Department for Farm Animals and Veterinary Public Health

University of Veterinary Medicine Vienna

Unit of Veterinary Public Health and Epidemiology
(Head: Univ.-Prof. Dr. med. vet. Annemarie Käsbohrer)

Epidemiology of bovine leptospirosis in Europe: Systematic literature review of the risk factors of infection

Diploma

University of Veterinary Medicine Vienna

submitted by

Julia Jöbstl

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Supervisor: Ass.-Prof. Amelie Desvars-Larrive, DVM, PhD.

Unit of Veterinary Public Health and Epidemiology / Teaching and Research Farm Kremesberg (VetFarm)

Department for Farm Animals and Veterinary Public Health

University of Veterinary Medicine Vienna

Reviewer: Dr. med. vet. Harald Pothmann

Clinical Unit of Herd Health Management in Ruminants

Department for Farm Animals and Veterinary Public Health

University of Veterinary Medicine Vienna

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Abstract

Leptospirosis is a zoonotic disease caused by pathogenic bacteria of the genus *Leptospira*. The infection in cattle can be subclinical, acute, or chronic and can severely impact reproduction performances. Typical signs include abortion, stillbirth, poor fertility, or decrease in the milk production. The disease can therefore have great economic effects on cattle farms.

Prevention and control of bovine leptospirosis requires interventions aiming at limiting the exposure of individuals or herds to risk factors of infection. However, risk factors of infection are often described at farm or regional level and no overview of the risk factors of leptospirosis in European cattle was available.

To fill this knowledge gap, a systematic search following the PRISMA guidelines was performed and 28 papers from twelve European countries, published between 2001 and 2021, were selected and summarized through a narrative text, tables, and figures.

Overall, 53 risk factors were investigated in the published studies. The most frequently investigated ones were environmental risk factors (e. g. climate, geographic localization), followed by herd management practices, and biosecurity measures. Bigger herd size, purchase of replacement cattle (i. e. open herds), access to pasture and natural water sources (e. g. river, ponds, wells), presence of other diseases on the farm (e. g. BVD, BHV-1, *N. caninum*, *Salmonella* spp.), no separation of age-groups, and the use of a stock bull were significantly associated with *Leptospira* infection in cattle.

This work highlights several research gaps, including an under-representation of beef herds in the studies, a lack of large-scale studies, a lack of data from several countries in Europe, a lack of study on the risk related to artificial insemination, and a limited investigation of the role of rodents as source of *Leptospira* infection in cattle. Furthermore, the heterogeneity of the methodological approaches and sample sizes challenges the generalization of the results of each study.

The results of this review can help to prevent infection with *Leptospira* spp. in cattle farms through the reduction of exposure to known risk factors. These findings can also advance the monitoring of the disease by identifying herds or animals at-higher-risk of infection and therefore support veterinarians in their diagnostic process.

Zusammenfassung

Leptospirose ist eine Zoonose, verursacht durch pathogene Bakterien der Gattung *Leptospira*. Bei Rindern kann die Infektion subklinisch, akut oder chronisch auftreten und kann einen schwerwiegenden Einfluss auf die Fruchtbarkeit haben. Typische Symptome sind Abort, Totgeburten, schlechte Fruchtbarkeit oder ein Abfall der Milchleistung. Daher kann die Krankheit große ökonomischen Verluste in Rinderbetriebe verursachen.

Für die Prävention und Kontrolle der bovinen Leptospirose werden Maßnahmen benötigt, die die Exposition von Einzeltieren oder Beständen gegenüber Risikofaktoren für eine Infektion vermindern. Allerdings werden diese Risikofaktoren oft nur für einzelne Betriebe oder Regionen beschrieben, ein Überblick über Risikofaktoren für Leptospirose für europäische Rinder war bisher jedoch nicht verfügbar.

Um diese Wissenslücke zu füllen, wurde ein systematischer Review nach den PRISMA Richtlinien erstellt. Achtundzwanzig Paper aus zwölf europäischen Ländern, veröffentlicht zwischen 2001 und 2021, wurden ausgewählt und in einem narrativen Text, Tabellen und Abbildungen zusammengefasst.

In den publizierten Studien wurden 53 Risikofaktoren untersucht. Die am häufigsten untersuchten Risikofaktoren waren umwelt-assoziierte Risikofaktoren (z. B. Klima, geographische Lokalisation), gefolgt von Betriebsmanagement und Biosicherheitsmaßnahmen. Zunehmende Betriebsgröße, Zukäufe (z. B. in offenen Betrieben), Zugang zu Weide und natürlichen Wasserquellen (z. B. Fluss, Teich, Brunnen/Quelle), Präsenz von anderen Krankheitserregern im Betrieb (z. B. BVD, BHV-1, *N. caninum, Salmonella spp.*), keine Trennung der Altersgruppen und der Einsatz von Zuchtstieren waren signifikante Risikofaktoren für eine Infektion mit Leptospiren bei Rindern.

Diese Arbeit hebt mehrere Forschungslücken hervor, einschließlich einer Unterrepräsentation von Fleischrindern in den Studien, ein Fehlen von groß angelegten Studien, Daten aus mehreren europäischen Ländern sowie der Erforschung des Risikos von künstlicher Besamung und eine eingeschränkte Untersuchung der Rolle von Nagern als Infektionsquelle für Leptospirose bei Rindern. Zusätzlich erschwert die Heterogenität der Forschungsmethoden und des Probenumfangs die Verallgemeinerung der Ergebnisse der einzelnen Studien.

Die Ergebnisse dieses Reviews können bei der Prävention von Leptospirose in Rinderbetrieben durch die Reduktion der Exposition gegenüber bekannten Risikofaktoren helfen. Diese Erkenntnisse können zusätzlich die Überwachung von Leptospirose fördern, da sie helfen Tiere oder Betriebe mit hohem Risiko zu identifizieren, wodurch Tierärzte und Tierärztinnen in der Diagnostik unterstützt werden.

Abbreviations

AGES	Österreichische Agentur für Gesundheit und Ernährungssicherheit
AI	Artificial Insemination
BHV-1	Bovine Herpes Virus 1
BVD	Bovine Viral Diarrhea
CABI	Center for Agriculture and Bioscience International
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CSF	Cerebrospinal Fluid
ELISA	Enzyme-Linked Immunosorbent Assay
IVET	Institut für Veterinärmedizinische Untersuchungen
L.	*Leptospira*
LORN	LeptOspirose bei Rindern in Niederösterreich
MAT	Microsopic Agglutination Test
N.	*Neospora*
OR	Odds Ratio
PCR	Polymerase Chain Reaction
p. i.	post infection
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
R.	*Rattus*
RR	Risk Ratio
WHO	World Health Organization
WP1	Work Package 1

1. Introduction

1.1. *Leptospira* spp.

1.1.1. Taxonomy

Leptospires are spirochaetes (i. e. spiral-shaped bacteria) belonging to the order Spirochaetales, family *Leptospiraceae,* which contains three genera: *Leptospira, Leptonema* and *Turneriella* (1). The type-genus of these three genera is *Leptospira* Noguchi (1).

Taxonomic classification of *Leptospira* spp.:

Phylum: Spirochaetes

Class: Spirochaetia

Order: Leptospirales

Family: *Leptospiraceae*

Genus: *Leptospira*

(2)

1.1.2. Classification

1.1.2.1. Serological classification

The first *Leptospira*-isolate was discovered in 1914 by Wolbach and Binger in freshwater lake and given the name *Spirocheta biflexa* (3). Through the course of the next decades many authors found similar organisms in humans all over the world, for example in rice-field-workers in China, in soldiers who fought in the trenches in France in 1914-18, or in miners from Japan who suffered from “infectious” jaundice (1,4).

Noguchi (1917) first suggested to name the genus “*Leptospira*” in 1917 (5). Serovars (i. e. leptospires grouped based on their antigenic relatedness) were already described between 1920s and 1930s (6). Two strains are considered to belong to different serovars if, after cross-absorption with adequate amounts of heterologous antigen, more than 10 % of the homologous titer regularly remains in at least one of the two antisera in repeated tests (7).

Between 1920 and 1950 clinicians, still with very little scientific microbiological knowledge about the bacteria, recognized the various clinical features of leptospirosis and their link with the different serovars and animal reservoirs. It was therefore important to know the serological types of leptospires causing the different clinical syndromes in humans or animals as well as the potential sources of infection to establish a diagnosis and develop control programs.

As new serovars were discovered, they were considered as “species” and in 1948 four species were recognized, *Leptospira (L.) icterohaemorrhagieae*, *L. hebdomadis*, *L. biflexa* and *L. canicola* (1).

However, in the 60s, experts were reluctant to recognize the serovars as species. Wolff & Broom (1954) (8) and a World Health Organization (WHO) Expert Group (9) recommended that all the non-pathogenic (saprophytic) strains should be included in a group, called “complex”, named the "biflexa complex", while the pathogenic *Leptospira* were included in a group named the "interrogans" complex (6). Pathogenic and saprophytic strains were distinguished via growth in the presence of divalent copper ions. Later the two species were differentiated by phenotypic features, for example *L. biflexa* was able to grow at 13 °C, in the presence of 8-azaguanine (225 µg/ml) and was not able to form spherical cells in 1 M NaCl (1,4). Serovars represented subspecific designations within each complex (6).

In the 7th edition of Bergey’s Manual of Determinative Bacteriology (10), leptospires were divided into two species. The first – *L. icterohaemorrhagiae* – contained all the pathogenic strains and was further divided into serotypes and the second one – *L. biflexa* – contained all saprophytic strains and was not split into serotypes (1).

Eventually, in 1982 the Taxonomic Subcommittee agreed to define the complexes as species. Within each species, serovars sharing common antigens were organized into serogroups (without formal taxonomic status), named from the type serovar of the serogroup (6).

At its 2002 congress, the Committee on the Taxonomy of *Leptospira* of the International Union of Microbiological Societies has approved the following nomenclature for *Leptospira* serovars: the genus and the species must be written in italics, the name of the serovar should not be italicized and should be capitalized (11): *Genus species* serovar Serovar_name, e. g. *Leptospira interrogans* serovar Australis. Currently, more than 300 serovars of *Leptospira* are known, among which 60 are saprophytic (12).

In Table 1 some of the serogroups and serovars of *L. interrogans* are shown.

Table 1: Serogroups and some serovars of *L. interrogans* sensu lato (4).

Serogroup	Serovar(s)
Icterohaemorrhagiae	Icterohaemorrhagiae, Copenhageni, Lai, Zimbabwe
Hebdomadis	Hebdomadis, Jules, Kremastos
Autumnalis	Autumnalis, Fortbragg, Bim, Weerashighe
Pyrogenes	Pyrogenes
Bataviae	Bataviae
Grippotyphosa	Grippotyphosa, Canalzonae, Ratnapura
Canicola	Canicola
Australis	Australis, Bratislava, Iora
Pomona	Pomona
Javanica	Javanica
Sejroe	Sejroe, Saxkoebing, Hardjo
Panama	Panama, Mangus
Cynopteri	Cynopteri
Djasiman	Djasiman
Sarmin	Sarmin
Mini	Mini, Georgia
Tarassovi	Tarassovi
Ballum	Ballum, Aroborea
Celledoni	Celledoni
Louisiana	Louisiana, Lanka
Ranarum	Ranarum
Manhao	Manhao
Shermani	Shermani
Hurstbridge	Hurstbridge

1.1.3. Molecular classification

Modern methods in molecular taxonomy have allowed the development of a genotypic classification of leptospires, with the species *Leptospira* (also referred as genomospecies) as the basic taxon. Phylogenetically, 68 *Leptospira* species are currently described. They are divided into two major clades called “Pathogens” (P) and “Saprophytes” (S) and four subclades (13,14).

There is a poor correlation between the two *Leptospira* classification schemes and antigen properties cannot be used to reliably predict to which species an isolate belongs. For example, one serogroup may contain serovars belonging to different *Leptospira* species while one genomospecies may include strains belonging to different serogroups (Table 2) (1,4).

The serological classification remains essential to support clinical diagnostics and vaccination programs (4) but phylogenomics have advanced the understanding of the biology and epidemiology of the bacteria in the environment and the different hosts (13).

