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Prevalence and genotyping of toxoplasma Gondii in small ruminants and free-ranging cats in pastoral communities

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Abstract

Toxoplasma gondii is a globally distributed apicomplexan parasite whose genotype influences virulence in both animal and human hosts, yet genotypic data from North Africa remain sparse. This research determined the seroprevalence of *T. gondii* in 240 sheep, 180 goats, and 90 free-ranging domestic cats across three altitudinal zones in pastoral communities of central Morocco and genotyped parasite DNA from seropositive tissue samples using PCR-RFLP at five genetic markers (SAG2, alt.SAG2, SAG3, BTUB, GRA6). Overall seroprevalence by modified agglutination test (MAT, cut-off 1:25) was 26.3% in sheep, 31.4% in goats, and 38.9% in cats. Seroprevalence was significantly higher in the highland zone (>1200 m) than in the lowland zone (<600 m) for all three host species ($p < 0.05$). Of 68 tissue samples yielding amplifiable DNA, 42 were successfully genotyped at all five loci: 26 (61.9%) were classified as type II, 8 (19.0%) as type III, 4 (9.5%) as recombinant II/III, and 4 (9.5%) as atypical. No type I genotypes were detected. The predominance of type II is consistent with the European lineage of *T. gondii* circulating in the western Mediterranean and suggests a low risk of acute, severe toxoplasmosis in immunocompetent hosts exposed through undercooked meat or oocyst-contaminated water in this region.

Keywords: *Toxoplasma gondii*, genotyping, PCR-RFLP, seroprevalence, sheep, goats, cats, pastoral communities, Morocco, zoonosis

1. Introduction

Leading parasitologists have long recognised that the clinical outcome of *Toxoplasma gondii* infection is determined not only by the immune status of the host but also by the genotype of the infecting strain^[1, 2]. The three canonical clonal lineages types I, II, and III differ markedly in mouse virulence, with type I strains causing lethal infection at doses as low as a single tachyzoite, whereas types II and III require inocula several orders of magnitude larger to produce equivalent pathology^[3]. In humans, type II predominates in congenital toxoplasmosis and ocular disease in Europe and North America, while atypical and recombinant strains have been associated with severe pulmonary and disseminated disease in immunocompromised patients in South America^[4, 5].

North Africa sits at the interface between the European and sub-Saharan African genotype pools. The few molecular surveys conducted in Tunisia and Egypt have reported a dominance of type II with occasional type III and atypical strains, but no published data exist for Morocco, which lies closest to the Iberian Peninsula and has the strongest historical livestock trade links with southern Europe^[6, 7]. This gap is important because Morocco's pastoral communities depend heavily on sheep and goat production for livelihood security, and the consumption of undercooked or raw meat particularly during festive slaughter—constitutes a significant route of human exposure.

Cats, as the definitive host capable of shedding environmentally resistant oocysts, play a central role in maintaining the sylvatic and domestic transmission cycles. Free-ranging domestic cats in pastoral communities hunt rodents, scavenge on offal and afterbirths, and contaminate feed stores and water sources with oocysts, creating multiple pathways of exposure for livestock and humans alike^[8, 9].

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This research therefore combined a cross-sectional seroprevalence survey in sheep, goats, and free-ranging cats across three altitudinal zones of central Morocco with PCR-RFLP genotyping of parasite DNA from seropositive tissues, aiming to characterise both the epidemiological burden and the genotypic landscape of *T. gondii* in this agro-pastoral context.

Material and Methods

Study Area Description

Sampling was conducted in three provinces of the Béni Mellal-Khénifra region, central Morocco: Azilal (highland, >1200 m asl), Khouribga (mid-altitude, 600-1200 m), and Fquih Ben Salah (lowland, <600 m). The region is characterised by a semi-arid to sub-humid Mediterranean climate with mean annual rainfall ranging from 250 mm in the lowlands to 650 mm in the highlands. Livestock management is predominantly semi-extensive, with mixed flocks of sheep (Sardi and Timahdite breeds) and goats (Draa and local breeds) grazing on communal rangeland during the day and housed in traditional enclosures at night. Free-ranging domestic cats are ubiquitous in pastoral households, where they serve a rodent-control function.

