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Let's trace: Leishmaniasis in Tuscany (Italy), tracking, research, analysis and continuous evaluation – a retrospective study protocol on underreporting of human cases, geolocation and public health implications

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ABSTRACT

Introduction Leishmaniases are a group of vector-borne diseases caused by parasites of the genus *Leishmania*, which are renowned for increasing global spread due to factors like climate change, globalisation, urbanisation and migration. Leishmaniasis is classified as a neglected tropical disease but is endemic in several areas of the Mediterranean Basin, including Italy, where *Leishmania infantum* is most involved as the parasite, phlebotomine sand fly as the vector and dog as the principal reservoir. Effective surveillance of communicable infectious diseases is a goal worldwide for organisations such as the WHO and for local and national governments but is an unfulfilled objective. Even in Italy and particularly in the region of Tuscany, despite mandatory reporting, significant gaps each year are identified between reported cases and hospital admissions. By estimating the underreporting of confirmed human leishmaniasis cases, this protocol aims to suggest actions to strengthen the current epidemiological surveillance system to enable timely and effective public health intervention in human and veterinary populations.

Methods and analysis This retrospective multicentre study, conducted in the Central Tuscany Health District, the most populous area of the Tuscany region with approximately 1.6 million inhabitants, is based on the analysis of data collected from 2014 to 2024 using diagnostic laboratory, hospital and regional information system sources. The primary objective is to estimate the degree of underreporting of leishmaniasis in this area through the application of capture-recapture models. The secondary objective is to analyse the clinical and demographic characteristics of individuals diagnosed as confirmed leishmaniasis cases between January 2014 and December 2024, as well as to perform a geolocation analysis of the cases. The study includes the entire population, both adult and paediatric, of the Central Tuscany Health District who underwent laboratory testing for leishmaniasis (serological tests identifying the presence of antibodies; parasitological examination with evidence of amastigotes in aspirates, smears or biopsy sections; culture examination of aspirates, biopsies and/or peripheral blood positive for the presence of promastigotes; identification of *Leishmania* nucleic acid in aspirates, biopsies and/or peripheral blood samples via molecular diagnosis).

Ethics and dissemination The study is being conducted in accordance with the protocol approved by the Ethics Committee of the Tuscany Region – Pediatrics Section, in November 2024. Ethics Committee opinion register number: 219/2024. Because the study uses only pseudonymised, routinely collected administrative and laboratory data with no direct patient contact or intervention, individual informed consent was not required, as confirmed by the Ethics Committee. Findings will be submitted to a peer-reviewed journal, presented at international conferences and presented at stakeholder workshops.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Multisource case ascertainment: linkage of laboratory, hospital discharge records and official notifications using predefined deterministic rules.
⇒ Three-source capture–recapture: log-linear models with two-way interaction terms to account for dependence between sources.
⇒ Potential information bias: reliance on routine data may miss episodes tested or hospitalised outside the study area, potentially reducing case ascertainment.
⇒ Geospatial assessment: standardised geocoding to municipality level and small-number suppression for confidentiality.

INTRODUCTION

Leishmaniasis is a vector-borne disease classified as a 'neglected tropical disease'.[1] It is endemic in 99 countries and territories worldwide that report data to the WHO, including several areas of the Mediterranean Basin, such as Italy.[2 3]

Leishmaniasis is caused by protozoan parasites of the *Leishmania* genus, with more than 20 species pathogenic to humans and whose main reservoirs vary. In Italy, the disease is caused by *Leishmania infantum* and is transmitted through the bite of infected female phlebotomine sand flies. In this case, the dog is the main reservoir host, although other domestic and wild mammals can act as reservoirs, such as cats, rodents and rabbits/hares.[4]

The clinical manifestations of leishmaniasis are diverse, with two main forms being prevalent worldwide: cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). In humans, *L. infantum* can cause both forms: CL, which is characterised by skin ulcers, and VL, also known as kala-azar, which is potentially fatal if untreated.[5 6] The infection may progress asymptomatically or evolve into severe complications, including hospitalisation and death.[7] The vector of *Leishmania*, the sand fly, is present in rural, peri-urban and urban environments.[8]

