Original Article

Seroprevalence and Environmental Determinants of *Toxoplasma gondii* Infection in Armenian Livestock: An Expanded Nationwide Analysis

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ABSTRACT

Background: Livestock exposure to *Toxoplasma gondii* is important for animal health, food safety, and One Health surveillance, but data from Armenia remain limited outside previously studied northern provinces.

Objectives: This study expanded the Gevorgyan 2026 open database by adding Armenian livestock samples and environmental covariates to estimate seroprevalence and evaluate host, geographic, and environmental determinants of exposure.

Methods: We analyzed a sample-level dataset of 3,581 livestock records, classifying those that matched the Gevorgyan (2026) open database separately from the expanded and new data. Seroprevalence was summarized overall and by dataset source, host species, and province. Logistic regression of cattle and sheep assessed associations with dataset source, host species, year, elevation, annual precipitation, and NDVI (normalized difference vegetation index).

Results: Overall, 740 samples were seropositive, giving a seroprevalence of 20.7% (95% CI, 19.4%, 22%). The open-database component had a seroprevalence of 15.6%, whereas the expanded/new data had a seroprevalence of 27%. Sheep had higher seroprevalence than cattle (33.2% vs 11.8%), and Syunik had the highest province-level prevalence (39.9%). In the selected environmental model, sheep remained strongly associated with seropositivity, the effect of the expanded/new data was no longer significant after adjustment, and elevation showed a nonlinear association.

Conclusion: Expanded sampling substantially changed the apparent livestock exposure landscape in Armenia. Sheep and high-prevalence regions, particularly Syunik, should be prioritized for future surveillance and food-safety-oriented studies.

Keywords: *Toxoplasma gondii*, Livestock, Seroprevalence, Armenia, Environmental epidemiology

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Introduction

Toxoplasma gondii is a globally distributed apicomplexan parasite with medical, veterinary, and food-safety importance. Felids are the definitive hosts and shed environmentally resistant oocysts, while a wide range of warm-blooded animals, including livestock, can become infected as intermediate hosts. Human and animal infection may occur by ingesting sporulated oocysts from contaminated soil, water, feed, or produce, or through the consumption of raw or undercooked meat containing tissue cysts (Hill & Dubey, 2013; Guo et al., 2015; Yan et al., 2016). This dual environmental and foodborne transmission pathway makes livestock surveillance relevant both for animal-health assessment and for One Health risk evaluation. Food animals differ in their exposure and epidemiological importance. Reviews of meat-animal toxoplasmosis have emphasized that infection is commonly detected in pigs, sheep, goats, poultry, and other food animals, while cattle often show lower seroprevalence than small ruminants or pigs (Guo et al., 2015; Hill & Dubey, 2013). Sheep are especially important because they are frequently exposed during grazing and because infection can have reproductive, economic, and public-health consequences. A global systematic review estimated substantial pooled seroprevalence in sheep and goats, with marked heterogeneity among regions and study designs (Ahaduzzaman & Hasan, 2022). Regional studies from Europe and Asia likewise show broad variation in sheep and ruminant seroprevalence, reflecting differences in climate, husbandry, host age, diagnostic methods, and environmental exposure (Deksne et al., 2017; García-Bocanegra et al., 2013; Gazzonis et al., 2015; Yang et al., 2024).

Environmental conditions are central to *T. gondii* transmission because oocyst survival outside the host is affected by moisture, temperature, soil conditions, and landscape context. Experimental work has shown that oocysts can persist longer under damp than dry soil conditions, and reviews have highlighted climate, water, vegetation, land use, and host ecology as drivers of the spatial distribution of *T. gondii* exposure (Lélu et al., 2012; Yan et al., 2016; Wilson et al., 2024). At the same time, environmental predictors are often interrelated, particularly in mountainous landscapes where elevation, temperature, precipitation, humidity, and vegetation covary. For this reason, ecological analyses of *T. gondii* should consider both biological plausibility and multicollinearity among environmental variables.

Armenia provides a useful setting for such analysis because its mountainous topography and contrasting climatic zones create substantial environmental heterogeneity over short distances. Until recently, however, livestock toxoplasmosis data from Armenia were limited. Gevorgyan et al. addressed this gap by reporting *T. gondii* seroprevalence in livestock from the northern Armenian provinces of Shirak, Lori, and Tavush (Gevorgyan et al., 2026). That study found an overall seroprevalence of 15.5% among cattle and sheep, higher prevalence in sheep than cattle, and an altitude-dependent pattern in sheep. These findings established an important regional baseline, but they did not cover all Armenian livestock-producing regions and therefore could not fully describe the national environmental and geographic structure of exposure.

The present study builds directly on the Gevorgyan et al. (2026) open database by integrating those records with additional livestock samples from other Armenian regions and expanded environmental covariates. The objectives were to cross-check and classify records from the previously published open database, estimate *T. gondii* seroprevalence overall and by dataset source, host species, and province, and evaluate whether environmental gradients explain the higher crude prevalence observed in the expanded dataset. By combining sample-level provenance, descriptive epidemiology, non-collinear environmental variable selection, and logistic regression modeling, this study aimed to provide a broader Armenia-wide baseline for livestock *T. gondii* exposure and to identify priorities for future surveillance.

Materials and Methods

Study design and data sources

This study was designed as an expanded, sample-level analysis of livestock *T. gondii* seroprevalence in Armenia. Samples were sourced from regional branches of the Food Safety Inspection Body of the Republic of Armenia (Ministry of Economy). This collection was facilitated through a collaborative agreement with the Scientific Center of Zoology and Hydroecology of the National Academy of Sciences of the Republic of Armenia (SCZH NAS RA) and was conducted in strict accordance with institutional ethics approval code N2459-124. No anesthesia, euthanasia, or any kind of animal sacrifice was part of the study. The dataset incorporated the open database associated with Gevorgyan et al. (2026), available as the PLOS One supporting dataset, and additional records compiled in the expanded Armenia-wide workbook. The Gevorgyan et al. (2026) open database included samples from the northern Armenian provinces of Shirak, Lori, and Tavush collected during 2021-2023 (Gevorgyan et al., 2026). The expanded workbook included these previously published

records, along with additional samples from other provinces and additional environmental covariates (Figure 1).

