Journal of Infection and Public Health 18 (2025) 102691

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: www.elsevier.com/locate/jiph

Review

An insight into brucellosis in wild boar and domestic pigs in Europe: A systematic review

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A R T I C L E I N F O

Article history:
Received 22 September 2024
Received in revised form 27 January 2025
Accepted 28 January 2025

Keywords:
Brucella
Sus scrofa
Sus scrofa domesticus
Swine
One health

A B S T R A C T

In some European countries, animal brucellosis is a concern that requires a comprehensive understanding of its distribution, hosts and transmission routes, particularly involving wild species, in which the disease is still neglected. This systematic review was designed to summarize the current knowledge on the epidemiology of brucellosis in wild boar and domestic pigs in Europe. A systematic search was conducted in four databases to identify relevant original research articles. Thirty-six articles were included. Brucellosis has been identified in 17 European countries and the most reported circulating *Brucella* species and biovar was *Brucella suis* biovar 2. *Brucella suis* biovar 1, *Brucella suis* biovar 3, *Brucella melitensis* biovar 3 and *Brucella microti* were also identified from molecular investigations. We highlight the diversity in the occurrence of this infection, with the presence of *Brucella* species with zoonotic potential, and emphasize the need for surveillance.

Contents

Introduction

Brucellosis remains an animal health concern with public health relevance [1]. Within the Mediterranean region, particularly affected by brucellosis in small ruminants [2], Spain and Croatia have practically eradicated the disease, while in Greece, Italy and Portugal

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https://doi.org/10.1016/j.jiph.2025.102691

continues to be a veterinary and public health concern [3]. Among the various species of *Brucella* (*B.*). *B. melitensis*, *B. abortus*, *B. canis* and *B. suis* are those that cause most impact on human health [3].

Efforts have been made to eradicate brucellosis in small ruminants in Europe [3], however swine brucellosis remains neglected [4]. In Europe, *B. suis* biovar 2 is endemic in wild boar (*Sus scrofa*) and they may act as sources of brucellosis contagion for domestic pigs (*Sus scrofa domesticus*) [5].

The transmission of *Brucella* spp. occurs through direct contact [6], indirectly via contaminated fomites [7] and through environmental contamination [8]. Biological secretions from infected animals, such as secretions from the reproductive tract, milk, aerosols and abortive materials are sources of transmission [6]. The prevalence of brucellosis should not be neglected, it is considered a major cause of reproductive failure in pigs worldwide [9–12]. The disease is characterized to be responsible for substantial reproductive disorders and production losses. Chronic inflammatory lesions in the reproductive organs and joints are common, because the bacteria infect and persist in many organs, including lymph nodes, joint fluids, bones, and reproductive organs [13].

Considering the significant impact of brucellosis on both veterinary and public health, it is important to investigate the present disease situation that can provide important insights, helping implement public and animal health control strategies from a One Health perspective. Few reviews and systematic reviews appraised and reported the distribution, genetic diversity and prevalence of brucellosis in wild boar and domestic pigs [14–18]. Thus, we conducted a systematic review with the following aims: (i) to update and compile current studies that reported brucellosis in wild boar and domestic pigs, (ii) to identify the European countries where brucellosis has been reported in these animals and (iii) to identify the species of *Brucella* spp. involved in these animals' cases of disease since the year 2000. This will improve our understanding of *Brucella* spp. range in wild boar and domestic pigs, their distribution in Europe and the diversity of species detected in these animal host.

Methods

Protocol and registry

The protocol used for this study has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the ID number CRD42024542009 at https://www.crd.york.ac.uk/Prospero.

Data sources and search strategy

Epidemiologically, brucellosis is a worldwide infection, which infects animals and humans. The research questions were "What is the current epidemiological situation of brucellosis in wild boar and domestic pigs in Europe? and "Which *Brucella* species are involved?".

To develop search expressions, the "PICO" method was established, as shown in Table 1**.**

The literature search strategy was developed systematically on the detection and diagnosis of circulating *Brucella* spp. and the geographical distribution of brucellosis in wild boar and domestic pigs in Europe. The databases included for search were PubMed, Scopus, Web of Science and ResearchGate. Synonyms for relevant search terms were identified using the Medical Subject Headings (MeSH). During an online search, the Boolean operators "AND/OR" were employed to find relevant findings by combining similar phrases/words. The search strategy is reported in Appendix A. Screening in the ResearchGate database was performed using the following keywords: *Brucella*, brucellosis, epidemiology, prevalence, occurrence, diagnostic, wild boar, pig, swine and Europe. We conducted a final search on 15th May 2024.

Table 1
Eligibility criteria used for the screening of studies.

Item	Definition
Population (P)	Wild boar and domestic pigs
Intervention/exposure (I)	Infection with *Brucella* spp.
Comparison (C)	No comparator
Outcome (O)	Swine brucellosis disease in Europe: diagnosis of the disease in European countries

All articles were imported into the online tool Ryyan (https://www.rayyan.ai/), to facilitate and organize the systematic literature review process [19].

Inclusion and exclusion criteria

Eligible articles were screened according to the inclusion and exclusion criteria below. Inclusion criteria were: (i) the study population was limited to wild boar and domestic pigs, (ii) published full-text on the diagnosis of brucellosis in these animals in European countries, (iii) any observational studies and case studies (iv) articles published between 1st January 2000 until 30th April 2024 to generate contemporary data on brucellosis, (v) articles written in English, Spanish and Portuguese.

Exclusion criteria were: (i) studies reporting results outside the scope of the study questions (including experimental studies and review articles), (ii) wrong population, (iii) studies conducted outside of Europe, (iv) no information about brucellosis, (v) written on other languages, (vi) articles published outside of study periods, (vii) duplicate articles, (viii) articles that cannot be downloaded and (ix) other types of publications that did not meet the inclusion criteria.

