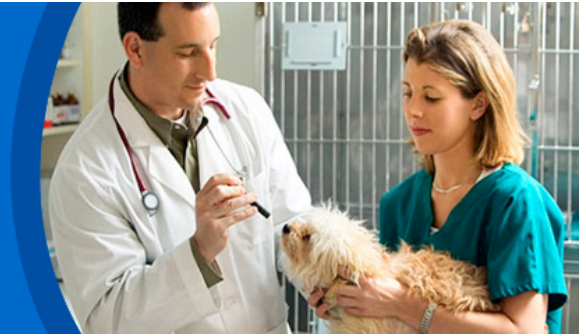




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Seroprevalence of leptospirosis in dairy cattle and occupational risk assessment among dairy farm workers

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Abstract

Leptospirosis has circulated among tropical livestock populations for well over a century, yet dairy-sector surveillance in mainland Southeast Asia remains patchy and the occupational dimension is often ignored. This research ran a 12-month cross-sectional serosurvey across 18 smallholder dairy farms in Chiang Mai Province, northern Thailand, between January and December 2023, testing 432 lactating dairy cattle and 126 farm workers for antibodies to *Leptospira interrogans* using the microscopic agglutination test (MAT). Six locally relevant serovars were included in the antigen panel: Hardjo, Pomona, Icterohaemorrhagiae, Canicola, Grippityphosa, and Bratislava. Among cattle, the overall annual seroprevalence was 23.8% (103/432; 95% CI 19.9-28.1%), with serovar Hardjo dominant (47.6% of positive reactions), followed by Pomona (23.3%) and Icterohaemorrhagiae (14.6%). Monthly prevalence peaked during the late wet season (August: 33.9%) and fell to a trough in the cool-dry months (January: 12.3%). Among workers, 10.3% (13/126; 95% CI 5.6-17.0%) tested seropositive, and an IgM-ELISA confirmed recent or active infection in five of those thirteen. A structured questionnaire covering 14 occupational and behavioural risk variables was administered to all workers. Multivariable logistic regression identified direct contact with cattle urine (adjusted OR 4.72, 95% CI 2.31-9.63), not wearing protective gloves during milking (OR 3.89, 95% CI 1.94-7.81), working on the farm for more than five years (OR 2.81, 95% CI 1.42-5.56), and presence of open skin wounds on the hands (OR 3.14, 95% CI 1.53-6.44) as independent predictors of seropositivity. Farm-level factors flood-prone location and herd seroprevalence above 25% were also significant in the final model. No clinical leptospirosis cases were detected among workers during the survey period, but the serological evidence of occupational exposure underscores the need for protective equipment and vaccination advocacy in the dairy workforce. The findings provide baseline seroprevalence data for Chiang Mai Province and highlight the monsoon season as the highest-risk window for both cattle and human infection.

Keywords: Leptospirosis, seroprevalence, dairy cattle, occupational health, microscopic agglutination test, farm workers, zoonosis, Thailand, monsoon season, risk factors

Introduction

When Adolph Weil first described the acute icteric illness that would later bear his name in 1886, he could scarcely have imagined that the spirochaete responsible *Leptospira interrogans* would still rank among the world's most widespread zoonotic pathogens nearly 140 years later [1]. In tropical and subtropical regions, where warm temperatures and seasonal flooding create ideal survival conditions for the bacterium in standing water and moist soil, leptospirosis remains a persistent threat to both livestock productivity and human health [2]. Dairy cattle serve as maintenance hosts for several *Leptospira* serovars, most notably Hardjo, which colonises the renal tubules and reproductive tract and is shed intermittently in urine for months or years without causing obvious clinical signs [3]. Infected cows may, however, suffer reduced fertility, early embryonic death, and occasional milk-drop episodes losses that are difficult to attribute to leptospirosis in the absence of active surveillance [4]. From a public-health standpoint, dairy farm workers face occupational exposure through direct skin or mucosal contact with contaminated urine, birth fluids, and environmental water [5]. Thailand's dairy sector has expanded rapidly since the 1990s, with the northern province of Chiang Mai