Records in the expanded workbook were matched to the Gevorgyan et al. (2026) open database using province, locality, host species, collection date, and nzyme-linked immunosorbent assay (ELISA) result. Because multiple animals could share identical locality/date/species/ELISA combinations, duplicate-order matching was used within each matching key to avoid over-assigning repeated records. Matched records were classified as the Gevorgyan 2026 open-database component (hereafter, open-database component), whereas all unmatched records were classified as expanded/new data. The complete sample-level dataset, including all variables used in this analysis and provenance columns identifying Gevorgyan 2026 matched records, is provided as Supplementary Table S1.

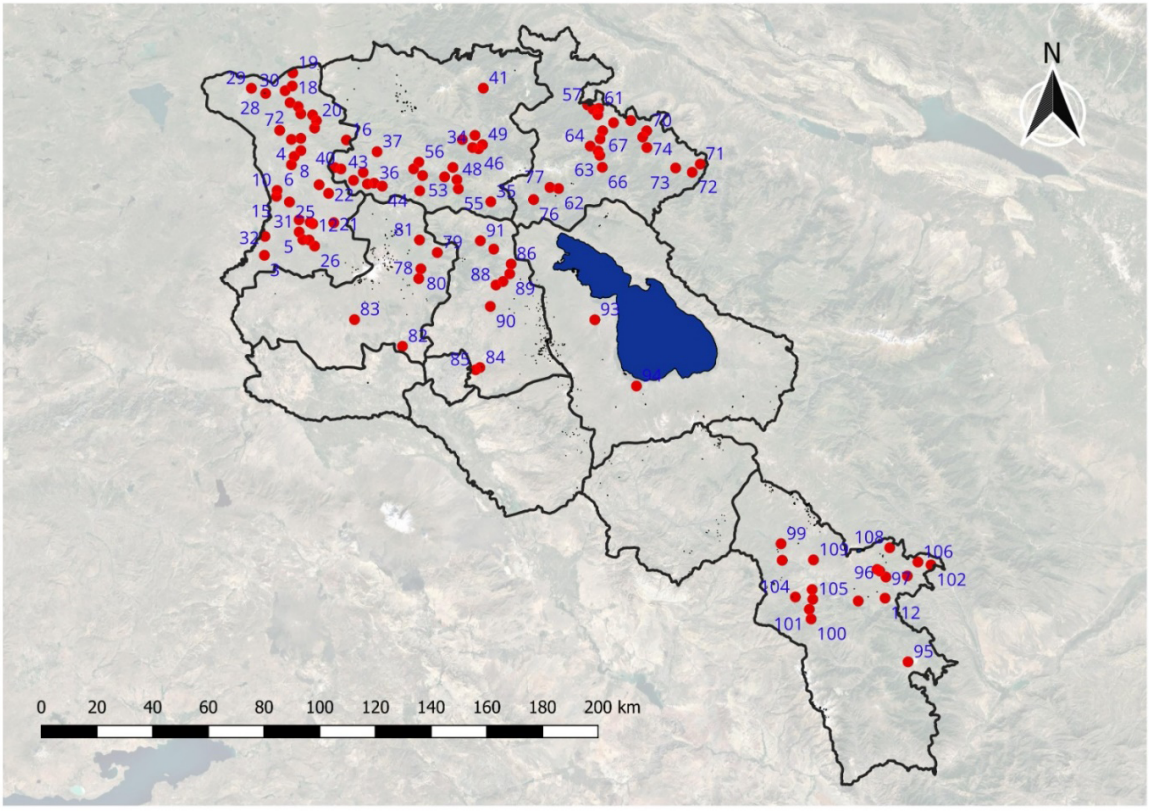


Figure 1. Geographical distribution of sampling sites:

1-33 Shirak: 1-Haykasar, 2-Mets Sepasar, 3-Sarakap, 4-Gogahovit, 5-Hayrenyats, 6-Mayisyan, 7-Amasia, 8-Keti, 9-Karmravan, 10-Voskehask, 11-Salut, 12-Nor Kyanq, 13-Azatan, 14-Torosgyugh, 15-Akhurik, 16-Dzorashen, 17-Ashotsk, 18-Sizavet, 19-Saragyugh, 20-Musayelyan, 21-Spandaryan, 22-Basen, 23-Karnut, 24-Pokrashen, 25-Vardakar, 26-Pemzashen, 27-Hartashen, 28-Ardenis, 29-Zorakert, 30-Tavshut, 31-Saratak, 32-Isahakyan, 33-Tufashen.

34-56 Lori: 34-Vahagni, 35-Margahovit, 36-Jrashen, 37-Gogaran, 38-Dzoragyugh, 39-Nor Khachakap, 40-Hartagyugh, 41-Alaverdi, 42-Tsakhkaber, 43-Katnajur, 44-Saramej, 45-Antaramut, 46-Yeghegnut, 47-Lernavan, 48-Gugark, 49-Debed, 50-Karaberd, 51-Khnkoyan, 52-Lernajur, 53-Lernapat, 54-Vanadzor, 55-Shahumyan, 56-Arjut.

57-77 Tavush: 57-Kirants, 58-Tsaghkavan, 59-Vazashen, 60-Sevkar, 61-Sarigyugh, 62-Haghartsin, 63-Ijevan, 64-Yenokavan, 65-Getahovit, 66-Gandzakar, 67-Aknaghbyur, 68-Azatamut, 69-Verin Tsaghkavan, 70-Varagavan, 71-Chinari, 72-Aygedzor, 73-Artsvaberd, 74-Chinchin, 75-Lusadzor, 76-Dilijan, 77-Teghut.

78-83 Aragatsotn: 78-Shenavan, 79-Ttu jur, 80-Kuchak, 81-Lusagyugh, 82-Oshakan, 83-Lernarot.

84-92 Kotayk: 84-Dzoraghbyur, 85-Jrvezh, 86-Vanatur, 87-Aghavnadzor, 88-Solak, 89-Hrazdan, 90-Fantan, 91-Meghradzor, 92-Kaghsi.

