

PROTOCOL OF STEP-BY-STEP INSTRUCTIONS FOR IDENTIFYING THE SOURCE OF TOXOPLASMOSIS PATHOGEN AND PREVENTING ITS SPREAD FOR JOINT PREVENTIVE AND LIQUIDATION MEASURES (REFERENCE DOCUMENT)

Developed under Activity 1.5. “Increasing awareness on emerging diseases and
the prevention of cross-border epidemics”
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“Development of screening and monitoring systems for emerging diseases and
prevention of cross-border epidemics in the current context” ROUA00136,
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PREAMBLE

This Protocol of step-by-step instructions for identifying the source of Toxoplasmosis pathogen and preventing its spread for joint preventive and liquidation measures (hereinafter – the Protocol) was developed by PI "Educational and research institute of biotechnology and biosafety" Agreement No. 151 dated September 22, 2026 (hereinafter – the Agreement), concluded with Odesa State Agrarian University (OSAU) as Partner 3 (PP3) of the international project “Development of screening and monitoring systems for emerging diseases and prevention of cross-border epidemics in the current context” ROUA00136, co-financed by the EU under the Interreg NEXT Romania – Ukraine 2021–2027 Programme (hereinafter – the Project).

The document was prepared in the framework of Activity 1.5. “Increasing awareness on emerging diseases and the prevention of cross-border epidemics” (Deliverable D.1.5.1.).

The purpose of this Protocol is to provide veterinary professionals, medical practitioners, food safety inspectors, local authorities, research institutions, and other stakeholders with practical step-by-step guidance for:

- identifying the sources and transmission pathways of *Toxoplasma gondii* within the cycle "felids (definitive host) – oocysts – water/soil/feed – livestock and companion animals – humans";
- assessing the role of food of animal origin (meat and milk) and water as transmission factors for toxoplasmosis in a cross-border context;
- conducting joint laboratory researches (serological and post-mortem) of livestock, companion, and stray animals in accordance with the One Health approach;
- harmonising approaches to toxoplasmosis monitoring in the food chain and water supply systems between Ukraine and Romania in the Lower Danube region.

The Protocol is based on:

- the results of joint field investigations and laboratory analyses;

- the Joint Terms of Reference (ToR) approved by the project partners, which establish unified methods for sample collection, transportation, preparation, and laboratory examination;
- previous experience in investigating the prevalence of toxoplasmosis in livestock;
- published scientific data on the prevalence of *Toxoplasma gondii* among animals in European countries (including Poland and France) and in several regions of Ukraine (particularly in cattle and sheep);
- guidance documents issued by EFSA, WHO, WOA (OIE), and FAO on the management of food-borne parasitic diseases.

Unlike the other diseases addressed by the Project (African swine fever, West Nile virus, and Avian influenza), toxoplasmosis is not included among transmissible animal diseases listed in Categories A–E under Regulation (EU) 2016/429 and is therefore not subject to mandatory notification through the ADIS system. Instead, its regulation within the European Union is focused on food safety, zoonoses monitoring, and drinking water quality.

The Protocol has been prepared in accordance with the requirements and provisions of the following documents:

- Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, under which toxoplasmosis is included among the pathogens reported annually in the EFSA/ECDC European Union One Health Zoonoses Report;
- Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law;
- Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for official controls on products of animal origin intended for human consumption (as amended by Commission Delegated Regulation (EU) 2025/687 of 30 January 2025 and Commission Implementing Regulation



(EU) 2025/2241 of 6 November 2025). Based on EFSA scientific opinions, *Toxoplasma gondii* has been identified as a priority biological hazard in meat inspection of cattle, small ruminants, pigs, and game meat;

- Regulation (EC) No. 178/2002 of 28 January 2002 laying down the general principles and requirements of food law (General Food Law);
- Regulation (EC) No. 853/2004 of 29 April 2004 laying down specific hygiene rules for food of animal origin;
- Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast), which requires drinking water to be free from microorganisms and parasites posing a risk to human health and introduces a strengthened risk-based approach to water supply monitoring;
- Law of Ukraine No. 1206-IX "On Veterinary Medicine and Animal Welfare";
- Law of Ukraine No. 771/97-VR "On the Basic Principles and Requirements for Food Safety and Quality";
- the joint FAO/WHO publication *Multicriteria-based Ranking for Risk Management of Food-borne Parasites*, which identifies *Toxoplasma gondii* as one of the priority food-borne parasites of global importance;
- ECDC/EFSA Joint Report: The European Union One Health 2024 Zoonoses Report.

The implementation of the SMS-RO-UA Project contributes to achieving the objectives of the EU Strategy for the Danube Region (EUSDR) in the fields of environmental protection and public health. Since toxoplasmosis is not subject to official registration in Ukraine, this Protocol represents one of the first steps towards establishing systematic monitoring of this disease within the livestock production and food supply chains of the region in accordance with the approaches adopted in the European Union.

RELEVANCE

Toxoplasma gondii infects approximately two billion people worldwide. Although only a small proportion of infected individuals develop severe disease, the widespread distribution of this parasite makes it one of the most important zoonotic pathogens globally. Toxoplasmosis is caused by the intracellular apicomplexan protozoan *Toxoplasma gondii* (phylum *Apicomplexa*, family *Sarcocystidae*).

There is substantial evidence that *Toxoplasma gondii* can be transmitted through contaminated drinking water, inadequate sanitation, and poor hygiene practices. It is recognised as a significant waterborne zoonotic pathogen, and numerous outbreaks of waterborne toxoplasmosis have been documented.

Domestic cats and other felids are the definitive hosts of *Toxoplasma gondii*. Under natural conditions, they become infected by preying on infected rodents and birds.

Toxoplasma gondii is transmitted mainly by two routes: the faecal–oral route through the consumption of food or water contaminated with oocysts, and the ingestion of tissue cysts present in raw or insufficiently cooked meat from infected animals. Non-oral routes of transmission are also possible, including vertical transmission from mother to child, organ transplantation, and blood transfusion.

Toxoplasmosis is usually asymptomatic in humans. When acute disease develops, it is generally mild and characterised by influenza-like symptoms accompanied by enlargement of the lymph nodes, liver, and spleen. However, asymptomatic infection may later result in ocular toxoplasmosis, while immunocompromised individuals are at risk of developing severe clinical manifestations. Congenital infection may lead to miscarriage, stillbirth, or fetal abnormalities. Ocular toxoplasmosis may also develop later in life, even in individuals who were asymptotically infected before birth.



Toxoplasmosis affects people of all ages and socioeconomic groups worldwide but occurs more frequently in warm and humid climates. Poor socioeconomic conditions increase the risk of infection.

