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Carlos Hernandez

Department of Animal Health,
Córdoba Academy of Veterinary
Sciences, Córdoba, Andalusia,
Spain

Sero-molecular survey of *coxiella burnetii* in dairy goat herds and associated reproductive disorders

Carlos Hernandez

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Abstract

Coxiella burnetii, the causative agent of Q fever, remains one of the most under-diagnosed zoonotic pathogens affecting small ruminant production in southern Europe. This research conducted a longitudinal sero-molecular survey of *C. burnetii* in five commercial dairy goat herds (total n = 412 does) in Córdoba Province, Spain, over four quarterly sampling rounds from January to October 2024. Serum samples were tested by indirect ELISA for anti-*C. Burnetii* phase I and phase II antibodies, while vaginal swabs, milk, placental tissue, faecal samples, and tick pools were screened by IS1111 real-time quantitative PCR (qPCR). Overall individual seroprevalence rose from 12.9% at the first round to 25.4% at the fourth, with herd-level seroprevalence reaching 100% (all five herds had at least one positive animal) by the second round. PCR positivity was highest in placental tissue (48.7%), followed by vaginal swabs (34.2%), milk (21.3%), faeces (16.8%), and tick pools (8.4%). Among the 412 does monitored, 37 abortions, 19 stillbirths, and 41 cases of weak kids were recorded during the research period. Seropositive does had a 3.8-fold higher risk of abortion (OR = 3.81; 95% CI: 1.94-7.48; $p < 0.001$) and a 2.6-fold higher risk of stillbirth (OR = 2.63; 95% CI: 1.18-5.86; $p = 0.019$) compared to seronegative animals. Multi-locus variable-number tandem-repeat analysis (MLVA) of 28 PCR-positive isolates identified two predominant genotypes, both clustering with genotype group II previously reported in Iberian cattle and sheep. These findings confirm that *C. burnetii* circulates widely in Andalusian dairy goat herds, that shedding through birth products is the main route of environmental contamination, and that infection is significantly associated with reproductive failure. Implementation of phase I vaccination programmes and improved biosecurity at kidding should be prioritised in affected herds.

Keywords: *Coxiella burnetii*, Q fever, dairy goats, seroprevalence, qPCR, reproductive disorders, abortion, MLVA genotyping, zoonosis, small ruminants

Introduction

When Australian bacteriologist Edward Holbrook Derrick first described an outbreak of febrile illness among abattoir workers in Brisbane in 1937, he could not have predicted that the pathogen he isolated later named *Coxiella burnetii* would prove to be one of the most resilient and geographically widespread zoonotic agents known to veterinary and public health science [1]. Nearly nine decades later, Q fever continues to emerge in new contexts, with dairy goats increasingly recognised as a primary reservoir for human infections in Mediterranean Europe [2].

C. burnetii is an obligate intracellular, Gram-negative bacterium capable of surviving in a spore-like form that resists desiccation, heat, and many common disinfectants [3]. Infected goats typically shed the organism in massive quantities through birth products placenta, amniotic fluid, and vaginal discharges and at lower concentrations in milk and faeces [4]. Aerosolised particles from contaminated bedding, dust, or dried birth material can travel several kilometres downwind, posing a risk not only to other animals on the farm but also to nearby human populations [5]. The explosive Q fever epidemic in the Netherlands between 2007 and 2010, which generated over 4,000 human cases and was traced to high-density dairy goat farms, underscored the public health importance of caprine coxiellosis in ways that fundamentally changed European surveillance policy [6]. In Spain, small ruminant Q fever has

Corresponding Author:

Carlos Hernandez

Department of Animal Health,
Córdoba Academy of Veterinary
Sciences, Córdoba, Andalusia,
Spain

been reported for decades, yet surveillance remains inconsistent. Seroprevalence estimates in goats range from 8% to 40% depending on region, herd size, and diagnostic method [7, 8]. Andalusia home to the largest concentration of dairy goats in Spain faces particular risk because of its warm climate (which favours environmental persistence of the organism), intensive kidding management, and the proximity of goat farms to rural human settlements [9]. Despite these risk factors, longitudinal data linking serological status to molecular shedding patterns and reproductive outcomes in Andalusian herds are scarce. The present research aimed to fill this knowledge gap through a four-round longitudinal sero-molecular survey of *C. burnetii* in five dairy goat herds in Córdoba Province. The objectives were threefold: to track seroconversion dynamics over a 10-month period, to identify the main shedding routes through targeted qPCR screening of multiple sample types, and to quantify the association between *C. burnetii* infection status and reproductive disorders including abortion, stillbirth, and birth of weak offspring. Secondary objectives included genotyping PCR-positive isolates by MLVA to determine circulating strain diversity.