Table 2: Leptospiral serovars found in multiple species (4).

Serovar	Species
Bataviae	*L. interrogans, L. santarosai*
Bulgarica	*L. interrogans, L. kirschneri*
Grippotyphosa	*L. kirschneri, L. interrogans*
Hardjo	*L. borgpetersenii, L. interrogans, L. meyeri*
Icterohaemorrhagiae	*L. interrogans, L. inadai*
Kremastos	*L. interrogans, L. santarosai*
Mwogolo	*L. kirschneri, L. interrogans*
Paidjan	*L. kirschneri, L. interrogans*
Pomona	*L. interrogans, L. noguchii*
Pyrogenes	*L. interrogans, L. santarosai*
Szwajizak	*L. interrogans, L. santarosai*
Valbuzzi	*L. interrogans, L. kirschneri*

1.2. Leptospirosis

1.2.1. Epidemiology

Leptospirosis is a zoonotic disease that is globally distributed, except in the polar regions. Human infection is often related to an exposure to the bacteria during professional or leisure activities (e. g. berry picking, hunting, water-related sports). Humans working in direct contact with animals present a higher risk of infection, for example farmers, veterinarians, slaughterhouse workers, rodent control workers and meat inspectors. People in contact with potentially contaminated water or soil are also more exposed to the bacteria, such as sewage workers, fish farmers, miners, or rice field workers (4,15).

A wide range of wild and domestic mammal species can carry and excrete the bacteria into the environment, usually intermittently and for a variable period (16,17). Animals usually remain symptom-free and little to no pathological changes are observed, except in animals which are immunodeficient at the time of infection, e. g. animals in late pregnancy or presenting concomitant infections (18).

Rodents, especially wild rats (i. e. the brown rat, *Rattus (R.) norvegicus*, and the black rat, *R. rattus*) are probably the most important reservoir of *Leptospira* spp. and the most common source of infection for humans and animals (19–21).

Several serovars exhibit preferential association with certain hosts. Furthermore, one animal species may host different serovars in different geographic areas, depending on the local epidemiology and context (22). An animal species may be (asymptomatic) reservoir host of some serovars but incidental host of others, which may cause severe to fatal disease (17,22).

Bovines are the maintenance host for *L. borgpetersenii* serovar Hardjo (type Hardjobovis) and *L. interrogans* serovar Hardjo (type Hardjoprajitno), both belonging to the serogroup Sejroe, which is widespread globally (17,18,23–25). However, *Leptospira* infection in cattle is not limited to these serovars, incidental infections with other serovars have also been reported, e. g. with serovars belonging to the serogroups Icterohaemorrhagiae, Canicola, Hebdomadis, Sejroe, Pyrogenes, Autumnalis, Australis, Javanica, Tarassovi and Grippotyphosa (18).

1.2.2. Pathogenesis and clinical signs

The bacteria enter the body via an abrasion or cut in the skin or via a mucosal membrane, e. g. the mouth, nose, eyes or genitals (4,18). Infection in humans and animals can occur through direct contact with the urine of an infected animal or indirectly, through contaminated water or soil (4,17). A transmission via ingestion of milk of infected females has been suggested in rats and humans (26,27) although this transmission path is not fully elucidated yet. In certain mammal species, a venereal transmission is also described, not only through natural mating but through artificial insemination and embryo transfer (28–30). Vertical transmission (in utero) of *Leptospira* is also possible (18,31,32).

One to two days after infection starts the leptospiremic (or bacteremic) phase, generally lasting for a week. During this phase, the bacteria can be isolated from blood and some organs, as well as from the cerebrospinal fluid. The bacteremia ends with the immune phase (i. e. when antibodies appear), which starts 10-14 days post infection (p. i.). In rare cases, a second bacteremic phase occurs after 15-26 days. Anti-leptospiral agglutinins reach a peak around 3-6 weeks. The antibody titer is greatly variable and high titers have been associated with severe forms of the disease (33). Thereafter the antibody level declines, although low titer levels can be detected for up to six years in human patients (18).

After the bacteremic period, leptospires migrate to the proximal renal tubules where they multiply and are excreted intermittently in the urine for a variable period (18).

Beside the main localization in the kidneys, leptospires can be found in the female and male genital tract. In pregnant females, genital colonization may induce abortions, stillbirths or infect

the foetus, leading to neonatal leptospirosis. Leptospires present in the female genital tract are shed through vaginal or uterine discharge while in some species, the bacteria have been detected in the semen of infected males and can therefore be transmitted through the venereal route (18,28–30). In some animals the bacteria can also persist in the eye, for example in horses, where it can cause recurrent uveitis and blindness (34).

Typical symptoms of *Leptospira* infection in humans and dogs include lethargy, fever, inappetence, polyuria and polydipsia, which may be followed by multi-organ dysfunction (mostly acute renal failure), cholestatic hepatic dysfunction (icterus), pancreatitis, pulmonary haemorrhage, myositis, and sometimes uveitis (17,18).

In cattle, leptospirosis rarely manifests with acute and/or severe symptoms. It presents usually in a subclinical and/or chronic form characterized by reproductive problems such as abortion, stillbirth, poor fertility, and birth of premature or weak calves, and reduced birth weight. It can also appear as an acute syndrome called “milk drop syndrome”, where cows experience a sudden decrease in milk production, which disappears with or without treatment after ten to fourteen days (17,18,29).

Acute and severe leptospirosis infections, which signs include hyperthermia, icterus, haematuria, high abortion rates, congenital icterus in the aborted foetuses, and mortality, rarely occur in cattle. In most cases they are caused by incidental infections with serovars that are not adapted to cattle, mostly belonging to the serogroups Pomona, Grippotyphosa and Icterohaemorrhagiae, which are most likely transmitted and maintained by pigs, rodents and wildlife (18,35).

1.2.3. Laboratory diagnostics

The type of sample and method of testing depends on the phase of infection. In the first ten days of infection, the bacteria can be evidenced from blood, cerebrospinal fluid (CSF) or some organs (e. g. lung, kidney, liver, pancreas, or heart). Aborted fetal tissues or the placenta can also be used to detect the presence of the bacteria. After the bacteremic phase, serological tests can be used to detect the presence of antibodies, in serum or milk. Samples from kidney tissue, urine, or cervico-vaginal mucus can also be used for direct detection of the bacteria during the chronic phase (a negative result on urine sample is difficult to interpret though since the bacteria is intermittently excreted) (18,36).

The “gold standard” for leptospirosis diagnostic is the microscopic agglutination test (MAT), in which patient (animal or human) sera are reacted with live strains of different *Leptospira* serovars (antigens). After incubation, the serum-antigen reaction is observed under dark field microscopy; agglutination is searched and the titer, for each serovar, is determined.

Interpretation of the MAT is complex because cross-reactions generally occur between different serogroups, particularly in blood samples collected during the acute phase of the disease. Paired sera, i. e. collected ~ 2 weeks apart, are necessary to confirm an ongoing infection (we usually consider that a four-fold increase in the titer allow to certify infection) (4). Very high titers (over 1,000) usually suggest a recent infection whereas lower titer levels indicate an older infection (18,36). The enzyme linked immunosorbent assay (ELISA) can be used to detect *Leptospira* antigens or antibodies against *Leptospira.* In contrast to the ELISA assays used for diagnosis of human infection, which are generally broadly reactive and can evidence a broad range of serogroups, few serovar-specific assays have been developed in veterinary medicine that can detect serovar-specific antibodies. For example, there is an ELISA assay to detect infection by serovar Hardjo from blood or milk sample in cattle (35,37–39).

The polymerase chain reaction (PCR) allows the detection of a specific DNA segment of *Leptospira* and amplifies it. The method is very sensitive and allows further sequencing of the DNA to determine the circulating *Leptospira* species. However, PCR and sequencing do not allow the identification of the circulating serovar (36).

Bacterial isolation via culture represents an alternative method to detect *Leptospira* spp. Leptospires have a doubling time of 6-8 hours. After inoculation in appropriate medium, under adequate temperature, and in the absence of contamination by other bacteria, the growth of *Leptospira* in culture may be observed within a week but it generally takes up to 13 weeks (6). The most used medium is called “EMJH”, named after Ellinghausen, McCullough, Johnson and Harris. The culture should be checked weekly to detect any growth of leptospires via dark field microscope (36,40). Overall, the culture of *Leptospira* is fastidious, time consuming, shows a low success rate and is not adapted to routine diagnosis.

1.3. Background and objectives

This work was performed in the framework of the LORN project: “LeptospirOse bei Rindern in Niederösterreich: Ein gezielter Ansatz zur Verbesserung der Veterinärdiagnostik und zur Verhinderung einer beruflichen Exposition gegenüber Zoonosen”, implemented by the VetFarm of the University of Veterinary Medicine Vienna and the Institute for Veterinary Medical Examinations Mödling of the Austrian Agency for Health and Food Safety (Institut für veterinärmedizinische Untersuchungen Mödling, Österreichische Agentur für Gesundheit und Ernährungssicherheit, IVET-AGES). The aim of the LORN project is to isolate regional pathogenic *Leptospira* strains from infected cattle in farms and slaughterhouses in Lower Austria to improve the sensitivity of routine serological diagnostics for humans and animals. We therefore simultaneously conducted a targeted and random sampling of symptomatic and

slaughtered cattle, respectively, in Lower Austria and collected urine and kidney tissues. These samples were investigated for the presence of pathogenic *Leptospira* species via PCR and culture. This study should provide an insight into the epidemiology of *Leptospira*-infections in cattle in Lower Austria, unveiling the circulating *Leptospira* species and potential risks at the cattle-human interface.

The present work benefited from a systematic literature search conducted within Work Package 1 (WP1) of the LORN project, which aimed to perform a systematic literature review on leptospirosis in cattle in Europe using scientific articles published between 2001 and 2021.

We hypothesise that the exposure to environmental risk factors and a lack of biosecurity measures increase the risk of *Leptospira* infection in European cattle. To confirm or deny this hypothesis, I have collected, analysed, and summarised 20 years of data pertaining to risk factors associated to *Leptospira* infection in cattle in Europe. This thesis describes the methodological approach and presents the results of this research.

Moreover, I have assisted with collecting kidney samples from cattle at the slaughterhouse and urine samples from symptomatic cows on farms in Lower Austria (results not shown here).

2. Methods

2.1. Selection of the scientific papers

In the framework of the systematic review on the epidemiology of leptospirosis in European cattle, scientific papers were selected through a systematic search using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (41). The systematic search was carried out from the 7th of June to the 26th of August 2021, using the electronic databases Scopus, PubMed, Web of Science and CABI (Center for Agriculture and Bioscience International). Keywords used for the search were “lepto*”, “cattle”, “cows” and “cow”. More papers were retrieved through web search or tracking of citations in already selected papers. Papers published in the last 20 years, i. e., between 1st January 2001 to the last day of search (26 August 2021) were considered for the systematic review.

Only studies conducted in European countries, as defined by the most common geographical definition of Europe, i. e*.,* the land bordered by the Arctic Ocean to the north, the Atlantic Ocean to the west, the Mediterranean Sea to the south, and the Ural Mountains to the east, were included. Case reports, outbreak descriptions, epidemiologic surveys, reviews or epidemiological reports were included. Publications in German, English and French language were considered for the review. All papers were inspected by two independent reviewers and included/excluded for the systematic review based on inclusion/exclusion criteria. Relevant information regarding the epidemiology of bovine leptospirosis in Europe was extracted by these two reviewers. The selection process and data extraction were not part of the present thesis.

For my thesis, I have first selected, from the list of included scientific articles, those which contained qualitative and/or quantitative data on risk factors of leptospirosis infection in European cattle. Then, I have curated and validated previously extracted data for these papers. Potential mistakes or anomalies as well as missing information or values were manually investigated by going back to the original papers and corrected when needed.

2.2. Data extraction and organization

Sixty-two papers were selected for the systematic review and from those, 28 papers (45.2 %) investigated risk factors for *Leptospira* infection. From these 28 papers the following data were entered into a table in MS Excel (version 2202): bibliographic information (citation, year of publication), study design, country of the study, sampling unit (animal or herd), sampling size, type of production (dairy, beef or mixed), investigated risk factor, dependent variable (used in the model(s)), statistical analysis (yes/no), association between the dependent variable and

the risk factor (positive, negative, not evidenced), statistical model used, statistics reported, value of the statistics, 95 % confidence interval (CI), and p-value.