The infectious dose of *Toxoplasma gondii* for humans has not been established, although it is assumed that even low numbers of oocysts or tissue cysts may be sufficient to cause infection.

Oocysts of *Toxoplasma gondii* can be detected in water using several methods, including microscopy and molecular techniques; however, no standardised detection methods are currently available. A limited number of studies have identified *Toxoplasma gondii* oocysts in wastewater, surface water, groundwater, drinking water, seawater, and on agricultural crops. Oocysts are capable of remaining viable in water for prolonged periods.

Since felids are the definitive hosts of *Toxoplasma gondii*, effective management of microbial risks associated with this parasite requires systematic approaches aimed at identifying and controlling contamination with feline faeces throughout sanitation systems and drinking water supply chains.

GENERAL CHARACTERISTICS OF THE PATHOGEN

Toxoplasma gondii is a protozoan parasite belonging to the class *Coccidia*. It exists in three infectious forms capable of infecting humans: the oocyst, the tissue cyst, and the tachyzoite. Toxoplasmosis has a complex epidemiology because the parasite is capable of infecting almost all warm-blooded animals and has a two-host life cycle [1].

Domestic cats and other felids are the definitive hosts. All non-felid animals, including dogs and humans, serve as intermediate hosts; however, *Toxoplasma gondii* is also capable of asexual reproduction in members of the family *Felidae*, which may therefore also act as intermediate hosts [2].



The life cycle of the parasite includes three developmental stages—tachyzoites, bradyzoites, and sporozoites—which determine its biological cycle [3].

In intermediate hosts, *T. gondii* undergoes two phases of asexual development. During the first phase, tachyzoites (endozoites) multiply rapidly through repeated endodyogeny (internal budding) within many different host cell types. The last generation of tachyzoites initiates the second developmental phase, resulting in the formation of tissue cysts. Within these tissue cysts, bradyzoites (cystozoites) multiply slowly by endodyogeny [4–6].

Tissue cysts show a marked affinity for nervous and muscular tissues. They are found predominantly in the central nervous system (CNS), the eyes, and the skeletal and cardiac muscles. To a lesser extent, they may also be detected in visceral organs, including the lungs, liver, and kidneys. Tissue cysts may persist throughout the lifetime of the host. Both tissue cysts and the oocysts shed by cats are potentially infectious when ingested by mammals (including humans) and birds [7].

IMPACT ON HUMAN HEALTH

Toxoplasmosis in humans is generally asymptomatic. In a small proportion of cases (approximately 10%), influenza-like symptoms together with enlargement of the lymph nodes, liver, and spleen develop 5–23 days after ingestion of tissue cysts or oocysts [8].

During chronic infection, dormant tissue cysts remain in the host's body. Immunocompetent individuals are at risk of developing ocular toxoplasmosis later in life [9, 10], whereas severely immunocompromised individuals (for example, transplant recipients or patients with HIV/AIDS) are at risk of developing more severe disease. Reactivation of tissue cysts may result in disseminated infection involving the central nervous system and lungs, leading to severe neurological disorders or pneumonia that may be fatal [7].



If a woman acquires primary infection during pregnancy, the parasite is transmitted to the fetus in approximately one-third of cases. Infection during early pregnancy may result in spontaneous abortion, stillbirth, or congenital fetal abnormalities [11–14]. Congenital toxoplasmosis may cause chorioretinitis, intracranial calcifications, hydrocephalus, severe thrombocytopenia, and seizures when infection occurs during the first trimester. If infection develops later in pregnancy, congenital toxoplasmosis may remain asymptomatic at birth; however, ocular toxoplasmosis may still develop years later [12].

EPIDEMIOLOGY AND EPIZOOTOLOGY

A number of international studies have reported that the prevalence of toxoplasmosis-positive cats ranges from 9% to 46% in European countries, South America, and the United States, while in Asia it ranges from 6% to 9% [15–20]. Up to 80% of urban cats have antibodies against *Toxoplasma gondii* [11]. Cats with acute toxoplasmosis shed oocysts in their faeces, contributing to environmental contamination and the infection of other animals and humans. For example, J. P. Dubey reported that coprological examinations identified oocyst shedding in 7–11% of cats [20].

According to J. Sroka et al. [21], in eastern Poland 66.9% of examined animals were seropositive for *Toxoplasma gondii*. The highest prevalence was recorded in cats (75%) and dogs (53.6%). Lower prevalence rates were reported in cattle (33.8%), pigs (17.9%), chickens (33.5%), and ducks (21.2%).

There is currently no official registration of toxoplasmosis in Ukraine. However, available studies indicate that the prevalence of *Toxoplasma gondii* infection in cattle from farms located in the Lviv, Khmelnytskyi, Zhytomyr, and Kyiv regions was 10.3%, while in sheep from farms in the Lviv, Zhytomyr, Kyiv, Odesa, Poltava, and Cherkasy regions, as well as the Autonomous Republic of Crimea, the prevalence reached 38.5%.



ROUTES OF INFECTION (TRANSMISSION)

Cats become infected by ingesting sporulated oocysts or meat containing tissue cysts; however, no more than 50% of infected cats shed oocysts. On average, oocyst shedding begins approximately 20 days after infection, or about 30 days after ingestion of meat containing tissue cysts.

Sporulated oocysts may contaminate hands, footwear, or clothing through contact with soil during gardening, agricultural work, or landscaping. Dogs may carry oocysts on their fur after walks, particularly if they roll on grass, soil, or occasionally in the faeces of other animals.

Herbivorous animals and birds are primarily infected by ingesting sporulated oocysts, which remain viable in the environment for long periods.

Toxoplasma gondii is transmitted through the consumption of raw or undercooked meat containing tissue cysts with bradyzoites [22]. Infection may also occur through ingestion of oocysts present in food, untreated or inadequately treated drinking water, or recreational surface waters [23, 24].

In addition, infection may result from the consumption of contaminated vegetables, fruits, and raw or insufficiently cooked shellfish [25, 26]. Contact with contaminated soil, for example during gardening or while children play in sandboxes, also represents a potential route of exposure [7]. Furthermore, several studies have identified cat ownership as a risk factor for *Toxoplasma gondii* infection [25].

Humans are intermediate hosts and do not shed oocysts; therefore, they are not considered a source of infection for others. Nevertheless, human-to-human transmission may occur through vertical transmission, resulting in congenital toxoplasmosis [27–30], as well as through organ transplantation or blood transfusion [31, 32].