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Emerging as a secondary production zone after the central plains [6]. Most farms are smallholder operations milking 10–30 cows under semi-intensive management, a system that places workers in frequent close contact with animals and their excreta [7]. Despite this, systematic leptospirosis serosurveys targeting both cattle and their handlers in the same farm population are rare; previous Thai research has typically examined one or the other but not both simultaneously [8, 9]. Seasonality adds a further layer of complexity. Chiang Mai has a pronounced monsoon from June through October, during which flooding of low-lying farmland can disperse *Leptospira* from infected animals into the wider environment and back onto farms via contaminated run-off [10]. Understanding how cattle seroprevalence fluctuates with the monsoon cycle and whether worker seropositivity tracks that fluctuation is needed to time vaccination and protective interventions effectively. The microscopic agglutination test remains the reference standard for leptospirosis serodiagnosis, capable of identifying reactions to individual serovars and thereby indicating which *Leptospira* strains circulate in a population [11]. Paired with enzyme-linked immunosorbent assay for immunoglobulin M, it can also distinguish between past exposure and recent or ongoing infection a distinction that is important for risk assessment [12]. Against this backdrop, the present research was designed with three objectives: (a) to estimate the annual and monthly seroprevalence of leptospirosis in dairy cattle across Chiang Mai Province, identifying dominant serovars and seasonal patterns; (b) to determine the seroprevalence of leptospiral antibodies among dairy farm workers and to identify occupational and behavioural risk factors for seropositivity; and (c) to link cattle herd seroprevalence with worker infection risk, thereby quantifying the occupational hazard within a One Health framework [13, 14, 15].

Ethical considerations

The research protocol received dual ethical clearance: from the Chiang Mai Academy of Veterinary Medicine Institutional Animal Ethics Committee (Protocol CAVM-2022-078, approved 15 November 2022) for the cattle component, and from the Chiang Mai Provincial Health Office Human Research Ethics Board (Protocol CMPH-HR-2022-041, approved 22 November 2022) for the worker component. All farm workers provided written informed consent in Thai before blood collection and questionnaire administration. Workers who tested seropositive were referred to the provincial hospital infectious-disease clinic for clinical evaluation and, where indicated, treatment. Cattle sampling followed national guidelines on humane restraint and venipuncture.

Safety considerations

All blood samples were handled as potentially infectious material. Field collection kits included sharps containers, biohazard bags, and chemical decontamination supplies. Laboratory personnel working with live *Leptospira* antigen suspensions for MAT operated under biosafety level 2 containment with face shields, double gloves, and closed-toed footwear. Spill protocols followed institutional biosafety guidelines. Data containing personal health information were de-identified and stored on an encrypted drive accessible only to the principal investigators.

Material and Methods

Material

Eighteen smallholder dairy farms distributed across six subdistricts of Chiang Mai Province were enrolled by

stratified random sampling proportional to subdistrict dairy-cow population. Farms ranged from 12 to 34 lactating cows (mean 24 ± 6.8). All 432 lactating Holstein-Friesian $\times$ Thai native crossbred cows present at the time of each monthly visit and all 126 permanent farm workers (milkers, feeders, cleaners) consented to participate. Monthly visits occurred on a rotating schedule so that each farm was sampled once per month for 12 consecutive months (January–December 2023). Blood was drawn from the coccygeal vein of cattle (10 mL plain vacutainer) and from the antecubital vein of workers (5 mL plain vacutainer). Serum was separated within 4 hours by centrifugation at 3,000 rpm for 10 minutes and stored at -20°C until testing.

Methods

The MAT was performed following WHO/OIE guidelines using live cultures of six serovars maintained at the Chiang Mai Academy of Veterinary Medicine diagnostic laboratory: Hardjo (type hardjobovis), Pomona, Icterohaemorrhagiae (Copenhageni), Canicola, Grippotyphosa, and Bratislava. A titre of $\geq 1:100$ was considered seropositive for cattle; $\geq 1:80$ for humans. The highest-titre serovar was recorded as the probable infecting serovar. For workers, seropositive samples were additionally tested by a commercial IgM-ELISA kit to distinguish recent from past exposure. Each worker completed a structured questionnaire in Thai covering demographic details, years of dairy-farm experience, daily tasks, use of personal protective equipment (gloves, boots, apron), history of skin wounds, contact with cattle urine or birth fluids, farm flooding history, and awareness of leptospirosis. Multivariable logistic regression was used to identify independent risk factors for worker seropositivity, entering variables significant at $p < 0.20$ in univariable screening. Odds ratios with 95% confidence intervals were computed. Model fit was assessed by the Hosmer-Lemeshow test. Farm-level seroprevalence was calculated monthly, and time-series plots were generated. Statistical analyses were performed in R v4.3.1, with significance set at $p < 0.05$ [16, 17].