Data extraction

A comprehensive search strategy in multiple databases was conducted for this systematic review to achieve precision in the retrieval of studies and to minimize potential selection bias. Data was collected independently by ZMR. Firstly, arranged systematically based on the title and abstract, title and abstracts derived through the primary electronic search were thoroughly assessed for the possibility of inclusion based on the study type. Unclear information in the title and abstract was a factor that led to a full reading of the text. The second stage involved checking the type of article against a list of inclusion and exclusion criteria. For this purpose, the citations were exported from the databases, in the format supported by the software, for subsequent import to Ryyan Systems Inc., web tool for efficient organization for screening, documenting the reasons for exclusion and deleting duplicate articles. Two researchers (ZMR and TLM) performed both screening independently in Ryyan Systems Inc. Disagreements between reviewers were discussed and resolved by consensus between ZMR, TLM and MVP. From each eligible article, data on the article title, authors, year of publication, country, studied species (wild boar and/or domestic pigs), sample type, number of animals tested, number of positives, diagnostic method, *Brucella* species identified, clinical presentation and pathological findings when applicable, were extracted and organized using Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA). For articles to which we didn't have access, the articles were requested from authors and articles that were not sent were considered excluded. References were saved in Mendeley Reference Manager software (Version 2.120.1, Elsevier).

To achieve an accurate review, our systematic review followed the criteria described in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Appendix B) [20].

Results

Search summary of the systematic review

The search strategy identified 7490 articles. The first screening was performed according to the title, abstract and year of publication and 247 articles were eligible for full-text screening. We were unable to retrieve five articles from the databases. The authors of these articles were asked by email whether they could provide the full text of their study, but no response was given.

The specific flow chart is shown in Fig. 1.

Application of inclusion and exclusion criteria allowed the identification of 36 articles potentially suitable for the systematic review. There was a clear dominance of articles carried out in wild boar. Out of the 36 included studies in the systematic review, nineteen studies were performed in wild boar, seven in domestic pigs and ten articles were performed in wild boar and domestic pigs.

The characteristics of the included articles are provided in Table 2.

Temporally, the pattern of multiple publications is shown in Fig. 2.

The countries for which we identified relevant data of brucellosis in wild boar and domestic pigs are shown in Fig. 3.

Italy was the region most frequently studied in wild boar and domestic pigs with 11 studies, followed by: Croatia (n = 6), Spain (n = 6), Germany (n = 3), Hungary (n = 3), Poland (n = 3), Portugal (n = 3), Switzerland (n = 3), Belgium (n = 2), France (n = 2), Denmark (n = 1), Finland (n = 1), Latvia (n = 1), Netherlands (n = 1), Romania (n = 1), Serbia (n = 1) and Slovenia (n = 1).

Brucellosis in wild boar publications were identified across 14 European countries: Italy (n = 9), Spain (n = 4), Croatia (n = 3), Germany (n = 3), Hungary (n = 3), Poland (n = 3), Portugal (n = 3), Belgium (n = 2), France (n = 2), Switzerland (n = 2), Finland (n = 1), Latvia (n = 1), Netherlands (n = 1) and Slovenia (n = 1). While

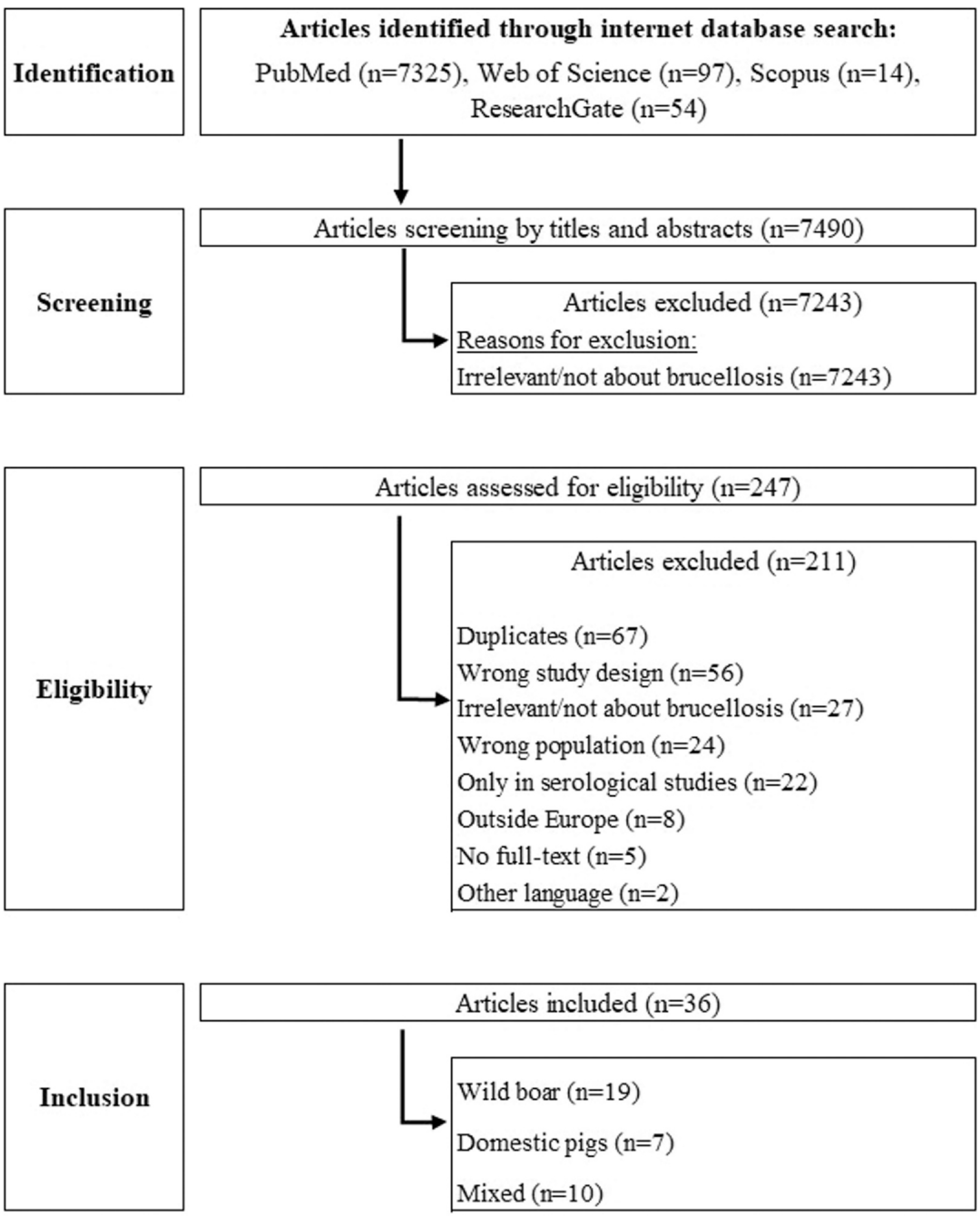

Fig. 1. Flow diagram of eligible studies for searching and selecting.