The presence and distribution of vectors are increasing globally due to various factors, including climate change, globalisation, the rise of global trade, international travel, migration and urbanisation.[9–12] These factors have led to an increase in the incidence of human leishmaniasis, even in areas where it was previously unknown.[4] VL, in particular, poses epidemic and mortality potential. The WHO estimates there are between 50 000 and 90 000 new cases globally each year, of which only 25%–45% are reported. For CL, 95% of global cases are concentrated in the Americas, the Mediterranean basin, the Middle East and Central Asia, with an estimated incidence of 600 000 to 1 million new cases annually, of which only about 200 000 are reported to the WHO.[13]

In the Tuscany region, the largest in central Italy and the fifth largest by surface in Italy,[14] there is a sustained circulation of the *L. infantum* parasite, with official reports indicating an increase in cases, particularly in the last 3 years.[15] Yet for at least the past 10 years, a discrepancy has been evident between the number of leishmaniasis-related hospitalisations that are reported and the number of official notifications. Despite the mandatory notification of human leishmaniasis cases in Italy, as established by Royal Decree in 1934,[16] official records report an average of only half the number of notifications compared with hospitalisations for both the cutaneous and visceral forms each year, indicating significant systemic under-reporting.[15] Leishmaniasis notifications in Tuscany are collected at the Local Health Unit (LHU) level, regionally aggregated and transmitted to the Ministry of Health. However, there is a reporting gap even at the WHO level, as evidenced by the observation that in 2022, for VL, 26 cases and 35 hospitalisations were reported in Tuscany,[15] while the WHO's Global Health Observatory reported only 33 cases for the entire country of Italy for the same year,[17] indicating evidently inaccurate surveillance. This highlights the significant gap in the surveillance of this disease. Epidemiological surveillance systems are one of the cornerstones of public health protection and the principal tool of prevention and control in the fight against transmissible infectious diseases. Health surveillance involves the systematic and continuous collection, analysis and interpretation of data to provide information for targeted interventions.[18] Detection of under-reporting is therefore a prerequisite to more efficient methods of surveillance. This is critical for monitoring disease trends, making targeted and effective public health policy and determining the impact of measures instituted. This need is even more compelling when, as with *L. infantum*, the pathogen cycle involves not only humans but also dogs and other mammals. Regarding regional data, the number of reported hospitalisations is the closest reflection of the actual confirmed cases identified. However, since many cases involve the cutaneous form, the disease does not always result in hospitalisation, which reduces the likelihood of notification. This can particularly be observed among children, who are treated almost solely on a day-hospital or outpatient basis. In any case, diagnosis is primarily established through laboratory tests and must be reported by the physician who issued the prescription. This case highlights the need for improved epidemiological surveillance and a composite system that includes not only hospital and official notification figures but also laboratory diagnosis.

The Tuscany region is administratively divided into three health macro-areas/districts,[19] comprising several provinces and served by an LHU. The most populous of them is the Central Tuscany Health District (figure 1) comprising the provinces of Florence, Prato, Pistoia and Empoli, with a population of approximately 1.6 million people.[20] It includes the hospitals of the Tuscany Central-LHU (TC-LHU), two university hospitals: Careggi University Hospital, a pole of high specialisation and clinical research, and Meyer Children's Hospital IRCCS (Istituto di Ricovero e Cura a Carattere Scientifico, Scientific Institute for Research - Hospitalization and Healthcare), a regional and national reference for child health. This territorial configuration represents the demographic and healthcare core of Tuscany. Involving all hospitals within the Central Tuscany Health District and covering the period from January 2014 to December 2024, the primary objective of this study is to estimate the degree of under-reporting through the analysis of laboratory tests used to identify confirmed cases of all types of leishmaniasis, comparing these results with official notification and hospitalisation data. The secondary objective is a descriptive analysis of the comorbidities and demographic characteristics of individuals who underwent any of the laboratory tests necessary to be identified as a confirmed case. In addition, geolocation analysis will be performed to better understand the spatial distribution of cases. This information will be invaluable to precisely mapping the