93-94 Gegharkunik: 93-Gavar, 94-Martuni.

95-112 Syunik: 95-Achanan, 96-Verishen, 97-Goris, 98-Khndzoresk, 99-Spandaryan, 100-Dastakert, 101-Torunik, 102-Kornidzor, 103-Shaghat, 104-Tasik, 105-Akhlatyan, 106-Tegh, 107-Tolors, 108-Vaghatur, 109-Ishkhanasar, 110-Akner, 111-Harzhis, 112-Khot.

Outcome and explanatory variables

The outcome variable was ELISA serostatus for anti-*T. gondii* antibodies, coded as negative (0) or positive (1). Host species, sample type, province, region, locality, sampling year, and geographic coordinates were retained for analysis. Text fields were cleaned by trimming extra spaces and harmonizing labels. Because goat and pig sample sizes were small, these species were included in descriptive summaries but excluded from inferential host-species and environmental logistic regression models. The laboratory processing, ELISA testing, and interpretation of serological results for the expanded/new samples followed the same procedures described by Gevorgyan et al. (2026), ensuring comparability between the previously published open-database records and the newly added records.

Collection season was derived from the available collection date for cattle and sheep records and categorized as winter (December-February), spring (March-May), summer (June-August), or autumn (September-November). Seasonal comparisons were treated as exploratory because ELISA seropositivity reflects previous exposure and may not indicate infection during the sampling season.

To examine whether regional differences were influenced by seasonal sampling, an additional sensitivity analysis compared Syunik with all other provinces before and after adjustment for collection season. These models were restricted to cattle and sheep records with parsable collection dates and were adjusted for host species, dataset source, and sampling year.

Environmental covariates included elevation, elevation zone, annual precipitation, mean daily precipitation, mean temperature, minimum temperature, maximum temperature, temperature range, mean relative humidity, slope, and normalized difference vegetation index (NDVI). Climate variables were obtained from the NASA POWER (prediction of worldwide energy resources) (NASA POWER, 2026) agroclimatology/meteorological database using the geographic coordinates and sampling year corresponding to each livestock record. Daily meteorological data were retrieved for each locality-year combination and aggregated into annual summary variables, including total annual precipitation, mean daily precipitation, mean annual temperature, minimum and maximum annual temperature, temperature range, and mean annual relative humidity (NASA POWER, 2026). Vegetation greenness was represented by annual mean NDVI derived in Google Earth Engine for the corresponding sampling year; Google Earth Engine provides a cloud-based platform for planetary-scale geospatial analysis. Elevation and slope were extracted from digital elevation/geospatial layers in QGIS and linked to each georeferenced sampling locality using QGIS Geographic Information System software (QGIS Development Team, 2024). For environmental modeling, continuous predictors were standardized before analysis; therefore, odds ratios for these variables represent the change in odds per one standard deviation increase.

Descriptive seroprevalence analysis

Descriptive seroprevalence was calculated overall and by dataset source, host species, province, sampling year, elevation zone, and sample type. Exact counts of the tested and seropositive animals were reported together with percentages and Wilson 95% confidence intervals (CIs). Differences in seropositivity between the open-database component and expanded/new data were first assessed using Pearson chi-square tests. Logistic regression was then used to estimate odds ratios for seropositivity in the expanded/new data relative to the open-database component, first unadjusted and then adjusted for host species and sampling year.

Environmental variable selection

Environmental predictors were selected before modeling using biological interpretability and pairwise correlation structure, taking into account previous evidence that climate, moisture, elevation, vegetation, and other ecological gradients can influence *T. gondii* oocyst survival and transmission (Lélu et al., 2012; Yan et al., 2016; Gevorgyan et al., 2026). Slope was excluded a priori because it was considered less directly interpretable for *T. gondii* oocyst survival and livestock exposure than climate, elevation, and vegetation measures. Candidate environmental variables included elevation, annual precipitation, mean temperature, temperature range, mean humidity, and NDVI. Strongly intercorrelated predictors were not retained together in the final multivariable model. In particular, mean humidity was strongly correlated with both annual precipitation and mean temperature; therefore, precipitation and humidity were evaluated as alternative moisture predictors rather than included together.

Environmental logistic regression modelling

Environmental modeling was restricted to cattle and sheep. Logistic regression models with a binomial error distribution and logit link were fitted to evaluate associations between environmental predictors and *T. gon-*

dii seropositivity. A baseline model containing dataset source, host species, and sampling year was compared with environmental models using Akaike information criterion (AIC) and likelihood-ratio tests. To account for the altitude-dependent pattern reported by Gevorgyan et al. (2026), elevation was included using both linear and quadratic terms. The selected non-collinear environmental model included elevation, elevation squared, annual precipitation, and NDVI, with adjustment for dataset source, host species, and sampling year. Model estimates were reported as odds ratios with 95% confidence intervals. Model-predicted probabilities were generated across the observed ranges of the selected environmental predictors to visualize fitted environmental gradients for cattle and sheep.

The primary model was specified as logit [P(seropositive)]=beta0 + dataset source + host species + sampling year + elevation_z + elevation_z squared + annual precipitation_z + NDVI_z. dataset source, host species, and year were retained for epidemiological adjustment, elevation terms were retained based on the previously reported altitude pattern, and precipitation and NDVI were retained as non-collinear environmental predictors selected for biological plausibility and model parsimony.

The selected environmental model was also refitted as a season-adjusted sensitivity model on the subset of cattle and sheep records with parsable collection dates. This model included collection season together with dataset source, host species, sampling year, elevation, elevation squared, annual precipitation, humidity, and NDVI.

As an additional clustering sensitivity analysis, the selected environmental model was evaluated using cluster-robust standard errors by province and province-locality and approximate mixed-effects logistic models with province or province-locality random intercepts; farm-level random effects could not be fitted because farm identifiers were not consistently available.

To further assess the counterintuitive inverse precipitation and NDVI associations, additional sensitivity analyses used alternative covariate specifications, province fixed effects, exclusion of Syunik, leave-one-province-out models, and species-specific cattle and sheep models.