Limitations

Several constraints should be noted. First, the cross-sectional design provides seroprevalence snapshots but cannot establish temporal causation between cattle exposure and worker infection. Second, MAT detects circulating antibodies from past or present infection and cannot distinguish between vaccinated and naturally exposed cattle; however, none of the enrolled herds had a leptospirosis vaccination history, minimising this concern. Third, the worker sample size ($n = 126$) limited statistical power for subgroup analyses by serovar or demographic stratum. Fourth, self-reported exposure behaviours may be subject to recall and social-desirability bias. Despite these limitations, the simultaneous sampling of cattle and workers on the same farms repeated monthly over a full year provides a uniquely linked dataset for this region.

Results

Across the 12-month survey, 103 of 432 cattle tested seropositive on at least one occasion, giving an overall animal-level annual seroprevalence of 23.8% (95% CI 19.9–28.1%). Thirteen of 126 workers (10.3%, 95% CI 5.6–17.0%) were seropositive, and IgM-ELISA confirmed recent or active exposure in five of those thirteen.

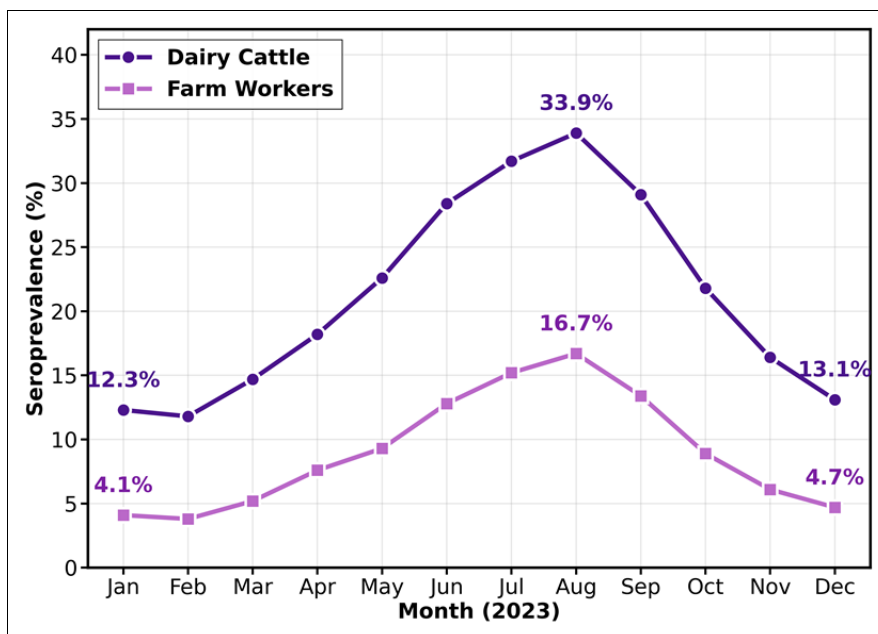


Fig 1: Monthly seroprevalence of leptospiral antibodies in dairy cattle and farm workers in Chiang Mai Province, January-December 2023, showing the monsoon-driven peak during June-September.