Table 2
Brucellosis in wild boar and domestic pigs in Europe.

Ref.	Year of publication	Country	Animals	Type of sample	Serological tests (positive reactions / tested)	Culture (positive / tested)	Detection method (positive / tested)	Genus, species and biovar	Clinical presentation	Necropsy / Pathological findings
[21]	2004	Croatia	Wild boar	Serum (n = 514), uterus (n = 38), testicles (n = 67) and fetuses (n = 5)	iELISA: (70/514) RBT: (59/514) CFT: (54/514) SAT: (46/514)	√ (18/106 wild boar)	PCR (18/18 wild boar)	*B. suis* biovar 2	-	-
[22]	2007	Switzerland	Wild boar	Serum (n = 1826), reproductive organs (n = 50) and spleen (n = 62)	iELISA: (180/1826)	√ (1 uterus and 1 accessory sexual gland/ 16; 1 spleen/28)	PCR-RFLP (1 uterus and 1 accessory sexual gland/1; 1 spleen/1)	*B. suis* biovar 2	-	-
[23]	2009	Croatia	Wild boar Domestic pigs	Serum (n = 424), uterus (n = 45), testicles (n = 48) and lymph nodes[1]) (n = 42) Serum (n = 93,107), uterus (n = 485), testicles (n = 54), lymph nodes[1]) (n = 66) and aborted piglets (n = 12)	iELISA, RBT and CFT: (98/424) iELISA, RBT and CFT: (766/93,107)	√ (7/93 wild boar) √ (88/151 serologically positive pigs)	PCR (7/7 wild boar) PCR (88/88 domestic pigs)	*B. suis* biovar 2 (6/7) *B. suis* biovar 3 (1/7) *B. suis* biovar 2 (86/88) *B. suis* biovar 3 (2/88)	-	√
[24]	2009	Italy	Wild boar	Serum (n = 2267). Uterus, testicles and spleen (n = 1841)	RBT and CFT: (448/2267)	√ (448/1841 wild boar)	PCR (448/448 wild boar)	*B. suis* biovar 2	-	-
[25]	2009	Poland	Wild boar	Lymph nodes (n = 235)	-	√ (25/235 wild boar)	PCR (27/235 wild boar) AMOS-PCR (0/235 wild boar)	*B. suis* biovar 2	-	-
[26]	2010	Portugal and Spain	Wild boar	Serum, uterus, testicles, spleen and lymph nodes[2]) (n = 4454)	iELISA: (1470/4454)	√ (104/589 wild boar)	Multiplex PCR and PCR-RFLP (104/104 wild boar)	*B. suis* biovar 2	-	-
[27]	2010	Croatia	Domestic pigs	Serum (n = 28,520). Uterus, testicles, spleen, lymph nodes[3]) and liver (n = 23 domestic pigs serologically positive)	iELISA: (178/4280) RBT: (178/28,520)	√ (9/23 serologically positive pigs)	PCR (9/9 domestic pigs)	*B. suis* biovar 2	-	-
[28]	2010	Spain	Wild boar	Serum (n = 256) and spleen (n = 69)	iELISA: (28/256)	√ (1/14 spleen from seropositive wild boar)	AMOS-PCR and PCR-RFLP (1/1 spleen)	*B. suis* biovar 2	-	-
[29]	2011	Switzerland	Wild boar	Serum, spleen, reproductive organs, urine and blood (n = 252)	cELISA: (45/238) RBT: (63/233)	√ (20/244) (14/242 spleen; 1/15 reproductive organs and 9/135 urine)	RT-PCR and MLVA (64/243) (43/228 spleen; 8/99 reproductive organs and 19/187 blood)	*B. suis* biovar 2	-	-
[30]	2012	Italy	Wild boar	Lymph nodes[4]) (n = 1)	-	√ (1/1 wild boar)	AMOS-PCR and PCR-RFLP (1/1 wild boar)	*B. suis* biovar 2	-	-
[31]	2012	Belgium	Wild boar	Serum (n = 1168). Spleen and tonsils (n = 389)	iELISA: (641/1168) RBT (255/752) CFT (316/739) SAT (188/767)	√ (31/381 spleen and tonsils)	PCR (84/389 spleen and tonsils)	*B. suis* biovar 2	-	-
[32]	2013	Poland	Wild boar Domestic pigs	25 *Brucella* strains 1 *Brucella* strains	-	-	Multiplex PCR and MLVA (25/25 wild boar and 1/1 domestic pigs)	*B. suis* biovar 2	-	-

(*continued on next page*)

Table 2 (*continued*)