Furthermore, each risk factor was attributed to a category allowing to group the numerous risk factors into broader themes, allowing a better overview and global understanding of the risk associated to *Leptospira* infection in European cattle. For example, icteric abortion was categorized as *clinical signs*; animal introduction (e. g. purchase) was categorized as *biosecurity*. All risk factors and their assigned risk categories are shown in Table 3.

Table 3: Risk factors and their allocated risk categories.

Risk category	Risk factors investigated
Environmental factors	Access to natural water source
	Access to pasture
	Exposure to flooding
	Extreme weather event
	Geographical location
	Grazing acres
	Out-winter fed in fields
	Percentage wet land grazed
	Season spring
	Season summer
	Season fall
	Straw-bed shed
	Surface of the grazing acres
Herd management practices	Calves grazing on cow pasture
	Calving season
	Cows and heifers separate at calving
	Herd size
	Housing of calves later in the year
	Percentage of first lactation animals
	Rearing of calves on out farms
Biosecurity	Animal introduction: purchase
	Bull management practice
	Bull management practice: hiring bull
	Bull management practice: stock bull
	BVD vaccination
	Minimizing numbers of visitors to the farm
	Movement of cattle onto and off the farm
	Oral drenching equipment was regularly cleaned

	Presence of rodents
	Time since last purchase of cattle
	Use of swine manure
	Using agricultural contractors without insisting that their equipment was cleaned and disinfected
Infectious diseases	History of leptospirosis
	Incidence of *Neospora caninum*
	Incidence of *Salmonella*
	Testing positive for BHV-1
	Testing positive for BVD
	Testing positive for *Anaplasma phagocytophilum*
Animal individual characteristics	Age
	Breed
	Sex
	Type of production
Clinical signs	Abortion year before
	Necropsy of aborted foetus: coppery liver
	Necropsy of aborted foetus: extended haemorrhagic pattern
	Necropsy of aborted foetus: icterus
	Necropsy of aborted foetus: icterus + splenomegaly + coppery liver
	Necropsy of aborted foetus: peri-renal haemorrhages
	Necropsy of aborted foetus: splenomegaly
	Symptom = icteric abortion
	Weak calves the previous year
Study design	Sample date
	Year of sampling

BHV-1: Bovine Herpes Virus 1; BVD: Bovine Viral Diarrhea.

Summary statistics, tables and figures presented in this work were computed in R (42) using RStudio (43) and the following R packages: dplyr (44), lubridate (45), ggplot2 (46), stringr (47), sf (48), rnaturalearth (49), viridis (50), RColorBrewer (51), ggalluvial (52), ggfittext (53), gridExtra (54), randomcoloR (55), collapsibleTree (56), lattice (57), and forestplot (58).

The citation software used was Mendeley Reference Manager.

All data and their categorization are available in the Annex.

3. Results

3.1. Year of publication of the selected studies

Figure 1 displays the distribution of the years of publication of the 62 studies included in the qualitative synthesis (WP1, LORN project). While the years of publication of the 28 (45.2 %) articles investigating risk factors of leptospirosis in cattle is shown in Figure 2.

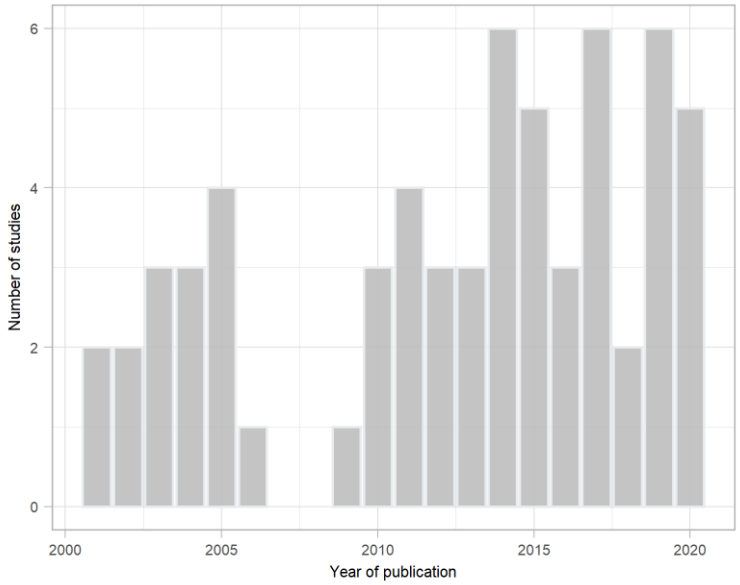

Figure 1. Barplot of the distribution of the years of publication of the 62 studies included in the review (WP1, LORN project), 2001-2021.

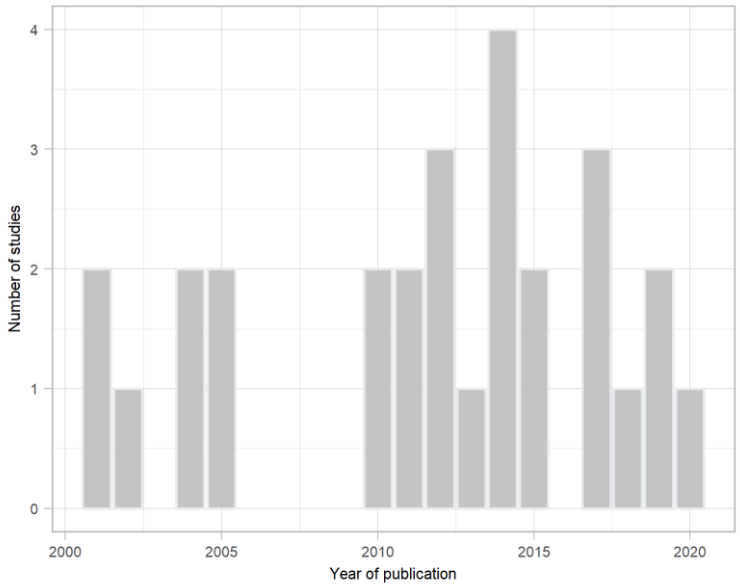

Figure 2. Barplot of the distribution of the years of publication of the studies addressing risk factors of leptospirosis in European cattle, 2001-2021.

3.2. Geographical distribution of the studies

The 62 studies were conducted in 18 countries in Europe (Figure 3, left). The 28 studies describing the risk factors associated with *Leptospira* infection were conducted in twelve different countries, namely Belgium, Bosnia and Herzegovina, Croatia, France, Germany, Ireland, the Netherlands, Poland, Spain, Sweden, Ukraine and the United Kingdom (Figure 3, right). The number of studies per country is detailed in Table 4.

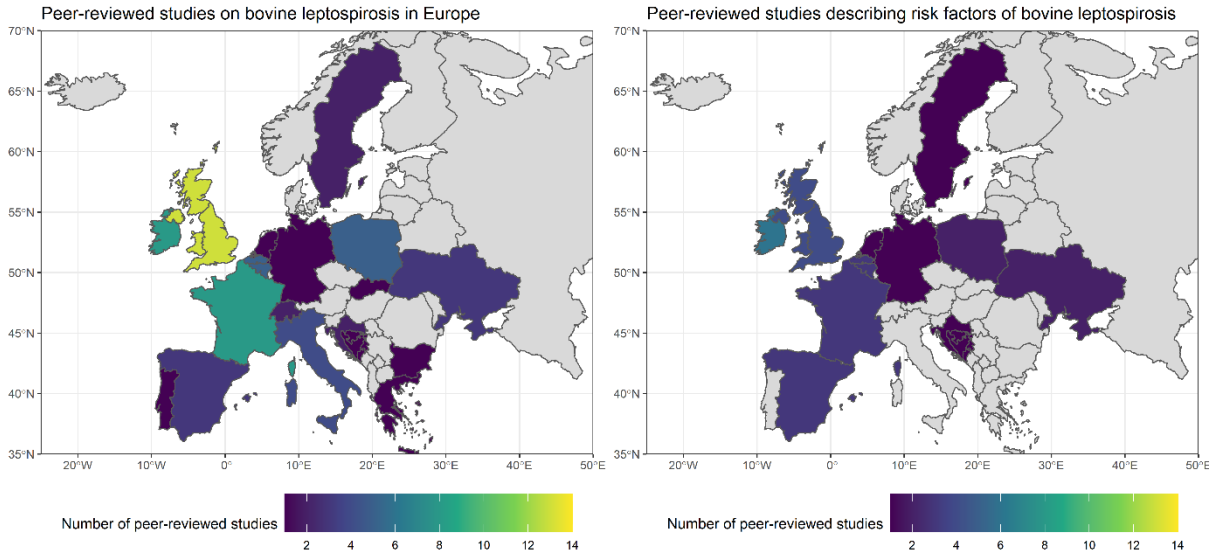

Figure 3. Left. Number of studies per country pertaining to bovine leptospirosis and conducted in Europe between 2001 and 2021. **Right.** Number of studies per country addressing risk factors of bovine leptospirosis and conducted in Europe between 2001 and 2021.

Table 4. Number of studies per country reporting risk factors of bovine leptospirosis in Europe (2001-2021) and associated references.

Country	Number of studies	References
Belgium	3	(31,59,60)
Bosnia and Herzegovina	1	(61)
Croatia	1	(62)
France	3	(63–65)
Germany	1	(66)
Ireland	6	(67–72)
Netherlands	1	(39)
Poland	2	(38,73)
Spain	3	(74–76)
Sweden	1	(77)
Ukraine	2	(78,79)
United Kingdom	4	(37,80–82)

3.3. Risk factors associated with leptospirosis in cattle in Europe, 2001-2021

3.3.1. Definition of risk factor

A risk factor is something that increases the chance (or probability) of a health event (e. g. disease, injury, or death) to occur, e. g. age, physiological or anatomical characteristics, genes, or environmental factors (83).

In epidemiology, the association between a health event and a risk factor is estimated through statistics, where the characteristics of two groups and the prevalence (or incidence) of a disease are evaluated and compared. The resulting statistics, e. g. relative risk, also called risk ratio (RR), or odds ratio (OR), shows how much more frequent the health event is occurring because of the presence of an exposition to the risk factor in comparison to a control group (84).

The RR is calculated by dividing the risk (e. g. incidence proportion, attack rate) in group 1 (exposed) by the same metrics of risk in group 2 (not exposed). A RR greater than 1 means that there is an increased risk in comparison to the control group, whereas a RR < 1 means a decreased risk for the exposed group. A RR = 1 indicates similar risk between the two group (84).

An OR quantifies the relationship between an exposure (exposed versus non-exposed) and health outcome (e. g. disease, injury, or death). Referring to the table below, the odds ratio is calculated as:

	Diseased	Healthy
Exposed	a	b
Not exposed	c	d

OR = (a / b) * (c / d) = ad / bc

Where *a* is the number of persons exposed and with disease; *b* is the number of persons exposed and without disease; *c* is the number of persons unexposed and with disease; and *d* is the number of persons unexposed and without disease. For example, an OR of 2 means there is a 100 % increase in the odds of an outcome with a given exposure; an OR of 1.5 means there is a 50 % increase in the odds of an outcome with a given exposure (84).

If the 95 % confidence interval (95 % CI) for the RR or OR includes 1, there is insufficient evidence to conclude that the groups are statistically significantly different.

3.3.2. Overview

Fifty-three different risk factors were studied in the 28 papers (Table 5). The most investigated risk factors were related to environmental factors (n = 16 studies), followed by herd management practices (n = 10), biosecurity (n = 9), occurrence of infectious diseases (n = 6), individual factors related to the animal (e. g. age, sex, breed) (n = 5), and clinical signs associated to leptospirosis (n = 4). Figure 4 summarises the risk factors studied per type of production (dairy, beef, or mixed) and in relation to their attributed broader categories.

Thirty-one risk factors were studied in dairy cattle while only 17 were investigated in beef herds, showing preferential investigations of leptospirosis in dairy production system. The effect of infectious disease on *Leptospira* occurrence was only investigated in dairy herds, as well as the influence of age and calve-raising-practices. The risk factors “geographic location” and “herd size” were preferentially investigated in dairy herds.