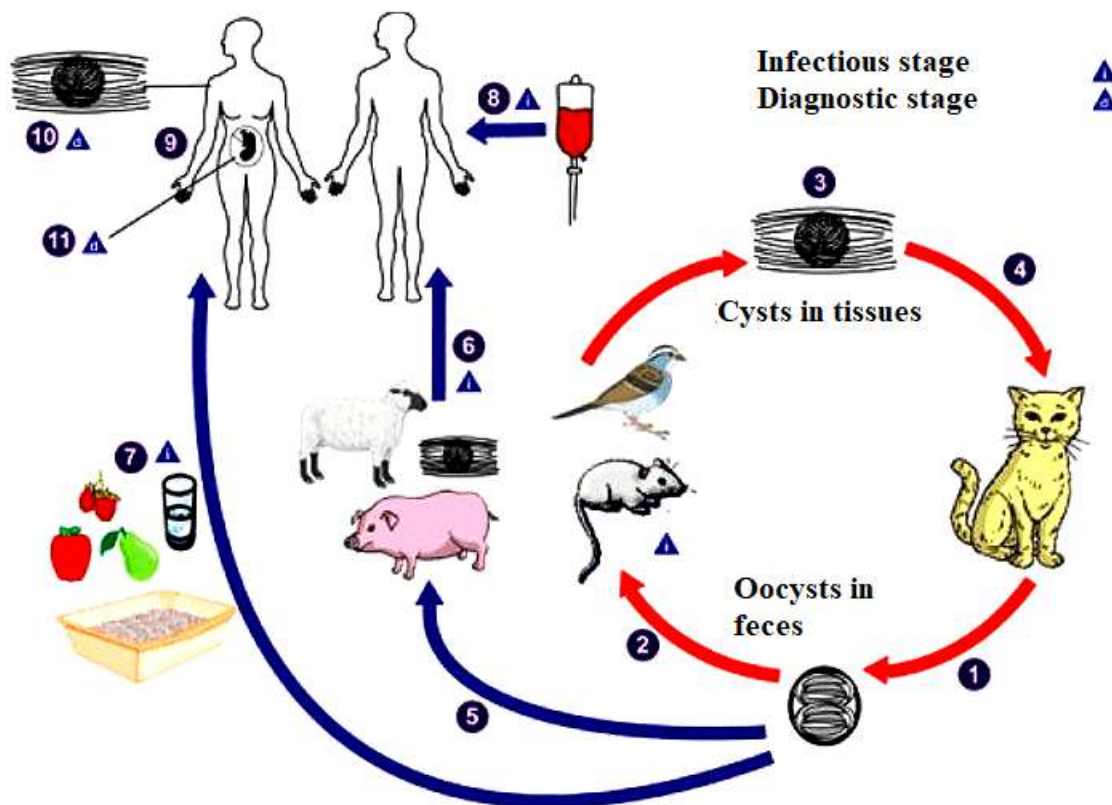


Figure 1. Life cycle of *Toxoplasma gondii*

IDENTIFICATION OF THE SOURCE OF TOXOPLASMA GONDII INFECTION

Diagnosis in Food of Animal Origin. Foodborne transmission is one of the major routes of *Toxoplasma gondii* infection, and consumption of raw or insufficiently cooked meat is recognized as a significant risk factor [33]. Although organizations such as the Food Standards Agency and the European Food Safety Authority have emphasized the importance of determining the role of different meat products in foodborne toxoplasmosis,

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relatively few studies have investigated the presence of *Toxoplasma gondii* in commercially available meat products [34, 35].

Consumption of raw or undercooked game meat has been identified as the source of *Toxoplasma gondii* infection in a number of toxoplasmosis cases [106–108]. More recently, an outbreak of acute toxoplasmosis occurred among a group of Canadian deer hunters who consumed undercooked venison steaks during a hunting trip in the United States [36].

As reported previously [37–40], the diagnosis of toxoplasmosis in animals and humans is established based on epizootiology, epidemiological, clinical, pathological, and laboratory findings, including serological, biological, microscopic, coprological, and other diagnostic methods.

In Switzerland, S. B. Glor et al. [41] evaluated a new commercial enzyme-linked immunosorbent assay (ELISA) kit for detecting antibodies against *Toxoplasma gondii* in serum, plasma, and muscle trypsinates. The assay was compared with the indirect fluorescent antibody test, the indirect agglutination test, and real-time polymerase chain reaction (PCR). The commercial ELISA kit proved to be an effective diagnostic tool for detecting toxoplasmosis in sheep both on farms and at slaughterhouses [41].

Although direct detection of the parasite by microscopy and bioassay remains the gold standard for confirming toxoplasmosis, clinical diagnosis is most commonly based on serological methods. Various serological assays have been developed to detect *Toxoplasma gondii*-specific antibodies or circulating antigens.

The enzyme-linked immunosorbent assay (ELISA) is now widely used in analytical biochemistry. It was first described by Engvall and Perlmann at Stockholm University, Sweden, in 1971 as a method for detecting the presence of a ligand in a liquid sample using antibodies. Today, ELISA is extensively applied in medicine, plant pathology, and biotechnology.

In its simplest form, ELISA involves immobilising antigens from the test sample on a solid surface. A specific antibody capable of binding to the antigen is then added. This antibody is conjugated to an enzyme, after which any unbound antibodies are



removed. Finally, a substrate containing the enzyme-specific reagent is added. If antigen–antibody binding has occurred, the resulting reaction produces a detectable signal, most commonly a color change.

Principle of the method. The assay is based on the interaction between at least one antibody and its corresponding antigen. The sample containing the antigen is immobilised on a solid support (most commonly polystyrene microplates), either non-specifically by adsorption onto the surface or specifically by capture with another specific antibody (sandwich format). After immobilization, an antibody is added to form an antigen–antibody complex. The antibody may either be covalently linked to an enzyme or detected using a secondary antibody. After each stage of the assay, the microplate is washed with special buffer solutions to remove proteins or antibodies that have bound non-specifically. Following the final washing step, an enzyme substrate is added to visualize the result. The intensity of the color produced is proportional to the amount of antigen present in the sample. Quantitative measurement is performed spectrophotometrically by determining the transmission of light of a specific wavelength through the liquid sample.

