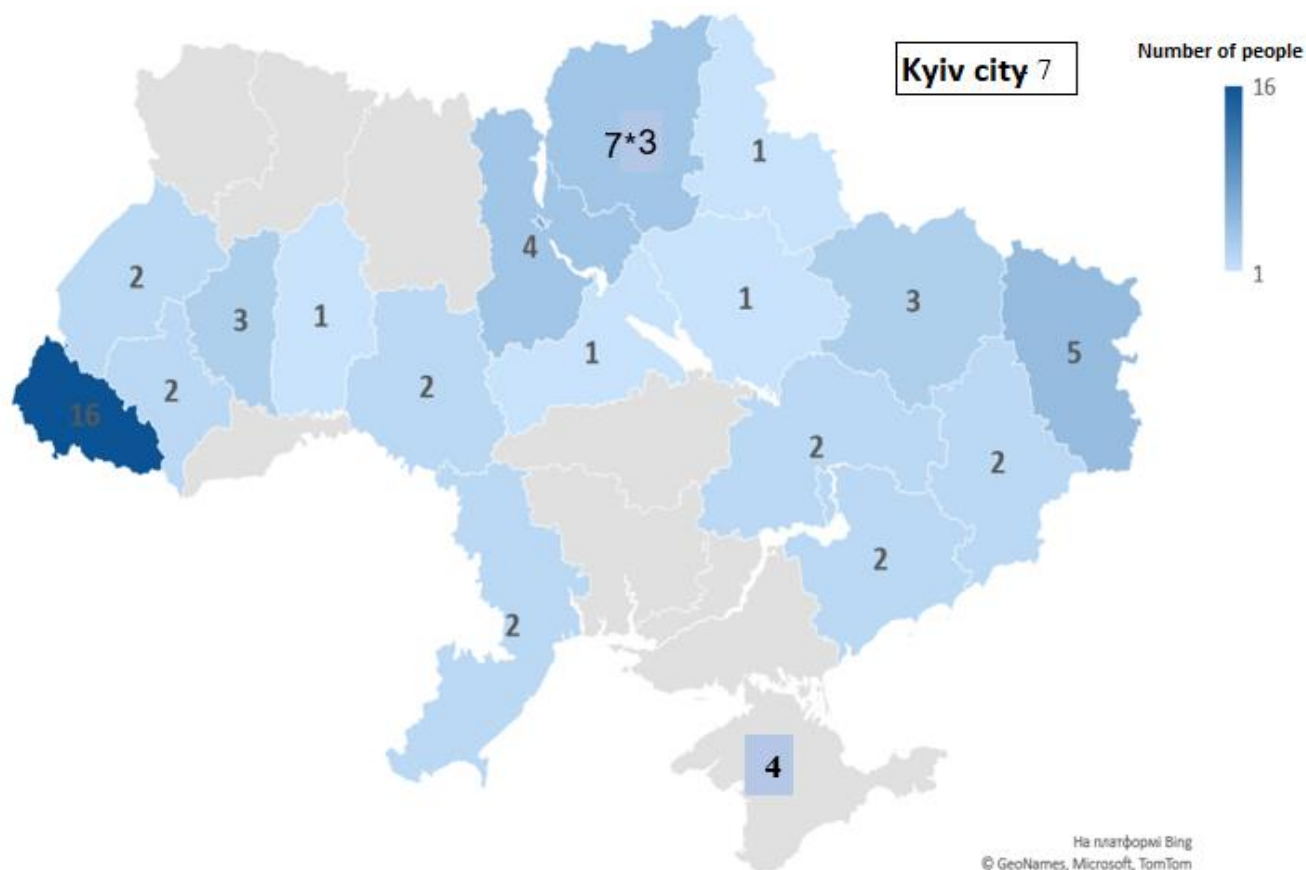


Fig. 1. Territorial distribution of diphtheria cases in Ukraine, 2010-2023

Table 2

Diphtheria incidence rates in residents of Kyiv oblast in 2012, 2018 and 2023

Year	Number of cases						Age group: children 15-17 years old		Place of residence (urban/rural)
	Total		including:				abs.	DI	
	abs.	DI*	adults		children (0-17)				
			abs.	DI**	abs.	DI***			
2012	1	0,06	1	0,06	0	0,00	0	0,00	urban
2018	1	0,06	0	0,00	1	0,30	1	2,4	urban
2023	1	0,06	0	0,00	1	0,06	1	0,06	rural
Total:	3	-	2	-	2	-	2	-	

The number of diphtheria cases in the Kyiv oblast, considering age groups, is associated with an insufficient level of vaccination of the population of all age groups, according to the National Vaccination Schedule. Analysis of data on preventive vaccinations in the Kyiv oblast for 2019-2023 shows that no age group has achieved the recommended level of immunization against diphtheria – 95%. In 2020–2023 the lowest vaccination coverage rates were recorded among the adult population of the oblast (table 3).

As of January 2024, a new case of diphtheria was reported in the Kyiv oblast in a 73-year-old female resident of the rural area of Vyshhorod district (citizen N.) who fell ill on December 22, 2023, but did not seek medical care and treated herself without antibiotics.

On the morning of December 29, 2023, at 10.30 a.m., the patient felt a deterioration in her health and was taken to a local hospital by ambulance with a primary diagnosis of "bilateral pneumonia?".

Table 3

**Rates of preventive diphtheria vaccinations with combined vaccines
in Kyiv oblast in 2019-2023**

Vaccination according to the schedule	Recommended level, %	Vaccination coverage, age, %				
		2019	2020	2021	2022	2023
AKDP (DTP) (before 1 year old: 2, 4, 6 months)	95	83,3	79,7	83,1	83,5	85,7
AKDP (DTP) (18 months)	95	84,3	78,3	84,3	81,9	89,4
ADP (DTP) (6 years old)	95	39,2	44,5	58,3	85,0	81,4
ADP-M (DTa) (16 years old)	95	94,8	83,5	80,9	77,4	82,0
ADP-M (DTa) (adults)	95	90,0	53,4	57,7	40,5	44,0

Note: AKDP - combined pertussis, diphtheria, and tetanus vaccine; ADP - combined diphtheria and tetanus vaccine.

During her hospitalization, citizen N. demonstrated signs of difficulty breathing, dry cough, and severe general weakness. After examination, the head of the therapeutic department diagnosed her with "Acute laryngitis. Laryngospasm. Acute bronchitis with obstructive syndrome. Coronary heart disease. Post-infarction atherosclerosis. Second-degree heart failure". For testing for respiratory infections, no samples were taken.

The patient was immediately transferred to the Anesthesiology and Intensive Care Ward, where she was examined by a related specialist (psychiatrist), who diagnosed her: "Malignant neuroleptic syndrome?". Citizen N. received detoxification infusion therapy, but the treatment did not work.

On the evening of December 29, 2023, at 10.15 pm. citizen N. was diagnosed with biological death. The body was sent for a pathological autopsy, where a preliminary diagnosis was made: "Diphtheria of the upper respiratory tract?" and sectional samples were taken for laboratory testing.

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According to the results of the pathological autopsy and laboratory tests of the sectional samples as of January 03, 2024, the final pathological diagnosis was made: "A36.8 Diphtheria of the upper respiratory tract. Infection and toxic shock". The results of laboratory tests are shown in Table 4.

According to the results of laboratory tests conducted by the virology laboratory of SI "Kyiv RDCPC of the Ministry of Health of Ukraine" RNA of the influenza A virus pathogen was detected in the sectioned material (pieces of trachea and lungs).

During microbiological studies, toxigenic strains of the diphtheria pathogen *Corynebacterium ulcerans* were isolated by the bacteriological method in pieces of the trachea, lungs, and pharyngeal swabs. The real-time PCR analysis showed specific nucleic acid fragments of the diphtheria toxin gene in the sectional samples.

The results of laboratory tests indicate a mixed infection caused by influenza A virus and the toxigenic bacterium *Corynebacterium ulcerans*.

Table 4

Laboratory testing of sectional material results

Sample	Method	Results	Date of results delivery	The laboratory that conducted the testing
Pieces of trachea and lungs	PCR real-time	Detected RNA of the influenza virus pathogen (Flu A)	01.01.2024	SI "Kyiv oblast rCDC of the Ministry of Health of Ukraine"
Pieces of trachea, lungs, and pharyngeal	PCR real-time	specific nucleic acid fragments of the diphtheria toxin gene were detected	02.01.2024	SI "PHC of the Ministry of Health of Ukraine"
Nasal and pharyngeal swabs	PCR real-time	specific nucleic acid fragments of the diphtheria toxin gene were not detected	02.01.2024	SI "PHC of the Ministry of Health of Ukraine"
Pieces of trachea, lungs, and pharyngeal	Bacteriological	<i>Corynebacterium ulcerans</i> tox+ was isolated	05.01.2024	SI "PHC of the Ministry of Health of Ukraine"
Nasal and pharyngeal swabs, pieces of trachea, and lungs	Bacteriological	<i>Corynebacterium ulcerans</i> tox+ was isolated	05.01.2024	SI "Kyiv oblast rCDC of the Ministry of Health of Ukraine"

The epidemiological investigation of the case and the implementation of anti-epidemic measures were carried out by epidemiological specialists of SI "Kyiv oblast rCDC of the Ministry of Health of Ukraine" from 12.30.2023 to 12.01.2024.

During the epidemiological investigation, it was found that citizen N. led a secluded life, had a mental illness, did not communicate with fellow villagers and relatives, did not attend the

local church, and was not served by social workers. She regularly visited the local market and used public transportation.

The circle of contact persons was established (16 people in total), including two of the fellow villagers (who had regular contact during care at the patient's place of residence, they brought food and medicine) and 14 medical workers in the hospital who had contact during medical care. The contact persons were monitored, and laboratory samples were tested for diphtheria. According to the results of laboratory tests and nasal and oropharyngeal swabs, no pathogen was detected. All contact persons were vaccinated according to the National Vaccination Schedule, they also got the emergency immunization against diphtheria. The source of the infection could not be identified.

Taking mentioned above into account, the problem of diphtheria incidence both in the Kyiv oblast and in Ukraine remains important, that requires increased vigilance of health care providers in timely diagnosis and identification of the source of the infectious agent, as evidenced by the described fatal case of diphtheria.

Conclusions.

1. The described fatal case and the late diagnosis are evidence of a lack of vigilance of healthcare providers regarding diphtheria.

2. The results of laboratory tests showed that the fatal case of diphtheria in a resident of the oblast was associated with infection with a toxigenic microorganism of the species *Corynebacterium ulcerans*. At the same time, it is appropriate to strengthen the laboratory link on the oblast level with additional molecular genetic methods that will allow us to identify in a short time toxigenic strains of microorganisms of the *Corynebacterium diphtheria* » "complex".

3. The vaccination coverage against diphtheria in the oblast is insufficient, given that the necessary condition for preventing this disease within the oblast is the vaccination coverage of at least 95% of the population according to the National Vaccination Schedule. There is an urgent need to strengthen communication work with healthcare workers and community members in Kyiv oblast, on the safety and necessity of vaccination against diphtheria (pertussis, diphtheria, tetanus (DPT) or diphtheria, tetanus (DT)) according to the National Vaccination Schedule.

4. During the epidemiological investigation, the source and routes of transmission of the pathogen were not established. At the same time, anti-epidemic measures and the examination of contact persons were carried out in full.

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ЕПІДЕМІОЛОГІЧНЕ РОЗСЛІДУВАННЯ СМЕРТЕЛЬНОГО ВИПАДКУ ДИФТЕРІЇ У МЕШКАНКИ КИЇВСЬКОЇ ОБЛАСТІ

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Резюме. Дифтерія залишається актуальною інфекційною хворобою в Україні. Провідну роль у розвитку даної інфекції відіграють токсини, які виділяють збудники

One Health Journal. 2024. Vol. 2. N 3

трьох видів коринебактерій, а саме: *Corynebacterium diphtheria*, *Corynebacterium ulcerans* та *Corynebacterium pseudotuberculosis*.

У січні 2024 року в Україні вперше за останнє десятиліття зареєстровано смертельний випадок дифтерії у мешканки Київської області. За результатами лабораторних досліджень доведено, що чинником хвороби став токсигенний мікроорганізм виду *Corynebacterium ulcerans*. Діагноз у померлої був встановлений за результатами патолого-анатомічного розтину та лабораторно підтверджений при дослідженні зразків секційного матеріалу. Незважаючи на клінічні прояви, при зверненні по медичну допомогу, матеріал у захворілої для досліджень на респіраторні патогени вірусного та бактерійного походження не відбирався, а первинний діагноз не містив інформації про підозру на дифтерію. Це свідчить про низьку настороженість лікарів щодо можливості виникнення та поширення дифтерії.

