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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).

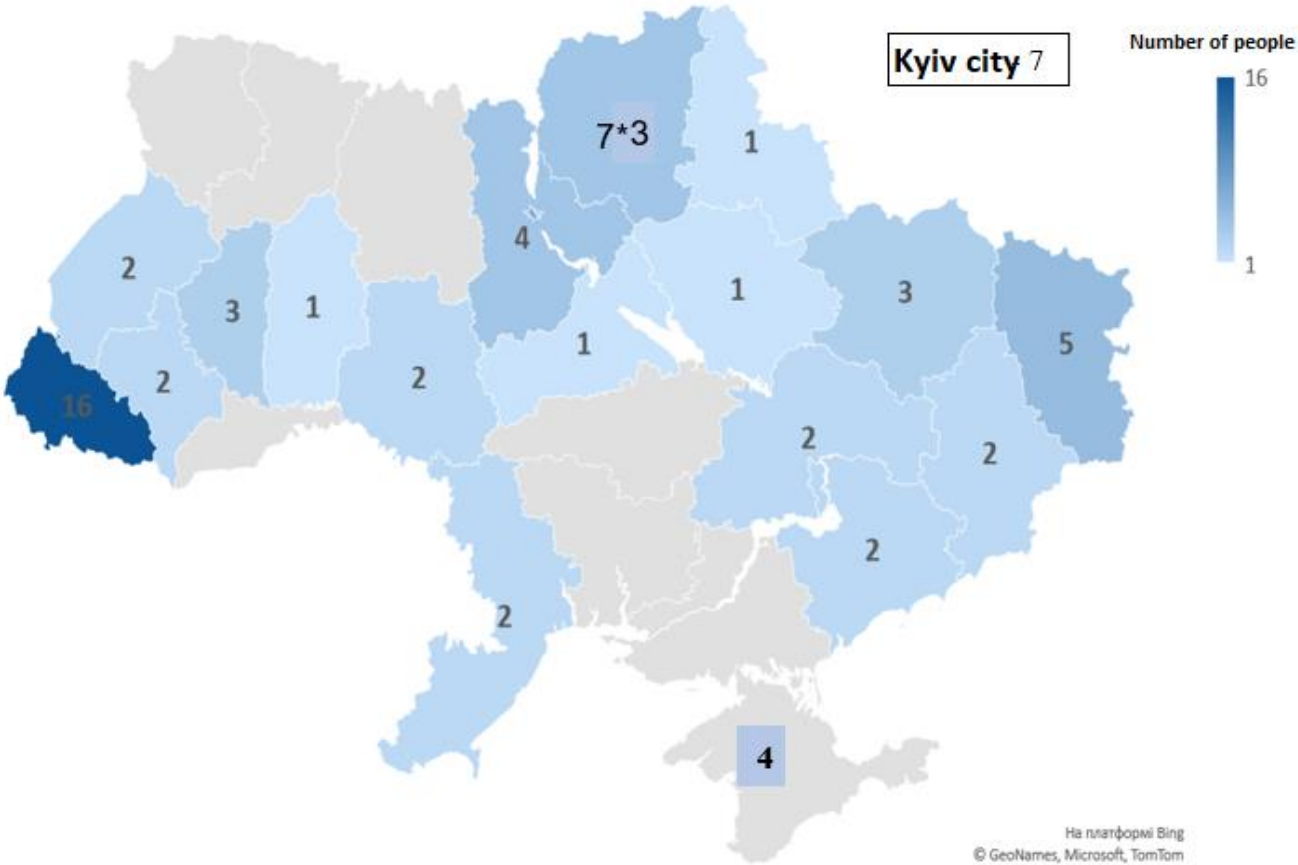


Fig. 1. Territorial distribution of diphtheria cases in Ukraine, 2010-2023

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:						
			adults		children (0-17)				
	abs.	DI*	abs.	DI**	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

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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

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