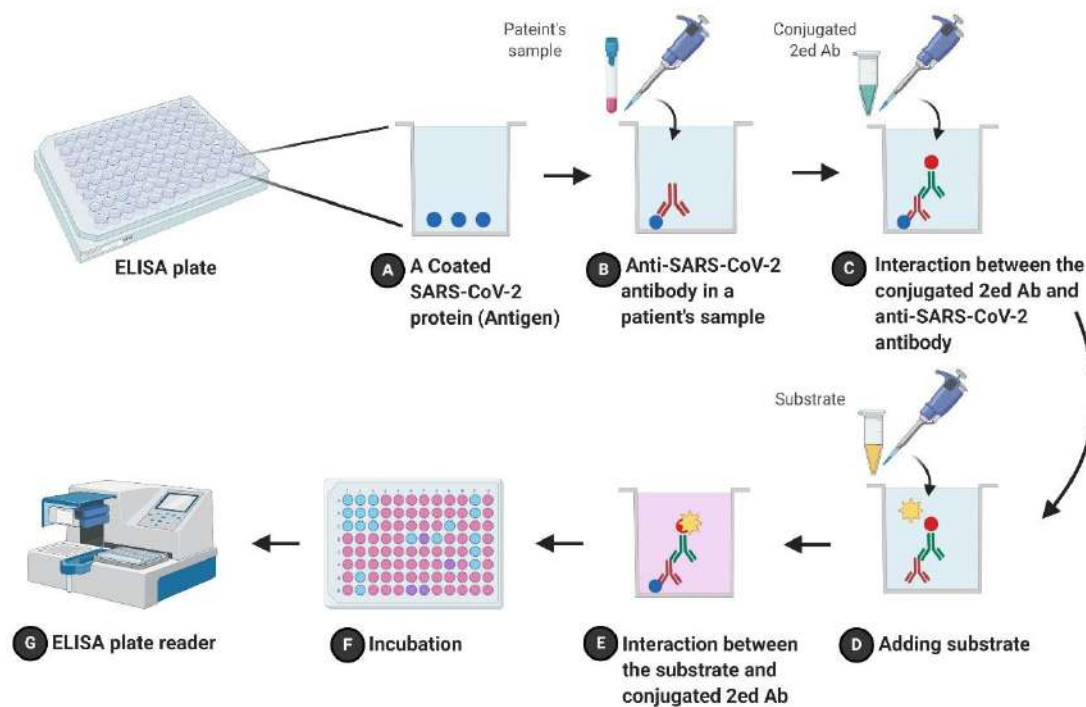


Figure 2. General scheme of the enzyme-linked immunosorbent assay (ELISA)

[43]

Several types of ELISA are available; however, the principal formats are the direct ELISA, sandwich ELISA, and competitive ELISA.

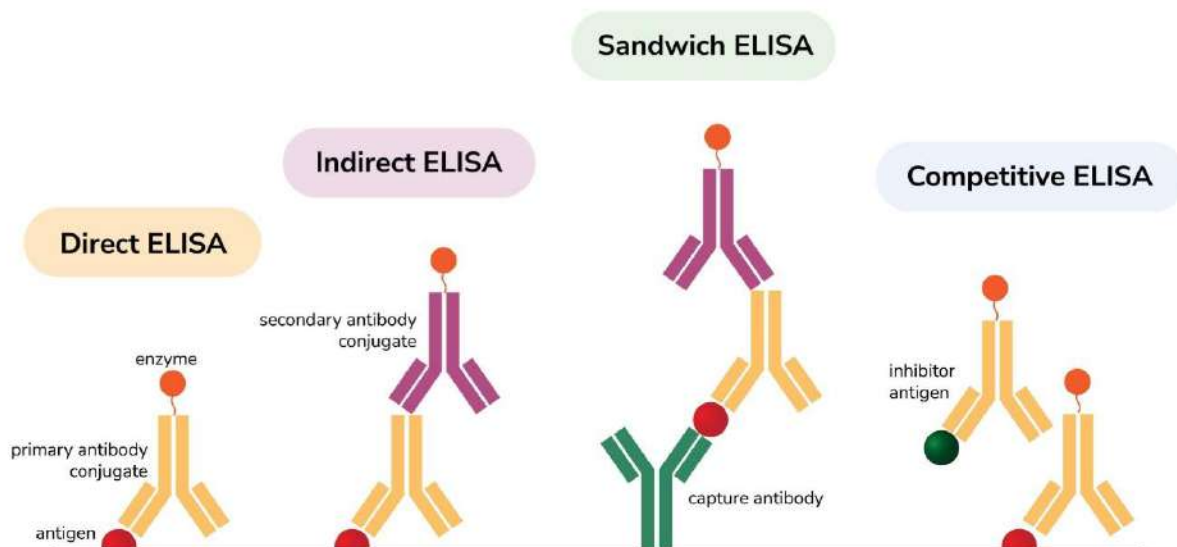


Figure 3. Main types of enzyme-linked immunosorbent assay (ELISA)

The wide variety of ELISA modifications mainly results from different combinations of antigen–antibody interactions and from which immunoreagent is immobilised on the solid phase.

Post-mortem (Post-slaughter) Diagnosis of Toxoplasmosis in Animals. Since meat from cattle and small ruminants is commonly used as food for companion animals, it should also be regarded as a potential source of toxoplasmosis infection for various animal species and humans.

The method for post-mortem (post-slaughter) diagnosis of toxoplasmosis in animals is based on detecting antibodies against *Toxoplasma gondii* in the supernatant of a diaphragm homogenate. The total analysis time is approximately 120 minutes.

The use of diaphragm tissue as the test material is justified by the higher concentration of antibodies against *Toxoplasma gondii* detected in this tissue, as confirmed by laboratory investigations (Table 1). Muscle samples from the diaphragm,

back, and thigh, together with tissues from the lungs, kidneys, and liver, were collected from animals with confirmed toxoplasmosis.

The highest antibody titres were detected in the diaphragm muscles.

The proposed method enables rapid and highly accurate post-mortem diagnosis of toxoplasmosis, reduces the consumption of materials and reagents required for testing, and contributes to the prevention of human infection with *Toxoplasma gondii* through contaminated meat and meat products.

Table 1. Antibody titres (IgM, IgG) in different tissues and organs of animals infected with toxoplasmosis

Animal species	Object of research					
	Muscles			tissues		
	Diaphragm	Back	Thigh	Lungs	Kidneys	Liver
Cattle	51.0	45.3	51.8	10.8	19.7	17.6
Sheep	44.7	39.1	43.6	17.4	15.0	10.3

Note. Interpretation of results (%): ≤ 40 – negative; 40–50 – doubtful; ≥ 50 – positive.

The method for post-mortem (post-slaughter) diagnosis of toxoplasmosis consists of sample preparation followed by enzyme-linked immunosorbent assay (ELISA).

Sample preparation. A 50 g sample of diaphragm tissue is minced using a meat grinder. The homogenate is then squeezed through four layers of gauze, and the collected liquid is centrifuged for 5 minutes at 3,000 rpm. Total antibodies against *Toxoplasma gondii* are subsequently detected in the resulting supernatant.

Assay procedure (*according to the instructions supplied with the ID Screen Toxoplasmosis Indirect Multi-species kit, IDvet*): Add 90 μ L of buffer solution into the antigen-coated microplate wells. Then add the positive and negative control samples, as well as 50 μ L of each supernatant sample.