Антивакцинальні настрої у населення та міграційні процеси в умовах воєнного стану сприяють зниженню рівня імунізації в Київській області і становлять загрозу виникненню спалахів дифтерії.

Ключові слова: дифтерія, епідеміологічний нагляд, діагностичні дослідження, імунопрофілактика

DOI: 10.31073/onehealthjournal2024-lil-03

CHALLENGES OF FASCIOSIS SPREADING IN CATTLE IN UKRAINE IN 2021-2023

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Abstract. *The paper presents a comparative analysis of the epizootic process of cattle Fasciolosis dynamics in Ukraine from 2021 to 2023. The data are broken down by region. The risk zones are determined, and the territory of Ukraine is conditionally divided into dangerous, threatened, and temporarily not-dangerous areas.*

Grazing ruminants' helminths are common worldwide and harm cattle productivity and food security. The concern that climate change can increase the frequency and intensity of helminth infections has been growing. In Ukraine, this concern stems from case reports and theoretical life-cycle models, which allow us to assess the impact of climate change on helminth epidemiology. We believe that this study is the first attempt to investigate geographic and climatic trends in cattle helminth infections spreading by region. In the total of 474209 conducted tests, 6967 cases had positive results, and the average infection rate of cattle with Fasciola from 2021 to 2023 was 1.5 % in the regions with mild climate.

Fasciolosis infection spread depends on the elevation of the farm and agricultural region. The studies show that meteorological changes can significantly contribute to understanding the impact of climate on infectious disease dynamics. If local environmental conditions are taken into account, the impact of climate change on disease dynamics can be clearer on a local scale. We recommend developing a strategy for extensive sample selection across Ukraine to monitor changes in helminthic disease risk and inform on adaptation strategies to increase productivity and protect the health of the cattle.

Keywords: Fasciolosis, spreading, epizootic process, territory

All agricultural mammalian animals can be infected with Fasciolosis. It is more severe in younger animals. If Fasciolosis is not treated on time or not at all, the frequency of Fasciolosis infection cases increases with age due to reinvasion and superinvasion. The course of Fasciolosis and all its specific epizootic are closely related to abiotic and biotic features, including anthropogenic factors of the region. These include the average annual temperature, humidity, soil, and water salinity, the number and type of infected animals, their migration from one area to another, the size and environment conditions of pastures, the number of mollusk habitats, as well as factors that ensure or impede the development of Fasciola larvae, including the degree of implementation of the proposed measures against Fasciolosis, and others (Charlier J, et al., 2014; Phelan P, et al., 2015).

The common liver fluke or sheep liver fluke (*Fasciola hepatica*) is a trematode parasite that infects a wide range of hosts, including ruminants, horses, rabbits, and humans (Gasbarre L.C., 2014). It causes the parasitic infection Fasciolosis and is widespread all over the world (Rose H., et al., 2015). The infection is often subclinical and can cause productivity losses due to liver damage (Vande Velde F., et al. 2015) and a decrease in weight, milk yield, and fertility (Sutherland I.A., et al., 2011).

Preventing the spread of helminthiasis among agricultural animals and taking measures against them is one of the basic principles of helminthology. Various scientists have been conducting research in this area for a long time, but it has not yet been possible to prevent completely infections caused by helminthiasis (Vercruysse J., et al., 2018; Matthews, K., et al., 2004; Smolynets, I.B., et al., 2016).

According to the analysis of scientists, it was found that Fasciolosis is quite widespread, which negatively affects the development of livestock and causes significant economic losses in agriculture. Animals affected by Fasciolosis show a decrease in the productivity of meat, milk, and wool; their meat quality becomes worse, their liver is rejected and they have their early death (Vande Velde F., et al. 2015).

For example, it has been proven that a cow infected with Fasciolosis loses at least 20-40 kg of live weight and a significant amount of milk. This causes significant losses to livestock farmers. Untimely treatment for Fasciolosis leads to premature slaughter of animals, which causes big losses to businesses. The carcasses of animals subjected to forced slaughter are thin and therefore have poor meat quality and the liver cannot be used, so we suffer significant economic losses (European Parliament and Council of European Union, 2016; Howell A., et al. 2015).

As a result of studies conducted around the world, it was found that the annual damage from Fasciolosis amounted to \$3 million (Chai, J. Y., 2019).

Thus, every year there is a decrease in the number of cattle and small ruminants due to the aggregated pathological effect of helminth infections, which cause large economic losses to livestock breeding.

The purpose of our study was to learn the dynamics of the epizootic process of cattle Fasciolosis by analyzing and comparing data from 2021 to 2023 in Ukraine.

Methods and materials. The material for the statistical analysis was the annual reporting form No. 2-Vet "Report on the Work of the State Service of Ukraine for Food Safety and Consumer Protection Laboratories".

The State Service of Ukraine on Food Safety and Consumer Protection controls and supervises the epizootic situation in Ukraine by conducting scheduled epizootic inspections. In 2021-2023, samples of slaughtered cattle of different ages and breeds from 24 different regions of Ukraine were collected and inspected against Fasciolosis (Vinnytsia, Volyn, Dnipropetrovs'k, Donetsk, Zhytomyr, Zakarpattia, Zaporizhzhia, Ivano-Frankivsk, Kyiv, Kirovohrad, Lviv, Luhansk, Mykolaiv, Odesa, Poltava, Rivne, Sumy, Ternopil, Kharkiv, Kherson, Khmelnytskyi, Cherkasy, Chernivtsi, Chernihiv). We researched cattle, considering both their age and the conditions they were kept in. Diagnostic tests have been conducted with microscopy methods in regional laboratories of the State Service of Ukraine for Food Safety and Consumer Protection, which are authorized and accredited in the ISO-17025 system.

Results. Cattle Fasciolosis is widespread in Ukraine. In the total of 474209 conducted tests, 6967 cases had positive results, the average infection rate of cattle with Fasciola from 2021 to 2023 was 1.5 %.

In 2021, the State Service of Ukraine for Food Safety and Consumer Protection laboratories conducted 201688 tests, 2957 cases had positive results, which totaled 1.5% of the infection rate. In 2022, 155319 tests were conducted, and 2017 cases had positive results, which totaled 1.3 %, in 2023, out of 117202 conducted tests 1993 cases were positive, which gave 1.7% (Fig. 1).

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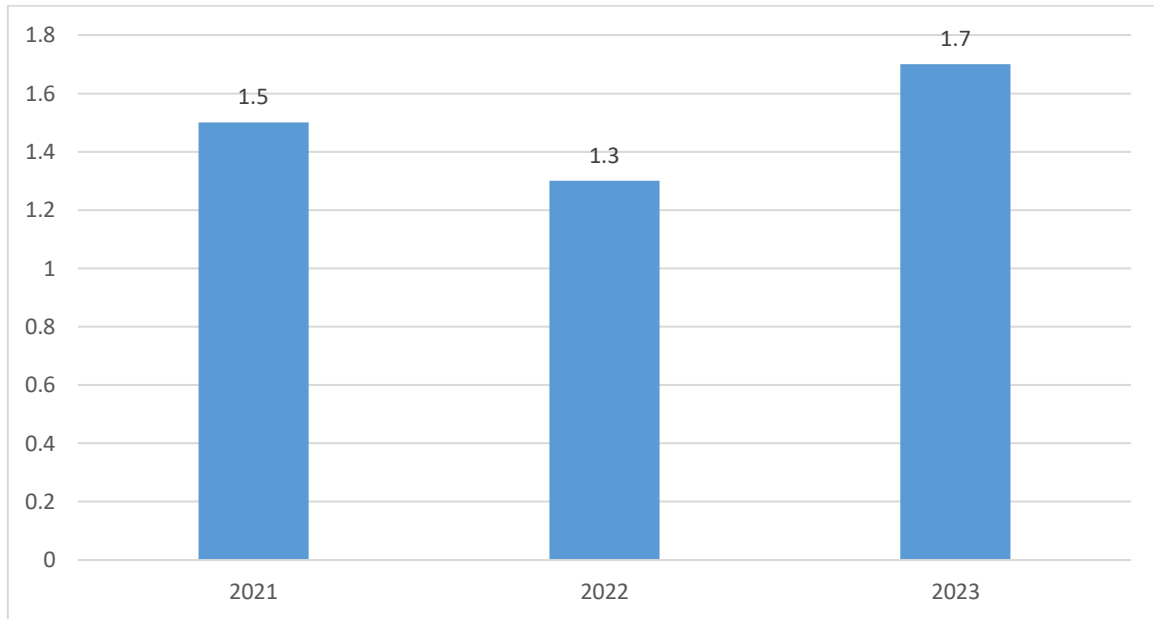


Fig. 1. Infection of cattle with Fasciolosis in Ukraine from 2021 to 2023

According to the development of the Fasciolosis epidemic, Ukraine can be divided into three risk areas: dangerous – with an infestation rate of 1 to 4.5%, threatening – with an infestation rate of 0 to 1%, temporarily not-dangerous – with an infestation rate of 0%.

The statistics analysis shows that from 2021 to 2023, 10 regions were included in the dangerous area: Volyn - 1.5%, Rivne - 4.5%, Odesa - 1%, Kirovohrad - 1.5%, Kharkiv - 1.6%, Chernihiv - 2%, Sumy - 3.1%, Lviv - 3.4%, Khmelnytskyi - 1.2%, Zhytomyr - 2% (Fig. 2).

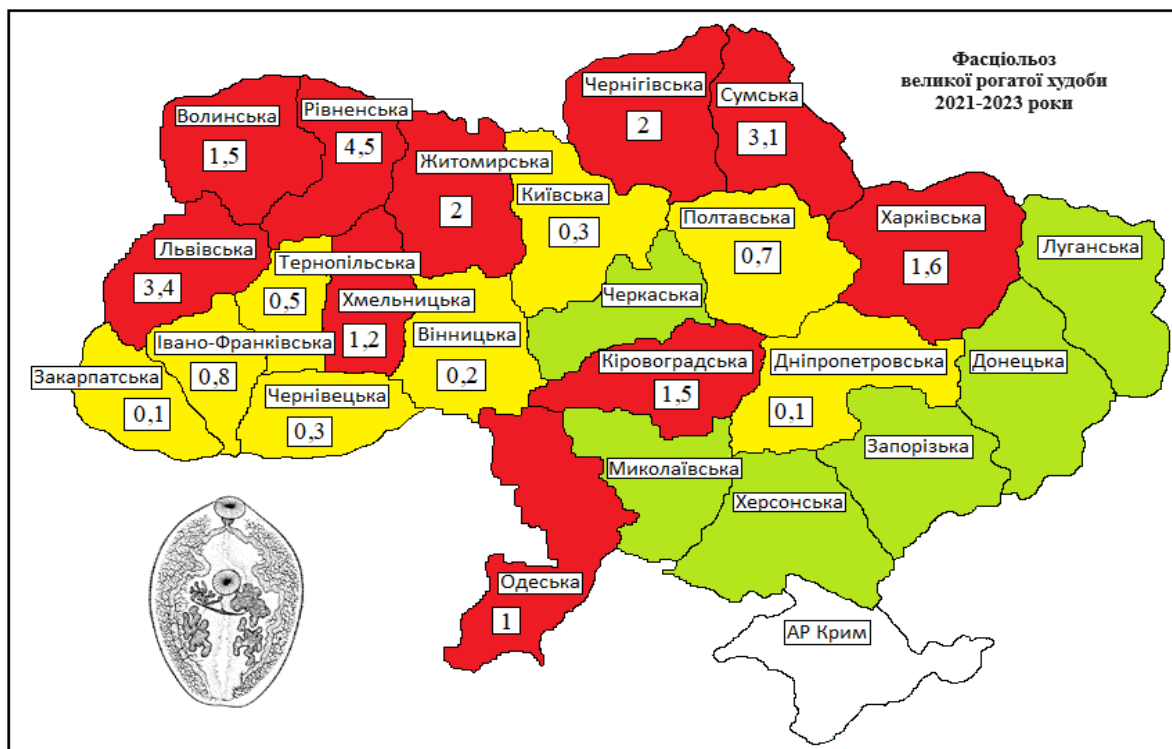


Fig. 2 Infection of cattle with Fasciolosis in Ukraine from 2021 to 2023

8 regions were included in the threatened risk area: Zakarpattia - 0.1%, Ivano-Frankivsk - 0.8%, Ternopil - 0.5%, Chernivtsi - 0.3%, Vinnytsia - 0.2%, Kyiv - 0.3%, Poltava - 0.7%, and Dnipropetrovsk - 0.1%,

6 regions had temporarily not-dangerous areas: Mykolaiv, Zaporizhzhia, Cherkasy, Kherson, Donetsk, and Luhansk.