Ref.	Year of publication	Country	Animals	Type of sample	Serological tests (positive reactions / tested)	Culture (positive / tested)	Detection method (positive / tested)	Genus, species and biovar	Clinical presentation	Necropsy / Pathological findings
[5]	2014	Hungary	Wild boar Domestic pigs	55 *Brucella* strains 8 *Brucella* strains	-	-	Multiplex PCR and MLVA (55/55 wild boar and 8/8 domestic pigs)	*B. suis* biovar 2	-	-
[33]	2014	Spain	Wild boar	Serum (n = 204), vaginal(n = 103) and testicular (n = 85) swabs	iELISA: (121/204)	√ (7/188 vaginal and testicular swabs)	Multiplex PCR and MLVA (7/7 vaginal and testicular swabs)	*B. suis* biovar 2	-	√
[34]	2015	Italy	Domestic pigs	Serum (n = 28), vaginal swabs (n = 24), fetuses (n = 2), stillborn piglets (n = 9). Spleen, abdominal lymph nodes (n = 2 domestic pigs with the highest CFT titers)	RBT: (25/28) CFT: (22/28)	√ (2/2 domestic pigs)	PCR (2/2 domestic pigs)	*B. suis* biovar 2	√	-
[35]	2015	Italy	Domestic pigs	Serum (n = 1251). Uterus, testicles, spleen, lymph nodes[5]), tonsils, fetuses (brain) (n = 285)	RBT: (406/1251) CFT: (292/748)	√ (6/139 pigs with a ICFTU/ml value ≥160)	PCR (12/54 domestic pigs)	*B. suis* biovar 2	√	-
[36]	2015	Hungary	Wild boar	Submandibular lymph node (n = 1)	-	√ (1/1 wild boar)	Multiplex PCR and MLVA (1/1 wild boar)	*B. microti*	-	-
[37]	2016	Croatia	Domestic pigs	Serum (n = 729). Uterus, testicles, spleen, lymph nodes[3]), liver (N = 50 domestic pigs)	iELISA: 239/729) RBT: (109/729) CFT: (708/729)	√ (15/50 domestic pigs)	PCR (15/15 domestic pigs)	*B. suis* biovar 2	-	-
[38]	2016	Belgium	Wild boar	20 *Brucella* strains			Multiplex PCR (20/20)	*B. suis* biovar 2	-	-
[38]	2016	Croatia	Domestic pigs	2 *Brucella* strains			Multiplex PCR (2/2)	*B. suis* biovar 1 *B. suis* biovar 2	-	-
[38]	2016	Denmark	Domestic pigs	1 *Brucella* strains			Multiplex PCR (1/1)	*B. suis* biovar 2	-	-
[38]	2016	France	Wild boar Domestic pigs	1 *Brucella* strains 7 *Brucella* strains			Multiplex PCR (1/1 wild boar and 7/7 domestic pigs)	*B. suis* biovar 2 *B. suis* biovar 1 *B. suis* biovar 2	-	-
[38]	2016	Germany	Wild boar	4 *Brucella* strains			Multiplex PCR (4/4)	*B. suis* biovar 2	-	-
[38]	2016	Italy	Wild boar	7 *Brucella* strains			Multiplex PCR (7/7)	*B. suis* biovar 2	-	-
[38]	2016	Portugal	Wild boar Domestic pigs	56 *Brucella* strains 33 *Brucella* strains			Multiplex PCR (56/56 wild boar and 33/33 domestic pigs)	*B. suis* biovar 2	-	-
[38]	2016	Spain	Domestic pigs	16 *Brucella* strains			Multiplex PCR (16/16)	*B. suis* biovar 2	-	-
[39]	2017	Croatia	Wild boar Domestic pigs	Serum (n = 3230), Serum (n = 641), uterus (n = 50), testicles (n = 18), spleen (n = 8), lymph nodes[1]) (n = 62), fetuses (n = 12) (N = 68 swine)	iELISA and RBT: (42/3230) iELISA, RBT, CFT and SAT: (34/641)	√ (45 domestic pigs and 2 wild boar/68 swine)	Multiplex PCR (47/47 swine)	*B. suis* biovar 2	√	-
[40]	2017	Serbia	Domestic pigs	Aborted fetuses (stomach) (n = 2)	-	√ (2/2 domestic pigs)	Multiplex PCR (2/2 domestic pigs)	*B. suis* biovar 2	√	-
[41]	2017	Italy	Wild boar	Fetuses, placenta, lymph nodes[6]) (N = 389)	-	√ (6/389 wild boar)	AMOS-PCRand PCR-RFLP (6/389 wild boar)	*B. suis* biovar 2	-	-
[42]	2018	Latvia	Wild boar	Serum, spleen, lymph nodes, tonsils and kidneys (n = 1044)	iELISA, RBT, CFT: (235/1044)	√ (21/29 seropositive wild boar)	PCR (42/78 seropositive wild boar)	*B. suis* biovar 2	-	-

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Table 2 (*continued*)

Ref.	Year of publication	Country	Animals	Type of sample	Serological tests (positive reactions / tested)	Culture (positive / tested)	Detection method (positive / tested)	Genus, species and biovar	Clinical presentation	Necropsy / Pathological findings
[43]	2019	Italy	Wild boar Domestic pigs	173 *Brucella* strains 11 *Brucella* strains			AMOS-PCR and PCR-RFLP (173/173 wild boar and 11/11 domestic pigs)	*B. suis* biovar 2 (170/173) *B. melitensis* biovar 3 (3/173) *B. suis* biovar 2	-	-
[44]	2019	France	Wild boar Domestic pigs	45 *Brucella* strains 104 *Brucella* strains			MLVA (45/45 wild boar and 104/104 domestic pigs)	*B. suis* biovar 2	-	-
[44]	2019	Germany	Wild boar Domestic pigs	22 *Brucella* strains 3 *Brucella* strains			MLVA (22/22 wild boar and 3/3 domestic pigs)	*B. suis* biovar 2	-	-
[45]	2019	Hungary	Wild boar Domestic pigs	55 *Brucella* strains 8 *Brucella* strains			MLVA (55/55 wild boar and 8/8 domestic pigs)	*B. suis* biovar 2	-	-
[44]	2019	Italy	Wild boar Domestic pigs	114 *Brucella* strains 9 *Brucella* strains			MLVA (114/114 wild boar and 9/9 domestic pigs)	*B. suis* biovar 2	-	-
[44]	2019	Poland	Wild boar Domestic pigs	5 *Brucella* strains 6 *Brucella* strains			MLVA (5/5 wild boar and 6/6 domestic pigs)	*B. suis* biovar 2	-	-
[46]	2019	Portugal	Wild boar Domestic pigs	56 *Brucella* strains 32 *Brucella* strains			MLVA (56/56 wild boar and 32/32 domestic pigs)	*B. suis* biovar 2	-	-
[44]	2019	Romania	Domestic pigs	27 *Brucella* strains			MLVA (27/27 domestic pigs)	*B. suis* biovar 2	-	-
[46]	2019	Spain	Wild boar Domestic pigs	48 *Brucella* strains 96 *Brucella* strains			MLVA (48/48 wild boar and 96/96 domestic pigs)	*B. suis* biovar 2	-	-
[47]	2019	Switzerland	Wild boar Domestic pigs	30 *Brucella* strains 7 *Brucella* strains			MLVA (30/30 wild boar and 7/7 domestic pigs)	*B. suis* biovar 2	-	-
[48]	2020	Finland	Wild boar	Spleen and kidneys (n = 366 wild boar)	iELISA and RBT: (8/87)	√ (4/5 seropositive wild boar)	PCR (4/4 seropositive wild boar)	*B. suis* biovar 2	-	-
[49]	2020	Netherlands	Wild boar	Serum (n = 2057), tonsils (n = 180)	iELISA: (131/2057)	√ (7/19 PCR-positive tonsils)	RT-PCR, MLVA and MLST (19/180 tonsils)	*B. suis* biovar 2	-	-
[50]	2021	Italy	Wild boar	Serum, uterus, testicles, epididymides, placentas, fetuses, spleen, mesenteric lymph nodes and liver (N = 287 wild boar)	RBT and CFT: (16/287)	-	RT-PCR (10/287 wild boar)	*B. suis* biovar 2	-	-