Incubate the plate for 45 minutes at 21°C, then wash it three times, adding 300 μ L of washing solution to each well during every washing cycle. Next, add 100 μ L of the

prepared conjugate to each well and incubate for 30 minutes at the same temperature. Wash the plate again using the same procedure, then add 100 μ L of substrate solution followed by the stop solution to each well. Read the results using a spectrophotometer at a wavelength of 450 nm.

Coprological Examination of Cat Faeces by the Centrifugal Flotation Method. A 33% zinc sulfate (ZnSO_4) solution is used as the flotation medium (330 g ZnSO_4 dissolved in 1,000 mL H_2O ; solution density 1.18–1.20). A fresh faecal sample weighing 5 g is mixed with a small volume of the ZnSO_4 flotation solution in a paper or plastic cup until a homogeneous suspension is obtained using a wooden stick or spatula.

The resulting suspension is filtered through a double layer of gauze or a nylon sieve (mesh size approximately 1 mm). The filtrate is transferred into a 15 mL centrifuge tube, which is then filled with the flotation solution until a reverse meniscus is formed and covered with a coverslip.

The sample is centrifuged in a bench centrifuge for approximately 5 minutes at 2,500 rpm. After centrifugation, the coverslip is carefully transferred onto a microscope slide and examined for the presence of *Toxoplasma gondii* using light microscopy at 400 $\times$ magnification. In positive samples, small spherical oocysts with a smooth wall and a diameter of approximately 10 μ m are observed.

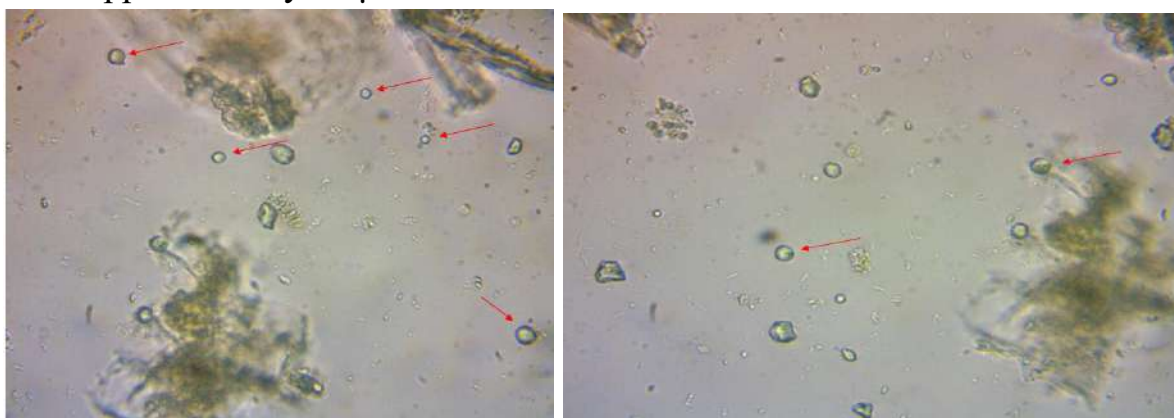


Figure 4. *Toxoplasma gondii* oocysts in a faecal smear (indicated by arrows) (400 $\times$ magnification)



It is generally accepted that cats may shed millions of oocysts during a relatively short period (2–3 weeks) following primary infection; however, only about 1% of cats in a population are capable of shedding oocysts after reinfection. Consequently, detection of oocysts in feline faeces is extremely rare [44].

Detection of Toxoplasma gondii in Water. There is substantial evidence linking the transmission of *Toxoplasma gondii* with contamination of drinking water. The first documented waterborne outbreak of toxoplasmosis associated with contamination of a municipal drinking water supply occurred in Vancouver, Canada, where a sudden increase in acute toxoplasmosis cases was observed; among the 100 identified patients, 19% subsequently developed ocular toxoplasmosis [45].

Because *Toxoplasma gondii* oocysts remain viable in the environment for extended periods and exhibit high resistance to chemical disinfectants, the absence of *Escherichia coli* (or, alternatively, thermotolerant coliform bacteria) is not considered a reliable indicator of the absence of *Toxoplasma gondii* in drinking water. Nevertheless, the presence of *E. coli* is regarded as an indicator of the potential presence of *Toxoplasma gondii*.

Sample Collection and Concentration. Large-volume water samples (10–100 L, depending on the water source) are collected for analysis. Water is passed through specialised membrane filters (for example, Envirochek filters) to retain oocysts. Material recovered from the filters is further concentrated by centrifugation and differential flotation (using sucrose or sodium nitrate solutions) to separate the parasites from heavier impurities.

DNA is extracted from the resulting sediment (concentrate) using commercial extraction kits specifically adapted for protozoan oocysts and capable of removing PCR inhibitors. The most commonly used primers target highly conserved and species-specific genomic regions, such as the B1 gene or the 529-bp repetitive element (529-bp repeat).

Because PCR detects only the presence of DNA, including DNA originating from non-viable oocysts, bioassays involving experimental infection of laboratory mice



followed by serological testing for specific antibodies are additionally employed to confirm the infectivity of the water sample.

CLINICAL MANIFESTATIONS AND BIOCHEMICAL CHANGES IN BLOOD PARAMETERS IN TOXOPLASMOSIS

A unique feature of *Toxoplasma gondii* is its ability to alter the behaviour of its host. One of the best-documented examples is the loss of the natural aversion of infected rodents to feline predators, which are the definitive hosts of *Toxoplasma gondii* [46]. Although some studies suggest that this behavioural alteration is highly specific, others have reported more general sensorimotor deficits [47].

In humans, most studies investigating the behavioural consequences of latent *Toxoplasma gondii* infection have demonstrated an association between seropositivity and an increased risk of certain neuropsychiatric disorders, including depression, self-harm-related disorders, and psychotic disorders, particularly schizophrenia [48]. Possible associations between chronic *Toxoplasma gondii* infection and subtle behavioural changes remain insufficiently studied and are currently under researches [49]. Nevertheless, it has been suggested that a range of psychomotor and cognitive functions may be altered in *Toxoplasma gondii*-seropositive animals [50].

Because of the ubiquitous distribution of *Toxoplasma gondii* and its complex epidemiology and epizootology, it is impossible to define universal control or prevention strategies that are equally effective worldwide or across all ethnic populations within a particular region. Recent multicentre case-control studies conducted in six European countries identified travel outside Europe as a significant risk factor for primary *Toxoplasma gondii* infection [51].

Pathological changes are mainly observed in chronically infected hosts and are characterised by degenerative lesions in multiple organs. *Toxoplasma gondii* exhibits a marked tropism for muscular and nervous tissues; therefore, the parenchymal organs,



skeletal muscles, brain, myocardium, and endocrine glands are most frequently affected [44].