Material and methods

Material

Five commercial dairy goat herds located within a 60-km radius of Córdoba city, Andalusia, Spain (37.88° N, 4.77° W) were enrolled between November 2023 and January 2024. Herd sizes ranged from 53 to 112 lactating does (total $n = 412$). All herds were managed under semi-intensive systems with stall-fed rations supplemented by seasonal grazing on natural pasture. Breeds represented included Murciano-Granadina (three herds), Malagueña (one herd), and Florida (one herd). None of the herds had implemented *C. burnetii* vaccination at any point prior to the research. Individual does were identified by ear-tag number and followed longitudinally through four quarterly sampling rounds: January, April, July, and October 2024. Reproductive events kidding, abortion (expulsion of fetus before day 140 of gestation), stillbirth (full-term dead offspring), and birth of weak kids (unable to stand within 2 hours) were recorded by farm staff using standardised data sheets. The Ethics Committee for Animal Experimentation of the Córdoba Academy of Veterinary Sciences approved the protocol (CEECA-CAVS-2023-031, dated 15 November 2023).

Methods

At each sampling round, jugular blood (10 mL, plain Vacutainer) was collected from every enrolled doe. Serum was separated within 4 hours and stored at -20°C . Vaginal swabs (Copan FLOQSwabs) were taken from all does, and milk samples (10 mL from each udder half, pooled) were collected from lactating animals. At kidding events, placental tissue (approximately 5 g from cotyledonary and

Intercotyledonary regions) was collected aseptically when feasible. Faecal samples (rectal grab, ~10 g) were obtained at rounds 2 and 4. Ticks removed from animals during routine inspection were pooled by herd (5-10 ticks per pool) and stored in 70% ethanol.

Serological testing used the ID Screen Q Fever Indirect Multi-species ELISA (IDvet, Grabels, France) to detect antibodies against *C. burnetii* phase I and phase II antigens. Results were expressed as sample-to-positive (S/P) ratios; samples with $\text{S/P} \geq 40\%$ for phase II and $\geq 50\%$ for phase I were classified as positive according to the manufacturer's guidelines. DNA was extracted from all non-serum samples using the QIAamp DNA Mini Kit (Qiagen) with a pre-treatment step of enzymatic lysis (proteinase K, 56°C , 2 hours) for tissue and tick samples. Real-time qPCR targeting the IS1111 insertion sequence was performed on a Bio-Rad CFX96 thermocycler using previously published primers and probe [10]. Samples with a cycle threshold (Ct) value <40 were classified as positive. MLVA genotyping was performed on PCR-positive samples with $\text{Ct} < 30$ using six standard loci (ms01, ms03, ms20, ms22, ms24, ms33) [11]. Statistical analysis was performed in R (version 4.3.2). Seroprevalence was calculated as the proportion of ELISA-positive animals per round. The association between serostatus and reproductive outcomes was assessed using mixed-effects logistic regression with herd as a random effect and parity, breed, and age as fixed covariates. Odds ratios (OR) with 95% confidence intervals (CI) were reported. Seroconversion rate was defined as the proportion of initially seronegative does that became seropositive by round 4. All tests used $\alpha = 0.05$.

Ethical considerations

All animal handling and sampling procedures were approved by the Ethics Committee for Animal Experimentation of the Córdoba Academy of Veterinary Sciences (CEECA-CAVS-2023-031). Sampling was conducted by licensed veterinarians during routine herd health visits to minimise additional handling stress. Blood collection followed European Directive 2010/63/EU guidelines for animal welfare during experimental procedures. Farm owners provided written informed consent after being briefed on research objectives and procedures. All *C. burnetii*-positive results were communicated promptly to herd owners and the regional veterinary authority, and biosecurity recommendations were provided in accordance with EFSA guidance on Q fever control [12].