Table 5. Risk factors of bovine leptospirosis investigated in European studies, 2001-2021.

Risk factor investigated	Number of studies	References
Geographical location	10	(38,61,66,68,71,72,77–79,81)
Herd size	10	(38,67,68,70–72,74,80–82)
Animal introduction: purchase	5	(39,67,71,80,82)
Age	4	(60,72,75,77)
Testing positive for BVD	4	(63,80–82)
Testing positive for BHV-1	3	(80–82)
Access to natural water source	2	(37,60)
Presence of rodents	2	(64,65)
Abortion year before	1	(75)
Access to pasture	1	(65)
Breed	1	(72)
Bull management practice	1	(82)
Bull management practice: hiring bulls	1	(80)
Bull management practice: use stock bull	1	(71)
BVD vaccination	1	(67)
Calves grazing on cow pasture	1	(70)
Calving season	1	(82)
Cows and heifers separate at calving	1	(71)
Exposure to flooding	1	(73)
Extreme weather event	1	(62)
Grazing acres	1	(71)
History of leptospirosis	1	(71)
Housing of calves later in the year	1	(70)
Incidence of *Neospora caninum*	1	(69)
Incidence of *Salmonella*	1	(69)
Minimizing numbers of visitors to the farm	1	(70)
Movement of cattle onto and off the farm	1	(70)
Necropsy of aborted foetus: coppery liver	1	(31)
Necropsy of aborted foetus: extended haemorrhagic pattern	1	(31)
Necropsy of aborted foetus: icterus	1	(31)
Necropsy of aborted foetus: icterus + splenomegaly + coppery liver	1	(31)
Necropsy of aborted foetus: peri-renal haemorrhages	1	(31)

Necropsy of aborted foetus: splenomegaly	1	(31)
Oral drenching equipment was regularly cleaned	1	(70)
Out-winter fed in fields	1	(71)
Percentage of first lactation animals	1	(70)
Percentage of wetland grazed	1	(71)
Rearing of calves on out farms	1	(70)
Sample date	1	(69)
Season fall	1	(76)
Season spring	1	(76)
Season summer	1	(76)
Sex	1	(72)
Straw-bed shed	1	(71)
Surface of the grazing acres	1	(71)
Symptom = icteric abortion	1	(59)
Testing positive for *Anaplasma phagocytophilum*	1	(63)
Time since last purchase of cattle	1	(82)
Type of production	1	(74)
Use of swine manure	1	(60)
Using agricultural contractors without insisting that their equipment was clean and disinfected	1	(70)
Weak calves the previous year	1	(71)
Year of sampling	1	(61)

BVD: Bovine Viral Diarrhea; BHV-1: Bovine Herpes Virus 1.

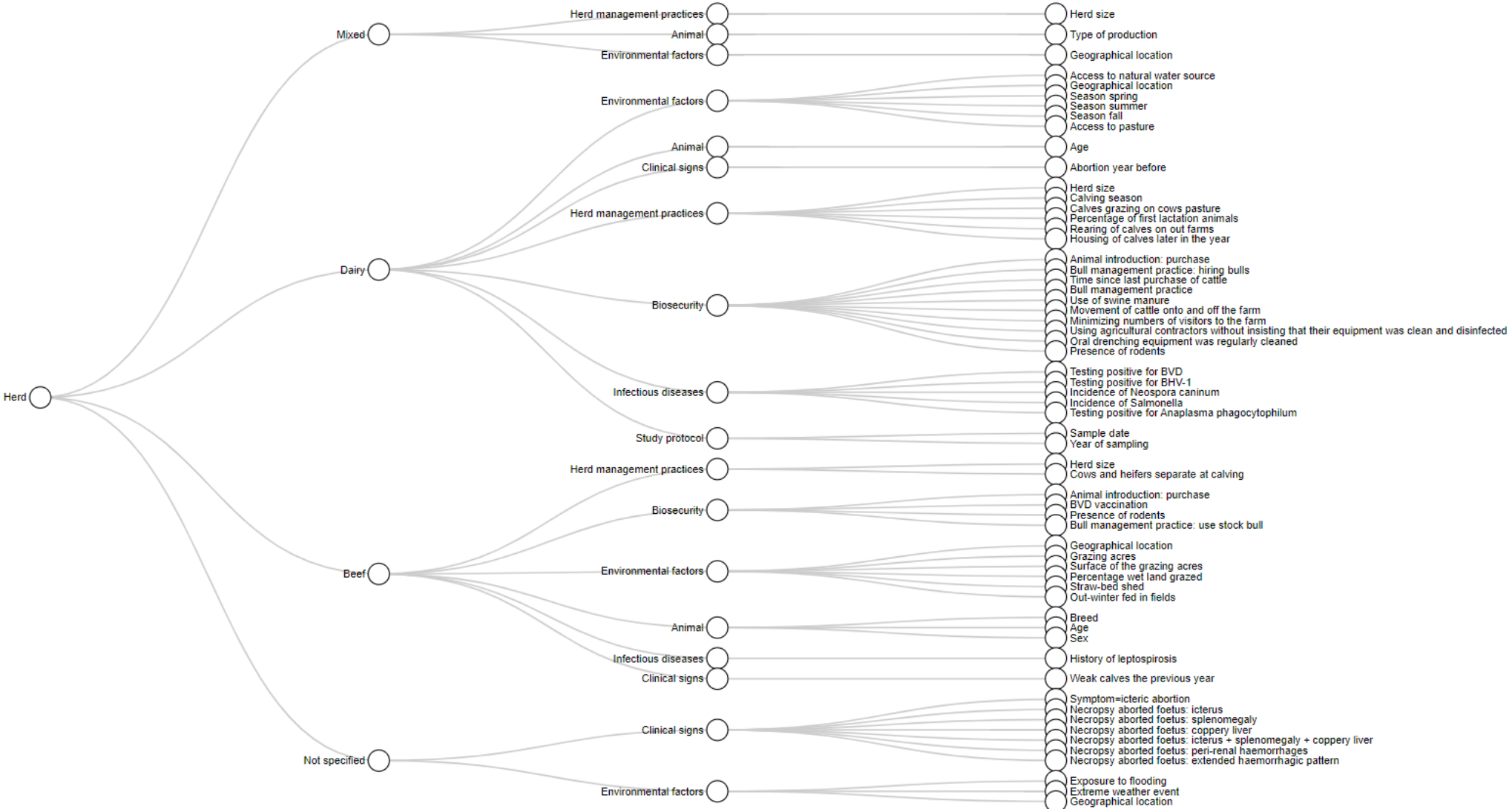

Figure 4. Risk factors of cattle leptospirosis investigated in the different types of production, Europe, 2001-2021. Risk factors are grouped into broader categories following a thematic analysis.

3.3.3. Dependent variables

In the different studies, each risk factor was assessed against various dependent variables (Table 6). For example, the effect of the herd size was tested on the herd (sero) positivity to *Leptospira* (70,71,80,82), within-herd antibody titer level (68) or within-herd (sero) positivity (38,67,72,74,81).

Table 6. Dependent variables used in the studies investigating risk factors of bovine leptospirosis in Europe, 2001-2021.

Dependent variable	Number of studies	References
Herd (sero)positivity to *Leptospira*	9	(66,68–71,73,80–82)
Within-herd seroprevalence	7	(38,67,72,74,80–82)
Seroprevalence	6	(61,62,72,77–79)
Animal (sero)positivity to *Leptospira*	5	(37,59,60,64,65)
Incidence of Leptospira	2	(39,69)
Animal (sero)positivity to *Leptospira* (serovar Bratislava)	1	(75)
Herd antibody titer level	1	(73)
Herd seroprevalence	1	(72)
Incidence of *Leptospira* Grippotyphosa	1	(76)
Incidence of *Leptospira* serogroup Australis (serovar Bratislava)	1	(76)
PCR positive in aborted foetus	1	(31)
Seropositivity to Leptospira in aborted dam (titer 1:100)	1	(31)
Severity of clinical symptoms	1	(63)
Within-herd antibody titer level	1	(68)
Within-herd seroprevalence (serovar Copenhageni)	1	(74)
Within-herd seroprevalence (serovar Grippotyphosa)	1	(74)
Within-herd seroprevalence (serovar Tarassovi)	1	(74)

3.3.4. Methods and statistics to evaluate risk factors of leptospirosis

Eighteen (31,38,59,61,67–77,80–82) out of 28 studies (64.3 %) performed a statistical analysis to quantify the risk of leptospirosis infection in cattle associated with the exposition to different risk factors. In the other studies (37–39,60,62–66,78,79), the risk was deduced through observation. The methods and models used were highly variable, including binary logistical regression (82), calculation of odds ratios (31,59,70,71,75,76,80–82), Chi-squared test

(38,61,75,82), Fisher's exact test (77), generalized estimating equations (69), Kruskal-Wallis analysis of variance (ANOVA) (82), linear regression (67), logistic regression (68,75,81), Mann-Whitney test (73), multivariable analysis (70), multivariable logistic regression (71), one-way ANOVA (72,82), Phi-correlation coefficient (81), proportional hazards regression method (76), Spearman correlation (68), Student's t-test (73), Wilcoxon rank-sum test (80), and univariate analysis (70,71) (names of the methods are reported here as mentioned in the articles).

3.3.5. Risk factors associated with *Leptospira* infection in European cattle

3.3.5.1. Environmental risk factors

Five studies showed a significant statistical association between the geographic location of the animal or herd and *Leptospira* infection (61,68,71,72,81) whereas one reported no significant statistical relationship between these two variables (77). Four studies observed an increase in seroprevalence associated with the geographic location of the animal or herd, although no statistical analysis was performed (38,66,78,79). Overall, the geographic location may have an indirect impact on the risk of infection, in relation with regional differences in mean herd size, soil type, local climate, and herd management practices (71).

Flooding was statistically significantly associated with higher anti-*Leptospira* antibody titers in Poland (73) while extreme weather conditions (i. e. warm/extremely warm or wet/extremely wet weather when compared to the past 30 years average) were hypothetically associated with a high seroprevalence of leptospirosis in Croatia (no statistical analysis performed) (62).

The influence of the seasons on infection with *Leptospira* serovar Bratislava and *Leptospira* serovar Grippotyphosa was studied in Spain. Only spring showed a significantly increased risk of infection with *Leptospira* serovar Grippotyphosa (76).

The risk factors access to pasture, grazing, and surface of grazing showed a significant positive association with *Leptospira* infection whereas increasing the percentage of wetland grazed significantly reduced the risk of infection (numbers are shown in Figure 6) (71). Moreover, the access to natural water sources might have been the source of infection in two studies, from the UK (37) and Belgium (60) (no statistical analysis performed).

3.3.5.2. Risks related to herd management practices

Seven studies showed that the herd size was significantly positively associated with an increased risk of *Leptospira* infection (67,68,70–72,80,82). However, in three studies, no statistical relationship was evidenced between herd size and *Leptospira* infection (38,74,81).

Neither calving in winter nor year-round showed an increased risk of *Leptospira* infection (4). However, segregating heifers and cows at calving showed a significant increase in the risk of infection (71). Similarly, rearing calves out of farm and co-grazing of calves and cows on the same pasture significantly increased the risk of leptospirosis in the herd. Housing the calves later in the year significantly increased the chance of infection (70). This herd management practice might be related to environmental exposure to *Leptospira* (longer exposure in the pasture when animals are housed late in the year). Furthermore, out-winter feeding in fields showed no change in the risk whereas the use of straw beds only approached significance (71). Finally, an Irish study revealed that the percentage of first-lactating cows in the herd was positively associated with the risk of herd infection by *Leptospira* (70).

3.3.5.3. Risk related to biosecurity

Two studies found that the purchase of animals significantly increased the risk of *Leptospira* infection in cattle (67,82), while the risk increased when time since purchase decreased (82). The observation of Van Schaik et al. (2002) supported these findings (no statistical analysis performed) (39). However, two studies reported no significant difference in the number of animals purchased between case and control herds (71,80).

