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TOXOPLASMOSIS IN ARMENIA (1961–2025): A CONCISE UPDATE AND "ONE HEALTH" RESEARCH AGENDA

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Toxoplasma gondii remains a significant zoonotic parasite in Armenia, however, the epidemiological data continue to be heterogeneous. Although Daryani et al. recently presented a comprehensive review of the Armenian scientific literature up to 2023, the present work is considered both an update to that review and a strategic agenda for future research. Readers interested in detailed descriptions of individual studies and analyses of seroprevalence prior to 2023 are encouraged to consult the aforementioned publication. Accordingly, this article provides a concise chronological overview (1961–2023), emphasizes a synthesized analysis of data generated after 2023, and further expands on prospective priorities within the One Health framework that are relevant to the current capacity of epidemiological surveillance in Armenia.

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Introduction. *Toxoplasma gondii* (*T. gondii*) is a zoonotic apicomplexan parasite that can infect virtually all warm-blooded animals, including birds, mammals, and humans. The parasite has a complex life cycle involving both definitive and intermediate hosts. Members of the family Felidae (including domestic cats) are definitive hosts, and environmentally resistant oocysts can only be shed with the feces of infected felids. Within intermediate hosts (all other warm-blooded animals, including humans), the parasite undergoes only asexual development [1].

Globally, toxoplasmosis is recognized as a major public health and veterinary problem due to its high prevalence, foodborne transmission, economic losses in animal production, and severe clinical consequences in immunocompromised individuals and during congenital infection. It is estimated that approximately one-third of the global human population has been exposed to *T. gondii*, with large differences between countries. Seroprevalence shows marked global variability; for example, in highly endemic regions, such as parts of Africa, seroprevalence can reach almost 90% in certain demographic groups, whereas in some European populations it can reach 60% [2]. Prevalence varies considerably depending on

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climate, dietary habits, hygiene practices, and cultural traditions [3, 4]. The disease is usually asymptomatic, but it is dangerous for pregnant women and the fetus, as well as for immunocompromised individuals (AIDS/HIV). Infected pregnant patients and newborns may experience severe outcomes including abortion, stillbirth, miscarriage, and congenital disease [5]. Recent studies have also suggested potential links between *T. gondii* infection and mental illness [6–8].

The parasite was first described in 1908 by Charles Nicolle and Louis Manceaux in Tunisia (from a North African rodent, *Ctenodactylus gundi*) and simultaneously by Splendore in Brazil (in a rabbit). In 1909, Nicolle and Manceaux distinguished the protozoan from *Leishmania* and named it *Toxoplasma gondii*, referring to the curved “bow-like” shape (Greek: “toxon” = bow) observed in infectious stages [1, 9]. *T. gondii* has long been considered a single species in the genus *Toxoplasma*, and parasite strains were classically grouped into genetic types I, II, and III [10]. The consequences of infection may depend on parasite genotype and host species [11]. Type I isolates are uniformly lethal to outbred mice, whereas type II and III isolates are significantly less virulent [12].

Humans become infected through the consumption of undercooked meat from animals (sheep, goats, etc.) infected with *T. gondii*, as well as through ingestion of soil or water contaminated with oocysts [13, 14]. Transmission can also occur via vertical transmission (mother to fetus), organ transplantation, and blood transfusion from infected donors [15].

Clinical symptoms of *T. gondii* infection are non-specific and unreliable for diagnosis [4]. Detection has traditionally relied on microscopy in fecal, water, environmental, and tissue samples [16], but light microscopy alone is less sensitive and may be unreliable. Diagnosis often depends on bioassays and serological tests, with limitations in detection and strain differentiation [17]. Serological methods include the Dye Test, Agglutination Test, Modified Agglutination Test, Latex Agglutination Test, ELISA, and Western blot. Molecular methods (PCR, nested PCR, real-time PCR, LAMP, multiplex PCR, and PCR-RFLP) are increasingly used, each with specific advantages and limitations [18, 19]. Molecular detection is appealing due to high sensitivity and specificity [20]. In Armenia, during the Soviet period, toxoplasmosis detection relied mainly on serological methods, whereas molecular genetic approaches have been applied only in the most recent studies.

Materials and Methods.

***Chronological Milestones in Armenia (1961–2023) – Compact Overview*:**

- 1961–1963: first clinical and parasitological confirmations of congenital and ocular toxoplasmosis; early use of complement fixation and parasite isolation.
- 1969–1975: population and animal surveys in Yerevan and other regions indicate widespread exposure with marked ecological variation.
- 1981–1994: large Soviet-era livestock and occupational studies document high seropositivity in cattle, sheep and high-risk professional groups; first attempts at regional mapping.
- 1999–2013: case reports and clinical studies emphasize congenital disease, ocular toxoplasmosis and HIV-associated complications.
- 2011–2023: renewed serology in humans and livestock using modern assays and limited data on cats and near-absence of environmental monitoring.