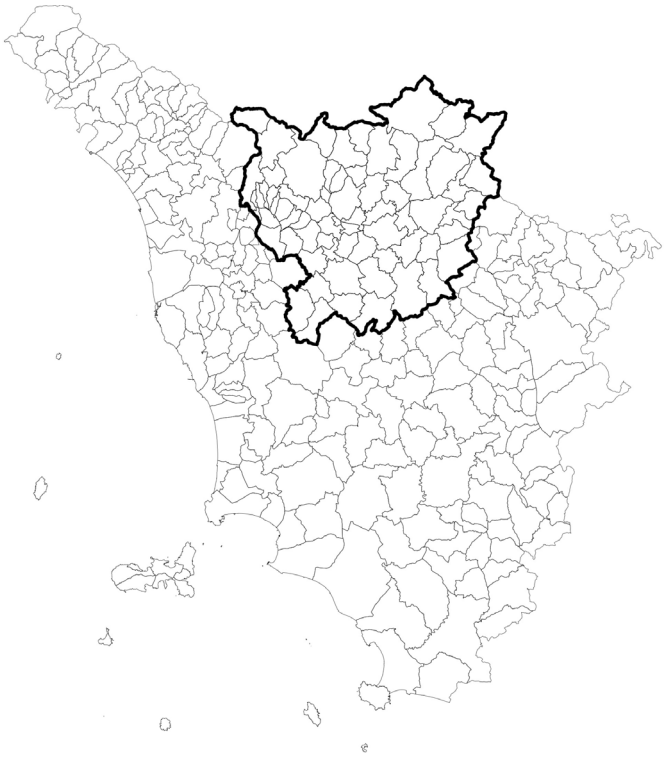

Figure 1 Study area: Tuscany (municipality boundaries) with Central Tuscany Health District outlined in bold.

epidemiological landscape of human leishmaniasis occurrence in the Tuscany region, with significant information regarding potential geographic distributions and risk zones. Consequently, it will provide a basis for evaluating the effectiveness of current prevention measures and identifying potential areas for enhancing strategies across human, animal and environmental health.

METHODS AND ANALYSIS

Study design

This is an observational, retrospective, multicentre study, conducted by the Department of Health Sciences at the University of Florence in collaboration with the Tuscany Central-LHU, Careggi University Hospital, Meyer Children's Hospital IRCCS and the Regional Health Agency of Tuscany (RHA). The planned study period runs from November 2025 to March 2026.

Patient and public involvement

None.

Study objectives

Primary objective: Estimate the extent of underreporting of leishmaniasis in the Central Tuscany Health District from January 2014 to December 2024 using capture-recapture models.

Secondary objective: Conduct a descriptive analysis of the comorbidities and demographic characteristics of individuals who underwent any of the laboratory tests required to be identified as a confirmed case from January 2014 to December 2024, along with a geolocation analysis to assess the spatial distribution of cases.

Study setting

The study will be conducted within the Central Tuscany Health District, which includes both adult and paediatric populations.

Study population

The study population consists of all individuals, both adults and children (of any age), who were identified as confirmed cases of leishmaniasis from January 2014 to December 2024 within the Central Tuscany Health District. Cases will be identified through at least one of the following three sources:

- Case notification;
- Laboratory analysis;
- Hospital discharge Records.

Surveillance case definitions

The definition of a 'confirmed case' of human leishmaniasis, according to the guidelines of the Italian Ministry of Health,[21] requires the presence of a laboratory diagnosis, specifically for:

- *VL*, the presence of at least one of the following elements: parasitological examination under a microscope showing amastigotes in aspirate or biopsy smears (after Giemsa or Wright-Giemsa staining); culture examination from aspirate, biopsy and/or peripheral blood positive for the presence of promastigotes; identification of *Leishmania* nucleic acid in aspirate, biopsy and/or peripheral blood samples by PCR; and positivity in quantitative serological tests for specific antibodies by ELISA or indirect ammunofluorescent antibody test.
- *CL*, the presence of at least one of the following elements: parasitological examination under a microscope showing amastigotes in smears or biopsy sections; culture examination from biopsy material positive for the presence of promastigotes; and identification of *Leishmania* nucleic acid in biopsy samples by PCR.

Data sources and criteria for inclusion and exclusion of cases

For the population within the care of TC-LHU, all individuals registered in the electronic health record system 'Concerto-Dedalus' who are identified as leishmaniasis confirmed cases following laboratory analysis will be included. Additionally, data for hospitalised individuals with a primary or secondary diagnosis of leishmaniasis, identified by the International Classification of Diseases, 9th revision, Clinical Modification (ICD9-CM) codes[22] 0851, 0852, 0853, 0854, 0855, 0859 (CL) and 0850 (VL), will be extracted from the HDRs.

For the population within the care of Careggi University Hospital, all individuals registered in the "Archimed" electronic system who are identified as leishmaniasis confirmed cases following laboratory analysis will be included. Hospitalised individuals with a diagnosis of

leishmaniasis, confirmed by the ICD9-CM codes for cutaneous and visceral forms, will also be included, with data extracted from the HDRs.