It was found that the farms had different terrain. Most farms are located on uplands, with small reservoirs and wetlands along the edges. The habitats of mollusks ponds are very diverse and are divided into permanent and temporary ones. Permanent habitats include small rivers with low flow rates and small ponds. Temporary ones include puddles, ditches, and indentations from animal hooves filled with rainwater (Довгій Ю.Ю., 2006; Lyngdoh D, et al. 2016; Lotfy, W. M., et al. 2013).

Based on registrations at slaughterhouses, the distribution of liver fluke *Fasciola hepatica* is more concentrated in the northwestern regions of Ukraine, with 1.7% positive registrations in 2023. The climate in this part of Ukraine is relatively mild with many precipitations. This is favorable for the parasite life cycle, in which the intermediate host, *Galba truncatula*, is common in small ponds in pasture. The effect of *F. hepatica* on cattle productivity and anthelmintic treatment procedures has not been studied (Ducheyne E, et al. 2015; Novobilský A, et al., 2015; Dube A, et al., 2023).

The spread of Fasciolosis agents from one area to another, from one region to another, or even from one country to another, depends on various environmental factors, including the movement of Fasciolosis-affected animals, as well as hay and other collected feedstock from dangerous to not-dangerous areas. The spread of Fasciolosis to other areas can also be caused by the water flow. Cercariae long-distance migration and mollusks infected with them lead to the emergence of new foci of Fasciolosis. Mollusks are also moved by flood waters to habitats in other regions. (Selemetas N, et al., 2015; Zárate-Rendón DA, et al., 2023)

Like many other trematodes, the *Fasciola* population is considered a free, complex, self-regulating, and dynamic system. The water factor plays an important role in the transmission of *Fasciola* during the spillage of rivers and water reservoirs, for both the conservation of flukes and their transmission.

The size of the *Fasciola* population depends on the regional differences and treatment frequency. The coastal zone, which is relatively warm and humid, is a more favorable environment for the development and survival of parasite larvae, and the grazing season is longer than in northern or highland areas. Many precipitations and marshy pastures are associated with an increased risk of exposure to *F. hepatica* (Howell A, et al., 2015; Довгій Ю.Ю., 2006). The homogeneity at the country level in the distribution and occurrence of *O. ostertagi* and *F. hepatica* may indicate that the two helminth infections share a common dependence on pastures as well as climate conditions (Bennema SC, et al., 2010), which is reflected in the responses of dairy farmers in our study (Charlier J, et al., 2014; Suleyman, Y., et al., 2006).

Thus, even though trematodes have existed for centuries, their complex life cycles still retain a huge reproduction potential that has not yet been fully explored. Therefore, monitoring studies in the field of trematode epidemiology, particularly *Fasciolas* should be conducted regularly.

Conclusions. It has been found that late detection of the disease in cattle suspected of having Fasciolosis and delayed treatment measures may lead to the occurrence of severe complications, on the one hand, and to other diseases, on the other. This finally ends in poor forecasts and even excessive additional costs and time spent on treating the disease.

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ПРОБЛЕМИ ПОШИРЕННЯ ФАСЦІОЛЬОЗУ ВЕЛИКОЇ РОГАТОЇ ХУДОБИ НА ТЕРИТОРІЇ УКРАЇНИ ЗА 2021-2023 РОКИ

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Резюме. У статті проведено порівняльний аналіз динаміки епізоотичного процесу щодо ураження великої рогатої худоби фасціольозом в Україні з 2021 по 2023

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pp. Наведено дані в розрізі областей. Визначено зони ризику з умовним поділом України на неблагополучну, загрозливу та тимчасово благополучну територію.

Гельмінти жуйних тварин, які випасаються, дуже поширені в усьому світі та негативно впливають на продуктивність тварин і продовольчу безпеку. Зростає занепокоєння, що зміна клімату збільшує частоту та інтенсивність гельмінтозів. В Україні ці занепокоєння впливають із звітів про випадки та теоретичних моделей життєвого циклу, що дає можливість оцінити впливу зміни клімату на епідеміологію гельмінтів. Ми вважаємо, що це дослідження є першим у дослідженні географічних та кліматичних тенденцій гельмінтозних інфекцій великої рогатої худоби в розрізі областей. Було проведено 474209 досліджень, з яких позитивний результат було отримано в 6967 випадках, середня інвазованість ВРХ фасціолами за період з 2021 по 2023 роки склала 1,5 відсотка у регіонах з помірним кліматом.

Зараження фасціольозом змінювалося залежно від висоти та сільськогосподарського регіону господарства. Ці дослідження показують, що метеорологічні зміни можуть значно сприяти розумінню впливу клімату на динаміку інфекційного захворювання. Якщо взяти до уваги місцеві умови навколишнього середовища, вплив зміни клімату на динаміку захворювань можна зрозуміти в більш локальних масштабах. Ми рекомендуємо створити стратегію широкого відбору проб по всій Україні, щоб відстежувати зміни ризику гельмінтозних захворювань та інформувати про стратегії адаптації для сприяння продуктивності тварин і захисту їх здоров'я.

Ключові слова: фасціольоз, поширення, епізоотичний процес, територія.

DOI: 10.31073/onehealthjournal2024-III-04

DOI: 10.31073/onehealthjournal2024-III-05

THE LABORATORY TESTING OF THE PCR-BASED PROTOCOL OF DETECTION OF THE RABBIT HAEMORRHAGIC DISEASE VIRUS RNAKorovin I.V.^{1,2}, Rusanova A.O.,¹ Gerilovych A.P.^{2*} (ORCID ID: 0000-0002-3280-4172)¹ - SRI 'Veterinary Biotechnologies', LLC, Kharkiv, Ukraine² - PSI "One Health Scientific and Research Institute" Kharkiv, Ukraine, * - antger2011@gmail.com

Abstract. *Rabbit Haemorrhagic Disease (RHD), also known as Rabbit Viral Haemorrhagic Disease (RVHD), is a highly contagious and often fatal viral disease that affects domestic and wild rabbits. It's caused by two related viruses: Rabbit Haemorrhagic Disease Virus (RHDV) and Rabbit Haemorrhagic Disease Virus 2 (RHDV2).*

The disease is endemic in many European, Asian and American countries, but the agent is still recognized as an emergent infection, associated with mass losses and extremely high mortality in rabbits of all breeds.

One Health Scientific and Research Institute, PSI in collaboration with SRI 'Veterinary Biotechnologies', LLC developed the in house PCR-based protocol for RHDV detection and RHDV-2 differentiation, that requires fast implementation. This diagnostics kit evaluation is described under OIE requirements with determination of the sensitivity, specificity, repeatability and domain-specificity. Detection kit is recommended for practical application.

Keywords: rabbit haemorrhagic disease virus, risk analysis, epidemiology, diagnostics, rabbits, PCR

Introduction. Rabbit Haemorrhagic Disease (RHD), also known as Rabbit Viral Haemorrhagic Disease (RVHD), is a highly contagious and often fatal viral disease in adult European rabbits (*Oryctolagus cuniculus*). This disease affects both wild and domestic rabbits. It's caused by two related viruses: Rabbit Haemorrhagic Disease Virus (RHDV) and Rabbit Haemorrhagic Disease Virus 2 (RHDV2). They belong to the family *Caliciviridae*, genus *Lagovirus* that causes rabbit haemorrhagic disease (RHD) in adult European rabbits (*Oryctolagus cuniculus*) (Abrantes, 2012, Tokarz-Deptuła, 2024).

RHD is primarily spread through direct contact with infected rabbits or their bodily fluids, such as saliva, urine, or feces. It can also be transmitted indirectly through contaminated objects or surfaces. The disease progresses rapidly, and affected rabbits may show symptoms such as fever, loss of appetite, lethargy, difficulty breathing, and bleeding from the nose or mouth. In some cases, rabbits may die suddenly without showing any symptoms (Prieto, 2000).

RHD particularly devastating in domestic rabbitries and can cause significant economic losses in commercial rabbit farming. Disease outbreaks can have a significant impact on rabbit populations, especially in areas where wild rabbits are abundant. The disease can spread quickly within a population, leading to high mortality rates (Hukowska-Szematowicz, 2012, Wang, 2012).

Vaccination is the most effective way to prevent RHD. Several vaccines are available, including ones specifically targeting RHDV and RHDV2 strains. Additionally, strict biosecurity measures, such as quarantining new rabbits and disinfecting equipment and facilities, can help prevent the spread of the disease (Mahar, 2018).

RHD has been reported in many parts of the world, including Europe, Asia, Australia, and the Americas. The emergence of RHDV2 in recent years has added complexity to disease control efforts, as this strain can affect previously vaccinated rabbits and has a wider host range (Wang, 2012, Mahar, 2018).

While RHD primarily affects rabbits, there is no evidence to suggest that it can infect humans or other animal species. However, strict biosecurity measures should still be followed

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to prevent the potential spread of the virus to other susceptible animals (Nicholson, 2017, Pedler, 2016).

Overall, Rabbit Haemorrhagic Disease is a significant concern for rabbit owners, breeders, and wildlife managers due to its high mortality rate and ease of transmission. Vigilance, vaccination, and good biosecurity practices are essential for controlling and preventing outbreaks of this disease (Smertina, 2024).

Polymerase Chain Reaction (PCR) is a molecular biology technique used to amplify specific DNA/cDNA sequences, and it's a valuable tool in detecting Rabbit Haemorrhagic Disease Virus (RHDV) in infected rabbits. First, samples are collected from suspected cases of RHDV infection. These samples can include tissue samples (such as liver or spleen), blood, feces, or nasal swabs from affected rabbits (Aguayo-Adán, 2022).

PCR-based methods offer several advantages for RHDV detection, including high sensitivity, specificity, and rapid turnaround time. These assays are crucial for early diagnosis, surveillance, and control of RHDV outbreaks in rabbit populations (Hukowska-Szematowicz, 2023, Hryniewicz, 2022).

One Health Scientific and Research Institute, PSI in collaboration with SRI 'Veterinary Biotechnologies', LLC were developed the methodical approach for detection of the RNA of RHDV and RHDV-2 differentiation using Real-Time PCR due the reason of enhancement of RVHD control measures in Ukraine.

The aim of the presented research is to study sensitivity, specificity, repeatability and reproducibility of PCR-based protocol for detection of the RNA of RHDV and RHDV-2 differentiation using Real-Time PCR.

Materials and methods.

During laboratory testing trials evaluated appearance, activity, specificity, sensitivity PCR-based protocol for detection of the RNA of RHDV and RHDV-2 differentiation using Real-Time PCR and reproducibility.

The *in house* kit samples from batch 1, control evaluation 1 were used, produced in SRI 'Veterinary Biotechnologies' LLC, in April 2024.

The following samples panel has been used for specificity and sensitivity study of the test-system:

- reference panel cDNAs (n = 3) of the RHDV/RHDV-2, received from partner lab, and its 1 to 10, 1 to 100, 1 to 1000 and 1:10000 dilutions;
- RNA/cDNA samples isolated from the livers of infected rabbits (n = 7).
- RNA/cDNA samples isolated from the livers of the intact animals (n = 10).
- positive control of the amplification (PC);
- negative control of the amplification (NC);
- non-template control (NTC).

Following heterologous templates were used to demonstrate the kit's species-specific specificity (genetic material of other cattle viruses):

- *Feline calicivirus virus* cDNA (vaccine);
- *Bovine herpesvirus* type 1 DNA (IBR virus);
- *Bovine diarrhea virus* cDNA (BVDV).

The polymerase chain reaction was managed under the recommended conditions described in the PCR-based protocol for detection of the RNA of RHDV and RHDV-2 differentiation Using Real-Time PCR. All testing was done without changes of the recommended features of reaction (table 1). To estimate the repeatability of testing this has been performed three times.

Table 1

Amplification cycle for RHDV/RHDV-2 RNAs detection

Stage	Temperature	Duration	Step	Number of cycles
1	50°C	10 min.	Reverse transcription	1
2	94°C	3 min.	Activation of the DNA-polymerase	1
3	94°C	10 sec	DNA denaturation	45
4	58°C	45 sec	Primer annealing/ elongation	

Results. Tests were conducted to verify the commission sensitivity and specificity of the test system and reproducibility obtained when using the results.