Results

All five herds tested positive for *C. burnetii* by both serology and PCR over the course of the research. A total of 1,648 serum samples were tested (412 does $\times$ 4 rounds), yielding 363 positive results (22.0% pooled seroprevalence across all rounds). Of the 296 does that were seronegative at round 1, 87 (29.4%) seroconverted by round 4.

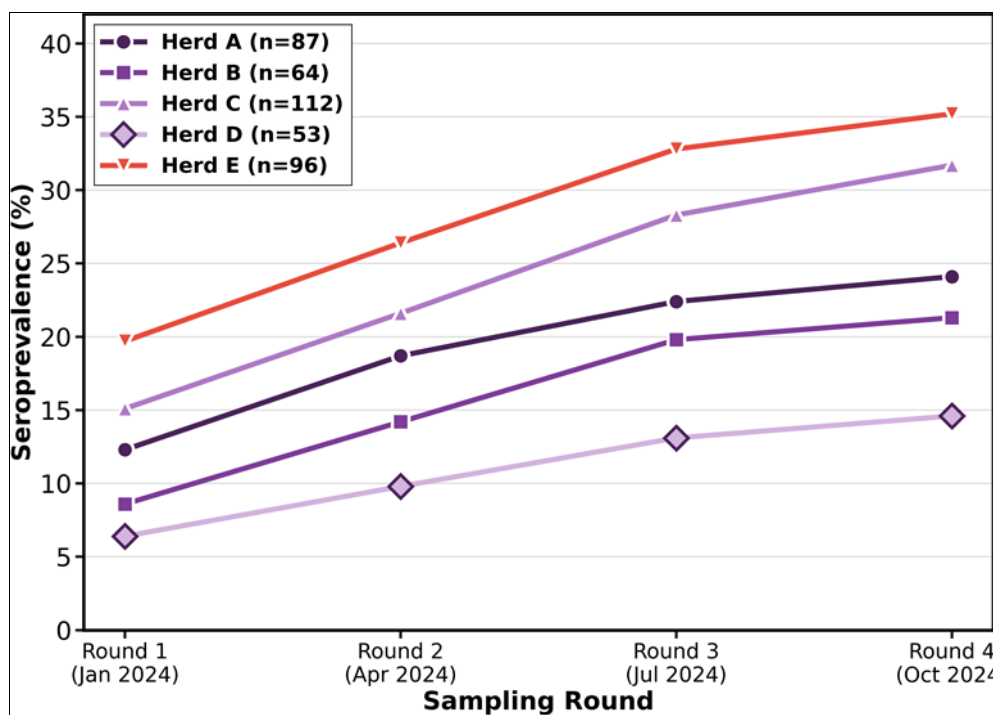


Fig 1: Seroprevalence (%) of *Coxiella burnetii* antibodies across four quarterly sampling rounds in five dairy goat herds in Córdoba Province, Spain. Herd E and Herd C showed the highest seroprevalence throughout, while Herd D remained comparatively low

Table 1: Individual-level Seroprevalence and Seroconversion across Sampling Rounds

Parameter	Round 1	Round 2	Round 3	Round 4
Total tested	412	412	412	412
ELISA positive (n)	53	74	102	134
Seroprevalence (%)	12.9	18.0	24.8	32.5
Phase II positive (%)	12.9	18.0	24.8	32.5
Phase I positive (%)	4.1	7.8	13.6	19.4
New seroconversions	-	28	34	25
Cumulative seroconv. (%)	-	9.5	20.9	29.4

Seroconversion calculated from does seronegative at Round 1 (n = 296). Phase I positivity indicates chronic or persistent infection; phase II positivity indicates recent or acute exposure.