All statistical analyses and figures were produced in R version 4.3.2 (R Core Team, 2023). Data handling and visualization used the readxl, dplyr, tidyr, stringr, janitor, ggplot2, broom, binom, forcats, purrr, scales, and readr packages.

Results

Dataset composition and descriptive seroprevalence

The expanded dataset included 3,581 livestock samples from seven Armenian provinces. Overall, 740 samples were seropositive for anti-*T. gondii* antibodies, corresponding to a seroprevalence of 20.7% (95% CI, 19.4%, 22.0%; Table 1).

Direct matching against the PLOS One supporting dataset from Gevorgyan et al. (2026) identified 1,996 records from the previously published open database. The remaining 1,585 records represented expanded/new data added in the present analysis.

Within the open-database component, 312 of 1,996 samples were seropositive (15.6%, 95% CI, 14.1%, 17.3%). When pigs were excluded, the cattle-and-sheep subset contained 308 positives among 1,982 samples (15.5%, 95% CI, 14.0%, 17.2%), matching the principal prevalence denominator reported by Gevorgyan et al. (2026). The expanded/new data showed higher seroprevalence, with 428 positives among 1,585 samples (27.0%, 95% CI, 24.9%, 29.2%). This difference was statistically significant (Pearson chi-square=69.69, df=1, $P=6.95\times10^{-17}$).

Among cattle and sheep, the expanded/new data were also associated with higher odds of seropositivity before environmental adjustment. The unadjusted odds ratio was 2.07 (95% CI, 1.75%, 2.44%, $P=6.81\times10^{-18}$), increasing to 2.34 after adjustment for host species (95% CI, 1.97%, 2.79%, $P=5.47\times10^{-22}$) and to 2.50 after additional adjustment for sampling year (95% CI, 2.09%, 2.99%, $P=1.32\times10^{-23}$).

Seroprevalence differed markedly by host species. Sheep had the highest seroprevalence, with 492 positives among 1,480 samples (33.2%, 95% CI, 30.9%, 35.7%), whereas cattle had 242 positives among 2,049 samples (11.8%, 95% CI, 10.5%, 13.3%). Pigs and goats were represented by small sample sizes and were interpreted descriptively: pigs had 5 positives among 47 samples (10.6%, 95% CI, 4.6%, 22.6%), and goats had 1 positive among 5 samples (20.0%, 95% CI, 3.6%, 62.4%). Similar host-species contrasts have been reported in multi-host livestock studies from Algeria, Egypt, Ethiopia, Malaysia, and China (Fereig et al., 2016; Tilahun et al., 2018; Abdallah et al., 2019; Sadiq et al., 2023; Yi et al., 2024).

In the host-adjusted logistic model restricted to cattle and sheep, sheep had substantially higher odds of seropositivity than cattle (OR=4.02, 95% CI, 3.37%, 4.8%, P=5.29 $\times 10^{-54}$). This association remained similar after adjustment for dataset source and year (OR=4.34, 95% CI, 3.57%, 5.28%, P=4.87$\times 10^{-49}$).

In the exploratory seasonal analysis, usable collection-season information was available for 3,161 cattle and sheep records. Cattle showed no significant seasonal difference in seroprevalence (chi-square=2.03, df=3, P=0.565), with estimates of 8.5% in winter, 11.2% in spring, 0.0% in summer (n=1), and 12.8% in autumn. Sheep showed a stronger seasonal pattern (chi-square=80.31, df=3, P<0.001), with the highest seroprevalence in spring (40.0%) compared with winter (18.2%), summer (15.7%), and autumn (17.2%). In the adjusted cattle-and-sheep model, spring collection was associated with higher odds of seropositivity relative to winter (OR 1.56, 95% CI, 1.04%, 2.34%, P=0.032), whereas summer and autumn were not significant.

Seroprevalence also varied geographically. Syunik had the highest observed prevalence, with 278 positives among 697 samples (39.9%, 95% CI, 36.3%, 43.6%). Intermediate prevalence was observed in Aragatsotn (23.8%, 95% CI, 18.5%, 30%), Tavush (23.1%, 95% CI, 20%, 26.6%), Kotayk (18.8%, 95% CI, 15.2%, 22.9%), and Shirak (16.2%, 95% CI, 13.5%, 19.3%). Lori had lower prevalence (93/975; 9.5%, 95% CI, 7.9%, 11.5%). No positive samples were detected in Gegharkunik, although the sample size was small (0/62; upper 95% confidence limit: 5.8%). Province-level estimates are presented in Table 1.

Because Syunik was sampled only in spring and autumn among records with parsable collection dates, we tested whether its higher prevalence was explained by sampling season. In Syunik, seroprevalence was 42.4% in spring (172/406) and 22.1% in autumn (43/195), compared with 20.5% and 12.4%, respectively, in other provinces. In the adjusted Syunik-versus-other-provinces model, Syunik remained associated with higher odds of

Table 1. Overall seroprevalence of anti-*T. gondii* antibodies and seroprevalence by dataset source, host species, and province

Group	Category	No.		Seroprevalence, % (95% CI)
		Tested	Positive	
Overall	All livestock	3581	740	20.7 (19.4, 22)
Dataset source	Expanded/new data	1585	428	27 (24.9, 29.2)
	Gevorgyan 2026 open database	1996	312	15.6 (14.1, 17.3)
Host species	Sheep	1480	492	33.2 (30.9, 35.7)
	Goat	5	1	20 (3.6, 62.4)
	Cattle	2049	242	11.8 (10.5, 13.3)
	Pig	47	5	10.6 (4.6, 22.6)
Province	Syunik	697	278	39.9 (36.3, 43.6)
	Aragatsotn	206	49	23.8 (18.5, 30)
	Tavush	635	147	23.1 (20, 26.6)
	Kotayk	394	74	18.8 (15.2, 22.9)
	Shirak	612	99	16.2 (13.5, 19.3)
	Lori	975	93	9.5 (7.9, 11.5)
	Gegharkunik	62	0	0 (0, 5.8)

CI: Confidence interval.

Note: Confidence intervals were calculated using the Wilson method. The open-database component was defined by direct matching to the PLOS One supporting dataset.