At the beginning of the commission were checked for completeness of PCR-based laboratory in house test-systems. All components necessary for operation and instruction were available.

In assessing the appearance found that the test system consists of the following components: RNA extraction kit (kit # 1):

- «extraction buffer» (lysis bufer) – 1 flack – 15 (30) ml, transparent non-coloured liquid;
- sorbent solution – 1 (2) tube - 1,5 ml, opalescentic fluid with white color;
- «Washing buffer» – 1 flack – 50 (100) ml, transparent non-coloured liquid;
- «Washing buffer ethanol» - 1 flack – 50 (100) ml, transparent non-coloured liquid;
- «solution 4» for the final washing – 1 flack – 30 (60) ml, transparent non-coloured liquid;
- TE-buffer – 1 flack – 20 (40) ml, transparent non-coloured liquid.

Kit for PCR amplification (kit # 2):

- «RT-One Step PCR MasterMix» – 1 (2) tubes, 0,5 ml, transparent non-coloured liquid;
- SYBR Green solution – 1 tube, 0,05 or 0,1 ml, transparent light green liquid;
- primer solution (20 pM/µl) – 1 tube of each (4 vials, 2 – for RHDV detection and 2 for RHDV-2 differentiation), 0,125 (0,25) ml, transparent non-coloured liquid;
- deionized water – 1 (2) tubes – 1,25 ml, transparent non-coloured liquid;
- positive control template (for 5 or 10 reactions) – 1 tube, 0,05 – 0,1 ml, transparent non-coloured liquid.

The PCR master mix was prepared using number of samples amount plus one under proportion per sample:

п/п	Component	Final concentration	1 x for reaction (µl)
1	Water for PCR		6,7
2	RT-PCR Master Mix- Path ID	1X	9
3	SYBR Green	n/a	1
4	Primer RHDV_F (RHDV-2_F) (20 pM/ µl)	400nM	0,4
5	Primer RHDV_R (RHDV-2_R) (20 pM/ µl)	400nM	0,4
	DNA (template or control)		2

The results of the sensitivity testing is the ability to identify all encrypted obviously positive samples, it was found that the test system is able to detect RNA of the RHDV and RHDV-2 virus in particular in both positive reference material samples, including their dilutions 1 to 10 – 1 to 10000 with Ct value 14.6-22.2. This is equal to the minimum titre of virus in the rabbit organism, we recorded an infected animal (in the liver and blood samples) in our previous research.

The specificity of the test system proved amplicon in the absence of any size of Ct value or Ct value over 38 in samples of rabbit origin tissues from non-infected animals.

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In addition, primers designed didn't hybridized with DNA samples from other viruses, including DNA extracts from *Bovine herpesvirus* of types 1, and cDNAs of *Feline calicivirus* and *BVDV* (Table. 2).

It was also marked by complete coincidence test results developed RHDV /RHDV-2 detection protocol in three repetitions under similar conditions and using different Thermocyclers same type.

Table 2

Testing results for PCR-based protocol for detection of the RNA of RHDV using Real-Time PCR with the panels of positive, negative and heterogenic samples

#	Material	1st repeat result	2nd repeat result	3rd repeat result
1.	Sample 1	Ct 14.6	Ct 14.8	Ct 14.6
2.	Sample 1 1:10	Ct 18.2	Ct 18.9	Ct 20.9
3.	Sample 1 1:100	Ct 22.4	Ct 21.1	Ct 22.2
4.	Sample 1 1:1000	Ct 24.3	Ct 24.4	Ct 24.9
5.	Sample 1 1:10000	Ct 26.2	Ct 26.1	Ct 26.0
6.	Sample 2	Ct 16.2	Ct 16.4	Ct 17.2
7.	Sample 2 1:10	Ct 18.7	Ct 18.8	Ct 18.7
8.	Sample 2 1:100	Ct 21.2	Ct 21.4	Ct 21.2
9.	Sample 2 1:1000	Ct 24.5	Ct 23.9	Ct 24.1
10.	Sample 2 1:10000	Ct 27.2	Ct 27.2	Ct 27.1
11.	Sample 3	Ct 18.6	Ct 18.8	Ct 18.9
12.	Sample 3 1:10	Ct 19.7	Ct 19.6	Ct 19.8
13.	Sample 3 1:100	Ct 22.4	Ct 22.3	Ct 22.6
14.	Sample 3 1:1000	Ct 25.3	Ct 25.2	Ct 24.9
15.	Sample 3 1:10000	Ct 27.2	Ct 26.7	Ct 26.7
16.	Liver 1	Ct 19.7	Ct 19.6	Ct 19.8
17.	Liver 2	Ct 14.2	Ct 14.8	Ct 14.4
18.	Liver 3	Ct 18.6	Ct 18.8	Ct 18.9
19.	Liver 4	Ct 18.2	Ct 18.4	Ct 18.3
20.	Liver 5	Ct 17.2	Ct 17.2	Ct 17.6
21.	Liver 6	Ct 18.4	Ct 18.2	Ct 18.5
22.	Liver 7	Ct 16.4	Ct 16.4	Ct 16.5
23.	Liver (intact) 1	N/d	N/d	N/d
24.	Liver (intact) 2	N/d	N/d	N/d
25.	Liver (intact) 3	N/d	N/d	N/d
26.	Liver (intact) 4	N/d	N/d	N/d
27.	Liver (intact) 5	N/d	N/d	N/d
28.	Liver (intact) 6	N/d	N/d	N/d
29.	Liver (intact) 7	N/d	N/d	N/d
30.	Liver (intact) 8	N/d	N/d	N/d
31.	Liver (intact) 9	N/d	N/d	N/d
32.	Liver (intact) 10	N/d	N/d	N/d
33.	IBRV	N/d	N/d	N/d
34.	FCV	N/d	Ct 42.0/ N/d	N/d
35.	BVDV	N/d	N/d	N/d

Amplification of RHDV-2 RNA demonstrated RNA presence in samples 2, 4, and 6 as well as in sample 1 of the reference panel. The Ct values for RHDV-2 amplification was observed on the levels 14.9 to 26.2.

Conclusions: 1. Specificity of the PCR-based protocol for detection of the RNA of RHDV detection using Real-Time PCR has the appropriate level of sensitivity and specificity, aligned with the detection of RHDV/RHDV-2 RNAs in clinical samples and their 1:10 to 1:10000 dilutions, in Ct values of 14.6-22.2 (detection), and 14.9 to 26.2 (differentiation) with its absence of Ct value over 38 in the “negative samples”. The developed kit doesn’t demonstrate the false positive reactions with heterogenic DNA and RNA specimens, such as BHV-1, BVDV, and FCV.

2. The sensitivity of the system can be considered satisfactory, since positive results were positive for samples with low concentrations of specific RNA of RHDV/RHDV-2.

3. Reproducibility and repeatability of PCR-based protocol for detection of the RNA of RHDV detection using Real-Time PCR has the appropriate level, in all three repetitions differences were observed results.

The perspectives for further application of the results. As the laboratory testing of PCR-based protocol for detection of the RNA of RHDV detection using Real-Time PCR developed by One Health Scientific and Research Institute, PSI in collaboration with SRI ‘Veterinary Biotechnologies’, LLC indicators of quality, such as sensitivity, specificity and reproducibility fit the requirements. In this regard, this protocol can be recommended for interestablishmental testing and using in practice of surveillance and diagnostics of RVHD.

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ЛАБОРАТОРНА АПРОБАЦІЯ ПРОТОКОЛУ ПЛР-ДЕТЕКЦІЇ РНК ВІРУСУ ГЕМОРАГІЧНОЇ ХВОРОБИ КРОЛІВ

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Резюме. Геморагічна хвороба кролів (ГХК), також відома як вірусна геморагічна хвороба кролів (ВГХК), є висококонтагіозним і часто смертельним вірусним захворюванням, що вражає домашніх і диких кролів. Викликається двома спорідненими вірусами: Вірус геморагічної хвороби кроликів (RHDV) та Вірус геморагічної хвороби кроликів 2 (RHDV2).

Захворювання є ендемічним у багатьох країнах Європи, Азії та Америки, але збудник все ще визнаний як емерджентна інфекція, пов'язана з масовими втратами та надзвичайно високою смертністю серед кролів усіх порід.

Науково-дослідний інститут єдиного здоров'я у співпраці з ТОВ "НДП "Ветеринарні біотехнології" розробив власний протокол на основі ПЛР для виявлення RHDV та диференціації RHDV-2, який потребує швидкого впровадження. Оцінка цього діагностичного набору описана відповідно до вимог МEB з визначенням чутливості, специфічності, повторюваності та доменної специфічності. Набір рекомендовано для практичного застосування.

Ключові слова: вірус геморагічної хвороби кролів, аналіз ризику, епідеміологія, діагностика, кролі, ПЛР

DOI: 10.31073/onehealthjournal2024-III-05

DOI: 10.31073/onehealthjournal2024-III-06

UDC: 614.2:614.3

ONE HEALTH MULTIMODAL SURVEILLANCE IN TIME OF CHANGE: LESSONS NOT LEARNT FROM CASE STUDY OF A/H5N1 SPILLOVER TO MAMMALS IN GDAŃSK METROPOLITAN AREA**Jarynowski A.**^{1, 2, 3}, (ORCID ID 0000-0003-0949-6674), **Romanowska M.**⁴,
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Abstract. *This case study of A/H5N1 spillover to mammals in the Gdańsk area underlines the complexities of managing emerging One Health threats in significant political and economic aspects. We compared the relatively successful rapid regional response with the utterly lost battle in communication and cooperation, emphasising the need for improved interdisciplinary regional and international cooperation and robust surveillance systems in an era of anthropogenic and natural change.*

Keywords: infodemic management, (re)-emerging diseases, early warning systems, one health, preparedness

Introduction. Anthropogenic, climatic, demographic and technological changes have altered the landscape of infectious disease risk as we saw during ASF epizootics (Jarynowski et al., 2024), COVID-19 pandemic and not only human refugee crisis (Jarynowski & Belik, 2022) due to Russian aggression in Ukraine. Our goal/aim is to show how to handle an unknown X disease (Al Asfoor et al., 2024).

The current (April 2024) A/H5N1 outbreak in cats/cattle in the U.S. (Ly, 2024) has shown that lessons have been learnt from an epizootiological standpoint (cats as a sentinel of a more general outbreak), but the same considerations, such as risk communication problems (e.g., contaminated milk), are valid. This paper is describing failures in improving the response to recent natural or anthropogenic crises in public and ONE health, such as the emergence of new pathogens (i.e. disease X) and the increasing occurrence of pandemics (Micah et al., 2023). Here we also discuss ethics and communication with the public in case of disease in companion animals (Makovska, 2020). Some of the mistakes made in the Gdansk region and the rest of Poland could be negative learning examples for ONE Health professionals around the world. To assess changing needs, multiple choices of public and ONE health tools are available, however their proper implementation can still be challenging (Gerilovych et al., 2023). There are assessments of the public and ONE health system's preparedness for emergencies (outbreaks and disasters), but they focus only on specific (known to the scientific community) aspects. This article addresses the gap in understanding how inspections actually respond to Disease X.

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History as life lesson. Gdańsk's - heart of so called Tri-city and Pomeranian Voivodeship - historical richness is built on connectivity and citizens paid a bill by also being first in the region to be affected by Plague, Smallpox and Cholera (Duży & others, 2012). Current One Health threats related to climate change, such as zoonotic diseases, antimicrobial resistance, food safety, vector-borne diseases, environmental pollution, pose new categories of risks to humans, animals and the environment and Gdańsk metropolitan area seems to be especially susceptible in this concept (Szkudlarek et al., 2021), especially during tourist season. I.e. in terms of possible spillovers and recombination of Avian Influenza (AI), northern Polish coastline and Gdańsk Bay was identified as one of the most important hotspots in whole Europe due to crossing of main bird migration paths (Waldenström et al., 2022). Since the beginning of 2024, Gdansk has become the fifth largest European freight hub, after Rotterdam, Antwerpen, Novorossiysk and Hamburg, being the undisputed leader in Central Europe with all the new sanitary risks (Waldenström et al., 2022). Moreover, the threat of biological or chemical hybrid aggression from Russia has become real since 2022 (Jarynowski, 2023) and the COVID-19 pandemic increased general understanding of (re-)emerging threats (Aarestrup et al., 2021). Spring of 2024, as expected, showed that A/H5N1 is a serious threat (Plaza et al., 2024).