Following oral infection, the ileum generally serves as the primary site of parasite entry. This is evidenced by a pronounced reaction of the mesenteric lymph nodes, where hyperplasia with the presence of multinucleated giant cells is observed.

Typical muscular lesions, particularly in the myocardium and, less frequently, in the skeletal muscles of other organs, consist of areas of productive interstitial myositis. Focal muscle necrosis is occasionally detected [52]. At sites where parasites are present in affected organs, small necrotic foci together with extensive inflammatory infiltrates composed of lymphocytes, histiocytes, plasma cells, neutrophils, and eosinophils are commonly observed. Necrotic lesions also develop within nervous tissue.

Clinical toxoplasmosis is considerably more common in cats than in dogs [44, 33], the latter being affected predominantly by neosporosis [53]. A substantial proportion of clinical *Toxoplasma gondii* infections develops in association with immunosuppressive chemotherapy [37]. Toxoplasmosis is also frequently accompanied by concurrent infections that aggravate clinical manifestations and prolong the course of the disease [54, 55].

Congenital toxoplasmosis is characterised by underdevelopment of the cerebral hemispheres. Damage to the ependyma, the formation of adhesions within the cerebral ventricles, and obliteration of the subarachnoid spaces result in hydrocephalus. During late fetopathy, foci of necrosis and calcification develop in the cerebral cortex, subcortical regions, and brainstem.

Acquired toxoplasmosis is characterised by productive inflammation of the brain and its meninges, manifesting as meningoencephalitis, meningoradiculitis, and paralysis of the limbs. Since the retina is composed of nervous tissue, necrobiosis with granuloma formation frequently develops in the vascular layer of the eye, resulting in productive necrotising endophthalmitis, chorioretinitis, and uveitis, which may ultimately lead to blindness [55].



The persistence of *Toxoplasma gondii* within the central nervous system and ocular tissues despite elimination of the parasite from other organs is explained by the slow diffusion of antibodies across the blood–brain barrier. This creates favourable conditions for parasite survival and cyst formation within the brain and ocular tissues [56].

Differentially expressed parasite genes involved in metabolic pathways exhibit marked alterations during the early stages of infection. These findings indicate a close relationship between gene expression in porcine kidney (PK-15) cells and their interaction with *Toxoplasma gondii* [57].

It has also been demonstrated that *Toxoplasma gondii* intensively utilises host cholesterol to maintain its survival within the host organism [58].

During replication of *Toxoplasma gondii*, several pathological changes have been observed in the liver, including hepatomegaly, granuloma formation, hepatitis with small necrotic foci, and inflammatory granulomas [59]. Histopathological examination revealed multifocal hepatocellular necrosis, extensive haemorrhage, and accumulation of fibrin, chromatin, and macrophages. Some hepatocytes contained several elongated basophilic bodies measuring approximately 3–4 µm in diameter, morphologically consistent with protozoan parasites [60].

Severe hepatic lesions may develop in both chronic and acute forms of toxoplasmosis, particularly in prolonged recurrent disease. Toxoplasmic hepatitis has no specific clinical signs and closely resembles viral hepatitis. Biliary dysfunction may also occur, leading to gallstone formation. The most commonly described lesion is interstitial hepatitis with small necrotic foci and marked portal inflammatory infiltration, which may subsequently progress to fibrosis [61].

Encephalomyelitis may be accompanied by neurological disorders including seizures, cranial nerve deficits, tremors, ataxia, paresis, and paralysis [62]. Paraparesis and tetraparesis progressing to lower motor neuron paralysis and spinal cord lesions have been reported in dogs with concurrent infection caused by *Sarcocystis neurona* and *Toxoplasma gondii* [54].



Cutaneous toxoplasmosis should be included in the differential diagnosis of skin lesions in dogs, particularly in immunocompromised animals [7]. Studies aimed at identifying possible associations between specific *Toxoplasma gondii* strains and particular clinical manifestations are also of considerable interest [63].

Thus, toxoplasmosis remains an important issue for both veterinary and medical fundamental and applied research in Ukraine and worldwide. The wide range of clinical manifestations and the specific features of disease progression, particularly in cats and dogs, highlight the need for educational and awareness-raising activities targeting both the general public and veterinary practitioners.

REDUCING THE RISK OF TOXOPLASMOSIS INFECTION IN HUMANS AND ANIMALS

To prevent the spread of toxoplasmosis, measures aimed at reducing the population of stray animals, particularly cats, are essential (Figure 5). Cats are the definitive hosts in the life cycle of *Toxoplasma gondii* and are responsible for the release of oocysts into water, soil, and feed intended for livestock, thereby contributing to the infection of animals. Meat and milk obtained from infected food-producing animals may subsequently become a source of infection for humans.

During recent decades, meat consumption habits have changed considerably, and meat is increasingly prepared using methods such as barbecue, which often do not ensure adequate heat treatment. The parasite is effectively inactivated by boiling or by freezing at -20°C for at least 72 hours.

Routine testing of food-producing animals for *Toxoplasma gondii*, both ante mortem and post mortem during veterinary-sanitary inspection of carcasses and organs, is also of great importance. For this purpose, enzyme-linked immunosorbent assay (ELISA) of diaphragm homogenates may be successfully applied.



The implementation of systematic monitoring programmes for toxoplasmosis in companion animals, stray animals, and livestock would enable timely preventive measures to be introduced and would contribute to reducing the spread of the disease.

Preventing contamination of water supplies and animal feed with faeces from definitive hosts that may contain *Toxoplasma gondii* oocysts is another essential preventive measure, as it interrupts the parasite's life cycle. Oocysts are highly resistant under environmental conditions and remain infective for up to 365 days at 4°C.

Preventing cats from entering feed storage and feed preparation areas is an effective way to reduce environmental contamination and interrupt further transmission of *Toxoplasma gondii*.

Public awareness campaigns highlighting the risks associated with toxoplasmosis should be promoted not only through veterinary institutions but also at points of sale of meat and meat products, as well as through modern communication media. Installing information boards describing the risks of toxoplasmosis near children's playgrounds and designated pet walking areas is also recommended.

Veterinary practitioners should provide owners of companion animals with professional guidance on avoiding the feeding of raw meat and preventing unrestricted outdoor access, thereby reducing the risk of infection through ingestion of oocysts during coprophagic behaviour.

Considering the widespread distribution of *Toxoplasma gondii*, it is necessary to establish, at the national level, an interagency programme involving both veterinary and public health authorities for systematic monitoring of toxoplasmosis in different animal species.