Table 2. Selected non-collinear environmental logistic regression model for *T. gondii* seropositivity in cattle and sheep

Predictor	Odds Ratio (95% CI)	P
Expanded/new data	1.12 (0.83, 1.53)	0.464
Sheep	4.7 (3.82, 5.79)	<0.001
Elevation, per 1 SD	1 (0.87, 1.14)	0.978
Elevation squared	1.17 (1.07, 1.28)	<0.001
Annual precipitation, per 1 SD	0.46 (0.37, 0.57)	<0.001
NDVI, per 1 SD	0.87 (0.77, 0.98)	0.027

CI: Confidence interval; NDVI: Normalized difference vegetation index.

Note: Continuous environmental predictors were standardized; odds ratios are per one standard deviation increase. The model was adjusted for dataset source, host species, and sampling year.

seropositivity after adding collection season (OR=2.52, 95% CI, 1.85%, 3.44%, P<0.001).

Together, these descriptive results showed that the expanded dataset substantially changed the epidemiological picture relative to the northern-Armenia open database, particularly through the inclusion of high-prevalence areas, such as Syunik.

Environmental associations with seropositivity

Pairwise correlations among candidate environmental variables showed strong relationships between mean humidity and mean temperature (r=-0.775) and between mean humidity and annual precipitation (r=0.744). Mean temperature was also moderately correlated with elevation (r=-0.634). To avoid including strongly intercorrelated predictors in the same model, slope and temperature variables were excluded from the main multivariable model, and precipitation and humidity were evaluated as alternative moisture predictors. The selected non-collinear model retained elevation, elevation squared, annual precipitation, and NDVI, with adjustment for dataset source, host species, and year.

Adding the selected environmental predictors improved model fit relative to the baseline model containing only dataset source, host species, and year. The baseline model had an AIC of 3270.65, whereas the selected precipitation-NDVI environmental model had an AIC of 3212.43. A likelihood-ratio test supported the addition of environmental predictors (deviance=66.22, df=4, P<0.001).

Host species remained the strongest predictor in the selected environmental model. Sheep had higher odds of seropositivity than cattle (OR=4.7, 95% CI, 3.82%, 5.79%, P<0.001). After environmental adjustment, the expanded/new data group no longer differed significantly from the open-database component (OR=1.12, 95% CI, 0.83%, 1.53%, P=0.464), indicating that the higher crude seroprevalence in the expanded data was largely explained by host, year, and environmental structure.

Among environmental predictors, the linear elevation term was not significant (OR=1.00, 95% CI, 0.87%, 1.14%, P=0.978), but the quadratic elevation term was significant (OR=1.17, 95% CI, 1.07%, 1.28%, P<0.001), supporting a non-linear altitude-related pattern similar to the altitude-dependent structure reported by Gevorgyan et al. (2026). Higher annual precipitation was associated with lower odds of seropositivity (OR=0.46, 95% CI, 0.37%, 0.57%, P<0.001), and higher NDVI was also associated with lower odds of seropositivity (OR=0.87, 95% CI, 0.77%, 0.98%, P=0.027). Model estimates are summarized in Table 2. Model-predicted probability curves for these environmental gradients are shown in Figure 2.

In the season-adjusted environmental sensitivity model, adding collection season improved model fit on the same 3,161-record subset (likelihood-ratio test P<0.001). The main environmental interpretation was retained: the quadratic elevation term remained significant (OR=1.28, 95% CI, 1.15%, 1.43%, P<0.001), annual precipitation remained negatively associated with seropositivity (OR=0.36, 95% CI, 0.22%, 0.59%, P<0.001), and NDVI was also negatively associated (OR=0.81, 95% CI, 0.7%, 0.94%, P=0.006).

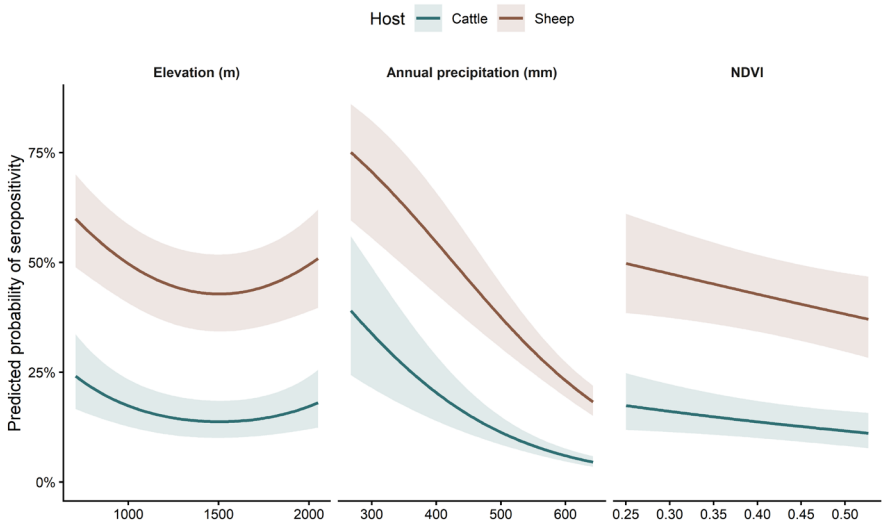


Figure 2. Model-predicted probability of *T. gondii* seropositivity across elevation, annual precipitation, and NDVI gradients in cattle and sheep.

Note: Shaded areas indicate 95% confidence intervals. Predictions were generated from the selected non-collinear environmental logistic regression model and are adjusted for dataset source and sampling year.

Geographic clustering was present in the modeling dataset, which included 3,529 cattle and sheep records distributed across 7 provinces and 112 province-locality clusters.

In clustering sensitivity analyses summarized in Supplementary Table S1, sheep remained strongly associated with seropositivity and dataset source remained non-significant, whereas some environmental terms, especially precipitation and NDVI, were less stable after locality-level clustering or approximate mixed-effects modeling.

Additional environmental sensitivity analyses showed that the inverse precipitation and NDVI associations were present in several model specifications but were not fully stable across species and province-exclusion checks; notably, both terms became non-significant when Syunik was excluded.