For the population within the care of Meyer Children's Hospital IRCCS, individuals registered in the 'DNAlab' and 'C7' electronic systems who are identified as leishmaniasis confirmed cases following laboratory analysis will be included. Data for hospitalised individuals with a diagnosis of leishmaniasis, using the ICD9-CM codes for CL and VL, will be reported from the HDRs.

For the notification flow, individuals registered in the Infectious Diseases Information System 'PREMAL' since 2019, and 'SIMI' for years prior to 2019, notified as leishmaniasis confirmed cases, will be included.

All individuals who cannot be identified as leishmaniasis confirmed cases using the 'Concerto-Dedalus', 'Archimed', 'DNAlab', 'C7', 'PREMAL/SIMI' or 'HDRs' systems, according to the inclusion criteria described above, will be excluded from the study.

If the same individual has been hospitalised multiple times, they will be included in the analysis only once (at the first hospitalisation; therefore, reference will be made to hospitalised patients rather than hospitalisations).

Since reported cases are recorded within the regional territory of Tuscany (which may include cases of disease reported for individuals not residing in Tuscany, but not those residing in Tuscany whose disease notification occurred in another region), in order to ensure a homogeneous comparison of data from different sources, only patients hospitalised within the regional territory will be selected, regardless of their place of residence.

Variables

The individual data for subjects identified as confirmed cases of leishmaniasis will be provided in pseudonymised form and will include the following variables:

- Sex (for all cases across all data sources).
- Date of birth (or age, if the date of birth is not available, for all cases across all data sources).
- Address (or residence, if the address is not available, for all cases across all data sources).
- Date of first positive result (for cases identified through laboratory testing).
- Description of the test (for cases identified through laboratory testing).
- Previous medical conditions (for cases identified through laboratory testing or hospitalisations).
- Notification date (for cases reported through the surveillance system).
- Date of diagnosis (for hospitalised cases).

Data management

The data will be stored in a database 'Research Electronic Data Capture' (REDCap platform)[23] with a personal code (Subject ID) assigned by the investigators. Access to REDCap is protected by temporary user credentials. The collected information will be stored in a way that ensures access is granted only to authorised individuals. The individuals responsible for pseudonymisation are the scientific supervisor and the investigators from the various participating centres, with a data processing officer appointed for RHA. The data will be processed in accordance with current regulations, transmitted via a data collection form on REDCap and stored on a password-protected removable disk. Prior to analysis, the datasets were subjected to standard data cleaning procedures. These included the removal of duplicate records, consistency checks across data sources (eg, matching on date of birth, sex and location) and removal of records that contained missing key variables required for case confirmation.

Statistical analysis

The main sociodemographic characteristics, comorbidities, laboratory diagnosis and geolocation data will be analysed using descriptive statistics. Specifically, frequency tables will be created for categorical variables, and tables containing the mean, SD, median, minimum and maximum values will be generated for continuous variables.

For each year (and for TC-LHU, Careggi University Hospital and Meyer Children's Hospital IRCCS), the number of cases and their incidence will be calculated (separately for each source). Using only laboratory data, the trend of the disease over time will be estimated by calculating the annual percentage variation in case notification rates. Specifically, a method commonly applied when working with surveillance data will be used, which involves performing linear regression with the logarithm of the annual incidence rates as the outcome and the year of observation as the only covariate.

Differences in cases between the various sources will be measured using statistical tests (analysis of variance/Kruskal-Wallis).

The records from the three data sources will be linked based on the date of birth (or age), gender, location and the period of data collection. The total number of cases will then be calculated, estimating the overlaps between the different databases. A Venn diagram will be created to present the number (and percentage) of leishmaniasis cases identified by the three sources from 2014 to 2024: subjects identified through laboratory tests from TC-LHU, Careggi University Hospital, Meyer Children's Hospital IRCCS, cases notified by PREMAL/SIMI, and the HDR flow, along with all overlaps.

We will use a deterministic, hierarchical linkage with blocking on date of birth and sex. The primary rule requires exact agreement on date of birth, sex and municipality plus temporal proximity of the index date (first positive test/diagnosis/notification). A secondary rule (for missing fields) will require agreement on ≥3 of 4 fields (date of birth/age band, sex, municipality/facility, index-date proximity). Borderline pairs will undergo independent clerical review with adjudication. Deduplication will collapse multiple records that refer to the same person and episode within sources. Across sources, linked

records will define a single case. Operational thresholds for temporal proximity will be pre-specified before analysis and explored in sensitivity analyses.