Unknown epizootics and reactions of inspections. In early June 2023, veterinarians from the Gdansk metropolitan area, together with the provincial veterinary inspection, observed a sudden increase in the mortality of cats (Jarynowski & Belik, 2023). After an outbreak investigation a spillover of A/H5N1 to mammals was confirmed and it was the biggest event of this kind in the last decades in Europe.

During the first days when the etiological cause of infection and its transmissibility (i.e. to humans)/leathility patterns were not known (European Food Safety Authority et al., 2023), Pomeranian sanitary inspectors placed cat owners under epidemiological surveillance. Local Pomeranian Veterinary and Sanitary Inspectorates acted without guidance, understanding that time can be of the essence in this type of event, as there had been no blueprints for such scenarios. The Chief Sanitary and Veterinary Inspectorates created the protocols in early July, when the local epidemic/outbreak was already in its declining phase. In the follow-up phase of the outbreak, the Chief Sanitary Inspectorate suggested that regional offices had performed unauthorised procedures and closed the file.

In addition, the cooperation between Pomeranian scientists (Rabalski et al., 2023) and Inspectorates was very complicated due to a lack of trust and interdisciplinary communication skills in a complex political area (including conflicting positions of the poultry industry and the cat-owning community), and as usual, an epidemic/epizootic was followed by an infodemic (Radziwon et al., 2023). Each interested scientific or government party used its own dataset [Fig. 1] and developed an analysis that ignores other sources of information (and reproduces many errors (Jarynowski & Belik, 2023)), and ultimately we had several competing hypotheses about the source of the virus (from contaminated poultry meat through separate introduction directly from migratory birds to endemicity in an unknown intermediate host between birds and cats).

Inspection officials appreciate agencies (such as WOAHA (World Organisation for Animal Health) and WHO/FAO) because they provide significant intellectual support and adequately communicate risks and uncertainties to the world. By contrast, EU institutions (such as EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control)) promoted many errors and flaws in their communications (Jarynowski & Belik, 2023), with very little institutional support, and most of the EU support came from personal connections between inspectors/researchers. Given such circumstances, the results of the outbreak investigation are officially inconclusive (source unknown).



Fig. 1. Artificial Intelligence generated graphs related to [left] the anecdote of a person searching for his lost keys at night, not where he dropped them, but under a streetlamp because that's where he can see and [right] the story of "The Blind Men and the Elephant": how individuals may understand and describe the same object differently based on their limited and subjective experiences.

Materials and methods.

Debriefing. Post-event or follow-up analysis often involves several scientific methods to understand what happened, why it happened and how similar events can be prevented or mitigated in the future. These analyses are crucial for developing lessons learnt and improving responses to future crises. We attempted to perform scientific methods in deliberative analysis after disasters called Lessons Learned Analysis (Paquay et al., 2022) widely used in military medicine. This is a process for collecting and analysing the experience gained from activities at events to identify successful behaviours and areas of improvement. This includes documenting what should be continued, stopped or adjusted. The first author attempted a professional approach using the Debriefing and Organisational Lessons Learned method, and even obtained verbal permission from local health inspectors and chief veterinary inspectors to conduct structured interviews with key Pomeranian ONE and public health officials involved in the outbreak investigation. However, upon official request for approval (which is required for this type of government work), the Chief Sanitary Inspector and the Pomeranian Voivodship Veterinary Inspector cancelled the appointments. It is also an interesting exercise to contrast the efforts of Pomeranian inspectors with other regions of Poland (Jarynowski & Skawina, 2021), where, for example, the quality of the outbreak investigation (prior to the issuance of guidelines by the chief secretaries from Warsaw) was questionable.

Unconventional data sources. There is a question of hierarchy of data sources, methods etc. In this outbreak investigation (probably first time in Poland in strictly veterinary aspect) real-time social media monitoring (Valentin et al., 2021)/participatory (Bispo Júnior & Morais, 2020) method was applied [Fig. 2].

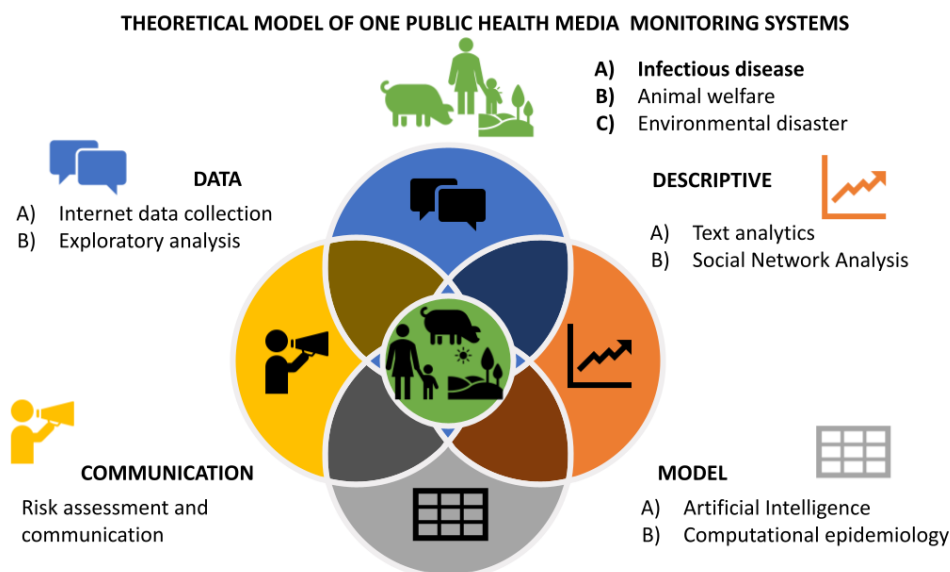


Fig 2. Concept of integration of infoveillance/infodemiology with crisis management and One public health

However, the usefulness of non-conventional surveillance and modelling approaches has not been normalised in terms of how to use this information in practice [Fig. 2]. Thus, for instance Veterinary Inspections did not mention un-official sources at all in their communication (i.e. the uncertainty of laboratory results out of certified systems was not known). As a result, there was a dissonance between the state and NGOs/researchers in the narrative, due to the use of different sources [Fig. 1]. The participatory and Internet trace of user activity are known to be biased, thus it must be interpreted together with an infodemiological approach (Eysenbach, 2020).

Crisis communication. Crisis communication involves the principles and practice of dynamic, effective, clear and persuasive techniques for verbal and written communication of up-to-date information about One Health risks (Steiner, 2024). An infodemic (Eysenbach, 2020) refers to the rapid and uncontrolled spread of information, misinformation, misleading narratives, and outright falsehoods surrounding a crisis – in this case, a disease outbreak. This deluge of information can be just as harmful as the disease itself, hampering effective response, eroding public trust, and leading to harmful behaviours. However, filtered non-official information was used (see subchapter before) in the analysis of source detection and the course of the outbreak (Jarynowski & Belik, 2023). To illustrate the problems of the discourse, we have selected some media reports (Table. 1).

Results.

Infodemic during the A/H5N1 Outbreak. While managing an infodemic is challenging, proactive strategies can mitigate its harm. Outbreak amongst cats in Poland exposed the nation's vulnerability to infodemic chaos. Governments and ONE health institutions did not have infodemic preparedness plans alongside their disease response protocols. The types of misinformation (e.g., hundreds of cat carcasses in Gdansk, the spread of disease to other mammals, toxic environmental contamination, or poultry meat as a source of outbreaks) could have been foreseen, and establishing partnerships with trusted experts and community leaders was possible (if prepared in advance).. A clear taxonomy (classification system) was not applied for misinformation and there was no designated authority to coordinate infodemic response across government agencies, academia, and the media. On the one hand poultry

businesses used public relation agencies to avoid panic among pets owners to their poultry/eggs products, on the other hand agenda setting and clickbait attitude of mainstream media were driving panic with titles such as “Deadly influenza from Butcher shop”. The public was not educated enough on critically evaluating information sources, identifying fake news, and relying on verified channels, although it is essential. Thus, many people wrongly trusted some influencers, who “knew” the origin of the outbreak from the beginning. The lack of official guidelines at the beginning of the outbreak (the first guidelines came from non-governmental veterinary associations) on pet safety during animal-borne disease outbreaks caused people to look for answers on unreliable platforms and fueled rumours of contaminated pet food. Conflicting or incomplete messages from the Ministry of Agriculture (focused on protecting poultry) and local news reporting cat deaths (rp.pl, gazetaprawna.pl) fostered mistrust. The response was fragmented, while well-intentioned, expert warnings (pulsmedycyny.pl) were easily taken out of context, adding fuel to fears about mutation and human risk. Some experts' indications were misused. The search for guidance (gdansk.pl, koty.pl) highlighted the public's need for clear information. This information void created space for predatory fake "cures" or conspiracy theories. The A/H5N1 outbreak is a stark reminder that managing the spread of information is as crucial as managing the spread of disease itself. Without preparation (Aslan et al., 2024), infodemic will continue to undercut public health efforts and erode trust in the institutions meant to protect us.

Table 1

Media Sources used in the article

Type of medium	URL	Summary
nationwide top3 general newspaper	rp.pl	Highlights the discrepancy between the Ministry's focus and public concerns.
nationwide business top15 newspaper	gazetaprawna.pl	News report highlighting cat deaths, demonstrating a need for clear communication.
nationwide medical top5 portal	pulsmedycyny.pl	Features expert opinion that could be misused to spread fear.
regional portal (local government owned)	gdansk.pl	Local authority providing practical pet safety advice, filling a potential information gap.
nationwide top3 cats portal	koty.pl	Offers cat care advice, demonstrating proactive response by some websites.
National Sanitary Inspection portal	gov.pl	Example of an official statement that is accurate but not reader-friendly for concerned pet owners.

Role of scientists. It is questionable how much information should be released to the public during the investigation of the outbreak. Let us compare two scientific discoveries known to sanitary/veterinary inspectors since the beginnings of investigation which could trigger panic in the society: 1) possible contamination of animal feed (Poland is the biggest poultry exporter in Europe (Jarynowski, 2024)); 2) nonfatal cases in dogs (Szaluś-Jordanow et al., 2024).

1) In the beginning of July a non-certified lab in Cracow found A/H5N1 animal feed (cat had typical AI symptoms around a month before) and virus was sequenced in Gdańsk later on (Rabalski et al., 2023). Back-tracking potentially contaminated food (frozen poultry meat) a month after purchase was impossible. In the first case the food researcher went on an early

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stage of investigation to the media “Bird Flu in a butcher store”, in second veterinarians instead of publishing their results in a highly cited scientific journal (when the story was “hot”) waited until the outbreak was finished. Inspectors were very upset with the attitudes and performance of researchers particularly from Gdańsk Medical University and Gdańsk University (not trained as veterinarians nor epizootiologists), who disclose this information quickly to the media, in consequence disturbing inspectors work. Moreover, similar complaints happened during SARS-CoV-2 outbreaks in mink in the Pomerania region around 2020/2021 (Rabalski et al., 2022). In informal chats (as described above formal post event debriefing was not possible), inspectors called them “scientific paparazzi”.

2) On the other hand, the information of the positive (Avian Influenza test) dogs stayed in the veterinary community not to cause panic (A/H5N1 course in dogs’ cases were never fatal) in country of dog lovers (there is ~8 million dogs and only 4 million cats in Poland, while usually in other European countries the ratio is moreover equal with some states with even opposite trend). Non-state veterinarians released findings to the general public about positive dogs, only when the outbreak was over (Szaluś-Jordanow et al., 2024) showing respect to their colleagues in Veterinary Inspection.

A question of responsible science can be emerged, because scientists are evaluated not by their input into society but by bibliographies scores. Thus, there is an incentive to use operational information (in statu nascendi) and share it to the world in an initial form (without discussion on uncertainty).

Thus, the mythomaniac role of scientists created by mass media such as Netflix (i.e., “Do not look up”¹, “High Water”²) builds a very wrong picture of crisis management. On the other hand, the old “Chernobyl”³ HBO series or “Epidemic”⁴ are much more realistic, conveying a realistic and intriguing take on the relationship between researchers, inspectors and the state (Kunicki, 2021).

Regional-National Coordination. In Poland crisis management is organised by county “powiat” (i.e. Gdańsk), province “voivodeship” (i.e. Pomerania) and nationwide level. Since the beginning, there were suspected cases from multiple counties around the city of Gdansk, so the coordination of the disaster should be considered at the level of Pomeranian voivodeship. Polish veterinarians were not in the same situation as their colleagues in the USA, who knew (because of Polish experience among others) that cats can be a sentinel of AI in mammals (Plaza et al., 2024). However, some intuition (i.e. using human rapid antigen tests) allowed them to follow into the right direction. Influenza virus was quickly found in the regional Veterinary Hygiene Institute and confirmed in national reference labs.