The model-predicted curves illustrated how the selected environmental gradients translated into estimated seropositivity risk for cattle and sheep. Across the observed elevation range, predicted seropositivity followed a shallow U-shaped pattern, consistent with the significant quadratic elevation term. Predicted seropositivity declined with increasing annual precipitation and showed a weaker decreasing trend with increasing NDVI. For all three environmental gradients, predicted probabilities were consistently higher in sheep than in cattle, reflecting the strong host-species effect observed in the regression model (Figure 2).

Discussion

Expanded dataset and descriptive seroprevalence

This study expands the epidemiological picture of *T. gondii* infection in Armenian livestock by combining the open database from Gevorgyan et al. (2026) with additional samples from other regions of Armenia and newly linked environmental variables. The overall seroprevalence in the combined dataset was 20.7%, but this aggregate value hides an important shift: the Gevorgyan 2026 open-database component showed 15.6% seropositivity, whereas the expanded/new data showed 27.0% (Figure 3). Because the previously published open dataset could be directly matched and because the expanded samples were analyzed using the same laboratory procedures as Gevorgyan et al. (2026), this contrast is unlikely to be explained simply by incompatible laboratory methods. Instead, it indicates that broadening the geographic frame changes the apparent burden of livestock exposure in Armenia.

The present estimate is higher than the 15.5% prevalence reported for cattle and sheep from northern Armenia by Gevorgyan et al. (2026), but the difference is epidemiologically informative rather than contradictory. The earlier study focused on Shirak, Lori, and Tavush, whereas the expanded dataset includes additional provinces, including high-prevalence Syunik. This supports the interpretation that the earlier northern-Armenia study provided a strong regional baseline, while the present analysis showed that national or multi-regional infer-

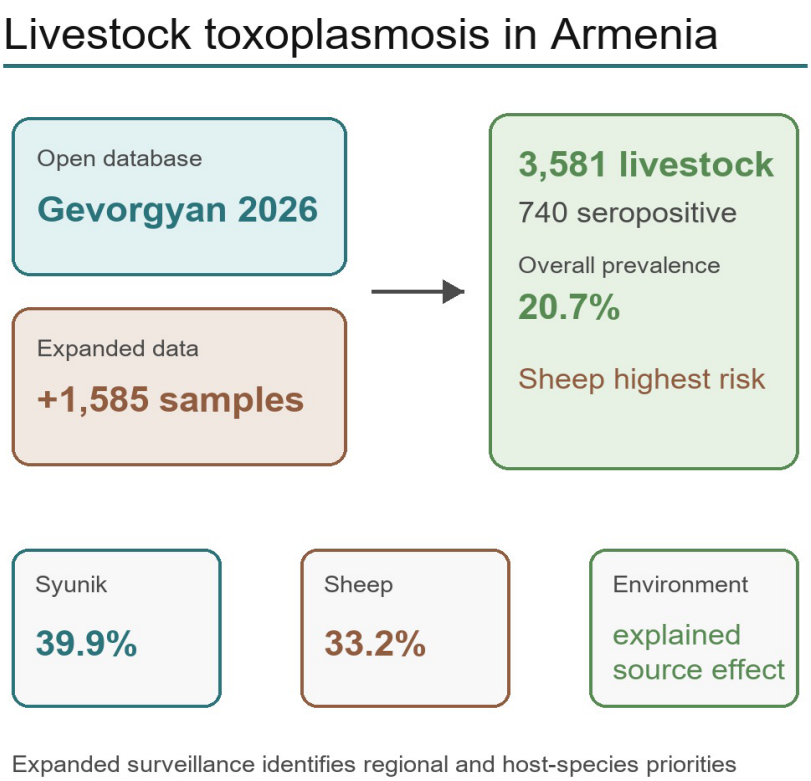


Figure 3. Graphical summary of livestock toxoplasmosis surveillance in Armenia

ence requires wider geographic representation. Similar spatial heterogeneity has been reported in geospatial and meta-analytical studies from other settings, where *T. gondii* prevalence differs substantially among regions, host populations, and ecological zones (Su et al., 2022; Wilson et al., 2024; Yang et al., 2024).

The host-species pattern was one of the most consistent findings. Sheep had much higher seroprevalence than cattle, both descriptively and in regression models. This agrees with Gevorgyan et al. (2026), who also found a higher prevalence in sheep than cattle in northern Armenia, and with the broader literature showing that sheep and other small ruminants are frequently exposed to *T. gondii* and are important food-animal hosts (Ahaduzzaman & Hasan, 2022; Guo et al., 2015). The sheep prevalence in the present study, 33.2%, is close to the global pooled estimate for sheep reported by Ahaduzzaman and Hasan (2022), although direct comparison across countries must account for differences in sampling design, husbandry, climate, and diagnostic tests.

The lower prevalence observed in cattle is also consistent with previous Armenian data and with reviews indicating that cattle often show lower *T. gondii* prevalence than sheep, pigs, or poultry in meat-animal systems (Gevorgyan et al., 2026; Guo et al., 2015). Nevertheless, cattle should not be dismissed as irrelevant, because seropositivity still indicates environmental exposure, and cattle may serve as sentinels of oocyst contamination in grazing landscapes. The pig and goat results should be interpreted only descriptively in this dataset because the sample sizes were small. Their inclusion is still useful for transparency and for Supplementary Table S1, but stronger species-specific conclusions will require targeted sampling.

Geographic heterogeneity was substantial. Syunik had the highest observed seroprevalence, while Lori had comparatively low prevalence and no positives were detected in the small Gegharkunik sample. This province-level pattern is important for two reasons. First, it explains why the expanded dataset has a higher crude prevalence than the previously published northern-Armenia dataset. Second, it suggests that infection risk is structured by local ecological, husbandry, or landscape conditions rather than being uniform across Armenia. The zero estimate for Gegharkunik should be treated cautiously because the sample size was limited and the confidence interval remained wide. Future sampling in low-count provinces would be valuable before interpreting apparent absence of infection as true low regional

risk. Geospatial studies from other countries similarly show clustered or regionally variable *T. gondii* prevalence rather than uniform national patterns (Li et al., 2021; Su et al., 2022; Yang et al., 2024).