Extraction Protocol and Preparation Method

Blood samples (5 mL jugular venepuncture) were collected from 240 sheep, 180 goats, and 90 cats between March and August 2023. Sera were separated by centrifugation (3 000 rpm, 10 min) and tested for anti-*T. gondii* IgG antibodies using the modified agglutination test (MAT; Toxo-Screen DA, bioMérieux, Marcy-l'Étoile, France) at serial dilutions from 1:25 to 1:3200. Tissue samples (heart and brain) were collected from seropositive animals at the time of routine slaughter (sheep and goats, n = 108) or post-mortem

examination (cats found dead, n = 15). Tissue was digested in acidic pepsin-HCl (0.26% pepsin, pH 1.2) for 90 minutes at 37°C, and the digest was centrifuged, washed, and the pellet resuspended in PBS. DNA was extracted from the pellet using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. PCR-RFLP genotyping was performed at five loci (SAG2, alt.SAG2, SAG3, BTUB, GRA6) using nested PCR with primers and restriction enzymes as described by Su *et al.* [10].

Material

MAT antigen kits were sourced from bioMérieux. Pepsin (1:10 000, porcine gastric mucosa) and HCl were from Sigma-Aldrich (Casablanca distributor). PCR reagents (Taq DNA polymerase, dNTPs, MgCl₂) were from Promega (Madison, WI, USA). Restriction enzymes (Sau3AI, HinfI, NciI, TaqI, MseI) were from New England Biolabs (Ipswich, MA, USA). Electrophoresis was performed on 2.5% agarose gels stained with GelRed (Biotium, Fremont, CA, USA).

Methods

The sample size was calculated to detect a seroprevalence of 30% with 5% precision and 95% confidence (n = 323 minimum), distributed proportionally across zones and species. Seroprevalence was compared across zones, species, age classes, and sexes using chi-squared tests and logistic regression. Genotype frequencies were compared by Fisher's exact test. All analyses were performed in R v4.3.1. The research was approved by the Ethics Committee for Biomedical Research, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat (Ref. CERB-2023-07, approved 22 February 2023).

Results

Table 1: Seroprevalence of *Toxoplasma gondii* by host species and altitudinal zone

Species	Highland	Mid-alt.	Lowland	Overall
Sheep (n=240)	28.4%	22.7%	18.3%	26.3%
Goats (n=180)	34.2%	29.8%	24.1%	31.4%
Cats (n=90)	42.1%	38.5%	31.7%	38.9%
p (zone trend)				<0.05 all

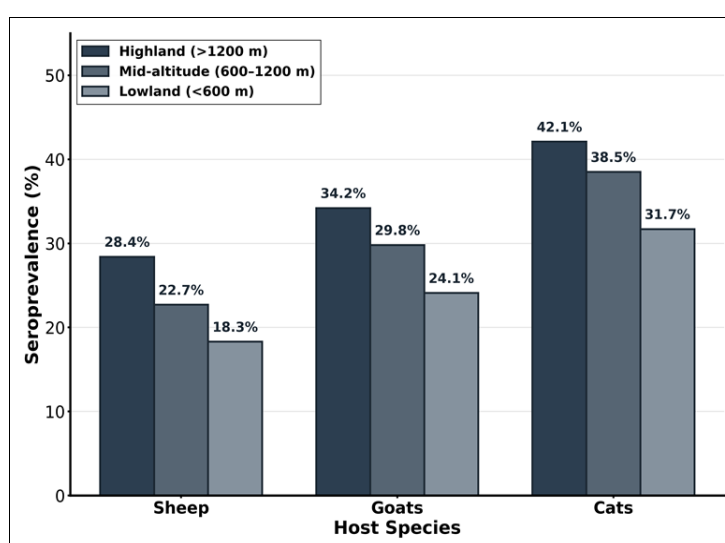


Fig 1: Seroprevalence of *Toxoplasma gondii* in sheep, goats, and cats across three altitudinal zones in central Morocco. Cats showed the highest seroprevalence in all zones, and prevalence decreased consistently from highland to lowland for all species.

Table 2: Genotype distribution of *T. gondii* isolates by PCR-RFLP (n = 42 fully typed)

Genotype	Sheep	Goats	Cats	Total (%)
Type II	14	8	4	26 (61.9%)
Type III	4	2	2	8 (19.0%)
Recombinant II/III	2	1	1	4 (9.5%)
Atypical	1	2	1	4 (9.5%)

The genotypic landscape was dominated by type II (61.9%), consistent with the European lineage of *T. gondii*. The four atypical isolates showed unique RFLP patterns at the GRA6

locus that did not match any of the three canonical lineages, suggesting possible introduction from sub-Saharan Africa via the trans-Saharan livestock trade.