After linkage, all overlap patterns (cases present in one, two or all three sources) will be summarised. Standard capture–recapture models will then be used to estimate how many cases were likely missed by each source and to derive overall and source-specific completeness with uncertainty ranges. To address key assumptions, analyses will be performed by calendar year (to approximate a closed population), with predefined quality checks on record linkage and sensitivity analyses using stricter and looser matching rules. Potential dependence between sources and differences in case detectability will be explored by comparing alternative model structures and by stratifying results (clinical form, age, sex, period). Specifically, we will fit three-source log-linear capture–recapture models. We will start from an independence model (no interactions) and then test pairwise interaction terms (laboratory × hospital, laboratory × notification, hospital × notification) to assess and, if needed, account for dependence between sources. Model selection will rely on Akaike Information Criterion, with Bayesian Information Criterion as a check, and goodness-of-fit tests, while retained interactions will be reported as evidence of dependence. The selected model will provide estimates of missed cases and overall and source-specific completeness with 95% CIs.

Variables used for linkage and modelling were available and harmonised across SIMI and PREMAL, so the 2019 platform migration is not expected to introduce structural changes in underreporting estimates.

In epidemiological studies, the capture–recapture method has been applied to estimate the true incidence of diseases by linking data from different sources, such as national surveillance databases and HDRs. This technique has been successfully used to assess underreporting in diseases like leishmaniasis in Spain and invasive pneumococcal disease in Italy.[24 25]

No imputation will be performed for missing data. Capture–recapture relies on observed overlaps. Imputing list membership or linkage fields may introduce false matches and bias underreporting estimates. Variables not used for linkage/modelling will have 'unknown' reported explicitly, with denominators stated. Records with missing stratifiers will contribute to overall analyses but not to that specific stratum.

The analyses will be conducted using the statistical software STATA 16.

Figure 2 provides a visual summary of sources, linkage/deduplication, capture histories, analyses and outputs.