Updates and New Evidence (2024–2025).

Molecular Detection in Wild Birds (Field Work 2013–2018, published 2024). Aghayan et al. reported the first PCR-based detection of *T. gondii* in wild birds sampled in Southern Armenia, showing that infection can be detected in migratory passerines (12%). The study observed higher positivity among long-distance migrants (16%) than among resident/short-distance migrants (2.86%) and documented several passerine species as intermediate hosts in the Armenian context. These findings support the hypothesis that migratory flyways may contribute to parasite circulation and highlight the need for genotyping to distinguish locally maintained strains from introduced lineage [21].

Molecular Detection in Small Mammals (Field Work 2018, published after 2023). PCR screening of rodents and shrews from multiple Armenian localities provided the first molecular evidence of *T. gondii* in small mammals (10.9%). Positivity varied by locality and host sex, indicating that exposure is heterogeneous at fine spatial scales. Because small mammals are key prey for felids, these data strengthen the case that sylvatic cycles can contribute to environmental oocyst contamination and potentially spill over to domestic settings [22].

Livestock Serology with Ecological Predictors (2021–2023 Datasets, Interpreted in Recent Publications). Recent ELISA-based surveys in Northern Armenia provide updated baseline seroprevalence in sheep (22.5%), cattle (10.1%) and pigs (28.6%) and enable exploratory modeling of ecological drivers. Available analyses suggest that altitude may be a predictor of infection in sheep in some datasets, whereas associations with temperature are less consistent. These results motivate standardized sampling across provinces, inclusion of farm management variables, and harmonized laboratory protocols to support comparable risk mapping [23].

*Experimental Anti-*Toxoplasma *Screening from Armenian Natural Products.* A newly submitted in vitro study from Armenia evaluated acetone, ethanol and methanol extracts of the lichen *Ramalina polymorpha* against the *T. gondii* RH strain in Vero cells, using pyrimethamine as a positive control. The work reports inhibitory activity across extracts, with the acetone extract showing the most promising selectivity profile, highlighting a potential pipeline for locally sourced lead compounds and the need for follow-up validation (e.g., parasite stage coverage, toxicity, in vivo models, and standardized reporting) [24].

Priority One Health Research and Surveillance Agenda for Armenia. Given the multi-host ecology of *T. gondii* and the importance of foodborne and environmental transmission, Armenia would benefit from a coordinated "One Health" framework that links public health, veterinary services, food safety authorities, and environmental monitoring. A practical agenda should prioritize standardized methods, comparable outputs, and clear pathways from evidence to intervention.

Environmental Contamination (Oocysts in the Environment). Initiate systematic monitoring of *T. gondii* oocysts in soil, irrigation and surface water, fresh produce, and high-risk urban sites (e.g., cat feeding areas, markets, parks, and hotspots around waste/containers). Priority should be given to validated concentration/recovery protocols followed by qPCR (and, where feasible, viability-oriented approaches), with routine reporting of sample volume/mass, recovery controls, and limits of detection. This stream would help identify seasonal patterns and "contamination corridors" relevant to produce and water reuse.

Genotyping and Source Attribution (Linking Human–Animal–Environment Transmission). Establish a national capacity (or a partner-supported pipeline) for multilocus genotyping and/or sequencing to connect infections across humans, livestock, cats, and wildlife, enabling source attribution and comparison with regional lineages. Genotyping should be prioritized for abortions in small ruminants, congenital and severe clinical cases, and representative isolates from cats and wildlife where feasible. Even partial genotyping, if standardized, would substantially improve interpretation of transmission routes and outbreak-like clusters.

Risk-factor Epidemiology (Comparable Evidence for Prevention). Future serosurveys and clinical studies should incorporate standardized questionnaires capturing key exposures: dietary habits (consumption of undercooked meat, unwashed produce), cat contact and household cat management, farm biosecurity, water source and treatment, and occupational risk (livestock handling, slaughter, gardening). Studies should report adjusted effect estimates (e.g., odds ratios with confidence intervals) using harmonized covariate definitions so results can support meta-analysis and inform targeted health messaging for vulnerable groups (pregnant women and immunocompromised patients).

Sentinel Wildlife (Sylvatic–Peri-domestic Connectivity). Continue targeted sampling of migratory birds and small mammals to understand how sylvatic cycles may seed peri-domestic transmission, especially along migratory corridors and in agro-ecological interfaces. Sentinel surveillance can be designed to answer specific questions (e.g., geographic connectivity, seasonal risk, and proximity to farms), while also supporting broader parasite biodiversity and pathogen co-infection studies.