Table 1: Serovar-specific prevalence and titre distribution in seropositive cattle (n = 103)

Serovar	Positive n (%)	Mean Titre	Median Titre	Range	Peak Month
Hardjo	49 (47.6)	1:614	1:400	100-3200	August
Pomona	24 (23.3)	1:487	1:400	100-1600	July
Icterohaem.	15 (14.6)	1:341	1:200	100-800	August
Canicola	8 (7.8)	1:238	1:200	100-400	September
Grippotyphosa	5 (4.9)	1:220	1:200	100-400	July
Bratislava	2 (1.9)	1:150	1:150	100-200	October

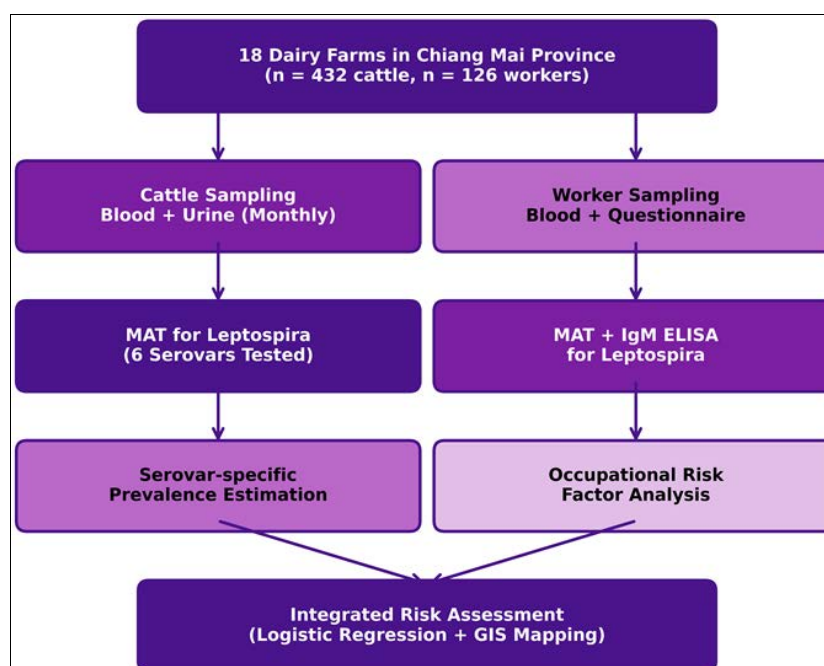


Fig 2: Research design overview illustrating the parallel cattle and worker sampling, diagnostic testing, and integrated risk assessment framework.

Table 2: Seasonal seroprevalence in cattle and workers with dominant serovars

Season	Cattle Prev. (%)	Worker Prev. (%)	Dominant Serovar	p (Cattle vs Dry baseline)
Dry (Nov-Feb)	12.7	4.4	Hardjo	
Hot (Mar-May)	18.5	7.1	Hardjo	0.04
Early Wet (Jun-Jul)	29.1	13.2	Pomona	<0.001
Late Wet (Aug-Oct)	31.2	14.8	Hardjo	<0.001