Regional veterinary inspection is the so-called “civil service” in Poland, so the inspectors have some capacity for unspecified tasks. Thus, in the first part of the outbreak the cost of laboratory tests were covered by Pomeranian Veterinary Inspectorates. Conversely, regional sanitary inspectors are not “civil service” and do not have these benefits (much lower status), hence are more inclined to wait for decision making Chief Sanitary Inspection (which only has “civil service” status). The situation got complicated in the later stage of the outbreak investigation outside of Pomerania, because there was no unified decision of the Chief Veterinary Inspectorate about who should pay for the tests. In some places (such as Pomerania) local inspectors paid, in the other the fees were paid by veterinarians or animal owners. Some non-state institutions such as university researchers, volunteer to analyse samples without payment. This was a huge mistake from an outbreak investigation perspective, because the owners in a great majority refused to pay for the post-mortem

¹ https://en.wikipedia.org/wiki/Don%27t_Look_Up

² [https://en.wikipedia.org/wiki/High_Water_\(TV_series\)](https://en.wikipedia.org/wiki/High_Water_(TV_series))

³ [https://en.wikipedia.org/wiki/Chernobyl_\(miniseries\)](https://en.wikipedia.org/wiki/Chernobyl_(miniseries))

⁴ [https://pl.wikipedia.org/wiki/Zaraza_\(film\)](https://pl.wikipedia.org/wiki/Zaraza_(film))

investigation of their pupils. Local veterinary authorities were complaining for lack of financial and meritoric support from the central institution (Jephcott, 2024).

Pomeranian Sanitary Inspection as being part of the healthcare system was in a much more complicated situation in paying for the test of humans in contact with sick animals (so they did not do it, just observed cases). Improvisation of Pomeranian Sanitary Inspectors was justified with guidelines around over two weeks from identifying that a source may have a zoonotic potential. Almost all sanitary inspectorates outside of Pomerania did not introduce any surveillance (even knowing to have animal cases in their territory) before getting the directive from Chief Sanitary Inspectorate.

Collaboration between local sanitary and veterinary inspectors did not work well in other regions of Poland. Often animal owners who trusted their vets and local veterinary inspectorate were afraid of sanitary inspectorates (they got bad fame during COVID-19 pandemic and first year full scale Russian invasion on Ukraine (Marek, 2022)). For instance, humans in contact did not want to be quarantined (even it was not the case).

International Bodies. We suffer from lack of support and misleading communication from EU institutions (similar filings were during the Ukrainian refugee crisis (Paradowski et al., 2023)).

Problems with ECDC:

- Errors in almost all communications (practical in technical reports and scientific publications - Eurosurveillance journal). All articles have multiple errors i.e. in maps Figure 1.b (Rabalski et al., 2023), Figure 1 (Domańska-Blicharz et al., 2023) suggest that there were cases in Lubuskie and Łódzkie voivodeships, which was not the case at least in the time period marked by authors. As trusted sources of information in the European Union, the Eurosurveillance journal may have asked authors for erratum as they claimed a false argument that spatial distribution of cases do not form any clusters (but they plot data wrongly). Throughout the COVID-19 pandemic rapid publishing caused retraction of some important epidemiological articles in The Lancet and The New England Journal of Medicine (Rzymiski et al., 2020). As of April 2024, Eurosurveillance did not publish any erratum. Moreover, in ECDC bulletin "Weekly threats reports" (at least in reports of weeks 26-28: the apogee of the outbreak investigation) the number of flaws was even higher. The geographical location of samples being negative were confused with positives and the numbers of positive cases were presented differently as these submitted by Polish authorities (ECDC, 2023). Thus, those who do not have contacted involved sanitary/veterinary inspectors from Poland and rely only on EU communicates (also in scientific press) may have a biased understanding of the outbreak.

- Lack of understanding of general context (i.e. agricultural and environmental constraints) by EU medical experts. In general understanding of ONE health concept (with stress on livestock supply chains) is low among epidemiologists at ECDC (who are supposed to support regional ONE public health officers) and usually ends at backtracking food-borne diseases.

Problem with EFSA /DG SANTE (Directorate-General for Health and Food Safety):

- Lack of possibility to use ADNS/ADIS (EFSA et al., 2017) systems with mammal as host (WOAH own EMPHRES allows that kind of data)

- Chaos in internal communication and guidelines in a situation without preparedness plans.

A serious problem, not only in ONE health, is the reluctance to talk about mistakes made, just as much as the reluctance of management to disclose them (Rosiński et al., 2019). On the other positive experience with UN bodies (WHO/FAO) and WOA, which are much more experienced is such a situation. It is important to underscore that collaboration with UN agencies in biomedical aspects during previous crises such as the flux of Ukrainian refugees was much better than with EU bodies (Jarynowski & Maksymowicz, 2024).

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Discussion

Infodemic management. Poland must learn from the A/H5N1 infodemic. Proactive measures are needed: such as developing robust digital monitoring tools to track narratives and identify emerging misinformation trends (Social Listening); creating a classification system for misinformation types, alongside clear regulations for addressing false narratives, especially on social media platforms (Taxonomy & Regulation).

Table 2.

The proposed taxonomy (Purnat et al., 2021) for health related narratives (adapted from (Romanowska, 2024))

Scope of Impact	Timeframe	Thematic Group	Examples of Misinformation	Consequences on Public Health
Individual & Public Health	Immediate	Vaccinations	False claims about adverse effects, conspiracy theories (mandatory vaccination of cats against AI)	Influences health behaviours and public health initiatives
Individual & Public Health	Immediate	Disease Information	Misinformation about disease origins, symptoms, prevention measures or about H5N1 transmission/sources of infection/ prevention and control of the disease etc.	Directly affects individual (or their animals) health decisions and public response, induced panic (some dogs owners)
Veterinary/ Healthcare Practice	Immediate	Disease Diagnostics	Inaccurate information on diagnostic test efficacy (i.e. use of human tests for animals) and safety	Impacts immediate healthcare/veterinary practices and public health strategies
Public Health Policy	Short to Medium Term	Antimicrobial Resistance (AMR)	False efficacy claims of antibiotics	Contributes to AMR, influencing healthcare practices and policy
Public Perception	Medium to Long Term	Alternative Therapies	Exaggerated claims about the effectiveness of unproven treatments (i.e. advertisements of immunomodulating supplements for cats)	Influences public perception and healthcare choices over time
Environmental Health	Long Term	Environmental Factors	False information about the health impacts of environmental factors (i.e. toxic water killing cats)	Affects long-term environmental health and policy

Environmental Health	Long Term	Climate Change	Misinformation regarding the health impacts of climate change	Impacts long-term public health strategies and environmental policies
Societal Behaviour	Long Term	Social, Economic, Geopolitical Factors	Misinformation about the health impact of socio-economic factors (i.e. false image that epizootic is equally distributed around the country)	Influences societal behaviour and long-term health policy
Public Health	Medium to Long Term	Nicotine and Nicotine Products	False safety and health effect claims of nicotine products	Influences public health initiatives and regulatory policies
Public Health	Medium to Long Term	Alcohol and Alcoholic Products	Misleading information about the health impact of alcohol	Affects public health measures and societal attitudes towards alcohol consumption
Emergency Response	Variable	Disasters	Misinformation about natural and man-made disasters	Impacts emergency preparedness and public safety measures
Societal Behaviour	Long Term	Elections	Misinformation about health policies and candidates' stances on public health issues	Influences voting behaviour, public trust in health systems, and health policy legislation
One Health	Long Term	One Health Mis- and Disinformation	False narratives disconnecting the interrelation of human, animal, and environmental health. False information about H5N1 transmission/impact/efficiency etc	Leads to misguided health practices and policies, overlooking ecosystem interconnectivity
Individual Health	Immediate to Long Term	Oral Health	Misconceptions about dental care, fluoridation, and oral hygiene links to overall health	Affects oral and overall health outcomes, can lead to systemic health issues

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Reproductive Health	Immediate to Long Term	Reproductive Health	False information regarding contraception, pregnancy, and reproductive rights	Influences reproductive choices and can impact public health and rights
Societal Behaviour	Long Term	Cultural/Racial Mis- and Disinformation	Stereotypes and false health narratives related to specific cultures or races	Exacerbates health disparities, affects healthcare delivery and societal inclusion
Societal Behaviour	Variable	Religious Mis- and Disinformation	False claims related to religious beliefs and health practices	Can lead to harmful health behaviours and undermine public health measures
Individual Health	Variable	Age-related Health Myths	Misinformation about ageing and associated health practices	Influences treatment of elderly, affects healthcare policy
Mental Health	Immediate to Long Term	Mental Health Stigmas	False narratives surrounding mental illness and treatment	Contributes to stigma, impacts mental health care seeking and provision
Public Perception	Immediate to Long Term	Celebrity Health Endorsements	Misleading health advice endorsed by celebrities without medical expertise	Influences public health behaviours, can lead to the adoption of unproven or harmful health practices
Public Perception	Immediate to Long Term	AI & Cybersecurity	Myths about AI (artificial intelligence) and cybersecurity risks on health	Causes distrust and fear of technology; may deter from adopting useful innovations
Environmental Health	Immediate to Long Term	Biotechnology, GMO & Genetic Engineering	False claims about the safety and impact of GMOs and genetic modifications (i.e. degenerated meat feed based on poultry feed with GMO grains)	Influences public opinion and policy, potentially rejecting beneficial technologies
Public Health & Safety	Long Term	Nuclear Power/Energy	Misinformation about the risks and benefits of nuclear energy (i.e. radiation from warzone in Ukraine)	Impacts energy policies and public support for nuclear solutions

Public Health & Safety	Immediate to Long Term	5G Technology	Conspiracy theories linking 5G technology to health issues such as cancer or COVID-19	Causes unwarranted fear and resistance to infrastructure development
Societal Behaviour	Immediate to Long Term	Robotics and Automation	Exaggerated fears about robots leading to mass unemployment (i.e. in farming) or loss of human autonomy	May influence public policy and economic decisions; fosters unnecessary fear and resistance to beneficial technology

The WHO document (WHO, 2023) outlines methods for identifying and classifying various types of health-related misinformation and disinformation, which has informed the categories and examples included in this taxonomy. It adheres to internationally recognized standards and practices.

The purpose of the taxonomy is not only to categorise health-related misinformation effectively but also to provide a practical tool that can be actively used by health professionals, educators, policymakers, journalists, and the public to understand, identify, and combat misinformation.

Structural and coordinating aspects. The lesson that decision-makers and organisers of the inspection system have not learned is the one that was learned long ago by scientists dealing with organisation theory - both sociologists and other theorists and practitioners in this field (Wisniewska et al., 2023). In these studies, organisations are seen as “competitive actors, striving to achieve their own goals” (Laumann et al., 1978). Therefore, each of them tries, in accordance with the assumptions of utilitarianism, to maximise their profits by minimising costs. What is important, “final allocation of resources is the product of a large number of small decisions, negotiated at the level of the interorganizational dyad. And these interorganizational organisations are often created by members themselves” (Galaskiewicz, 1985). And as with any organisation, the decisions made depend on power relations. Undoubtedly, “cooperation, coordination and collaboration are the basis of inter-organizational activities” (Castañer & Oliveira, 2020). But they are marked by complex networks of relationships, the understanding of which allows for better organisation and control of the flow of information.

We learnt that for the most unpredictable threats one public health responsibilities and challenges stand on the shoulders of regional stakeholders. It was the case of Legionella outbreak in Subcarpathia (Krzowski & Ostrowska, 2023), Ukrainian refugees wave (Madej et al., 2023) and also Oder river disasters (Free et al., 2023). In each of these cases, analyses revealed deficiencies in coordination at the international and central level and some positive aspects at the local level. As in the case of the ecological catastrophe in the Oder River in 2022 (Jarynowski & Maksymowicz, 2024), when most of the activities were initiated and carried out by smaller local groups, with an ongoing international dispute between Poland and Germany.

And such coordination was also missing in the case analysed in this article. The reason is both the silled operation (organisational silos) of separate inspections and their inability to coordinate and communicate. The reason for this is the fact that the veterinary inspection is a civil service, so it is a more independent structure, and its local veterinary inspector has high prerogatives, comparable to the mayor or the head of the national tax administration, so he is at the top of the official hierarchy. However, the situation is completely different in the case of the sanitary service, which is subordinated to the central authority and is not able to act independently, always waiting for decisions from the capital of the country. This power

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imbalance, known in organisational theory, causes the mentioned lack of coordination and prevents adequate communication between unequal actors.