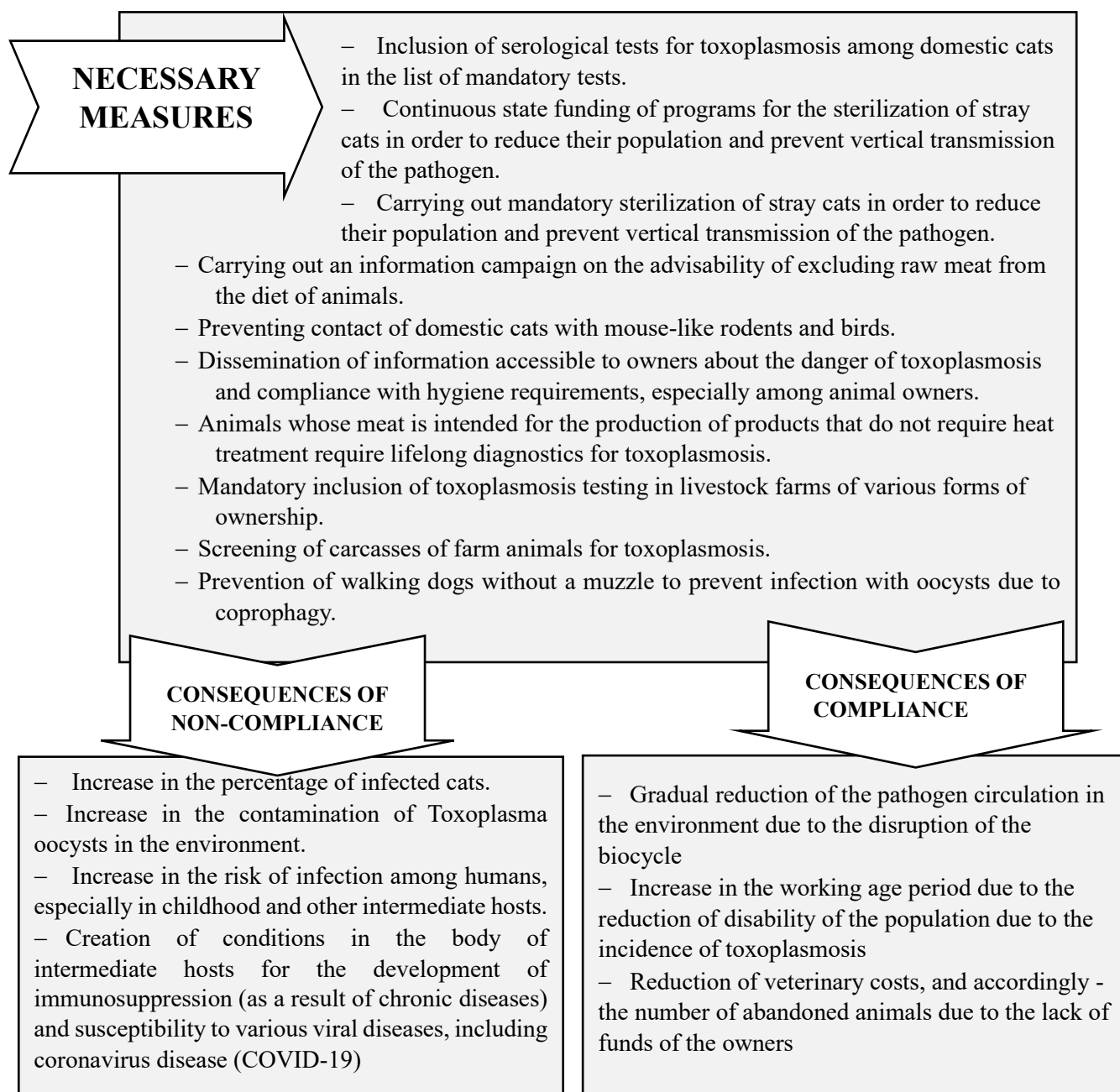


Figure 5. Scheme for reducing the risk of *Toxoplasma gondii* infection in humans and animals



Such a programme should include assessment of species-specific infection risks and the incorporation of toxoplasmosis testing into the list of mandatory examinations performed during veterinary-sanitary inspection of meat carcasses at food markets, as well as into routine antiparasitic control programmes implemented on livestock farms.

From the perspective of public health, diagnosing toxoplasmosis in food-producing animals and implementing appropriate treatment protocols are essential for eliminating one of the major sources of human infection, namely the consumption of meat from infected animals.

Therefore, the proposed toxoplasmosis prevention strategy includes several key components. First, measures should be implemented to prevent infection of animals through contaminated feed. For livestock, the principal source of infection is grain contaminated with *Toxoplasma gondii* oocysts, whereas for companion animals the main risk is the consumption of raw or inadequately heat-treated meat.

Systematic mandatory monitoring of blood serum from livestock and companion animals, together with routine coprological examinations of cats, should be introduced to prevent further spread of the parasite.

Educational programmes aimed at different population groups should also be implemented to promote safe practices regarding the consumption of meat and milk and to improve awareness among pet owners about proper hygiene and animal husbandry.

Effective management of microbial risks, including those associated with *Toxoplasma gondii*, requires systematic approaches for detecting and controlling faecal contamination throughout sanitation systems and drinking water supply chains.

PREVENTION OF HUMAN TOXOPLASMOSIS



- Wear protective gloves when gardening or whenever handling soil or sand that may be contaminated with cat faeces containing *Toxoplasma gondii*. Wash hands thoroughly with soap and water after gardening or any contact with soil or sand.
- Clean the cat litter box daily. *Toxoplasma gondii* becomes infective only 1–5 days after being shed in cat faeces. Daily removal of faeces prevents oocysts from becoming infective. Outdoor sandboxes should be kept covered.
- Have domestic cats regularly examined for toxoplasmosis at a veterinary clinic.
- Prevent cats from hunting mice and rats, as these are major sources of infection.
- Feed cats only commercially prepared canned or dry food, or thoroughly cooked household food. Cats should not be fed raw or undercooked meat.
- Avoid consuming raw or undercooked oysters, mussels, and other shellfish, as they may be contaminated with *Toxoplasma gondii* originating from seawater.
- Oocysts may be disseminated by coprophagous insects capable of contaminating vegetables, animal feed, and other foodstuffs. Flies may also transfer oocysts to feed and drinking water. Proper hand hygiene and effective protection of feed against fly contamination are important preventive measures.
- Wash fruits and vegetables thoroughly under running water before consumption.
- Do not consume unpasteurised milk and avoid drinking untreated water.
- Individuals with immunodeficiency (including patients with HIV infection or those undergoing chemotherapy) should avoid all potential sources of *Toxoplasma gondii*. They should refrain from consuming raw meat or unpasteurised milk and avoid contact with stray cats.
- Dogs should preferably be walked while wearing a muzzle to prevent oral contact with soil that may be contaminated with cat faeces.