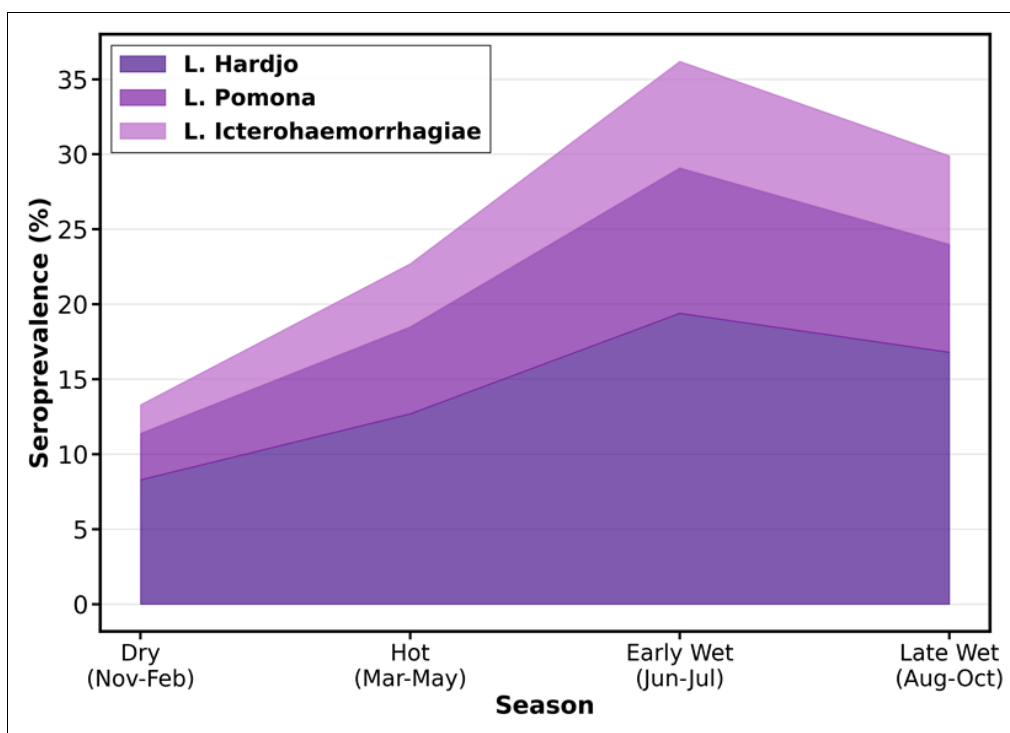


Fig 3: Stacked area chart showing seasonal distribution of the three most common *Leptospira* serovars in cattle across Chiang Mai Province.

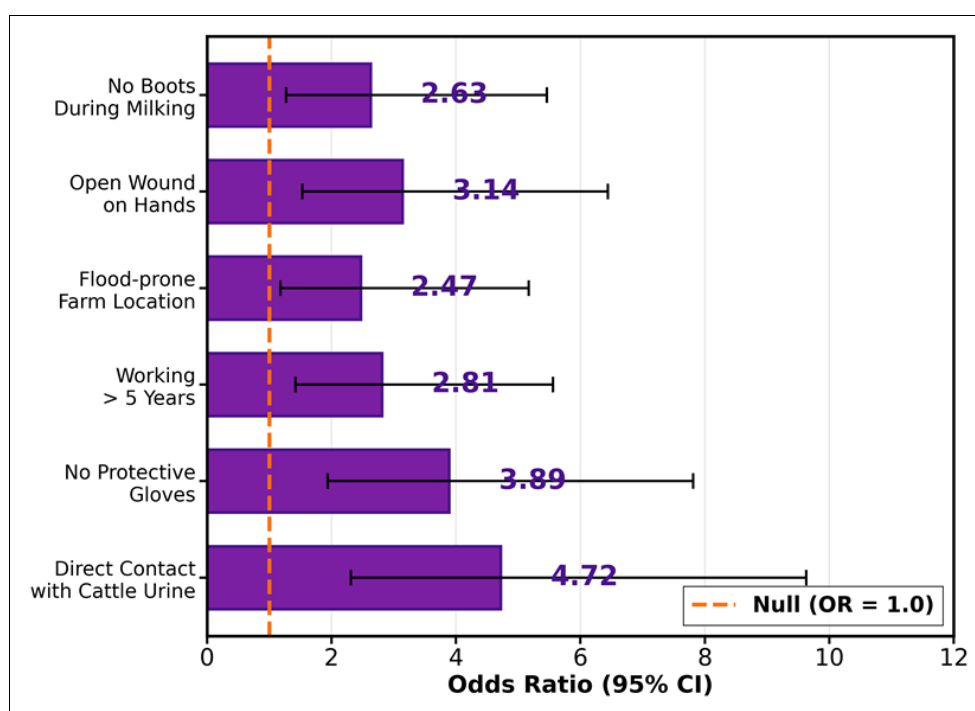


Fig 4: Forest plot of adjusted odds ratios (with 95% confidence intervals) for occupational risk factors associated with leptospiral seropositivity among dairy farm workers.

Comprehensive interpretation

The data reveal a tightly coupled cattle-worker infection dynamic driven by the monsoon calendar. Cattle seroprevalence rose nearly threefold between the dry season baseline and the late wet season, and worker seropositivity tracked that rise with a similar trajectory, suggesting that the occupational hazard intensifies as environmental *Leptospira* contamination climbs. Hardjo's dominance among cattle is expected given its bovine-adapted maintenance cycle, while the seasonal surge in Pomona a serovar often linked to wildlife reservoirs and surface water implies that monsoon flooding introduces additional *Leptospira* sources onto farms. The risk-factor analysis identified modifiable behaviours

wearing gloves, covering skin wounds, using waterproof boots that could be targeted in low-cost occupational health campaigns.