The movement of cattle onto and off the farm, e. g. for shows or temporary grazing, induced a significant increase of seropositivity in cattle herds (70). Three studies explored the use of a bull, hired *versus* stock bull, as a risk factor (71,80,82). Only one study evidenced that the use of a stock bull was significantly associated with the presence of *Leptospira* (71).

The employment of agricultural contractors who were not instructed to clean the equipment between visits to different farms was evidenced as a risk factor for *Leptospira* infection in cattle. Minimizing the number of visitors was shown to decrease the probability of *Leptospira* infection. Surprisingly, one study reported that herds where cleaning of drenching equipment was performed were more likely to test positive for antibodies to *Leptospira* serovar Hardjo, which is counterintuitive. The authors pointed out that one possible reason for the higher probability of a positive test is that cleaning of oral drenching equipment was only carried out in response to the presence of *Leptospira* serovar Hardjo or other infectious diseases in the herd (70).

A significant positive association was found between vaccination against the BVD virus and seroprevalence of *Leptospira* (67). Also, the use of swine manure for the cow pasture was considered as a possible source of *Leptospira* infection in cattle herds (not tested statistically)

(60). In two studies, the presence of rodents (e. g. mice, coypus) was considered as the source of infection of the cows (not tested statistically) (64,65).

3.3.5.4. Risk factors related to infectious diseases

Past or co-occurring infections by other pathogens generally increased the risk of *Leptospira* infection. Detection of circulating antibodies to BVD and BHV-1 increased the probability of detecting antibodies to *Leptospira* serovar Hardjo in the bulk milk (80–82). In one study from France, the authors hypothesized that the investigated outbreak of acute leptospirosis in a cattle herd was aggravated by the co-occurrence of *Anaplasma phagocytophilum* and the BVD virus (63). Similarly, in Ireland, the incidence of *Salmonella* showed a significant statistical association with the incidence of *Leptospira* serovar Hardjo whereas a relationship between the incidence of the serovar Hardjo and *Neospora* (*N.*) *caninum* could not be evidenced (69). Finally, the relationship between a previous *Leptospira* infection and an increased risk was tested but did not exhibit significant impact (71).

3.3.5.5. Risk factors related to the animal individual characteristics

Animal characteristics such as age, sex, breed, or type of production had an impact on *Leptospira* infection in cattle. Alonso-Andicoberry et al. (2001) showed that dairy herds were significantly more at risk than beef herds to be seropositive for *Leptospira* serovar Copenhageni, Grippotyphosa and Tarassovi (74). A significant statistical difference in the risk among breeds was demonstrated in Ireland: between Aberdeen Angus and Belgian Blue, between Aberdeen Angus and Charolais, and between Aberdeen Angus and Limousine, with Aberdeen Angus having the lowest seroprevalence (72).

The age and sex of the cattle showed inconsistent effect on the risk of infection by *Leptospira*. Whereas one study from Ireland did not evidence any relationship between age or sex and leptospirosis (72), only animals older than two years were found seropositive in two other studies from Sweden (77) and Belgium (60) (not statistically confirmed). On the contrary, a negative association between seropositivity to *Leptospira* serovar Bratislava and age was reported from Spain. The authors hypothesised that the antibody levels may decline with increasing age of the cows or that a resistance may appear in older cows following prolonged exposure to *Leptospira* (75).

3.3.5.6. Clinical signs

Two studies evidenced that icteric abortion was a strong predictor of *Leptospira* infection of the aborted dams (determined via serology) (59) and foetuses (determined by molecular detection on several organs) (31). Besides icterus, coppery liver and splenomegaly in the aborted foetuses were also significantly associated with infection by *Leptospira* (31). On the opposite, neither significant association was found between the presence of peri-renal

haemorrhages in the foetuses and a positive serology in dams nor positive PCR in foetuses. An extended haemorrhagic pattern was significantly associated with high antibody titers against *Leptospira* (1/1,000), although only for dams with non-icteric abortions (numbers are shown in Figure 6) (31).

Recent history of abortion (i. e. abortion the year before testing) was significantly associated with higher odds to test seropositive for *Leptospira* serovar Bratislava (75).

3.3.5.7. Factors related to the study design

Two studies assessed the influence of the sample date on the risk of infection. Whereas the study from Ireland did not evidence any effect of the sample date (69), the six-year longitudinal study conducted in Bosnia and Herzegovina showed a significant effect of the sampling year on the seroprevalence (61). Certainly, date of sampling could be a confounding factor that may reflect the influence of e. g. the season, weather, or specific conditions at time of sampling.

An overview of the statistically significant results presented in the 18 studies using statistical analyses is given in Figure 5, along with type of production investigated and the number of studies that investigated each risk factors.

Nine studies have calculated the OR, generally in a case-control study (31,59,70,71,75,76,80–82). I have chosen to present them because OR are generally easier to comprehend and provide a metrics that is, to some extent, comparable between studies when models and dependent variable are provided (85). The quantitative results of these nine studies are displayed in Figure 6.

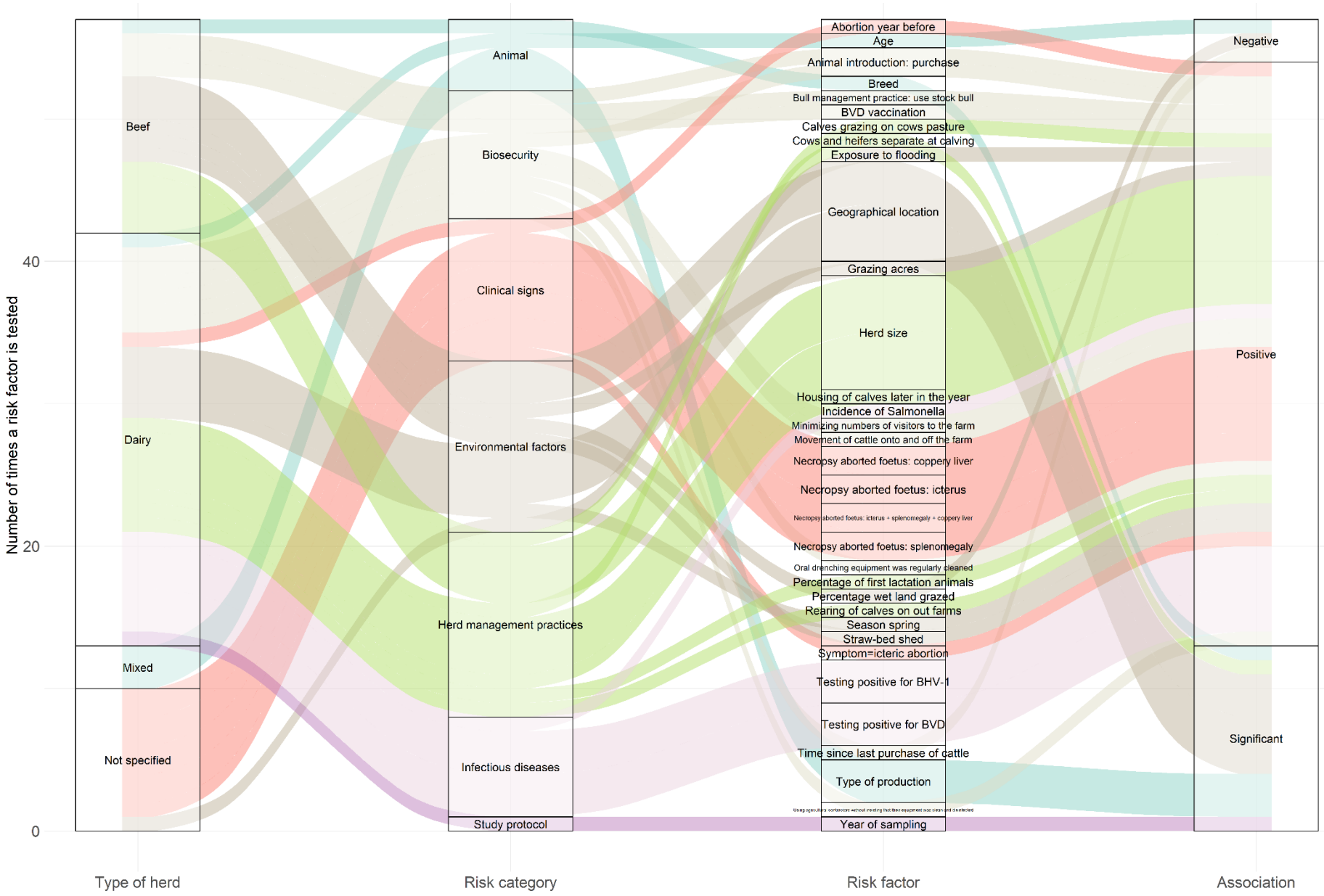
Number of times a risk factor is tested
0
20
40
Beef
Dairy
Mixed
Not specified
Animal
Biosecurity
Clinical signs
Environmental factors
Herd management practices
Infectious diseases
Study protocol
Abortion year before
Age
Animal introduction: purchase
Breed
Bull management practice: use stock bull
BVD vaccination
Calves grazing on cows pasture
Cows and heifers separate at calving
Exposure to flooding
Geographical location
Grazing acres
Herd size
Housing of calves later in the year
Incidence of Salmonella
Minimizing numbers of visitors to the farm
Movement of cattle onto and off the farm
Necropsy aborted foetus: coppery liver
Necropsy aborted foetus: icterus
Necropsy aborted foetus: icterus + splenomegaly + coppery liver
Necropsy aborted foetus: splenomegaly
Oral drenching equipment was regularly cleaned
Percentage of first lactation animals
Percentage wet land grazed
Rearing of calves on out farms
Season spring
Straw-bed shed
Symptom=icteric abortion
Testing positive for BHV-1
Testing positive for BVD
Time since last purchase of cattle
Type of production
Using agricultural contractors without insisting that their equipment was cleaned and disinfected
Year of sampling
Negative
Positive
Significant
Type of herd
Risk category
Risk factor
Association

Figure 5. Sankey diagram showing the type of production, the category of the risk factor investigated (Table 6), the risk factors investigated and the direction of the association with *Leptospira*-infection, i. e. positive or negative ("significant" means that the association is statistically significant but no direction is provided), Europe, 2001-2021. Only studies that have performed a statistics analysis are included and only significant risk factors are displayed. Colours represent the risk categories.

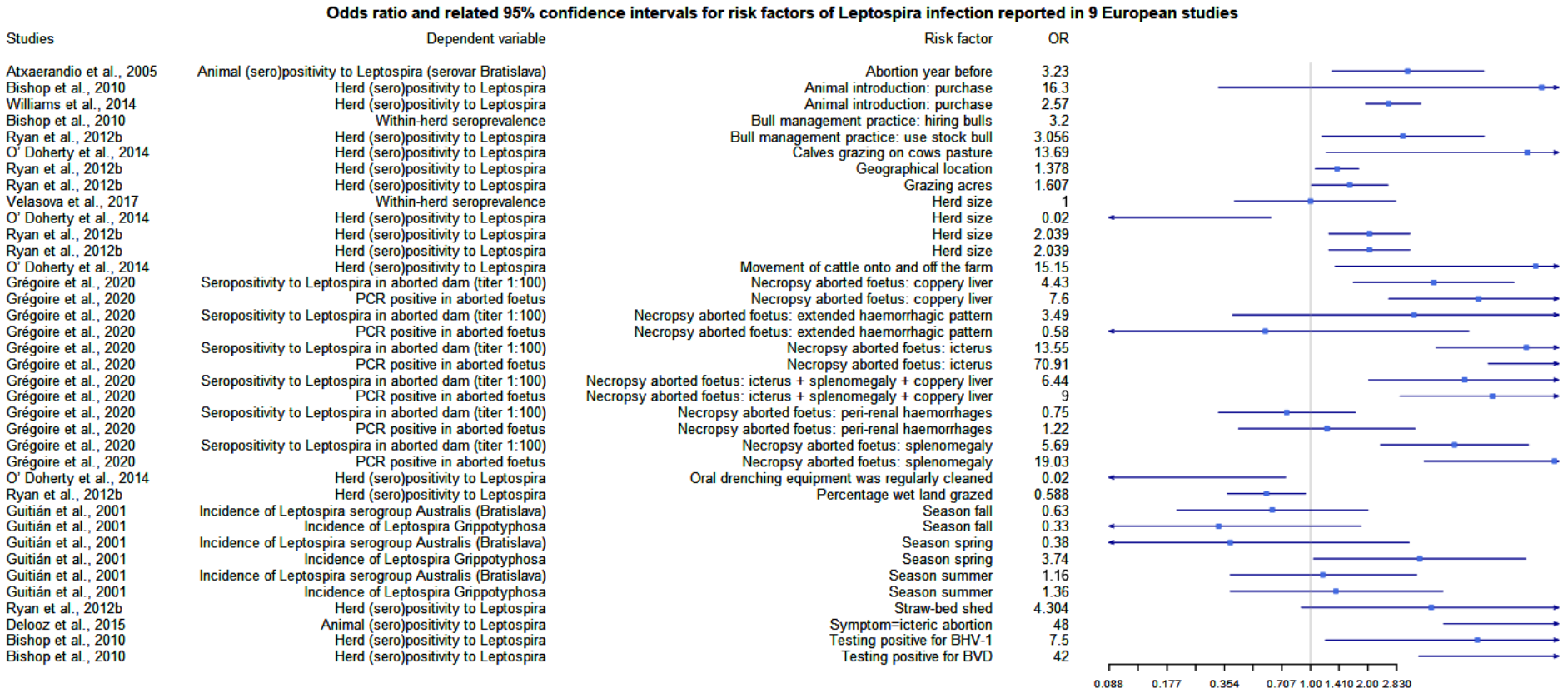

Figure 6. Forest plot of the risk factors of bovine leptospirosis, Europe, 2001-2021. The figure presents the odds ratios (OR) and 95 % confidence interval (95 % CI) for nine European studies. Note that if 95 % CI contains the value 1, the OR is not significant.