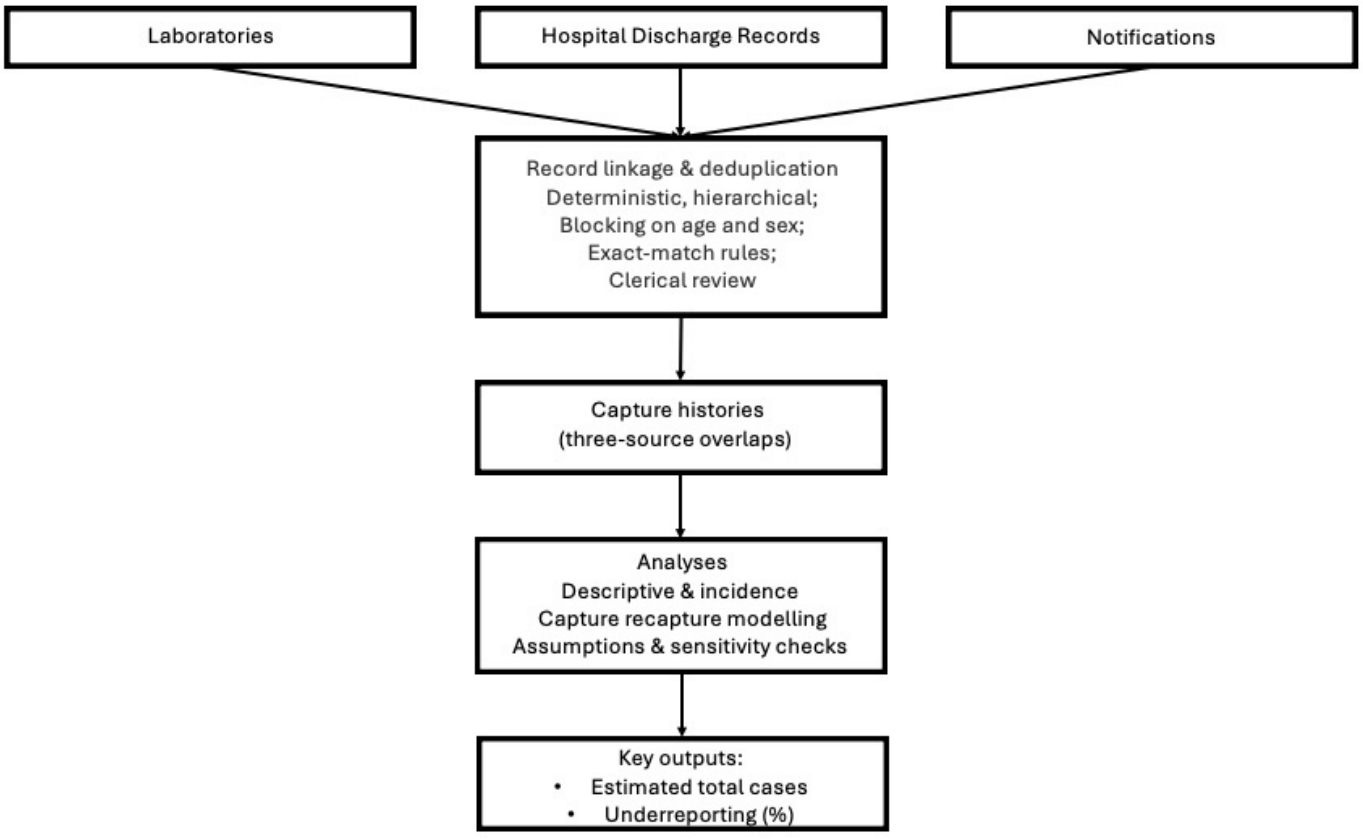

Figure 2 Study flow and record linkage. Records from laboratories, hospital discharges and notifications are deduplicated and linked deterministically to build three-source capture histories, which feed descriptive/incidence analyses and capture–recapture estimates of total cases and notification/source completeness.

Ethics and dissemination

The study is being conducted in accordance with the protocol approved by the Ethics Committee of the Tuscany Region – Pediatrics Section, in November 2024. Ethics Committee opinion register number: 219/2024. Because the study uses only pseudonymised, routinely collected administrative and laboratory data with no direct patient contact or intervention, individual informed consent was not required, as confirmed by the Ethics Committee. Findings will be submitted to a peer-reviewed journal, presented at international conferences and presented at stakeholder workshops.

Expected results

Our study hypothesises that the systematic underreporting of human leishmaniasis cases leads to official statistics that significantly underestimate the true extent of the disease. The use of capture–recapture models is expected to provide clearer insights into the true incidence of the disease, suggesting that the actual number of cases could potentially be at least double the currently reported figures.

Additionally, the descriptive analysis should offer a detailed overview of the sociodemographic and comorbidities of patients, allowing the identification of risk factors associated with the disease and revealing trends related to geographic distribution, age, sex and comorbidities. The extent of the gaps in the current surveillance system could contribute to the development of more effective and targeted health strategies.

DISCUSSION

The results of this study will be essential for accurately assessing the true epidemiological situation of confirmed leishmaniasis cases in Tuscany, as well as evaluating the effectiveness of the current surveillance system in the region. Confirmation of significant underreporting in Tuscany would place a priority on having multiple and combined surveillance systems that incorporate laboratory data consistently with official and hospital data. This can also be applied to other infectious conditions, strengthening the overall monitoring and response capability of the healthcare system.

Leishmaniasis is a disease that affects humans, as well as other animals. In Italy, *L. infantum* has one of its main reservoirs in dogs, and its vector, the phlebotomine sand fly, is expanding its boundaries not only geographically but also temporally, remaining active for a longer period during the year.[26] For this reason, it is fundamental that the human case surveillance system is not only efficient, but also integrated with equally robust veterinary and entomological surveillance systems: this would create a highly responsive, dynamic integrated surveillance system, useful for protecting both human and animal health. In this regard, it is important to stimulate debate among public health professionals, clinicians, veterinarians and entomologists to work in a coordinated manner to implement the best policies for protecting the health of local citizens and animals.[4]

A striking example of the importance of effective surveillance is the leishmaniasis outbreak that occurred between 2009 and 2012 in the suburbs of Madrid, in Fuenlabrada. Without timely surveillance, the epidemic could have persisted longer, delaying the implementation of effective control measures.[27]

With a better understanding of the actual burden of leishmaniasis, our results could encourage local health authorities to reorganise resources allocated for prevention efforts, to protect not only the local population but also animals, particularly dogs. Targeted interventions could include awareness campaigns for the public and those in close contact with animals, prevention and treatment programmes, enhancements to diagnostic pathways and environmental measures focused on entomological monitoring and control. It is indeed proven that there is a need for the general population to become more aware of the disease in humans, as European studies confirm that the disease in humans is less known compared with that in dogs.[28] All these interventions together would be essential for reducing the incidence of the disease and limiting its health and economic consequences. Moreover, identifying potential geographical or socioeconomic disparities in the distribution of cases could provide useful insights for more equitable health policies. For instance, identifying transmission hotspots could facilitate targeted interventions, such as reinforcing healthcare infrastructure in affected areas.