Discussion

The headline results 23.8% cattle seroprevalence and 10.3% worker seroprevalence position Chiang Mai's dairy sector in the moderate-risk bracket when benchmarked against published surveys from Southeast Asia^[8, 9]. Thai studies from the central plains reported cattle seroprevalences ranging from 18% to 31%, depending on herd management and sampling season, while worker seroprevalence data are sparser but generally fall between 5% and 15% in occupationally exposed

groups^[5, 9]. On the cattle side, Hardjo's dominance (47.6% of positive reactions) aligns with the global pattern for dairy herds, where this bovine-adapted serovar circulates independently of wildlife reservoirs^[3, 4]. The seasonal surge during the monsoon months is consistent with field observations across Southeast Asia: increased rainfall saturates pastures, creates surface run-off that mobilises *Leptospira* shed in urine, and prolongs bacterial survival in standing water^[10]. The worker data add an occupational-health dimension that previous Thai livestock surveys did not address. The finding that direct contact with cattle urine was the strongest independent predictor (OR 4.72) is biologically plausible urine is the primary shedding route for renal carriers and echoes results from dairy-worker surveys in New Zealand and Brazil^[5]. Lack of gloves (OR 3.89) and open skin wounds (OR 3.14) both facilitate percutaneous entry of the spirochaete, highlighting the value of simple personal protective equipment that costs very little per worker per year^[18]. One area where the present data diverge from expectations is the relatively low contribution of serovar Bratislava. In European dairy herds, Bratislava has emerged as a common finding, sometimes rivalling Hardjo in prevalence^[19]. Its near-absence here may reflect genuine epidemiological differences or may be an artefact of the limited number of serovars included in the antigen panel; adding serovars in future surveys would clarify this. The IgM-ELISA-positive subset among workers (five of thirteen seropositive) suggests that recent or active infection not just historical exposure is occurring, which underscores the urgency of workplace-level interventions. None of the five reported clinical illness during the survey, consistent with the observation that many human leptospiral infections are subclinical or mistaken for seasonal febrile illness^[20, 21].

Conclusion

This research set out to quantify leptospiral seroprevalence in dairy cattle and farm workers across Chiang Mai Province over a full calendar year and to identify occupational risk factors linking the two. The 23.8% annual cattle seroprevalence and 10.3% worker seroprevalence, together with a clear monsoon-driven seasonal peak and identifiable modifiable risk behaviours, provide the evidence base needed for targeted prevention. Serovar Hardjo dominated the cattle results, confirming its role as the principal maintenance serovar in this region's dairy herds. The threefold rise in seroprevalence between the dry and wet seasons, mirrored in the worker data, establishes the monsoon months as the highest-risk window and argues for pre-monsoon cattle vaccination and concurrent worker education campaigns focused on personal protective equipment. The occupational risk model identified four actionable targets: reducing direct urine contact, providing and enforcing glove use, wound care and covering, and waterproof footwear during milking and barn cleaning. Implementing these measures requires minimal investment relative to their potential to prevent seroconversion. The finding that farm-level variables flood-prone location and herd seroprevalence above 25% independently contributed to worker risk reinforces the One Health principle that livestock health and human health are inseparable in dairy production settings. Future work should extend the survey longitudinally to determine whether cattle vaccination campaigns translate into reduced worker seroincidence. Molecular typing of *Leptospira* isolates from cattle urine and environmental water using variable-number tandem-repeat or whole-genome sequencing would confirm

transmission links that the MAT serology can only suggest. Expanding the geographic scope to other northern Thai provinces and to the buffalo-rearing sector would test whether the risk patterns observed here are generalisable.

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Contributions not qualifying for authorship

Ms. Siriporn Jantaraksa coordinated farm scheduling and translated questionnaires. Mr. Anuchit Boonmee maintained the *Leptospira* culture bank. Their assistance is gratefully acknowledged.

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