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Table 2 (*continued*)

Ref.	Year of publication	Country	Animals	Type of sample	Serological tests (positive reactions / tested)	Culture (positive / tested)	Detection method (positive / tested)	Genus, species and biovar	Clinical presentation	Necropsy / Pathological findings
[8]	2022	Spain	Domestic pigs	Serum and vaginal swabs (n = 1140) Environmental surveillance – gauze sponges (n = 175)	iELISA: 1stround: (180/195) 2nd round: (373/468) 3rd round: (179/588) BST: 1stround: (285/558) 2nd round: (368/468) 3rd round: (202/588)	√ (69 vaginal swabs/1140 and 46 environmental gauze sponges/175)	PCR (69 vaginal swabs/1140 and 46 environmental gauze sponges/175)	*B. suis* biovar 2	√	-
[51]	2023	Slovenia	Wild boar	17 *Brucella* strains			Multiplex PCR and MLST (17/17)	*B. suis* biovar 2	-	-
[52]	2023	Germany	Wild boar	Serum (n = 11,956). Uterus, testicles, lymph nodes, spleen (n = 681)	iELISA: (2141/11,956)	√ (5/681 wild boar)	RT-PCR and MLST (8/681 wild boar)	*B. suis* biovar 2	-	-
[53]	2023	Italy	Wild boar	Uterus, testicles and spleen (n = 8864)	-	√ (44/8864 wild boar)	PCR (210/8864 wild boar)	*B. suis* biovar 2	-	-
[54]	2024	Italy	Wild boar	Serum (n = 6440), uterus (n = 42), testicles (n = 50), spleen (n = 92) submandibular lymph nodes (n = 95) (N = 95 wild boar)	cELISA: (378/6440)	√ (18/95 wild boar)	RT-PCR and PCR-RFLP (18/18 wild boar)	*B. suis* biovar 2	-	√

Abbreviation: AMOS-PCR: abortus melitensis ovis suis-polymerase chain reaction; BST: brucellin skin test; cELISA: competitive enzyme-linked immunosorbent assay; CFT: complement fixation test; ICFTU/ml: international complement fixation test units per ml; iELISA: indirect enzyme-linked immunosorbent assay; MLST: multi-locus sequence typing; MLVA: multi-locus variable number tandem repeat analysis; PCR: polymerase chain reaction; RBT: rose bengal test; RFLP: restriction fragment length polymorphism; RT-PCR: real tyme polymerase chain reaction; SAT: serum agglutination test.

1) parotid, submandibular, retropharyngeal, portal, subiliac, mesothelial and supramammary

2) cranial and iliac lymph nodes

3) supramammary, inguinal, mandibular and mesenteric lymph nodes

4) submandibular, retropharyngeal and mammary lymph nodes

5) retropharyngeal, mesenteric, mediastinal and parotid lymph nodes

6) Submandibular and retropharyngeal lymph nodes

Necropsy/Pathological findings:
[23] - Domestic pigs: Orchitis, epididymitis, metritis, hyperaemic and oedematous placentas
[33] - Orchitis with adherences and purulent exudates in the scrotum
[54] - Chronic orchitis, miliary metritis
Clinical presentations:
[34] – Herd with reproductive problems
[35] - Infertility, abortion, small litter size, stillbirths, fetal mummification, decreased farrowing rates and poor conception rates
[39] – Domestic pigs: abortion, infertility and stillbirths
[40] – Abortions
[8] - Abortions and reproductive problems