Food-chain Interventions (from Prevalence to Risk Reduction). Prioritize evidence generation in high-consumption meats, especially sheep and goat, by measuring seroprevalence and, where possible, estimating tissue cyst burden in relevant tissues. Parallel work should evaluate realistic mitigation strategies, including consumer cooking guidance, farm rodent control, and cat management around feed storage and lambing/kidding areas. Where feasible, link farm-level practices with animal outcomes (abortions, seroconversion) to identify the most cost-effective intervention packages.

Data Integration and Open Reporting (Accelerating National Capacity). Maintain a national toxoplasmosis evidence hub that compiles published and grey literature, tracks ongoing studies, and archives standardized protocols. Making de-identified datasets, metadata (sampling design, geography, diagnostic methods), and analysis scripts available would reduce duplication, enable cross-sector comparisons, and improve Armenia's readiness for multi-institutional One Health projects and external funding opportunities.

Conclusion. Armenian toxoplasmosis research has moved from predominantly serological studies to the first molecular detections in wildlife and renewed livestock surveillance. The new wildlife data underscore the importance of integrating ecological connectivity with classic foodborne and domestic risk pathways. Coupled with the comprehensive 1961–2023 synthesis [25], the present update supports a practical One Health roadmap for Armenia focused on environmental monitoring, genotyping, and standardized risk-factor studies.

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Ռ. Հ. ԳԵՎՈՐԳՅԱՆ

ՏՈՔՍՈՊԼԱԶՄՈԶԸ ՀԱՅԱՍՏԱՆՈՒՄ (1961–2025)՝ ՀԱՄԱՌՈՏ ԹԱՐՄԱՑՈՒՄ ԵՎ ՀԵՏԱԶՈՏԱԿԱՆ ԾՐԱԳԻՐ «ՄԵԿ ԱՌՈՂՋՈՒԹՅՈՒՆ» ՀԱՅԵՑԱԿԱՐԳԻ ՇՐՋԱՆԱԿՆԵՐՈՒՄ

Toxoplasma gondii-ն շարունակում է մնալ կարևոր զոոնոզային մակաբույծ Հայաստանում, սակայն համաճարակաբանական տվյալները դեռևս բնութագրվում են անհամաչափությամբ: Չնայած նրան, որ Դարյանի և համահեղինակները վերջերս ներկայացրել են Հայաստանի վերաբերյալ գիտական գրականության համապարփակ ակնարկ մինչև 2023թ., սույն աշխատանքը դիտարկվում է և՛ որպես դրա թարմացում, և՛ որպես հետագա հետազոտությունների ռազմավարական ծրագիր: Այն ընթերցողներին, ովքեր հետաքրքրված են առանձին հետազոտությունների մանրամասներով և մինչև 2023թ.շճավարակվածության վերլուծությամբ, խորհուրդ է տրվում դիմել նշված հրապարակմանը: Համապատասխանաբար, այս հոդվածը ներկայացնում է սեղմ ժամանակագրական ակնարկ (1961–2023 թթ.), շեշտադրում է 2023թ.-ից հետո ստացված տվյալների համակցված վերլուծությունը, ինչպես նաև ընդլայնում է «Մեկ առողջություն» (One Health) հայեցակարգի հեռանկարային առաջնահերթությունների քննարկումը, որոնք համապատասխան են Հայաստանի համաճարակաբանական հսկողության ներկայիս կարողություններին:

Р. Г. ГЕВОРКЯН

ТОКСОПЛАЗМОЗ В АРМЕНИИ (1961–2025 гг.): КРАТКОЕ ОБНОВЛЕНИЕ И ПРОГРАММА ИССЛЕДОВАНИЙ В РАМКАХ КОНЦЕПЦИИ "ЕДИНОЕ ЗДОРОВЬЕ"

Toxoplasma gondii остается значимым зоонозным паразитом в Армении, однако эпидемиологические данные по-прежнему характеризуются неоднородностью. Несмотря на то, что Дарьяни и соавт. недавно представили всесторонний обзор научной литературы до 2023г. по Армении настоящая работа рассматривается как его обновление и одновременно как стратегическая программа дальнейших исследований. Читателям, заинтересованным в деталях отдельных исследований и анализе серопревалентности за период до 2023 г., рекомендуется обратиться к указанной публикации. Соответственно, данная статья представляет сжатый хронологический обзор (1961–2023 гг.), делает акцент на сводный анализ данных, полученных после 2023г., а также расширяет обсуждение перспективных приоритетов концепции «Единое здоровье» (One Health), актуальных для текущего потенциала эпидемиологического надзора в Армении.