This study could, therefore, serve as a model for other regions. There are systematic information gaps in the surveillance system, not only in Tuscany but also among the data flows from other Italian regions, as well as between the Ministry of Health's data and the final data available in the WHO Global Health Observatory. This situation highlights the widespread nature of underreporting at the national level, with notable rates of underreporting of leishmaniasis commonly observed across the Mediterranean Basin.[29 30]

Our study is multicentric, which provides a major strength by integrating data from various sources, offering the most comprehensive possible view of the epidemiology of leishmaniasis. Moreover, the adoption of the capture-recapture method to estimate underreporting represents an innovative approach, well-established in other sectors but still rarely used for vector-borne diseases in Italy.

This method is preferable to conventional single-source counts or simple ratios because it uses the overlaps between sources to estimate the cases that none of the sources captured, and it quantifies how complete each source is. In a setting like Tuscany, where laboratories, hospital records and notifications contribute different pieces of information, capture–recapture provides a transparent estimate of unseen cases and a practical basis to improve surveillance.

However, there is a risk of information bias, mainly due to missing data: some patients may have undergone tests

or hospitalisation in a different area from the Central Tuscany Health District.

We expect that the findings from this study will encourage further research and investment in the surveillance sector: it is from this point that public health decisions regarding investments and the organisation of resources for disease prevention and control are made. In the case of leishmaniasis, it is also crucial to promote a 'One Health' approach that considers human, animal and environmental health as interconnected systems and acts accordingly through an efficient and integrated surveillance system.

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Contributors CC and MDR contributed to the conception, overall design and planning of the study. MDR, CC and FP contributed to the conception and design of the methods section. MDR, CC and FP contributed to the design of the methods section and primarily focused on how the data should be collected and interpreted. CC, CM, GB, PB, LB, TB, MI, MM, FV, MS, LZ, AB, FM, EV, SR, LG, FP, FV and MDR contributed to writing the protocol. All authors revised this version of the protocol and gave final approval for it to be published. All authors ensure that questions related to the accuracy or integrity of any part of this protocol are appropriately investigated and resolved. CC is the guarantor of the work, taking responsibility for the integrity of the study and the accuracy of the data and analysis.