4. Discussion and conclusion

The systematic review on the epidemiology of leptospirosis in European cattle performed in the framework of the LORN project retrieved 62 articles published between 2001 and 2021. Almost half of the studies (45.2 %) investigated risk factor of bovine leptospirosis. With six studies, the Republic of Ireland was the country with the highest number of published papers reporting risk factors of leptospirosis in cattle.

The hypothesis, that environmental risk factors and a lack of biosecurity measures increase the risk of Leptospira infection could be confirmed. Additionally, we found these two factors to be were closely entangled with other risk factors of infection.

This review provides a comprehensive overview of the risk factors for *Leptospira* infection in cattle in Europe. In this work, I evidenced a great heterogeneity of the approaches used to assess the risk factors of infection, i. e. regarding study design, diagnostic method, statistical model, dependent and explanatory variables, sample size, and epidemiological unit. In general, most reported significant risk factors of leptospirosis were related to biosecurity measures, e. g. purchase and introduction policy, rodent control, separation of age groups, mixing with other species, or use of a stock bull.

4.1. Comparison with results from other regions

When comparing the results of this review with studies from tropical regions, where the disease is generally endemic, many similar results appear. In the tropics and subtropics, presence on the farm of other animals species, or contact with them (mainly pigs, dogs and rodents) (86–89), introduction of new animals to the herd (86), grazing on pasture (88,90), access to natural water sources (e. g. river, weir, stagnant water) (86,88,90,91), geographic location (86,92), and increased herd size (89,90) were similarly found to be risk factors for *Leptospira* infection. Moreover, the use of a stock bull was found to increase the risk of *Leptospira* infection (71) in Laos (91) and Chile (92). In Colombia artificial insemination (AI) and use of certified semen has been found to be a protective measure against *Leptospira* infection (87), yet in Brazil, *Leptospira* infection through artificial insemination was described, highlighting the importance of using certified *Leptospira*-free semen (29,93,94). Although a study from Chile reported year-round calving to be a risk factor of *Leptospira* infection in cattle, this was not reported in Europe (92).

Climatic conditions play a major role in the risk of infection in cattle in Europe (36,60,65,67,70,71,77,78,80), e. g. precipitations or occurrence of extreme weather events (62,73). The relationship between weather conditions and outbreak occurrence has been areported multiple times from tropical regions, where cattle are more likely to test positive for *Leptospira* during the wet season than during the dry season (91), most probably because the wet (and warmer) season favours the survival of the bacteria in the environment and increases water-borne transmission (4).

Older cows are more at risk for *Leptospira* infections than younger ones in Europe, which is confirmed in studies from tropical areas (86,89). Similarly, variable susceptibility of animals from different breed was evidenced not only in Europe (72) but also in Colombia, where Holstein presented an increased risk (87) and in Brazil, where Jersey and crossbreeds showed higher risk of infection (88).

As shown in different studies across Europe, the presence of other pathogens on cattle farms (e. g. BVD virus, BHV-1, *N. caninum, Salmonella*, etc.) increases the risk for *Leptospira* infection and vice versa (63,69,80–82). These findings are supported by results from Laos where seropositivity to *Leptospira* was associated with higher antibody titers against *N. caninum* and BVD virus (90). We can hypothesize that a lack of biosecurity measures (e. g. quarantine of newly purchased animals) and hygiene on infected farms favours not only infection by *Leptospira* but also by other pathogens. It is also likely that the studied pathogens share similar risk factors of infection (80). Another hypothesis is that farms infected with multi-pathogens have low-frequency veterinary visits; for example, in Ecuador and Brazil, farms with no or limited veterinary services have a higher risk of infection with *Leptospira* (86,90).

4.2. Risk factors of *Leptospira* infection in cattle: a local epidemiology?

Local or even regional environmental conditions influence the presence of *Leptospira* in the environment as well as host-bacteria interactions. Furthermore, several factors related to the soil and water pH, temperature, or composition of the environmental microbiome may determine the possibility of persistence of *Leptospira* in the environment (95–97).

In this review many different risk factors were investigated with varying results. This may be due to the different environmental conditions and management practices in the study countries. Europe has a wide variation of climates and each country presents specific temperature ranges as well as precipitation and seasonal patterns, which all have an impact on the chances of

survival of *Leptospira* (98). Another reason for the wide variety of investigated risk factors may be the differences in local farming practices. We can hypothesize that the risk factors studied in the publications reflect the increased research interest for locally relevant parameters. This might explain the diversity of the risk factors and dependent variables explored in the 28 reviewed papers.

4.3. Leptospirosis in cattle: an emerging disease in Europe?

The local and regional climatic context plays a major role in the risk of infection, e. g. climatic conditions or occurrence of extreme weather events are generally linked with outbreaks of *Leptospira* in animals, including cattle, as evidenced in the review, but also in humans (99–101). Climatic conditions in Europe are becoming increasingly suitable for the survival and transmission of water- and rodent-borne diseases, among them, leptospirosis (102). Extreme weather events and natural disasters intensify the direct and indirect (i. e. via the environment) contacts between leptospires, humans, maintenance and susceptible animals (101,103,104), therefore increasing the risk for public and animal health. Leptospirosis may become a more apparent disease in Europe due to global warming, compounding the impact of land-use change (especially urbanisation). Indeed, temperatures are getting warmer and extreme weather events happen more frequently, providing optimized living environment for *Leptospira* spp. in regions where the bacteria was previously not present or at low prevalence (4,105,106)

Moreover, intensification of livestock farming in Europe will also certainly play a major role in the future incidence of leptospirosis in farm animals. Individual farms are, in general, becoming bigger, which increases the risk for *Leptospira* infection due to bigger herd size and more animal purchase (107–109). Higher density of animals in one area also increases the risk of infection for both animals and humans (110). On the other hand, bigger farms often have a better hygienic and health status, because they are sometimes more automatized with different robots (e. g. milking, cleaning and feeding robots) and sensors (e. g. sensors for activity, rumen pH, rumen acidity, rumination time, milk yield, somatic cell count, body condition score). These sensors help with monitoring the animal health status and may be useful to detect early infection in the herd (111).

4.4. Limitations

The 28 studies included in this work presented different study designs, diagnostic methods, statistics, dependent and explanatory variables, sample size and epidemiological unit, among others. For example, some papers presented case reports, while others reported longitudinal or transversal studies; some paper investigated prevalence at herd level (one herd is positive if one positive animal is present in the herd) whereas others investigated seroprevalence at individual level. The detection method as well as sample material used were different across studies, for example, blood, bulk milk, foetal organs, or urine were tested via MAT, PCR or ELISA. The samples were either tested for all available serovars or just a few selected ones (sometimes only *Leptospira* serovar Hardjo) and studies used different cut-off values for determining positivity. Those methodological elements have a major influence on the estimation of risk factors. The most noticeable difference among the reviewed studies is the statistical methods used to estimate the risk. In addition, some risk factors were deduced without performing statistical analysis while in some studies, the sample size was too small to achieve a sufficient statistical power. Finally, the same risk factor could be tested against different outcomes (dependent variables) and results may be different, which limits the interpretation of the results and prevents us from deciphering global patterns.

Overall, the heterogeneity of the methodological approaches and sample sizes, in addition to the very local epidemiology of the disease, challenge the interpretation and comparison of the results and limit the identification of general risk factors for *Leptospira* infection in European cattle. To objectively compare risk factors among the 28 studies, a meta-analysis would be the preferred approach (112).

Finally, some of the studied factors presented surprising results. For example, the cleaning of drenching equipment seems to increase the probability to test positive for antibodies against *Leptospira* serovar Hardjo (70). In this specific case, very likely, the cleaning of oral drenching equipment was carried out in response to the presence of *Leptospira* and other infectious diseases in the herd and should therefore be considered as a confounding factor (70). Another study examined the effect of the percentage of wetland grazing on infection risk among others and the result showed a decrease in risk (71). This result seems counterintuitive since leptospires can survive longer in a wet environment (4). Possible explanations include a low environmental contamination of the pasture with the studied bacteria, suboptimal environmental conditions for the survival of the leptospires, or a massive dilution of the bacteria in the wetland, therefore decreasing the risk of infection (113).

4.5. Applications

Knowledge on the risk factors of leptospirosis in European cattle will support the development and implementation of improved, evidence-driven prevention and surveillance programmes of *Leptospira* infections in cattle herds and can help reducing the incidence of the disease in cattle, and therefore in the exposed human population (especially farmers, veterinarians, and slaughterhouse workers). From this perspective, knowledge about risk factors of infection can facilitate the development of adapted biosecurity measures and farming practices to reduce the exposure to the bacteria and then reduce the economic losses related to the disease (including subclinical infections), as well as the impact on animal health, animal welfare, and public health (18).

This review demonstrated the multiplicity and complexity of the different risk factors associated with cattle infection with *Leptospira.* Such findings should motivate the combined application of biosecurity measures to prevent infection. Notably, our findings showed that the quarantine of newly purchased animals before introduction into the herd and testing for *Leptospira* infection during the quarantine period would be an efficient measure to limit introduction of the pathogen into the herd. A similar procedure should be applied to the handling of bulls used for natural mating. If a stock bull is used, it may be tested for *Leptospira* infection and treated or removed when positive. As for artificial insemination, the use of certified semen should be recommended.

A reduction of the environmental exposure of cattle to *Leptospira* spp. could be achieved by restricting the access of cattle to high-risk, potentially infected places where they may encounter high density of bacteria (e. g. infected pastures) or infected cattle from other farms (e. g. at shows, auctions, or on communal pastures). Moreover, our results highlighted the importance of age segregation on pasture and that calves should not graze on pastures previously used by adult cattle.

The study of Grégoire et al. (2020) highlighted that the necropsy of aborted foetuses could be used as an indicator of *Leptospira*-induced abortion in cattle, for example in surveillance programmes. When aborted foetuses are presented with icterus, coppery liver, splenomegaly or a combination of the tree symptoms, there should be a suspicion of *Leptospira* infection of the dam and/or the foetus (31) that should be further confirmed by laboratory tests. Similarly, control measures of rodent populations via trapping may be combined with a surveillance programme of *Leptospira* (and other rat-borne pathogens) on the trapped rodents, to

determine the presence/absence of the bacteria and eventually identify the circulating serovar(s).