The regional season-sensitivity analysis indicates that Syunik's high prevalence was not fully explained by spring sampling. However, because Syunik records with parsable dates were concentrated in spring and autumn, the province effect should still be interpreted as a regional signal that may combine local ecology, herd management, host composition, and seasonal sampling structure.

Interpretation of environmental associations

The environmental analysis strengthens the main descriptive interpretation. Before environmental adjustment, the expanded/new data had substantially higher odds of seropositivity than the open-database component. After adjustment for host species, sampling year, elevation structure, precipitation, and NDVI, the dataset-source effect was no longer significant. This suggests that the higher crude prevalence in the expanded dataset was largely explained by the composition and environmental context of the added samples, rather than by an unexplained difference between data sources. In other words, adding new regions did not merely increase sample size; it added ecological variation that changed the estimated risk landscape.

The season-adjusted environmental sensitivity model similarly suggests that the precipitation, NDVI, and non-linear elevation associations were not simply artifacts of seasonal sampling. At the same time, season was informative in the model; thus, the environmental findings should be interpreted as adjusted ecological associations rather than direct causal effects of climate variables alone.

The clustering sensitivity analysis supports the robustness of the host-species finding but indicates that environmental associations should be interpreted cautiously because part of the signal may reflect unmeasured province- or locality-level structure.

Because farm identifiers were unavailable, residual farm-level clustering could not be assessed directly.

The decision to exclude slope and strongly intercorrelated climate variables from the main model was important. Mean humidity was strongly correlated with mean temperature and annual precipitation, and mean temperature was also correlated with elevation. Including all of these predictors together would have made regression coefficients unstable and difficult to interpret. The selected model therefore emphasized a smaller set of biologically interpretable and less redundant variables: elevation, precipitation, and NDVI, with humidity evaluated as an alternative/sensitivity variable. This approach follows the same logic used in ecological epidemiology studies, where environmental predictors often represent overlapping climatic gradients rather than independent causal exposures (Yan et al., 2016; Wilson et al., 2024).

The significant quadratic elevation term indicates a non-linear altitude-related pattern. This is especially relevant because Gevorgyan et al. (2026) reported an altitude-dependent pattern in sheep from northern Armenia, with infection probability decreasing from low to mid altitudes and increasing slightly at higher elevations. The present combined analysis is not identical, because it includes a larger geographic frame and adjusts simultaneously for host species, year, precipitation, and NDVI. However, the persistence of a non-linear elevation signal supports the idea that altitude is not simply a monotonic risk factor. Elevation may summarize multiple unmeasured features, including temperature regime, grazing season, settlement structure, pasture use, cat density around farms, and hydrological conditions.

The negative associations of annual precipitation and NDVI with seropositivity should be interpreted carefully. Experimental and field evidence generally supports the biological importance of moisture for oocyst survival. For example, Lélu et al. found markedly higher *T. gondii* oocyst survival in damp than dry soil conditions (Lélu et al., 2012), and studies from Iran and Pakistan have linked higher humidity or meteorological conditions with increased ovine exposure (Khademi et al., 2021; Rahman et al., 2023). At first glance, the inverse precipitation and NDVI associations observed here may seem inconsistent with those studies. A more cautious interpretation is that, in this Armenian dataset, precipitation and NDVI may act as regional ecological proxies rather than direct measures of oocyst survival at the farm level. Areas with higher precipitation or greener landscapes in the dataset may correspond to provinces, elevations, management systems, or host compositions with lower observed prevalence, whereas drier or less vegetated areas may include high-prevalence sampling locations. Studies comparing humid and other ecological zones demonstrate that moisture-related predictors may be important, but their direction and interpretation can depend on scale, host system, and local environmental context (Lélu et al., 2012; Khademi et al., 2021; Rahman et al., 2023).

This distinction matters for causal inference. *T. gondii* transmission depends on environmental oocyst contamination, survival, host exposure, and the density and behavior of definitive hosts. Local rainfall or vegetation indices can influence some of these processes, but they cannot capture all farm-level mechanisms. Reviews emphasize that climate, water, soil, animal management, food habits, and host ecology jointly shape *T. gondii* transmission (Hill & Dubey, 2013; Guo et al., 2015; Yan et al., 2016). Therefore, the environmental model should be read as evidence that seropositivity is geographically and ecologically structured, not as proof that precipitation or NDVI directly protects animals from infection.

Strengths, limitations, and implications

A major strength of this study is the integration of a published open database with expanded sample-level data and environmental variables. A direct cross-check against the PLOS One supporting dataset confirmed that the Gevorgyan 2026 open-database records were reproduced correctly. Supplementary Table S1 also preserves sample-level provenance by identifying whether records came from the Gevorgyan 2026 open database or from the expanded/new data. These features improve transparency and make the analysis reproducible. The study also moves beyond simple prevalence estimation by evaluating whether the expanded pattern remains after accounting for host species, year, and environmental structure.

Several limitations should be considered. The dataset was not generated from a single nationally randomized sampling design, and sampling intensity differed among provinces, years, and host species. Goats and pigs were represented by very small sample sizes, limiting species-specific inference. Important individual- and farm-level variables—including age, sex, breed, cat contact, farm biosecurity, grazing intensity, feed and water sources, and reproductive history—were not consistently available across all samples. Serology indicates exposure to *T. gondii* but does not demonstrate viable tissue cysts in meat. Finally, environmental predictors were linked at the locality level and may reflect broader landscape gradients rather than precise farm-level exposure conditions. Similar limitations, including uneven sampling, age or herd effects, and diagnostic heterogeneity, are common in livestock toxoplasmosis surveys and meta-analyses (Ahaduzzaman & Hasan, 2022; Dámek et al., 2023; Deksne et al., 2017).

Age or age-class data were not available in the source datasets, including the expanded workbook and the open-database component from Gevorgyan et al. (2026); therefore, age-adjusted or age-stratified analyses could not be performed. Future studies should record age or age class prospectively because cumulative grazing exposure may influence *T. gondii* seropositivity.