Such action is therefore consistent with the analyses which indicate that: 1) the more that other organisations are dependent upon the focal organisation for the resources they need, the more likely that organisations are going to view the focal organisation as being influential and 2) those organisations less dependent upon the local system for resources tended to interact less with local agencies and had more disagreements with locals than organisations more dependent upon local systems. Thus, strong (scientifically and man-powered), locally networked and independent (financially and administratively) integrated ONE health inspection on a level of region with ~few millions citizens/tens of thousands km² seems to be the best structure for unknown threats (Al Asfoor et al., 2024).

The matter becomes even more complicated when we add one more necessary element that is an independent organisation related to a separate field: science (e.g. university structure). Scientists should be added to this organisational puzzle, who, although have the knowledge necessary to solve the problem. Yet they are not included in the process, or it happens accidentally. Meanwhile they should be included in the critical process as a natural and independent actor, complementing the entire crisis prevention system (although not with a leading role). But also, in this dimension there is a conflict regarding resources: scientists' goal is scientific work culminating in a high-scoring publication, so their interest is often limited to topics that have publication potential. This, in turn, may lead to a limitation of their scientific interest in a situation that is important from the inspection point of view but has little publication potential. Summarising to avoid mistakes happened in Poland, organisers or ONE health response system should take into consideration: 1) decentralisation in ONE health system, that will promote the transfer of decision-making power from central governments to local entities, enhancing responsiveness to community health needs and streamlining the management and distribution of resources; 2) local integration and networking facilitate cooperation among various ONE health sectors and actors, to create a unified framework for rapid information sharing and collective decision-making at local, and central/international levels, thereby improving the overall effectiveness of ONE health protection.

Conclusions: *The future?*

A/H5N1 in cats is just an example of an institutional system response during a crisis (Meletis et al., 2024), thus we don't know where new problems will arise, as it could be an invasive species spread from a cargo, a new or AMR (antimicrobial resistant) pathogen, etc. Thus, our example revealed many vulnerabilities to emerging (i.e. zoonotic (Grzybek et al., 2023)) threats at the regional, national and international levels, so we recommend the following for the future:

- Joint outbreak investigation and monitoring/surveillance schemes (Jarynowski & Belik, 2023) of veterinary and sanitary inspections (with the support of local scientists) should be encouraged at the regional level, and supported logistically and financially (at least in the case of Gdansk, the government benefits from increased customs duties, which should offset the risks of the hub's position).

- EU agencies such as ECDC and DG SANTE need to adjust better to new challenges (i.e. disease X) from more experienced players such as WHO or CDC (Bravo-Laguna, 2023).

- Mid-level regional ONE health inspectors should have the possibility to use public financial sources to provide microbiological tests in ONE health problems of unknown origin.

- Unconventional data sources (i.e., participatory epidemiology and infoveillance) should be incorporated into the surveillance/risk assessment scheme (Meletis et al., 2024; Radziwon et al., 2023).

- A national and/or regional Epidemic Intelligence System (Meletis et al., 2024) should operate in real time, aiming to identify, monitor and analyse One Health threat signals through early warning schemes.

- Perform infodemic management and use taxonomy recognized by global experts and global health organisations (if possible) when conducting a surveillance to identify information voids and threats (WHO, 2024). Uncontrolled infodemic should not repeat in the next crises. Pre-established channels for information exchange between government bodies, scientific experts, veterinarians, and reputable media outlets can foster consistent messaging that counters the confusion exploited by bad actors.

- Built resilience system (Aarestrup et al., 2021), educate and implement integrated environmental and behavioural health analytics. It is recommended to build an open ecosystem of tools, reference databases and communities.

- Cultural aspects must be included to trade off panic and outbreak/crisis management (here caution Poles are much more into dogs than cats), which can only be understood by locals (Jephcott, 2024).

Acknowledgement: AJ and VB were supported by DFG (458528774) and BMBF (031L0299A) grants. Authors declare no conflicts of interest, except AJ who was involved in the outbreak in investigation under confidentiality, thus only publicly available information may be included in the article. We thank civic/military veterinary and sanitary inspectors, researchers, journalists and volunteers who (sometimes anonymously) helped us with understanding the phenomenon.

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МУЛЬТИМОДАЛЬНИЙ ЕПІДНАГЛЯД В СИСТЕМІ ОХОРОНИ ЗДОРОВ'Я В ЧАСИ ЗМІН: УРОКИ, ЯКИХ НЕ БУЛО ЗАСВОЄНО З ПРИКЛАДУ РОЗПОВСЮДЖЕННЯ А/Н5Н1 СЕРЕД ССАВЦІВ У ГДАНСЬКІЙ АГЛОМЕРАЦІЇ

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Резюме. *Це тематичне дослідження поширення A/H5N1 на ссавців у районі Гданська підкреслює складність управління новими загрозами "Єдиному здоров'ю" у важливих політичних та економічних аспектах. Ми порівняли відносно успішне швидке регіональне реагування з абсолютно програною битвою у сфері комунікації та співпраці, підкресливши необхідність покращення міждисциплінарної регіональної та міжнародної співпраці та надійних систем епіднагляду в епоху антропогенних і природних змін.*

Ключові слова: управління інфодеміями, (знову) виникаючі хвороби, системи раннього попередження, єдине здоров'я, готовність

DOI: 10.31073/onehealthjournal2024-III-06

CURRENT STATUS OF THE LEGAL FRAMEWORK IN THE PLANT PROTECTION AND ECOLOGY AND HYGIENE MONITORING DOMAIN IN UKRAINE

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Abstract. *Presently chemical plant protection products are an inseparable part of agriculture. They have not only their main purpose of plant assistance, but they also have the potential risk of negative impact on biocenosis species (birds, bees, soil microflora, algae, etc.) and the human body and its health respectively.*

The purpose of our study was to aggregate data on the existing legal framework of plant protection products in Ukraine and assess their ecology and hygiene monitoring.

For analysis of the plant chemical protection, we used as the basic documents the regulatory framework of domestic legislation in toxicological and hygiene, ecology assessment, and ecology and hygiene monitoring domains.

Currently, many laws and legal acts regulate the use of pesticides by state and private agricultural farms in Ukraine. This number of documents covers not only pre-registration studies of pesticides but also their post-registration monitoring in the environment. The key entities that control potential negative risks of these products through the regulations are the State Emergency Service, Ministry of Environmental Protection, Ministry of Health, Ministry of Housing and Communal Services, Ministry of Agriculture Policy, State Agency of Water Resources, State Committee of Land Resources, State Agency of the Forest Resources. However, the impact of xenobiotics on non-target species of the ecosystem is currently quite underestimated. The decline in biodiversity directly depends on the condition of the environment and the negative impact on it. Instances of acute oral, inhalation, or dermal poisoning of birds, bees, and aquatic invertebrates with pesticides are quite common and among the factors that affect public health. That is why ecology and hygiene monitoring is essential in line with the assessment of the risks of the inappropriate release of pesticides. These should be treated as a critical component of managing environmental sustainability and safety for public health.

The implementation of global approaches to monitoring and controlling the post-registration impact of pesticides on the ecology and hygiene in Ukraine can also take into account the far-reaching consequences of their negative impact, accumulation, and environmental pollution. As a result, this will help to avoid adverse impacts on animal, insect, and bird populations, as well as human health.

Key words. **Government monitoring, agriculture, pesticides, eco-system, negative impact.**

Pesticides play a key role in modern agriculture, helping to increase yields and food production. However, the widespread use of plant protection products (PPP) raises concerns about their potential negative impact on the environment, ecosystems, and human health. The use of pesticides on agricultural lands can have far-reaching consequences for the environment and the population as a whole. For example, if the PPP from cultivated areas penetrates the groundwater or surface water basins they can impact aquatic ecosystems;

polluted air from cultivated areas and contaminated soil spread pesticides beyond the treated areas, affecting non-target species (birds, bees, soil microflora) and disrupting the ecological balance (the State service for food safety and consumers protection, 2024; Pathak V.M., 2022).

The purpose of the paper is to aggregate data on the existing legal framework of plant protection products in Ukraine and assess their ecology and hygiene monitoring.

Methods and materials. The analysis was based on the scientific literature and national regulatory documents in the domain of toxicology and hygiene, ecology assessment, and ecology and hygiene monitoring.

Results and discussions. The current legal framework of the plant protection domain consists of (Garmash S., 2023; the State Service for Food Safety and Consumers Protection, 2024):

- The Constitution of Ukraine
- The Law of Ukraine "On plants protection".
- The Law of Ukraine "On pesticides and agricultural chemicals",
- The Law of Ukraine "On the system of permits in the economic domain",
- The Law of Ukraine "On the Number of Permission Documents in the Economic Domain",
- The Law of Ukraine "On seeds and planting material",
-
- The Law of Ukraine "On the plant variety protection",
- Law of Ukraine "On sanitary and hygiene wellbeing of the population",
- the number of legal acts on the protection and quarantine of plants,
- international agreements of Ukraine,
- The Cabinet of Ministers decrees "On approval of assessment criteria of the rate of risk from agricultural activity in the plant protection domain and determination of the frequency of planned measures of government supervision (control) by the State service for food safety and consumers protection",
- Customs code of Ukraine and other legal acts.

The domain of ecology and hygiene monitoring has not yet had such a diverse, full-fledged, and clear regulatory framework. Most of the documents relate to pre-registration studies, risk and hazard assessment of new active substances and dosage forms.

In the ecology and hygiene monitoring domain, it is more complicated currently everything is limited to pre-registration assessment. Ukrainian research institutions, such as the Institute of Plant Protection of the National Academy of Agrarian Sciences (Ministry of Environmental Protection, 2024) and other acknowledged scientific institutions, research the pesticides' impact on the environment, on non-target organisms, obtaining valuable data for regulatory resolutions (Kovalenko Yu.L., 2020). However, they use scientific and methodological approaches that are different from European practices. For example, the Organization for Economic Cooperation and Development has elaborated guidelines and recommendations for risk assessment and mitigation of negative impact of pesticides (OECD Guidelines, 2024) together with the principles of good laboratory practice, in particular in the context of pesticide testing (GLP, 2024), which ensures the quality and integrity of preclinical laboratory tests, providing a basis for regulatory decisions. This includes methods for assessing the ecology and impact of pesticides, as well as their influence on human health. Recommendations for determining the biological effectiveness of pesticides have been developed, focusing on pest control efficiency. The recommendations also cover the calculation of pesticide content in food and the environment.

There is an extensive scientific and regulatory framework for toxicological and hygiene inspection and control of pesticides, and a functional system of sequencing the operation of organizations for research, pre-registration testing, and post-registration monitoring (the Cabinet of Ministers decree, 2024).

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The state toxicological and hygiene test of pesticides is carried out by accredited research institutions (the Cabinet of Ministers decree, 2024). The State Service of Ukraine on Food Safety and Consumer Protection plays a key role in the supervision of pesticide monitoring procedures (the State Service of Ukraine on Food Safety and Consumer Protection, 2024). It carries out regular inspections and assesses compliance with safety standards for pesticide content in food.

And most important thing is that the post-registration evaluation, control, and monitoring are not considered and conducted. The tests are run only in cases of emergencies, such as massive bee envenomation, etc. But these rare incidents of course cannot give a full picture of a particular pesticide circulation in the environment or the long-term, cumulative impact on non-target species.

Presently, there is an aggregate environment monitoring system that is supposed to register all pollutants. It has its structure and its operation is regulated by a specific framework (Environment e-monitoring, 2024).

The Law of Ukraine "On Environment Protection" (Articles 20, 22) stipulates for creation of the State Environmental Monitoring System (SEMS) and the conducting of observations of the condition and pollution of the environment (Environment e-monitoring, 2024). National executive authorities are responsible for this task, in particular the Ministry of Environmental Protection and Natural Resources of Ukraine, which are the constituents of the State Environmental Monitoring System, as well as the institutions, companies, and organizations whose main activities may potentially or lead to environmental degradation, pollution and denaturation (Environment e-monitoring, 2024).

Each of the eight entities responsible for monitoring (the State Emergency Service, Ministry of Environmental Protection, Ministry of Health, Ministry of Housing and Communal Services, Ministry of Agriculture Policy, State Agency of Water Resources, State Committee of Land Resources, State Agency of the Forest Resources) monitor environment as defined by the Regulation on the State Environmental Monitoring System and the procedures and regulations on State monitoring of certain environmental components (Environment e-monitoring, 2024; Kovalenko Yu.L., 2020).