4.6. Identified data and research gaps

This work highlights several research and data gaps concerning risk factors associated with leptospirosis in cattle in Europe. Notably, I observed a limited investigation of beef herds, a lack of large-scale studies, a lack of data from several countries in Europe, a lack of data on the risk related to artificial insemination, and a limited investigation of rodents as source of *Leptospira* infection in cattle herds.

Most risk factors in this review were studied in dairy herds whereas studies of bovine leptospirosis in beef cattle are underrepresented. To get a general understanding of risk factors for leptospirosis in cattle and more specifically assessing the risk for beef herds, further research is necessary in beef production systems. From the risk factors we have identified through this review, beef herds seem to be at high risk of exposure to the bacteria since they often spend a lot of time on pasture where wild rodents may be abundant, where cattle from different herds may be present, and where they have access to natural water sources. Furthermore, calves and adults are grazing on the same pasture, which represents an additional risk factor for *Leptospira* infection. In Brazil for example, beef herds presented a higher risk of infection than dairy or mixed herds (90).

Vaccination of cattle against *Leptospira* in not a common practice in Europe and there is an evident lack of research and data regarding the impact on the risk of infection, because in many studies of risk factors for leptospirosis vaccinated herds were excluded from the study. For example, in Ecuador and Chile, herd vaccination against *Leptospira* was reported as a protective measures against infection (86,92).

4.7. Conclusion

Overall, my research showed that the epidemiology of leptospirosis in cattle strongly depends on local or regional conditions, i. e. climate factors, epidemiological context, or farming practices. Therefore, local studies are very important to advance our understanding of the risk factors of infection at small scale (i. e. farms, municipality, region) and be able to develop and implement relevant, adapted prevention and control strategies. Nevertheless, a global overview of the epidemiology of the disease at continental scale, like presented in this review,

can yield novel insights into the epidemiological features of the disease, especially, by unfolding common determinants of disease events in specific animal populations.

5. References

1. Levett PN. Systematics of Leptospiraceae. In: Adler B, editor. Leptospira and Leptospirosis [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2015. p. 11–20. Available from: https://doi.org/10.1007/978-3-662-45059-8_2
2. NCBI Taxonomy browser [Internet]. 2022 [cited 2022 Sep 18]. Available from: https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Tree&id=170&lvl=3&lin=f&keep=1&srchmode=1&unlock
3. Wolbach SB, Binger CAL. Notes on a filterable spirochete from fresh water. Spirocheta biflexa (new species). J Med Res. 1914 Mar;30(1):23–6.
4. Levett PN. Leptospirosis. Clin Microbiol Rev [Internet]. 2001 Apr;14(2):296–326. Available from: https://doi.org/10.1128/CMR.14.2.296-326.2001
5. Noguchi H. Spirochaeta icterohaemorrhagiae in american wild rats and its relation to the japanese and european strains: First paper. J Exp Med [Internet]. 1917 May 1;25(5):755–63. Available from: https://doi.org/10.1084/jem.25.5.755
6. Faine S, Adler B, Bolin C, Pérolat P. Leptospira and leptospirosis. Second ed. Melbourne, Australia: MediSci; 1999.
7. Stallman ND. International committee on systematic bacteriology subcommittee on the taxonomy of Leptospira: Minutes of the meeting, 5 and 6 September 1986, Manchester, England. Int J Syst Bacteriol [Internet]. 1987 Oct 1;37(4):472–3. Available from: https://doi.org/10.1099/00207713-37-4-472
8. WOLFF JW, BROOM JC. The genus Leptospira Noguchi, 1917; problems of classification and a suggested system based on antigenic analysis. Doc Med Geogr Trop. 1954 Mar;6(1):78–95.
9. Current problems in leptospirosis research. Report of a WHO expert group. World Health Organ Tech Rep Ser. 1967;380:1–32.
10. Breed RS, Murray EGD, Smith NR. Bergey's manual of determinative bacteriology [Internet]. 7th Edition. Baltimore: The Williams & Wilkins Company; 1957. Available from: https://doi.org/10.5962/bhl.title.10728
11. Resources – international leptospirosis society [Internet]. [cited 2022 Dec 19]. Available from: https://leptosociety.org/resources/

12. Thibeaux R, Iraola G, Ferrés I, Bierque E, Girault D, Soupé-Gilbert ME, et al. Deciphering the unexplored Leptospira diversity from soils uncovers genomic evolution to virulence. Microb Genom [Internet]. 2018 Jan 1;4(1). Available from: https://doi.org/10.1099/mgen.0.000144

13. Vincent AT, Schiettekatte O, Goarant C, Neela VK, Bernet E, Thibeaux R, et al. Revisiting the taxonomy and evolution of pathogenicity of the genus Leptospira through the prism of genomics. PLoS Negl Trop Dis [Internet]. 2019 May 1;13(5). Available from: https://doi.org/10.1371/JOURNAL.PNTD.0007270

14. Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M. List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. Int J Syst Evol Microbiol [Internet]. 2020 Nov 1;70(11):5607–12. Available from: https://doi.org/10.1099/ijsem.0.004332

15. Haake DA, Levett PN. Leptospirosis in Humans. In: Adler B, editor. Leptospira and Leptospirosis [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2015. p. 65–97. Available from: https://doi.org/10.1007/978-3-662-45059-8_5

16. Goarant C. Leptospirosis: Risk factors and management challenges in developing countries. Res Rep Trop Med [Internet]. 2016 Sep;Volume 7:49–62. Available from: https://doi.org/10.2147/RRTM.S102543

17. Sykes JE, Haake DA, Gamage CD, Mills WZ, Nally JE. A global one health perspective on leptospirosis in humans and animals. J Am Vet Med Assoc [Internet]. 2022;260(13):1589–96. Available from: https://doi.org/10.2460/javma.22.06.0258

18. Ellis WA. Animal leptospirosis. In: Adler B, editor. Leptospira and Leptospirosis [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2015. p. 99–137. Available from: https://doi.org/10.1007/978-3-662-45059-8_6

19. Boey K, Shiokawa K, Rajeev S. Leptospira infection in rats: A literature review of global prevalence and distribution. PLoS Negl Trop Dis [Internet]. 2019 [cited 2023 Jan 30];13(8):e0007499. Available from: https://doi.org/10.1371/JOURNAL.PNTD.0007499

20. Obiegala A, Woll D, Karnath C, Silaghi C, Schex S, Eßbauer S, et al. Prevalence and genotype allocation of pathogenic Leptospira species in small mammals from various habitat types in Germany. PLoS Negl Trop Dis [Internet]. 2016 Mar 25;10(3). Available from: https://doi.org/10.1371/JOURNAL.PNTD.0004501

21. Cordonin C, Turpin M, Bringart M, Bascands JL, Flores O, Dellagi K, et al. Pathogenic Leptospira and their animal reservoirs: Testing host specificity through experimental infection. Sci Rep [Internet]. 2020 Apr 29;10(1):7239. Available from: https://doi.org/10.1038/s41598-020-64172-4

22. Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, et al. Leptospirosis: A zoonotic disease of global importance. Lancet Infectious Diseases [Internet]. 2003;3(12):757–71. Available from: https://doi.org/10.1016/S1473-3099(03)00830-2

23. Ellis W, Thiermann A, Marshall R. Genotypes of Leptospira Hardjo and their role in clinical disease. In: Proceedings of 14th World Congress on Diseases of Cattle, Dublin. 1986. p. 966–70.

24. Ellis W. Leptospirosis - A review of veterinary aspects. Irish Vet News. 1990;12:6–12.

25. Ellis W. Diagnosis of leptospirosis in farm animals. Current topics in veterinary medicine and animal science. 1986;

26. de Oliveira D, Figueira CP, Zhan L, Pertile AC, Pedra GG, Gusmão IM, et al. Leptospira in breast tissue and milk of urban Norway rats (Rattus norvegicus). Epidemiol Infect [Internet]. 2016 Aug 1;144(11):2420. Available from: https://doi.org/10.1017/S0950268816000637

27. Bolin CA, Koellner P. Human-to-human transmission of Leptospira interrogans by milk. J Infect Dis [Internet]. 1988 Jul;158(1):246–7. Available from: https://doi.org/10.1093/infdis/158.1.246

28. Cilia G, Bertelloni F, Piredda I, Ponti MN, Turchi B, Cantile C, et al. Presence of pathogenic Leptospira spp. in the reproductive system and fetuses of wild boars (Sus scrofa) in Italy. PLoS Negl Trop Dis [Internet]. 2020 Dec 1;14(12):e0008982. Available from: https://doi.org/10.1371/journal.pntd.0008982

29. Loureiro AP, Lilenbaum W. Genital bovine leptospirosis: A new look for an old disease. Theriogenology [Internet]. 2020 Jan 1;141:41–7. Available from: https://doi.org/10.1016/j.theriogenology.2019.09.011

30. di Azevedo MIN, Lilenbaum W. Equine genital leptospirosis: Evidence of an important silent chronic reproductive syndrome. Theriogenology [Internet]. 2022 Oct 15;192:81–8. Available from: https://doi.org/10.1016/j.theriogenology.2022.08.029

31. Grégoire F, Bakinahe R, Petitjean T, Boarbi S, Delooz L, Fretin D, et al. Laboratory diagnosis of bovine abortions caused by non-maintenance pathogenic Leptospira spp.: Necropsy, serology and molecular study out of a belgian experience. Pathogens [Internet]. 2020;9(6):413. Available from: https://doi.org/10.3390/pathogens9060413

32. Nogueira DB, da Costa FTR, de Sousa Bezerra C, Soares RR, da Costa Barnabé NN, Falcão BMR, et al. Leptospira sp. vertical transmission in ewes maintained in semiarid conditions. Anim Reprod Sci [Internet]. 2020 Aug;219:106530. Available from: https://doi.org/10.1016/j.anireprosci.2020.106530

33. Limothai U, Lumlertgul N, Sirivongrangson P, Kulvichit W, Tachaboon S, Dinhuzen J, et al. The role of leptospiremia and specific immune response in severe leptospirosis. Sci Rep [Internet]. 2021 Jul 16;11(1):14630. Available from: https://doi.org/10.1038/s41598-021-94073-z

34. Wollanke B, Gerhards H, Ackermann K. Infectious uveitis in horses and new insights in its leptospiral biofilm-related pathogenesis. Microorganisms [Internet]. 2022 Feb 1;10(2):387. Available from: https://doi.org/10.3390/microorganisms10020387

35. Delooz L, Czaplicki G, Gregoire F, Dal Pozzo F, Pez F, Kodjo A, et al. Serogroups and genotypes of Leptospira spp. strains from bovine aborted foetuses. Transbound Emerg Dis [Internet]. 2018;65(1):158–65. Available from: https://doi.org/10.1111/tbed.12643

36. Human leptospirosis: Guidance for diagnosis, surveillance and control. World Health Organization; 2003.

37. No author listed. Milk drop due to leptospirosis in dairy cows. Veterinary Record [Internet]. 2015;176(10):247–50. Available from: https://doi.org/10.1136/vr.h829

38. Rypuła K, Płoneczka-Janeczko K, Bierowiec K, Chorbiński P, Pearce MC, Lesiak M. Prevalence of antibodies to Leptospira Hardjo in bulk tank milk from unvaccinated dairy herds in the south-west region of Poland. Berl Munch Tierarztl Wochenschr [Internet]. 2014;127(5–6):247–50. Available from: https://doi.org/10.2376/0005-9366-127-247

39. van Schaik G, Schukken YH, Nielen M, Dijkhuizen AA, Barkema HW, Benedictus G. Probability of and risk factors for introduction of infectious diseases into Dutch SPF dairy farms: A cohort study. Prev Vet Med [Internet]. 2002;54(3):279–89. Available from: https://doi.org/10.1016/S0167-5877(02)00004-1

40. Koizumi N, Picardeau M, editors. Leptospira spp.: Methods and protocols [Internet]. Vol. 2134. New York, NY: Springer US; 2020. Available from: https://doi.org/10.1007/978-1-0716-0459-5

41. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ [Internet]. 2021;372. Available from: https://www.bmj.com/content/372/bmj.n71

42. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2022 [cited 2022 Dec 15]. Available from: https://www.R-project.org/

43. RStudio Team. RStudio: Integrated development environment for R [Internet]. Boston: RStudio, PBC; 2020 [cited 2022 Dec 15]. Available from: http://www.rstudio.com/