Herd movement and grazing distance were also not recorded. Therefore, NDVI or vegetation availability should not be interpreted as a direct proxy for shorter or longer animal travel. In Armenia, grazing management can differ substantially among villages, ranging from daily movement to nearby pastures with evening return to seasonal movement through progressively higher pastures. Future studies should record these management patterns directly.

Seasonal findings should also be interpreted cautiously. The season variable represents the sample collection date rather than the date of infection, and antibodies may persist after exposure. The sheep pattern, with higher spring seroprevalence, may reflect accumulated exposure from earlier grazing or regional management differences rather than a direct effect of spring conditions alone. The very small number of cattle sampled in summer also prevents meaningful interpretation of summer cattle prevalence. Nevertheless, the analysis supports the reviewer's point that hot, dry summer conditions, especially in southern areas, should be considered in future longitudinal studies that record grazing movements, water sources, and the timing of exposure.

This seasonal pattern is biologically plausible but should not be interpreted as direct evidence of infection during spring. The ecology of *T. gondii* is sensitive to climate because oocyst sporulation, survival, and infectivity depend partly on temperature and humidity, while seasonal precipitation can influence movement of oocysts from contaminated land into water or grazing areas (Yan et al., 2016). Experimental soil work also supports that oocyst viability can persist for long periods under suitable soil-moisture conditions, making exposure possible after earlier contamination rather than only at the moment of sampling (Lélu et al., 2012). Studies in ruminants further indicate that grazing systems, contaminated pasture, and water sources can increase contact with oocysts, and that seasonal pasture or season may be associated with seropositivity (Abdallah et al., 2019; Dahmane et al., 2025). Therefore, the higher spring seroprevalence observed in sheep may reflect accumulated exposure from previous grazing and management periods, together with regional differences in pasture and water use, rather than a simple effect of spring climate alone.

Despite these limitations, the findings have clear epidemiological implications. The expanded dataset indicates that livestock exposure to *T. gondii* in Armenia is not confined to the northern provinces previously studied and that sheep, especially in high-prevalence regions, such as Syunik, should be prioritized in future surveillance. From a food-safety perspective, the high sheep seroprevalence is relevant because infection in meat animals can contribute to human exposure through raw or undercooked meat, while environmental oocyst contamination can expose both livestock and humans through soil, water, and feed pathways (Hill & Dubey, 2013; Guo et al., 2015). Future work should combine targeted province-level sampling, balanced host-species coverage, farm-management data, and finer environmental measurements to distinguish direct ecological mechanisms from regional sampling structure.

Conclusion

This expanded Armenia-wide analysis shows that livestock exposure to *T. gondii* is higher and more geographically heterogeneous than suggested by the previously published northern-Armenia open database alone. Sheep were consistently at substantially higher risk than cattle, and Syunik emerged as a high-prevalence province in the expanded dataset. Environmental modeling indicated that the higher crude prevalence in the expanded/new data was largely explained by host composition, sampling year, and environmental structure, with evidence for a non-linear elevation pattern and inverse associations with annual precipitation and NDVI in the selected model. These findings support targeted surveillance in high-prevalence regions, balanced sampling of underrepresented host species and provinces, and future studies combining serology with farm-level management data, cat exposure, and finer environmental measurements.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization and supervision: Hasmik Gevorgyan and Sargis A. Aghayan; Methodology: Hasmik Gevorgyan and Ahmad Daryani; Investigation: Ruzanna Gevorgyan, Oleg Scherbakov, Jon Mkrtchyan, and Emil Adamyan; Data collection and curation: Oleg Scherbakov, Ruzanna Gevorgyan, Emil Adamyan, Jon Mkrtchyan, Tigran Abgaryan; Data analysis: Sargis A. Aghayan; Funding acquisition and resources: Hasmik Gevorgyan; Writing the original draft: Ruzanna Gevorgyan and Sargis A. Aghayan; Review and editing: All authors.

Conflict of interest

The authors declared no conflict of interest.

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Supplementary Table S1. Livestock samples tested for *T. gondii* antibodies in Armenia, by province and host species: Aggregated summary of 3,581 livestock samples from 112 province-specific sampling localities

Province	Host Species	Sampling Years	No.			%
			Localities	Tested	ELISA Positive	Seroprevalence
Aragatsotn	Cattle	2022	4	126	17	13.5
	Sheep	2023	2	80	32	40.0
Aragatsotn total	All hosts	2022, 2023	6	206	49	23.8
Gegharkunik	Cattle	2022	2	62	0	0.0
Gegharkunik total	All hosts	2022	2	62	0	0.0
Kotayk	Cattle	2021, 2023	6	221	27	12.2
	Sheep	2023	4	172	46	26.7
	Goat	2023	1	1	1	100.0
Kotayk total	All hosts	2021, 2023	9	394	74	18.8
Lori	Cattle	2022, 2023	15	547	53	9.7
	Sheep	2022, 2023	18	396	39	9.8
	Pig	2025	1	32	1	3.1
Lori total	All hosts	2022, 2023, 2025	23	975	93	9.5
Shirak	Cattle	2021, 2022, 2023	23	347	38	11.0
	Sheep	2022, 2023	17	261	61	23.4
	Goat	2023	2	4	0	0.0
Shirak total	All hosts	2021, 2022, 2023	33	612	99	16.2
Syunik	Cattle	2021, 2023	11	349	61	17.5
	Sheep	2022, 2023	8	347	217	62.5
	Pig	2023	1	1	0	0.0
Syunik total	All hosts	2021, 2022, 2023	18	697	278	39.9
Tavush	Cattle	2021, 2022, 2025	13	397	46	11.6
	Sheep	2021, 2022, 2023	11	224	97	43.3
	Pig	2021	1	14	4	28.6
Tavush total	All hosts	2021, 2022, 2023, 2025	21	635	147	23.1
Overall total	All hosts	2021, 2022, 2023, 2025	112	3581	740	20.7

Note: Seroprevalence = ELISA-positive/tested × 100. Locality counts in animal rows are species-specific and are therefore not additive; province totals count unique localities within each province. The complete sample-level dataset (n = 3,581; 23 variables) is supplied separately as Supplementary Data S1 in Excel format.