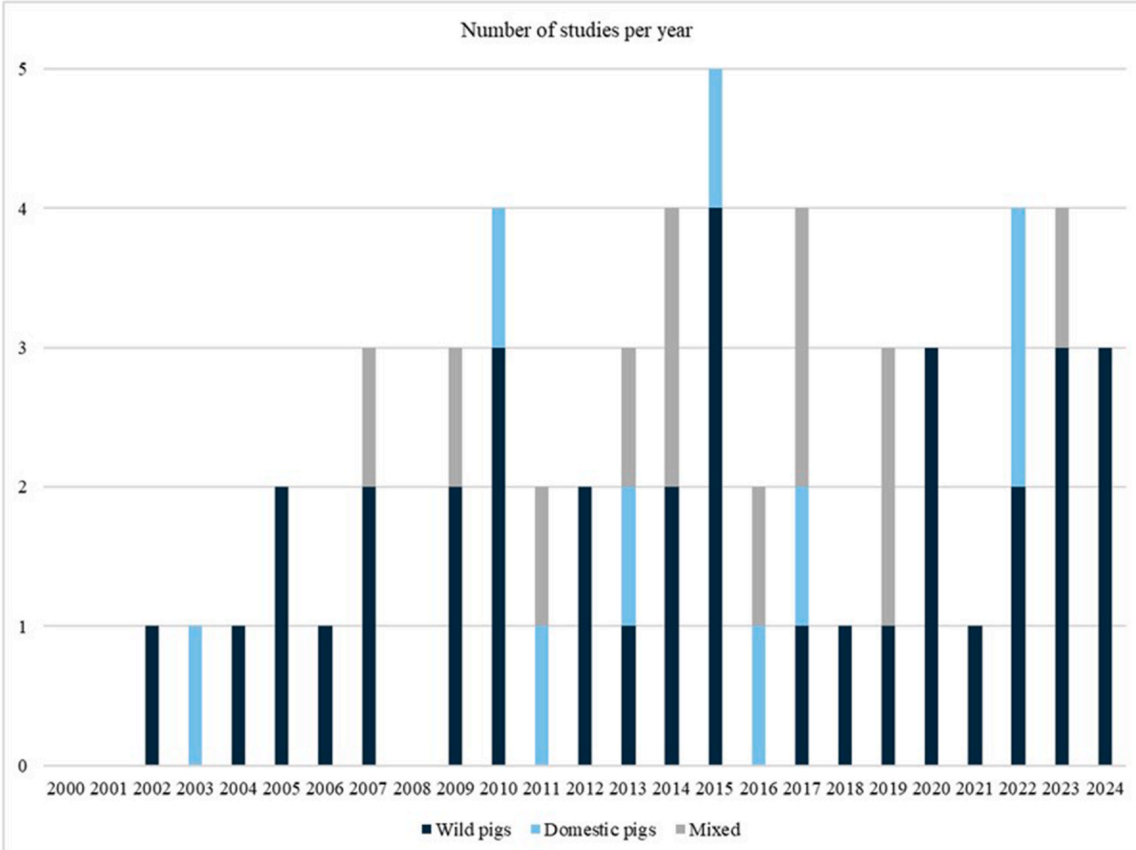

Fig. 2. Number of publications per year, between 1st January 2000 and 30th April 2024.

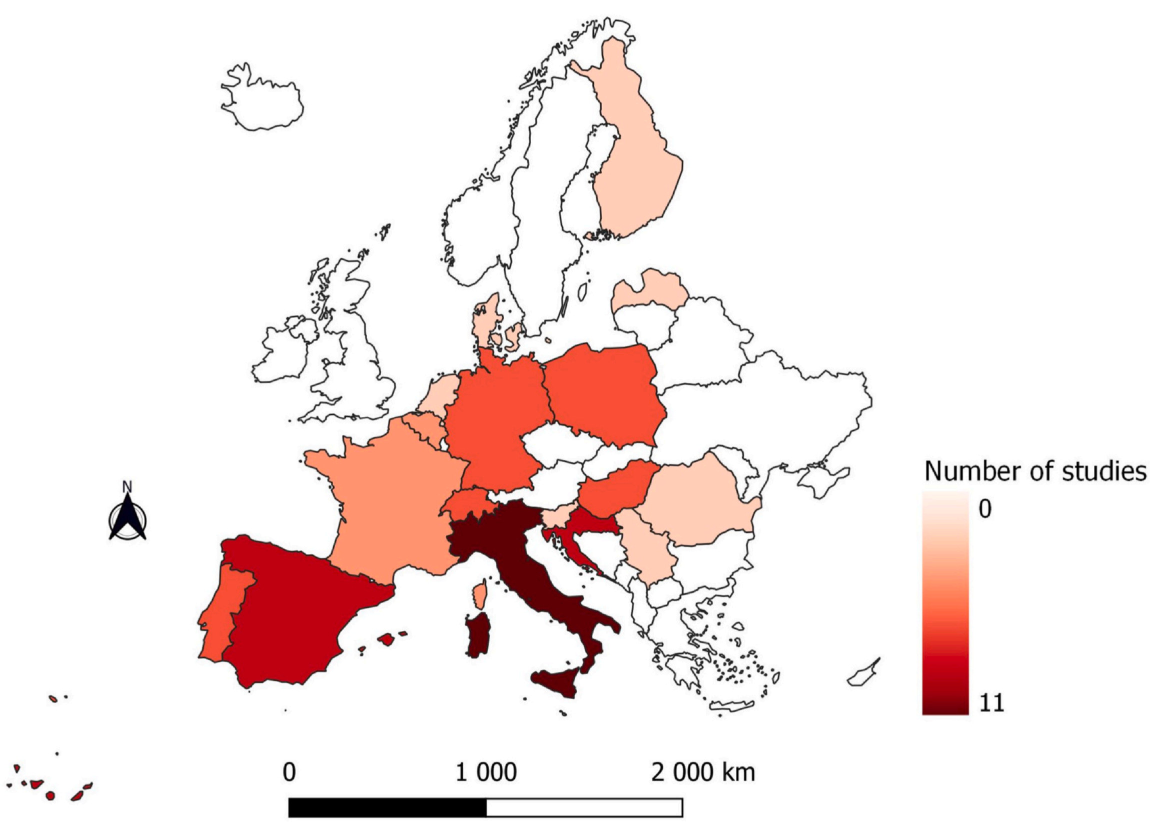

Fig. 3. Country wise distribution of the studies included in the systematic review.

publications on brucellosis in domestic pigs were found in only 12 European countries: Croatia (n = 5), Italy (n = 4), Spain (n = 3), France (n = 2), Hungary (n = 2), Poland (n = 2), Portugal (n = 2), Switzerland (n = 2), Denmark (n = 1), Germany (n = 1), Romania (n = 1) and Serbia (n = 1).

For the diagnostic of *Brucella* spp., the scientific community seems to give greater importance to the collection of reproductive organs, spleen and lymph nodes, mainly submandibular, retropharyngeal, mesenteric, inguinal and supramammary. However, environmental samples from closed-loop production can also be used for routine screening and considered for the diagnosis of brucellosis [8].

Brucella suis biovar 2 has been most frequently reported in wild boar and domestic pigs. In addition to *B. suis* biovar 2, *B. suis* biovar 1 and *B. suis* biovar 3 were also found in wild boar and domestic pig. *Brucella melitensis* biovar 3 has been reported. Also *B. microti* was found in one wild boar.