The basic regulatory documents of the State monitoring of the environment are (Environment e-monitoring, 2024; Kovalenko Yu.L., 2020):

- The Cabinet of Ministers decree as of 20.07.1996 #815 "On approval of the Procedure of State monitoring of waters";
- The Cabinet of Ministers decree as of 09.03.1999 # 343 "On approval of the Procedure of State monitoring of the air protection";
- The Cabinet of Ministers decree as of 26.02.2004 # 51 "On approval of the Statement of monitoring of the soils in agricultural land";
- The Cabinet of Ministers decree as of 20.08.1993 #661 "On approval of the Statement of monitoring of lands".

The presented system of State environment monitoring is based on the fulfillment of distributed functions by individual entities of the system that includes subordinate subsystems. Each subsystem is at the level of separate entities of the State Monitoring System that has its own scientific, methodological, structural, organizational, and technical basis (Environment e-monitoring, 2024; Kovalenko Yu.L., 2020).

It is worth mentioning that one of the deficiencies of the regulatory framework for organizing and implementing State Environmental Monitoring is the vague statement of the powers of its State System of Environment Monitoring components in the provisions on the relevant environmental objects (Environment Monitoring, 2024).

Apart from the main monitoring spheres (air, water, and soil), there are several special monitoring cases: forests, genetically modified organisms, and waste management (Environment e-monitoring, 2024; Environment Monitoring, 2024). Unfortunately, such an

important subdivision as ecology and hygiene monitoring of pesticides in the environment is lacking. It cannot be included in any of the existing groups, as the use, distribution, migration, metabolism, and impact on the ecosystem (target and non-target species) of plant protection products have several specific features.

We also should bear in mind the unique situation with pesticides: on the one hand, today it is impossible to do without them to ensure high sustainable yields sufficient to overcome famine in the world (Tkachenko, I.V., 2021; K. Chatzimichael, 2022) on the other hand, they are toxicants that can cause not only acute poisoning but also significant long-term effects (embryo and reproductive toxicity, carcinogenicity, endocrine disrupting effect, etc.) on the human body (Ibrahimova I.V., 2022; Antonenko A.M., 2019) and also a negative impact on so-called non-target organisms (birds, insects, soil worms, algae, etc.) (N.-F. Wan, 2023; A. Mitra, 2021; F.Sanchez-Bayo, 2021).

Presently to study the impact on non-target species, the so-called ecotoxicological studies are conducted, but only at the pre-registration stage. The effect on birds (quail, duck), bees, aquatic invertebrates (e.g., *Daphnia magna*), algae, earthworms soil microorganisms, etc. is studied for the formulation and its active ingredients. The median lethal concentrations and doses (LC50 and LD50) and half maximal effective concentrations (EC50) are measured in acute exposure studies, subthreshold, and threshold doses (NOEL and NOAEL) in subacute exposure experiments.

The accredited institutions also conduct these studies, and their results affect the possibility and status of pesticide registration. However further studies and monitoring have not been conducted. The accumulation and dynamic impact on these ecosystems are not monitored or studied, and that can have significant negative consequences, as changes in the life of these non-target species are directly related to the well-being of the human population and public health.

Many species of wild birds live in agricultural and forest areas where pesticides are often used. Birds can either inhale air contaminated with pesticides or have skin contact during and after pesticide spraying (T. Katagi, 2021). Contact with pesticide content on crops and weeds through feathers can lead to oral absorption of pesticides. Since crops, weeds, and insects are the main food for birds, the most likely route of pesticide content is oral (B. Mohanty, 2024). When pesticides are used to treat seeds, birds can be exposed to higher concentrations through ingestion of the treated seeds. In the case of the granular formulation, birds mistake the granules for seeds or swallow them instead of sand (T. Katagi, 2021; B. Mohanty, 2024). Considering these possible routes of exposure and the feeding habits of birds, the toxicological effects of pesticides should be assessed based on the impact when ingested with direct administration.

That is why ecology and hygiene monitoring of off-target pesticide exposure is a critical aspect of environment and public health management.

Discussion. Post-registration ecology and hygiene monitoring and control over the use, and accumulation of pesticides, and assessment of negative impact on environment and the human body are too scarce and poor in Ukraine in comparison with international European and United States practices. (Tkachenko, I.V., 2021; K. Chatzimichael, 2022). Besides the pre-registration studies, the focus should be made on conducting experiments on the long-term effects of chemical plant protection and its impact on water basins, soils, non-target species of biocenosis, and public health. ((Postanova Kabinetu Ministriv, 2024; N.-F. Wan, 2023; A. Mitra, 2021; F.Sanchez-Bayo, 2021; T. Katagi, 2021; B. Mohanty, 2024). After all, the assessment of the negative impact of drugs only at the time of registration is imperfect, as the acute impact may be renewed in the future and unacceptable, even if there aren't long-term consequences. (K. Chatzimichael, 2022; Ibrahimova I.V., 2022).

State testing and registration of pesticides and agrochemicals are carried out to minimize the negative impact of pesticides and agrochemicals and to meet the requirements for high

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biological effectiveness in terms of direct use, and safety for human health and the environment. But, when a pesticide is registered, its monitoring and assessment should not be over. It is of paramount importance to elaborate a comprehensive system of ecology classification of pesticides and agrochemicals based on global best practices and to introduce the possibility and methodology of extrapolating data from the active substance to the formulation of the pesticide.

It is also important to introduce global approaches to post-registration of ecology and hygiene monitoring and control in Ukraine to prevent the accumulation of pesticides, the long-term effects of their impact on the ecosystem, and on human health, and the possibility of timely response to changes in the conditions of animal, bird, insect populations, and the state of the water, soil and air environment.

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СУЧАСНИЙ СТАН ЗАКОНОДАВСТВА У СФЕРІ ЗАХИСТУ РОСЛИН ТА ЕКОЛОГО-ГІГІЄНИЧНОГО МОНІТОРИНГУ В УКРАЇНІ

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Резюме. Хімічні засоби захисту рослин, без яких наразі важко уявити сучасне сільське господарство, крім своєї цільової допомоги для рослинництва мають потенційний ризик негативної дії на представників біоценозу (птахів, бджіл, ґрунтової мікрофлори, водоростей тощо) і відповідно, на організм людини та її здоров'я.

Проведення узагальнення даних щодо наявного державного регулювання засобів захисту рослин в Україні та оцінка їх еколого-гігієнічного моніторингу.

Основними документами, які були взяті для аналізу сфери хімічного захисту рослин стали нормативна база вітчизняного законодавства в галузі токсиколого-гігієнічної, екологічної оцінки та еколого-гігієнічного моніторингу.

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В Україні, на сьогоднішній день, існує безліч законів, положень, які займаються регулюванням використання пестицидних препаратів державними сільськогосподарськими і фермерськими приватними господарствами. Ця низка документальної бази охоплює не тільки передреєстраційні дослідження пестицидів, а й постреєстраційний моніторинг їх в навколишньому середовищі. МНС, Міндовкілля, МОЗ, Мінжитлокомунгосп, Мінагрополітики, Держводгосп, Держкомзем, Держкомлісгосп – основні суб'єкти, які контролюють відповідними своїми положеннями потенційні негативні ризики препаратів. Проте, дія ксенобіотиків на нецільові види екосистеми, наразі, є досить неоціненою. Так, як зменшення біорізноманіття напряму залежить від стану довкілля та засобів впливу на нього. Випадки гострого перорального, інгаляційного чи нашкірного отруєння птахів, бджіл, водних безхребетних пестицидами є не поодинокими та одними із чинників від яких залежить громадське здоров'я. Тому, важливо виконувати еколого-гігієнічний моніторинг та проводити оцінку ризиків нецільового впливу пестицидів, що потрапляють у навколишнє середовище з точки зору критичного компоненту управління екологічною стійкістю та безпечністю для організму населення.

Запровадження в Україні світових підходів до моніторингу та контролю післяреєстраційного впливу пестицидів на еколого-гігієнічний стан довкілля може враховувати далекосяжні наслідки їх негативної дії, накопичення, забруднення об'єктів навколишнього середовища. В кінцевому результаті це сприятиме уникненню несприятливих наслідків для популяції тварин, комах, птахів, а також для здоров'я людини.

Ключові слова. Державний моніторинг, сільське господарство, пестициди, екосистема, негативний вплив.

DOI: 10.31073/onehealthjournal2024-III-07

The One Health SRI and One Health Institute, NGO present the Ukrainian translation of the WHO Laboratory Biosafety Manual

 ПНУ "Науково-дослідний інститут єдиного здоров'я"
ГО "Інститут Єдиного Здоров'я"



ПОСІБНИК ВООЗ З ЛАБОРАТОРНОЇ БІОБЕЗПЕКИ
ЧЕТВЕРТЕ ВИДАННЯ

Харків - 2024

Against the backdrop of the full-scale military aggression of the Russian Federation against the people of Ukraine and Ukrainian statehood, as well as the growing threat of military terrorist attacks on critical infrastructure, threats to food and energy security, aspects of biological security and biological defense are becoming extremely important.

The globalization of the modern world, the increase in the volume of international trade operations, transportation of people, animals, and agricultural products, the risks of the emergence and spread of infectious diseases, as well as the spread of pathogens that cause them, are significantly exacerbated. This explains the fact that biological security is a key component of national security.

International organizations deal with biosecurity issues in the world: The World Health Organization (WHO), the International Office for Epizootics - World Health Organization (OIE) and the Food and Agriculture Organization of the United Nations (FAO) in the context of implementing the One Health approach, which considers the processes of homeostasis in the living world as a whole.

The key role in the list of biological threats today belongs to emergent infections, which are accompanied by emergencies in a country or region, posing a threat to biological and food security, with certain economic and social consequences.

A critical aspect of the development of modern biosafety regulatory systems is the implementation of good practices to prevent the spread of pathogens and safety in laboratories and bioproduction facilities.

It is important that the draft Law of Ukraine "On Biological Safety and Biological Security" is being considered, and experts of the One Health Institute are involved in its development. There is a lot of work ahead to create bylaws for the future law and develop institutional policies.

Today, there is a need to strengthen the system of state regulation of biological safety and biological protection in Ukraine, including aspects of controlling the circulation of pathogens, creating conditions for safe diagnostic work and production of specific protection products for humans, animals and plants.

Today, with the kind permission of the World Health Organization, a team of specialists from the One Health SRI and the One Health Institute, NGO have completed an adapted translation of the WHO Biosafety Manual. By presenting this document and related monographs, we sincerely hope that this initiative will benefit the biomedical research and practice community in order to develop and strengthen national and subnational standards of biological safety and biological protection in Ukraine.

Sincerely yours

Anton Gerilovych,

Director General of the One Health
Scientific and Research Institute, PSI

For more information, please follow the link:

<https://www.onehealthinstitute.org.ua/publications/>

Журнал заснований 25 жовтня 2022 р. Державним науково-дослідним інститутом з лабораторної діагностики та ветеринарно-санітарної експертизи та Громадською організацією “Інститут Єдиного Здоров’я”. Видання зареєстроване Міністерством юстиції України, Свідоцтво КВ № 25382-15322Р від 10.01.2023 р. Включено до Переліку наукових фахових видань України (Категорія «Б») для ветеринарних наук (наказом МОН України № 768 від 20 червня 2023 р.).

Матеріали номера схвалено редколегією до друку 01.07.2024 р.

Видається чотири рази на рік, щоквартально.

НАУКОВЕ ВИДАННЯ

УДК 636.09:614:606

ISSN (print) 2786-7420

ISSN (online) 2786-7439

One Health Journal. Збірник наукових праць. Том 2. Номер 3. – Харків, **Державний науково-дослідний інститут з лабораторної діагностики та ветеринарно-санітарної експертизи та ГО “Інститут Єдиного Здоров’я”, 2024 – 70 стор.**

SCIENTIFIC EDITION

УДК 636.09:614:606

ISSN (print) 2786-7420

ISSN (online) 2786-7439

One Health Journal. Collected papers. Volume 2. Number 3. - Kharkov, **State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertize and One Health Institute, NGO, 2024 – 70 pages.**

Підписано до друку 05.07.2024

Формат 84x108/32

Папір офсетний. Друк офсетний.

Умов. друк. арк. – 8,2. Облік. від арк. – 8,8

Наклад 300 прим. Зам. №05-07/24

Віддруковано:

«ФОП Напольська А.В.»

Виписка з ЄДР ЮО та ФОП

№ 2 480 000 0000 152491 від 01.10.2013 р.

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