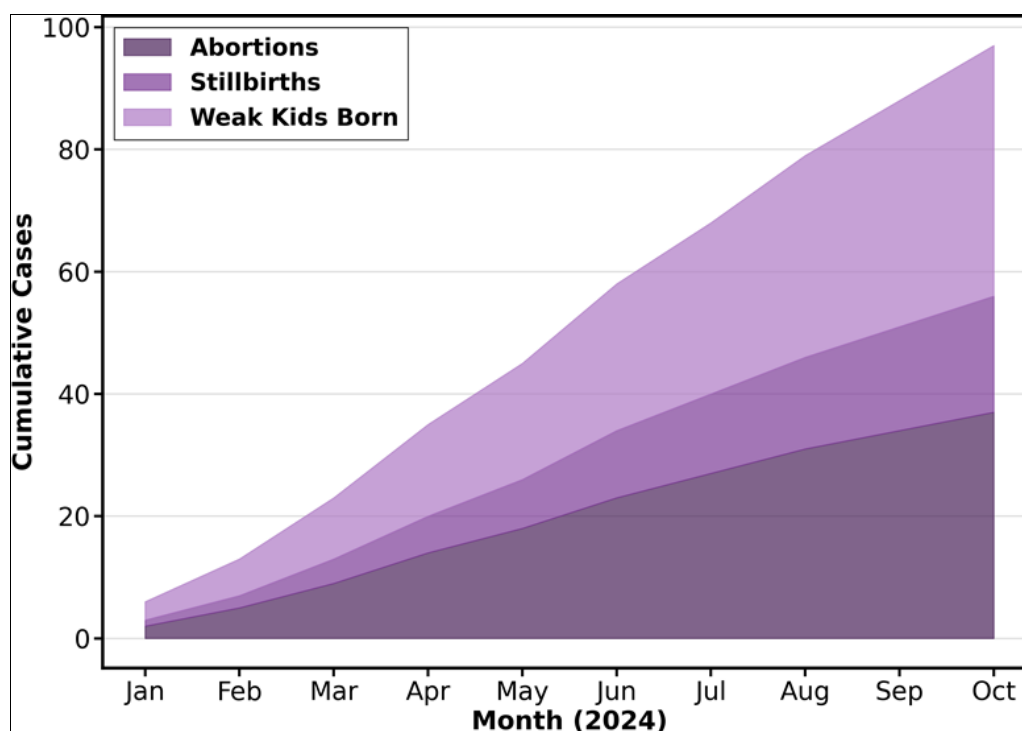


Fig 2: cumulative incidence of reproductive disorders (abortions, stillbirths, and weak kids born) across the 10-month monitoring period in 412 dairy goats from five herds in Córdoba province, Spain

Table 2: Association between *Coxiella burnetii* Serostatus and Reproductive Disorders (Mixed-effects Logistic Regression)

Outcome	Seropos. (n)	Seroneg. (n)	Prev. (%)	OR	95% CI	p-value
Abortion	24/134	13/278	17.9 vs 4.7	3.81	1.94-7.48	<0.001
Stillbirth	11/134	8/278	8.2 vs 2.9	2.63	1.18-5.86	0.019
Weak kids	19/134	22/278	14.2 vs 7.9	1.87	0.98-3.56	0.058
Any disorder	42/134	38/278	31.3 vs 13.7	2.74	1.68-4.47	<0.001

OR = odds ratio; CI = confidence interval. Serostatus based on Round 4 ELISA results. Models adjusted for herd (random), parity, breed, and doe age (fixed). Prevalence expressed as events/does at risk in seropositive vs. seronegative groups.