Ten studies described clinical signs and/or histological findings associated with brucellosis. Clinical signs have been reported in domestic pigs that tested positive for brucellosis. Pig farmers reported poor appetite and reproductive symptoms, such as abortion, stillbirths, irregular estrus intervals and poor conception rates.

Pathological findings were described in three studies on wild boar and two studies in domestic pigs. During necropsies, the authors of the studies observed orchitis and/or epididymitis, metritis and hyperemic and edematous placentas [23,33,54,55].

Discussion

Brucellosis remains a major global public health concern in several European countries, particularly in the Mediterranean region, where small ruminant brucellosis is still endemic [2].

Europe has many wild animal species, with the wild boar being one of the most numerous species [56]. They are considered a reservoir of *Brucella* spp., which poses a threat to livestock with potential adverse public health and economic consequences [57]. Germany, France, Austria, Belgium, Latvia, Poland, Portugal and Spain are among the European countries with the highest wild boar's density [58] and the proximity of wild boar to humans and domestic animals is notorious, pointing at the existing risk of brucellosis spillover [58,59]. There is no data indicating the wild boar population in European countries. It is expected that the number has been increasing, e.g. in 2012, at least 2.2 million wild boar were harvested in 18 European countries, compared with 864,000 harvested in 1992 [60].

Also, in Europe, domestic pigs represent the largest category of livestock. In 2023, the pig population in Europe was around 132,861.77 [61] with Germany, Spain, Poland, France and Denmark leading in the number of pigs [61]. Outdoor pig farms are a traditional form of pig farming in certain areas of southern Europe, such as the south-west of the Iberian Peninsula [62], and these pigs may come into contact with wildlife.

Therefore, the efforts of this systematic review were focused on providing up-to-date evidence on the presence and distribution pattern of the disease in wild boar and domestic pigs in Europe and on knowing which *Brucella* species are being diagnosed in these animals.

This review covered data from 36 publications originating from various European countries.

Over the years, there have been more studies published on wild boar than on domestic pigs. Interest in brucellosis in wild boar is growing in the scientific community. However, brucellosis in these animals continues to be neglected [4]. Brucellosis in wild boar was identified in 14 European countries. During the study period, only two articles based on *Brucella* strains were found in wild boar in France, one of the countries with an abundant population of wild boar [38,44]. In addition, in France, infection by *B. suis* biovar 2 was reported in seven humans exposed to wild boar hunting. However, according to the authors, these patients had potentially immunocompromising medical conditions that may be responsible for their increased susceptibility to infection [63]. Croatia and Italy are also among the European countries with the highest density of wild boar [58] and perhaps for this reason there is interest in studying swine brucellosis. Germany is a country with the largest number of wild boar [58] and domestic pigs [61], but the scientific community was more interested in studying wild boar than domestic pigs. This lack of interest in research on swine brucellosis can be explained by the fact that in Germany, outdoor holdings are by law required to obtain written permission from the local veterinary authority and are required to fulfil a defined set of biosecurity measures aimed at avoiding interaction between wild boar and domestic pigs [64]. The same source also states that in Austria, pigs are housed mainly indoors. Pregnant sows and piglets are generally kept outdoors in Denmark and France [64].

In addition to wild boar, the European brown hare (*Lepus europaeus*) is also an important reservoir of *B. suis* biovar 2 and may play a significant role in the spread of disease by serving as potential sources of infection for pigs or other domestic animals, even in the absence of wild boar [65].

The most common clinical manifestation of brucellosis in animals is abortion [66]. In this study, abortions were reported in domestic pigs in Italy [35], Croatia [39], Serbia [40] and Spain [8]. In studies outside Europe, for example in Southern India, clinical symptoms, particularly abortions, were also associated with seropositivity for swine brucellosis [67]. Other manifestations found include infertility, stillbirths, small litter size, fetal mummification, decreased farrowing rates and low conception rates [8,34,35,40,55,68]. These clinical symptoms were one of the reasons for testing swine brucellosis. For example, Zutic et al. [40] diagnosed swine brucellosis in Serbia, after the animals' owner notified the veterinary services of the presence of two aborted fetuses.

Compatible lesions, such as infertility, abnormal estrus, abortions, lameness or orchitis [13], have been identified in wild boar and domestic pigs [23,33,54,55]. The same is described in the review by Pinn-Woodcock et al. [66].

Swine infected with *Brucella* spp. may not transmit the disease to other animals. The occurrence of abortions, behavior during parturition and management practices are important risk factors for the spread of the disease [66]. In Switzerland, Koppel et al. [69] concluded that the prevalence of antibodies was significantly higher in pigs kept in outdoor systems where there are interactions with wild boar [64].

Based on the included articles, our study indicates that wild boar can be considered an important reservoir of *Brucella* spp., mainly for domestic pigs raised outdoors [8,29,34,37,39,40,69–71]. The prevalence of swine brucellosis has increased considerably in many countries where domestic pigs have contact with wild animals [69,71].