CONTROL MEASURES



Where toxoplasmosis is suspected, the following measures should be implemented on the holding before laboratory confirmation is obtained:

- isolate animals suspected of infection;
- submit stillborn offspring and aborted fetuses to a laboratory immediately for diagnostic examination;
- place all animals suspected of toxoplasmosis under veterinary surveillance and perform serological testing of blood serum once a month for six consecutive months.

Following confirmation of toxoplasmosis on the holding:

- animals with confirmed toxoplasmosis should be slaughtered. Meat obtained from such animals may be used only after heat treatment (internal temperature of at least +66°C) or deep freezing (internal temperature of –18°C). Hides obtained from slaughtered diseased or suspected animals may be released without restriction after preservation;
- premises where infected animals were kept should be disinfected using appropriate disinfectant solutions;
- personnel handling acutely ill animals, pathological material, or carcasses suspected of toxoplasmosis must strictly comply with biosafety requirements, including the use of protective clothing, face masks, rubber gloves, and protective footwear;
- after slaughter of infected animals and isolation of animals suspected of toxoplasmosis, the movement, sale, and use of other animals and all livestock products from the affected holding may continue without restriction;
- newly introduced animals should be quarantined for 30 days, and serological testing for toxoplasmosis should be performed when indicated;
- animals suspected of infection should remain isolated. Veterinary observation should be maintained, and serological examination of blood serum should be performed once monthly for six consecutive months. If, during this period, no infected animals are detected, serological reactions become negative, or antibody titres decrease to 1:5–1:10, such animals may be transferred to the healthy group;
- clinically healthy animals on holdings affected by toxoplasmosis should undergo serological examination once a month until the holding is declared free of the disease. If



clinical signs develop together with positive serological reactions in two consecutive tests and increasing antibody titres are observed, the animals should be transferred to the suspected group. In cases of mortality, stillbirths, abortions, or other pregnancy disorders, appropriate samples should be submitted to a laboratory;

- milk obtained from infected animals may be used only after pasteurisation or boiling;
- in holdings affected by toxoplasmosis, following the slaughter of infected animals and isolation of suspected animals, the sale and movement of other animals and the use of all livestock products may continue without restriction;
- a holding in which toxoplasmosis has been confirmed shall be considered free of the disease if, for one year, no infected animals are identified and no pronounced clinical signs of toxoplasmosis, including reproductive disorders or pregnancy-related pathology, are observed.

CONCLUSIONS AND OUTLOOK

1. Toxoplasmosis remains a "silent" disease within Ukraine's official surveillance system. Unlike leptospirosis or rabies, Ukraine currently has neither an effective national guideline nor an official monitoring programme for animal toxoplasmosis, and the disease is not subject to mandatory notification. This creates a substantial gap between the actual epidemiological situation and the existing state surveillance system.

2. Foodborne transmission through meat represents the principal priority for control in accordance with European Union approaches. Scientific opinions issued by EFSA consistently identify *Toxoplasma gondii* as one of the priority biological hazards in meat inspection of cattle, small ruminants, pigs, and game animals.

3. The cat population is the key risk factor. The high seroprevalence among both stray and owned cats results in continuous shedding of oocysts into the environment, contaminating soil, water, and animal feed. Without effective control of the stray cat population, interruption of the transmission cycle is impossible.



4. Waterborne transmission represents an underestimated risk for public water supply systems. The Danube Delta, which serves as a partial source of drinking water for the region, provides environmental conditions in which *T. gondii* oocysts may remain viable for prolonged periods. Conventional microbiological indicators, such as *Escherichia coli*, are not reliable markers of the absence of *T. gondii* in water, which is consistent with the provisions of Directive (EU) 2020/2184, emphasising the need for direct assessment of parasitic contamination.

5. Post-mortem diagnosis using diaphragm homogenate is a practical method for implementation at slaughterhouses. Detection of antibodies in diaphragm homogenate supernatants by ELISA (analysis time approximately 120 minutes) is economically feasible and can be incorporated into routine veterinary inspection of carcasses without substantial additional costs.

Future development of toxoplasmosis surveillance in the cross-border region should focus on integration into the European Union risk-based food chain control system and implementation of the One Health approach.

1. Inclusion of *T. gondii* in risk-based meat inspection programmes (Food Chain Information). In accordance with Commission Implementing Regulation (EU) 2019/627 (2025 edition), modern meat inspection within the European Union is gradually shifting from traditional palpation and incision towards epidemiologically based assessment using Food Chain Information (FCI). Post-mortem diagnosis based on diaphragm homogenate represents a practical tool for generating such epidemiological information and may become the first step towards introducing this approach at slaughterhouses throughout the region.

2. Integration of *T. gondii* surveillance into the national zoonoses reporting system. Harmonisation with Directive 2003/99/EC requires Ukraine, as a candidate country for EU membership, to gradually establish a zoonoses reporting system comparable to the EFSA/ECDC European Union One Health Zoonoses Report. Data generated within the **SMS-RO-UA Project** may serve as the initial contribution to a national toxoplasmosis database.

3. Inclusion of parasitological monitoring in drinking water quality programmes. In accordance with Directive (EU) 2020/2184, risk-based water safety management requires assessment of specific parasitic hazards relevant to each water abstraction source. For water supply systems dependent on surface waters of the Danube Delta, *T. gondii* should be included among the hazards subject to periodic risk assessment.

4. Development of a regional stray cat population management programme in cooperation with veterinary authorities. Population control and health management of stray cats, including sterilisation and registration programmes, represent one of the most effective long-term preventive measures.

5. Expansion of scientific cooperation on food-borne parasitic diseases within the WHO–FAO–WOAH framework. Joint FAO/WHO recommendations on the prioritisation of food-borne parasites identify *T. gondii* as a pathogen of global importance requiring the development of new, sensitive, and cost-effective diagnostic methods.

6. Strengthening educational activities at the interface of veterinary and human medicine. Considering that toxoplasmosis is asymptomatic in most individuals but may have severe consequences for pregnant women and immunocompromised patients, awareness campaigns should target not only animal owners but also maternal and child healthcare services, including antenatal care clinics, in accordance with the intersectoral One Health concept.

Recommendations for Future Activities

- Expand toxoplasmosis surveillance to include representative numbers of both stray and owned cats.
- Introduce pilot implementation of ELISA testing using diaphragm homogenates to evaluate the practical applicability of the method under official veterinary control.
- Include *T. gondii* in drinking water monitoring programmes by collecting samples from water abstraction points supplying centralised water systems in border communities.

- Develop educational materials for antenatal clinics and primary healthcare facilities on the prevention of toxoplasmosis during pregnancy, harmonised with veterinary recommendations for cat owners.
- Prepare proposals for incorporating toxoplasmosis surveillance into the national zoonoses reporting system following the model of the EFSA/ECDC European Union One Health Zoonoses Report.

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