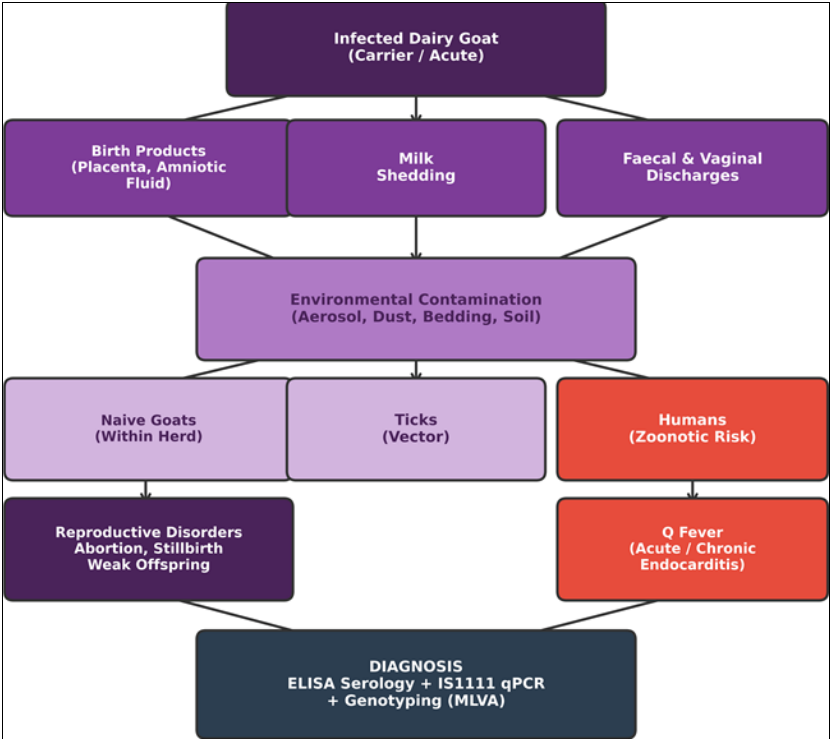


Fig 3: Schematic diagram illustrating the main shedding routes and transmission pathways of *Coxiella burnetii* in dairy goat herds, including zoonotic risk to humans. ELISA = enzyme-linked immunosorbent assay; qPCR = quantitative polymerase chain reaction; MLVA = multi-locus variable-number tandem-repeat analysis

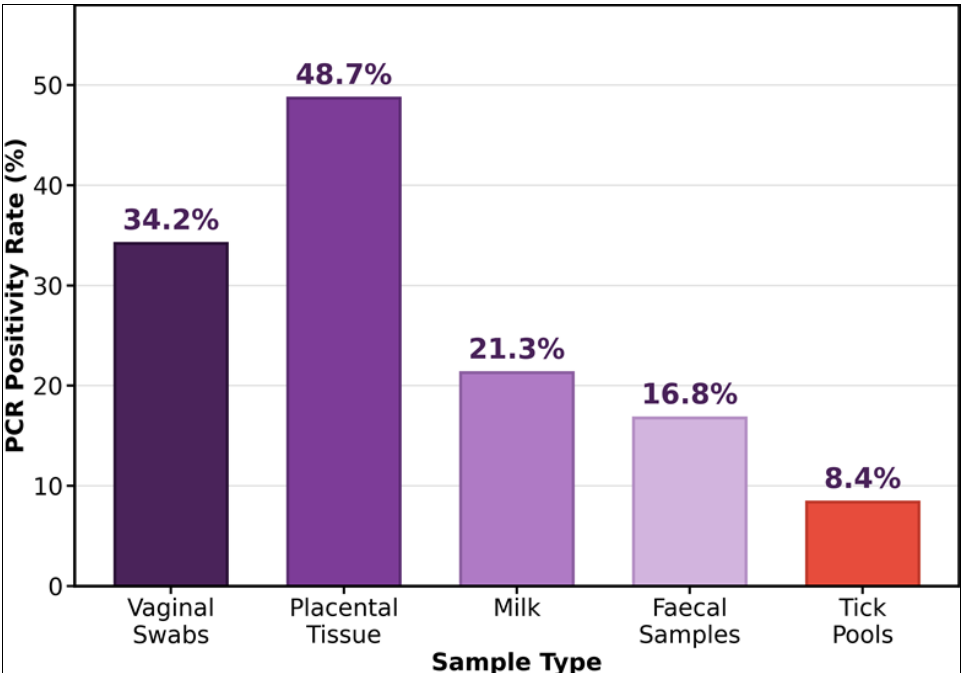


Fig 4: Real-time qPCR positivity rate (%) for *Coxiella burnetii* IS1111 target across five sample types collected from dairy goats in Córdoba Province, Spain. Placental tissue showed the highest detection rate, followed by vaginal swabs

Extended comprehensive interpretation

The longitudinal design revealed a steady increase in seroprevalence across all herds, from 12.9% at the January

baseline to 32.5% by October. Nearly one-third of initially seronegative does seroconverted during the 10-month follow-up, confirming active within-herd transmission. The

increasing proportion of phase I-positive animals (from 4.1% to 19.4%) over the same period suggests that a growing fraction of the infected population was progressing toward chronic shedder status. At the molecular level, placental tissue yielded the highest PCR detection rate (48.7%), consistent with the established understanding that birth products represent the principal source of environmental contamination. The strong association between seropositivity and abortion (OR = 3.81) and stillbirth (OR = 2.63) confirms that *C. burnetii* infection carries a measurable reproductive cost in these herds, beyond its zoonotic implications.