Italy was the country with the most studies reporting diagnosis of swine brucellosis. This can be explained due to the circulation of the bacteria in this area, included in the Mediterranean region, and the fact that pigs are commonly raised outdoors [64], for example, in Sardinia, pigs are raised in mountainous areas [35]. Some studies have also been published in the countries of the Iberian Peninsula [26]. The high prevalence of brucellosis in Portugal and Spain can also be explained by outdoor pig farming [64], the majority autochthonous breeds [72,73]. Several variables (as the presence and density of wild and domestic pigs' populations, the prevalence of disease, fences characteristics among others) may influence the transmission of the pathogen between wild boar and especially outdoor-reared pigs [74]. There are several tests available for the diagnosis of *Brucella* spp. infection, including serological, bacteriological and molecular tests [75]. Serology can help identify a possible exposure to *Brucella* spp. in swine [77]. However, due to the common infection with *Yersinia enterocolitica* serotype O:9 in swine and the polysaccharide chain shared with *Brucella,* current serological tests cannot reliably distinguish between the two pathogens and false-positive reactions are frequent [31]. Animals that have not yet developed antibodies can also compromise results by causing false-negative results [76]. Therefore, serological tests are suitable for surveillance and epidemiological investigations. To diagnose the disease, it is essential to use highly specific tests. The isolation and identification of *Brucella* bacteria offers a definite diagnosis of brucellosis. *Brucella* spp. persist in lymph nodes, joint fluids, bones and reproductive organs [13]. Reproductive organs, lymph nodes and spleen are the most collected samples for the diagnosis of the disease. Tonsils were collected in three studies [31,35,42]. Detection of DNA by PCR is considered an excellent tool for diagnosis of brucellosis [4,77]. Grégoire et al. [31] concluded that the sensitivity of PCR was higher on tonsils than on spleen. The interest of this systematic review was also to understand whether other species of *Brucella* were being diagnosed in these animals. *Brucella suis* biovars 1, 2 and 3 mainly occur in wild boar and domesticated pigs. Biovars 1 and 3 of *B. suis* occur worldwide, biovar 2 has mainly been reported in Europe [78]. In this study, the results of molecular investigations have demonstrated that *B. suis* biovar 2 as the principal agent reported in wild boar and domestic pigs in Europe, however, other species have been diagnosed. *Brucella suis* biovar 1 and 3 were also detected in wild boar and domestic pigs in Croatia [23,38]. *Brucella suis* biovar 1 is highly pathogenic to humans when compared to biovar 2 and biovar 1 is not endemic in Europe, however, some cases have appeared in Europe in other animal species and humans. In 2018, *B. suis* biovar 1 was diagnosed in a German patient without a travel history to endemic areas [79]. Also, in Germany in 2021, a dog was diagnosed with *B. suis* biovar 1. This dog also had no history of travel to endemic areas. In areas where *B. suis* biovar 1 is endemic in wildlife, canine infection with the pathogen is common [80]. Part of Turkey's territory is in the Mediterranean region and *B. suis* biovar 1 was also diagnosed in that country in a sheep and cattle breeder and wild boar hunter, which points to the presence of the disease in swine [81].

Brucella abortus and *B. melitensis* can infect both wild boar and domestic pigs [82]. During the study period, *B. melitensis* biovar 3 [43] and *B. microti* [36] were reported in wild boar. *Brucella melitensis* biovar 3 has been diagnosed in wild boar in Italy. The disease remains endemic in domestic ruminants in several Italian provinces [3,83]. Worldwide, *B. melitensis* is the most prevalent species causing human brucellosis [77] and their presence of *B. melitensis* biovar 3 in wild boar is a potential source of spillover and spillback infection in wildlife, human and domestic animals, namely domestic ruminants. *Brucella melitensis* has also been detected in pigs outside Europe [84]. *Brucella microti* has been identified in a rodent with widespread distribution in Europe, and its detection in wild boar may be associated with the fact that they fed on infected rodent [36]. Our research, since 1st January 2000, indicates that most European countries have reported swine brucellosis and the circulation of zoonotic *Brucella* species. Therefore, with these results and given that the number of wild boar has been increasing in Europe and domestic pigs represent the largest category of livestock, preventive measures are necessary. Having a comprehensive understanding of the occurrence of brucellosis is crucial to formulate and implement effectual measures, programs, and interventions aimed at preventing and managing disease in these animals.

There were limitations in this systematic review: (1) although an explicit search strategy was designed to retrieve published studies from online databases, and some articles was manually searched for additional relevant data, some data may still be missed; (2) the study does not estimate the prevalence of swine brucellosis and (3) lack of risk of bias assessment.

The conclusions presented in this study are derived from a comprehensive analysis from a systematic review of published articles pertaining to brucellosis in wild boar and domestic pigs.

Conclusion

This systematic review aims to provide updated knowledge on brucellosis in wild boar and domestic pigs in Europe. Although the scientific community has conducted research in this area, the disease remains neglected in these species.

Wild boar pose a spillover and back infection threat that needs to be controlled. It is important to have more information on the disease due to the business cycles of the pig industry.

In Europe, the presence of *Brucella* species, namely *B. suis* biovar 1, *B. suis* biovar 3 and *B. melitensis*, poses public health threats. These species are of zoonotic importance. Further studies are needed to investigate pathogenicity and virulence for humans. Therefore, in addition to small ruminant brucellosis, surveillance and control of these diseases in wild boar and domestic pigs is essential under the "One Health" concept.

List of Abbreviations

AMOS-PCR	Abortus melitensis ovis suis-polymerase chain reaction
B.	*Brucella*
BST	Brucellin skin test
cELISA	Competitive enzyme-linked immunosorbent assay
CFT	Complement fixation test
DNA	Deoxyribonucleic acid
ICFTU/ml	International complement fixation test units per ml
iELISA	Indirect enzyme-linked immunosorbent assay
MeSH	Medical subject headings
MLST	Multi-locus sequence typing
MLVA	Multi-locus variable number tandem repeat analysis
MVP	Madalena Vieira-Pinto
PCR	Polymerase chain reaction
PICO	Population, intervention/exposure, comparison, outcome
PROSPERO	Prospective register of systematic reviews
RBT	Rose bengal test
RFLP	Restriction fragment length polymorphism
RT-PCR	Real tyme polymerase chain reaction
SAT	Serum agglutination test
TLM	Teresa Letra Mateus
ZMR	Zita Martins Ruano

Funding

This work was supported by the projects UIDB/00772/2020 (DOI: 10.54499/UIDB/00772/2020) funded by FCT. The participation of Teresa Letra Mateus was funded by national funds through FCT within the scope of project UIDB/05937/2020 with DOI identifier 10.54499/UIDB/05937/2020 and UIDP/05937/2020 with DOI identifier 10.54499/UIDP/05937/2020.

CRediT authorship contribution statement

Conceptualization: ZMR, TLM and MVP; Data curation, Resources, Software: ZMR; Formal analysis, Methodology: ZMR; TLM and MVP; Supervision and Writing-review: TLM and MVP; Editing: ZMR, TLM and MVP. All authors contributed to the article and approved the submitted version. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper. The authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2025.102691.

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