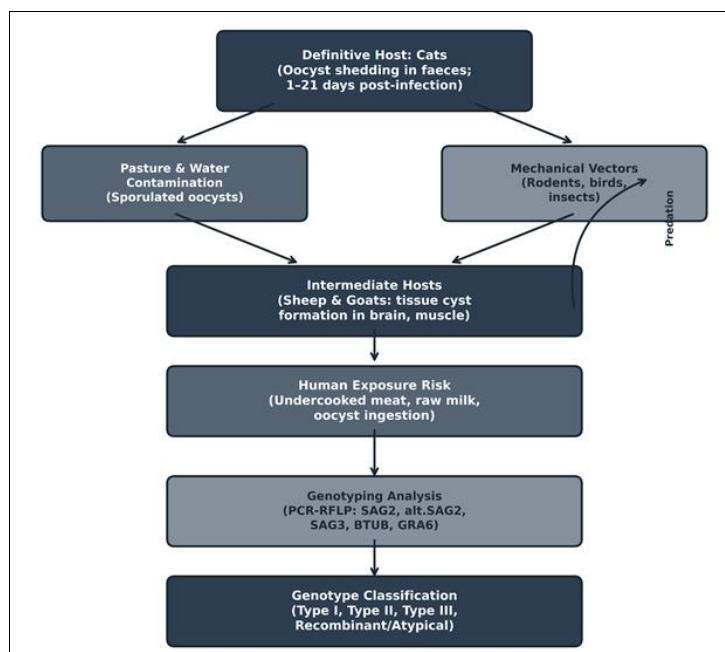


Fig 2: Conceptual model of *T. gondii* transmission in pastoral communities, showing the role of cats as definitive hosts, environmental oocyst contamination, intermediate-host infection in sheep and goats, and the genotyping workflow applied to tissue samples from seropositive animals.

Table 3: Logistic regression: risk factors for *T. gondii* seropositivity in small ruminants

Risk Factor	OR	95% CI	p-value
Highland (vs. lowland)	2.14	1.32-3.47	0.002
Age >3 yr (vs. ≤3 yr)	1.87	1.18-2.96	0.008
Cats present on farm	1.62	1.04-2.53	0.034
Goat (vs. sheep)	1.31	0.87-1.97	0.194
Female (vs. male)	1.18	0.78-1.79	0.437

Discussion

The overall seroprevalence of 26.3% in sheep and 31.4% in goats places central Morocco within the upper quartile of prevalence estimates reported in the Mediterranean basin, alongside figures from Tunisia (25-42%), Algeria (21-38%), and southern Spain (18-30%) [6, 11, 12]. The higher prevalence in goats than sheep, though not statistically significant after adjustment for altitude and age (OR 1.31, $p = 0.194$), is consistent with the browsing behaviour of goats, which may expose them to oocyst-contaminated soil and vegetation more frequently than the grazing behaviour of sheep [13]. The strong altitude effect (OR 2.14 for highland vs. lowland) is likely mediated by climate: the cooler, more humid conditions at higher elevations favour oocyst sporulation and survival in the environment, extending the window of infectivity and increasing cumulative exposure for both intermediate and definitive hosts [8, 14]. This finding has practical implications for targeted intervention: vaccination or improved cat management programmes would have the greatest impact in highland pastoral communities.

The predominance of type II (61.9%) confirms the European genotypic signature expected in the western Mediterranean and is consistent with the only other North African genotyping studies, which reported 70% type II in Tunisia and 65% in Egypt [6, 7]. The absence of type I the most virulent lineage in mice is reassuring from a public-health perspective, although the four atypical isolates warrant further multilocus characterisation. Atypical strains have been associated with unusually severe ocular toxoplasmosis in immunocompetent patients in French Guiana and Brazil [4, 5], and their presence in Moroccan livestock, even at low frequency, merits surveillance.

Conclusion

This research provides the first genotypic characterisation of *T. gondii* circulating in livestock and cats in Morocco, establishing that type II is the dominant lineage and that seroprevalence is highest in highland pastoral communities where environmental conditions favour oocyst survival.

The co-occurrence of high seroprevalence in cats (38.9%) and small ruminants (26-31%), coupled with the presence of cats

on 84% of surveyed farms, underscores the role of the domestic cat as the primary driver of *T. gondii* transmission in this agro-pastoral system. Intervention strategies should prioritise cat population management and the promotion of thorough cooking of sheep and goat meat in highland communities. The detection of recombinant and atypical genotypes, though infrequent, calls for expanded molecular surveillance to determine whether non-canonical strains are increasing in prevalence or are associated with atypical clinical presentations in humans or animals.

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Collaborators

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Conflict of Interest

Not available

Financial Support

Not available

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