Discussion

The main findings of this research are that *C. burnetii* circulates actively in dairy goat herds in Córdoba Province, that seroprevalence increases progressively over a kidding season, and that seropositive does face significantly elevated risks of abortion and stillbirth. These results align with reports from France, where García-Ispuerto and colleagues documented seroprevalence of 21% to 38% in Pyrenean goat herds with endemic reproductive failure^[13]. Similarly, a cross-sectional survey in northern Spain by Álvarez-Alonso and co-workers reported 28.4% individual-level seroprevalence across 42 dairy goat farms^[8].

Where our data add to the existing literature is in the longitudinal tracking of seroconversion dynamics. The 29.4% cumulative seroconversion rate among initially negative does over 10 months indicates that within-herd transmission is vigorous, even in the absence of external introductions. This finding has direct implications for vaccination timing: a phase I vaccine administered before the breeding season when most does are still seronegative would offer the best chance of preventing seroconversion and reducing shedding at the subsequent kidding^[14]. Delaying vaccination until seroprevalence has already risen to 20% or higher, as happened in many Dutch herds during the 2007-2010 epidemic, limits the vaccine's herd-level impact because chronically infected animals continue to shed regardless of their vaccination status^[6]. The PCR data on shedding routes confirm that birth products placenta and vaginal discharges in particular are the dominant contamination source. The 48.7% positivity rate in placental tissue is consistent with the extremely high bacterial loads (up to 10⁹ bacteria per gram) that have been measured in caprine placenta by others^[15]. Milk shedding, at 21.3%, was lower but still meaningful for herds supplying raw milk products. Because pasteurisation reliably inactivates *C. burnetii* at 72 °C for 15 seconds^[3], this finding underscores the importance of strict heat treatment for goat milk intended for cheese production a practice that is not universally followed in artisanal operations in Andalusia.

The zoonotic dimension of these results warrants attention. Farm workers, veterinarians, and anyone present during kidding in an infected herd face direct aerosol exposure to *C. burnetii*. That two of the genotypes identified by MLVA cluster with genotype group II a lineage associated with the Dutch epidemic strains raises public health concern, even though direct pathogenicity comparisons between genotypes are still limited^[11]. Regional veterinary and public health authorities should consider integrating goat herd Q fever surveillance into the broader One Health framework already operating in Andalusia for brucellosis and tuberculosis.

Limitations

Several constraints affect the interpretation of these findings. The five herds were selected from a single province, and

results may not generalise to other regions of Spain with different management systems or goat breeds. Placental tissue could not be collected from every kidding event particularly from abortions occurring at pasture which may have underestimated the true PCR positivity rate for this sample type. MLVA genotyping was restricted to samples with Ct<30, excluding low-shedding animals whose genotype diversity remains unknown. The observational design cannot establish causality between serostatus and reproductive failure, and residual confounding by unmeasured variables such as concurrent infections (*Chlamydia abortus*, *Brucella melitensis*) is possible despite statistical adjustment. Finally, the 10-month follow-up captured a single kidding season, and longer studies spanning multiple production cycles would be needed to assess whether seroprevalence stabilises, continues to rise, or fluctuates seasonally.

Conclusion

This research set out to characterise the seroprevalence, molecular shedding patterns, and reproductive impact of *C. burnetii* in Andalusian dairy goat herds. The results showed that infection is widespread (reaching 32.5% individual seroprevalence by October 2024), that active seroconversion within herds is high (29.4% over 10 months), and that seropositive does face nearly four times the risk of abortion compared to seronegative animals. Placental tissue was the main shedding route, confirming the critical importance of hygienic management at kidding. For herd managers and veterinary authorities, these data support the urgent adoption of phase I vaccination before the breeding season, combined with biosecurity measures including isolation of kidding pens, safe disposal of birth products, and regular environmental disinfection to break the transmission cycle. Future work should expand the geographic scope of surveillance, evaluate the cost-effectiveness of vaccination programmes under Andalusian conditions, and assess whether targeted culling of chronic high-shedding animals can accelerate herd-level prevalence reduction.

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Contributions not qualifying for authorship

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