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REFERENCES

1 World Health Organization. Neglected tropical diseases. Available: https://www.who.int/health-topics/neglected-tropical-diseases#tab=tab_1 [Accessed 20 Mar 2025].
2 World Health Organization. The global health observatory. Leishmaniasis. Available: https://www.who.int/data/gho/data/themes/topics/gho-ntd-leishmaniasis [Accessed 20 Mar 2025].
3 Maia C, Conceição C, Pereira A, *et al*. The estimated distribution of autochthonous leishmaniasis by Leishmania infantum in Europe in 2005-2020. *PLoS Negl Trop Dis* 2023;17:e0011497.
4 Cosma C, Maia C, Khan N, *et al*. Leishmaniasis in Humans and Animals: A One Health Approach for Surveillance, Prevention and Control in a Changing World. *TropicalMed* 2024;9:258.
5 Europe Centre for Disease Prevention and Control. Phlebotomine sand flies - factsheet for experts. Available: https://www.ecdc.europa.eu/en/disease-vectors/facts/phlebotomine-sand-flies [Accessed 20 Mar 2025].
6 van Griensven J, Diro E. Visceral Leishmaniasis. *Infect Dis Clin North Am* 2019;33:79–99.
7 Goto H, Lindoso JAL. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. *Expert Rev Anti Infect Ther* 2010;8:419–33.
8 Pradhan S, Schwartz RA, Patil A, *et al*. Treatment options for leishmaniasis. *Clin Experimental Derm* 2022;47:516–21.
9 World Health Organization. Leishmaniasis. Available: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis [Accessed 20 Mar 2025].
10 Pavli A, Maltezou HC. Leishmaniasis, an emerging infection in travelers. *Int J Infect Dis* 2010;14:e1032–9.
11 Hotez PJ. The rise of leishmaniasis in the twenty-first century. *Trans R Soc Trop Med Hyg* 2018;112:421–2.
12 Trájer AJ, Grmasha RA. The potential effects of climate change on the climatic suitability patterns of the Western Asian vectors and parasites of cutaneous leishmaniasis in the mid- and late twenty-first century. *Theor Appl Climatol* 2024;155:1897–914.
13 Carvalho BM, Maia C, Courtenay O, *et al*. A climatic suitability indicator to support *Leishmania infantum* surveillance in Europe: a modelling study. *Lancet Reg Health Eur* 2024;43:100971.
14 Istituto Nazionale di Statistica. Superficie territoriale e urbanizzazione. n.d. Available: https://www.istat.it/non-categorizzato/principali-dimensioni-geostatistiche-e-grado-di-urbanizzazione-del-paese/
15 Agenzia Regionale di Sanità Toscana. La sorveglianza epidemiologica delle malattie infettive in toscana. 2022. Available: https://www.ars.toscana.it/images/pubblicazioni/Rapporti/2023/Rapporto_malattie_infettive_2022.pdf https://www.who.int/news-room/fact-sheets/detail/leishmaniasis [Accessed 20 Mar 2025].
16 Presidency of the Council of Ministers. Royal decree 1934, n. 1265. 1934. Available: https://www.normattiva.it/uri-res/N2Ls?urn:nir:stato:regio.decreto:1934-07-27;1265!vig= [Accessed 20 Mar 2025].
17 World Health Organization. Leishmaniasis. Status of endemicity of cutaneous leishmaniasis. Available: https://apps.who.int/neglected_diseases/ntddata/leishmaniasis/leishmaniasis.html [Accessed 20 Mar 2025].
18 Regione Toscana. Aziende sanitarie. Available: https://www.regione.toscana.it/sst/organizzazione/aziende-sanitarie [Accessed 20 Mar 2025].
19 Agenzia Regionale di Sanità Toscana. Popolazione residente. Available: https://ars.toscana.it/banche-dati/dettaglio_indicatore-1442-popolazione-residente?dettaglio=ric_anno_geo_ausl&par_top_geografia=201&provenienza=comuni_elenco_indicatori_sintesi&utm [Accessed 20 Mar 2025].
20 Commissione Europea. Surveillance and early warning. Available: https://health.ec.europa.eu/health-security-and-infectious-diseases/surveillance-and-early-warning_it?utm_s [Accessed 20 Mar 2025].
21 Italian Ministry of Health. Prevenzione e Controllo Della Leishmaniosi in Italia. Available: https://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2020&codLeg=77839&parte=1%20&serie=null [Accessed 20 Mar 2025].
22 Gaspari V, Gritti T, Ortalli M, *et al*. Tegumentary Leishmaniasis in Northeastern Italy from 2017 to 2020: A Neglected Public Health Issue. *Int J Environ Res Public Health* 2022;19:16047.
23 Europe Centre for Disease Prevention and Control. Surveillance, prevention and control of leishmaniases in the European union and its neighbouring countries. Available: https://www.ecdc.europa.eu/sites/default/files/documents/leishmaniasis-surveillance-eu.pdf [Accessed 20 Mar 2025].
24 CDC Archive. International classification of diseases, ninth revision, clinical modification (ICD-9-CM). Available: https://archive.cdc.gov/www_cdc_gov/nchs/icd/icd9cm.htm#:~:text=ICD%2D9%2DCM%20is%

20the,10%20for%20mortality%20coding%20started [Accessed 20 Mar 2025].
25 Research electronic data capture. Available: https://project-redcap.org [Accessed 20 Mar 2025].
26 Humanes-Navarro AM, Herrador Z, Redondo L, *et al*. Estimating human leishmaniasis burden in Spain using the capture-recapture method, 2016-2017. *PLoS One* 2021;16:e0259225.
27 Quattrone F, Donzelli G, D'Arienzo S, *et al*. Invasive Pneumococcal Disease in Tuscany Region, Italy, 2016-2017: Integrating Multiple Data Sources to Investigate Underreporting. *Int J Environ Res Public Health* 2020;17:7581.
28 Maia C. Sand fly-borne diseases in Europe: epidemiological overview and potential triggers for their emergence and re-emergence. *J Comp Pathol* 2024;209:6–12.
29 Arce A, Estirado A, Ordobas M, *et al*. Re-emergence of leishmaniasis in Spain: community outbreak in Madrid, Spain, 2009 to 2012. *Euro Surveill* 2013;18:20546.
30 Rocha R, Conceição C, Gonçalves L, *et al*. Knowledge and practices regarding leishmaniasis in Portugal - Comparison between blood donors and health students/professionals. *One Health* 2024;18:100697.