44. Wickham H, François R, Henry L, Müller K. dplyr: A grammar of data manipulation. R package version 108 [Internet]. 2022 [cited 2022 Dec 15]. Available from: https://dplyr.tidyverse.org

45. Grolemund G, Wickham H. Dates and times made easy with lubridate. J Stat Softw [Internet]. 2011;40(3):1–25. Available from: https://www.jstatsoft.org/index.php/jss/article/view/v040i03

46. Wickham H. ggplot2: Elegant graphics for data analysis [Internet]. Springer New York, NY. Springer-Verlag New York; 2016 [cited 2022 Dec 15]. Available from: http://ggplot2.org

47. Wickham H. stringr: Simple, consistent wrappers for common string operations [Internet]. 2022 [cited 2022 Dec 15]. Available from: https://cran.r-project.org/package=stringr

48. Pebesma E. Simple features for R: Standardized support for spatial vector data. R Journal. 2018 Dec;10:439–46.

49. South A. rnaturalearth: World map data from natural earth [Internet]. 2017 [cited 2022 Dec 15]. Available from: https://cran.r-project.org/package=rnaturalearth

50. Garnier S, Ross N, Rudis boB, Filipovic-Pierucci A, Galili T, timelyportfolio, et al. sjmgarnier/viridis: viridis 0.6.0 (pre-CRAN release). 2021 Apr 11 [cited 2022 Dec 15]; Available from: https://zenodo.org/record/4679424

51. Neuwirth E. RColorBrewer: ColorBrewer palettes [Internet]. 2022 [cited 2022 Dec 15]. Available from: https://cran.r-project.org/package=RColorBrewer

52. Brunson JC, Read QD. ggalluvial: Alluvial plots in “ggplot2” R package version 0.12.3 [Internet]. 2020 [cited 2022 Dec 15]. Available from: http://corybrunson.github.io/ggalluvial/

53. Wilkins D. ggfittext: Fit text inside a box in “ggplot2” R package version 0.9.1 [Internet]. 2021 [cited 2022 Dec 15]. Available from: https://cran.r-project.org/package=ggfittext

54. Auguie B. gridExtra: Miscellaneous functions for “Grid” graphics R package version 2.3 [Internet]. 2017 [cited 2022 Dec 15]. Available from: https://cran.r-project.org/package=gridExtra

55. Ammar R. randomcoloR: Generate attractive random colors R package version 1.1.0.1 [Internet]. 2019 [cited 2022 Dec 15]. Available from: https://cran.r-project.org/package=randomcoloR

56. Khan, Adeel. collapsibleTree: Interactive collapsible tree diagrams using “D3.js”. R package version 0.1.7 [Internet]. 2018 [cited 2022 Dec 15]. Available from: https://CRAN.R-project.org/package=collapsibleTree

57. Sarkar D. Lattice: Multivariate data visualization with R. New York, NY: Springer New York; 2008.

58. Gordon M, Lumley T. forestplot: Advanced forest plot using “grid” graphics. R package version 3.1.0 [Internet]. 2022 [cited 2022 Dec 15]. Available from: https://CRAN.R-project.org/package=forestplot

59. Delooz L, Mori M, Petitjean T, Evrard J, Czaplicki G, Saegerman C. Congenital jaundice in bovine aborted foetuses: An emerging syndrome in southern Belgium. Transbound Emerg Dis [Internet]. 2015;62(2):124–6. Available from: https://doi.org/10.1111/tbed.12326

60. Mori M, Bakinahe R, Vannoorenberghe P, Maris J, de Jong E, Tignon M, et al. Reproductive disorders and leptospirosis: A case study in a mixed-species farm (cattle and swine). Vet Sci [Internet]. 2017;4(4):64. Available from: https://doi.org/10.3390/vetsci4040064

61. Rifatbegović M, Maksimović Z. Serological study of leptospirosis among dairy cattle in Bosnia and Herzegovina. Turk J Vet Anim Sci [Internet]. 2011;35(6):459–62. Available from: https://doi.org/10.3906/vet-1006-4

62. Habus J, Persic Z, Spicic S, Vince S, Stritof Z, Milas Z, et al. New trends in human and animal leptospirosis in Croatia, 2009–2014. Acta Trop [Internet]. 2017;168:1–8. Available from: https://doi.org/10.1016/j.actatropica.2017.01.002

63. Lebœuf C, Hodiesne J, Leclercq H. Acute leptospirosis, BVD and Anaplasma phagocytophilum [Leptospirose aiguë, BVD et Anaplasma phagocytophilum]. Point Veterinaire. 2004;35(251):62–5.

64. Marquez A, Ulivieri T, Benoit E, Kodjo A, Lattard V. House mice as a real sanitary threat of human and animal leptospirosis: Proposal for integrated management. Biomed Res Int [Internet]. 2019;2019. Available from: https://doi.org/10.1155/2019/3794876

65. Ventejou B, Mascaron L. Bovine leptospirosis in a dairy farm in Normandy: sharp decrease of the milk yield, re-infection and vaccination [Leptospirose bovine dans un élevage laitier en Normandie: chute de lait, ré-infections et vaccination]. Bulletin des GTV. 2010;55(7):75–9.

66. Schmid M, Wolf G, Kaaden OR, Reischl U, Meyer P, Kopp H. Seroprevalence of leptospira in bavarian cattle herds and detection of Leptospira in miscarriaged fetuses. Tierarztl Umsch. 2005;60(5):262–7.

67. Barrett D, Parr M, Fagan J, Johnson A, Tratalos J, Lively F, et al. Prevalence of bovine viral diarrhoea virus (BVDV), bovine herpes virus 1 (BHV 1), leptospirosis and neosporosis, and associated risk factors in 161 irish beef herds. BMC Vet Res [Internet]. 2018;14(1):8. Available from: https://doi.org/10.1186/s12917-017-1324-9

68. Leonard N, Mee JF, Snijders S, Mackie D. Prevalence of antibodies to Leptospira interrogans serovar Hardjo in bulk tank milk from unvaccinated irish dairy herds. Ir Vet J [Internet]. 2004;57(4):226–31. Available from: https://doi.org/10.1186/2046-0481-57-4-226

69. O' Doherty E, Sayers R, O' Grady L. Temporal trends in bulk milk antibodies to Salmonella, Neospora caninum, and Leptospira interrogans serovar Hardjo in irish dairy herds. Prev Vet Med [Internet]. 2012/11/03. 2013;109(3–4):343–8. Available from: https://doi.org/10.1016/j.prevetmed.2012.10.002

70. O' Doherty E, Berry DP, O' Grady L, Sayers R. Management practices as risk factors for the presence of bulk milk antibodies to Salmonella, Neospora caninum and Leptospira interrogans serovar Hardjo in irish dairy herds. Animal [Internet]. 2014/03/26. 2014;8(6):1010–9. Available from: https://doi.org/10.1017/s175173111400055x

71. Ryan EG, Leonard N, O'Grady L, Doherty ML, More SJ. Herd-level risk factors associated with Leptospira Hardjo seroprevalence in beef/suckler herds in the Republic of Ireland. Ir Vet J [Internet]. 2012 Mar 26;65:6. Available from: https://doi.org/10.1186/2046-0481-65-6

72. Ryan EG, Leonard N, O'Grady L, More SJ, Doherty ML. Seroprevalence of leptospira Hardjo in the irish suckler cattle population. Ir Vet J [Internet]. 2012;65:8. Available from: https://doi.org/10.1186/2046-0481-65-8

73. Wasiński B, Sroka J, Wójcik-Fatla A, Zajac V, Cisak E, Knap JP, et al. Occurrence of leptospirosis in domestic animals reared on exposed or non-exposed to flood areas of eastern Poland. J Vet Res [Internet]. 2012;56(4):489–93. Available from: https://doi.org/10.2478/v10213-012-0086-1

74. Alonso-Andicoberry C, García-Pea FJ, Pereira-Bueno J, Costas E, Ortega-Mora LM. Herd-level risk factors associated with Leptospira spp. seroprevalence in dairy and beef cattle in Spain. Prev Vet Med [Internet]. 2001;52(2):109–17. Available from: https://doi.org/10.1016/S0167-5877(01)00249-5

75. Atxaerandio R, Aduriz G, Ziluaga I, Esteban JI, Maranda L, Mainar-Jaime RC. Serological evidence of Leptospira interrogans serovar Bratislava infection and its association with abortions in cattle in northern Spain. Veterinary Record [Internet]. 2005;156(12):376–80. Available from: https://doi.org/10.1136/vr.156.12.376

76. Guitián FJ, García-Peña FJ, Oliveira J, Sanjuána ML, Yusa E. Serological study of the frequency of leptospiral infections among dairy cows in farms with suboptimal reproductive efficiency in Galicia, Spain. Vet Microbiol [Internet]. 2001;80(3):275–84. Available from: https://doi.org/10.1016/S0378-1135(01)00306-6

77. Lindahl E, Boqvist S, Artursson K, Magnusson U. A field-study on Leptospira seroprevalence in dairy cows in four geographical areas in Sweden. Acta Vet Scand [Internet]. 2011;53(1):53. Available from: https://doi.org/10.1186/1751-0147-53-53

78. Pyskun A, Ukhovskyi V, Pyskun O, Nedosekov V, Kovalenko V, Nychyk S, et al. Presence of antibodies against Leptospira interrogans serovar Hardjo in serum samples from cattle in Ukraine. Pol J Microbiol [Internet]. 2019;68(3):295–302. Available from: https://doi.org/10.33073/pjm-2019-031

79. Ukhovskyi VV, Kucheryavenko OO, Kulykova VV, Alekseeva GB, Gerilovych AP, Nedosekov VV, et al. Prevalence and dynamics of the etiological structure of leptospirosis in cattle in Ukraine. Vet Glas [Internet]. 2014;68(1–2):23–30. Available from: https://doi.org/10.2298/vetgl1402023u

80. Bishop H, Erkelens J, van Winden S. Indications of a relationship between buying-in policy and infectious diseases on dairy farms in Wales. Veterinary Record [Internet]. 2010;167(17):644–7. Available from: https://doi.org/10.1136/vr.c5256

81. Velasova M, Damaso A, Chengat Prakashbabu B, Gibbons J, Wheelhouse N, Longbottom D, et al. Herd-level prevalence of selected endemic infectious diseases of dairy cows in Great Britain. J Dairy Sci [Internet]. 2017;100:9215–33. Available from: https://doi.org/10.3168/jds.2016-11863

82. Williams D, Winden S v. Risk factors associated with high bulk milk antibody levels to common pathogens in UK dairies. Veterinary Record [Internet]. 2014;174(23):580. Available from: https://doi.org/10.1136/vr.102049

83. Risk factors in health and disease - EUPATI toolbox [Internet]. [cited 2022 Dec 4]. Available from: https://toolbox.eupati.eu/resources/risk-factors-in-health-and-disease/

84. Principles of epidemiology | Lesson 3 - Section 5 [Internet]. [cited 2022 Oct 26]. Available from: https://www.cdc.gov/csels/dsepd/ss1978/lesson3/section5.html

85. Norton EC, Dowd BE. Log odds and the interpretation of logit models. Health Serv Res [Internet]. 2018 Apr 1 [cited 2022 Dec 4];53(2):859. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5867187/

86. Ruano MP, Burgos Macías DI, Goicochea CAB, Zambrano Aguayo MD, Sandoval Valencia HP, Falconi Flores MA, et al. Seroprevalence and risk factors of bovine leptospirosis in the province of Manabí, Ecuador. Comp Immunol Microbiol Infect Dis [Internet]. 2020 Oct;72:101527. Available from: https://doi.org/10.1016/j.cimid.2020.101527

87. Bulla-Castañeda D, Buitrago H, Lancheros-Buitrago D, Díaz-Anaya A, Garcia-Corredor D, Tobón-Torreglosa J, et al. Seroprevalence and risk factors associated with the presence of bovine leptospirosis in the municipality of Sotaquirá, Colombia. Open Vet J [Internet]. 2022;12(5):668. Available from: https://doi.org/10.5455/OVJ.2022.v12.i5.11

88. Fávero JF, de Araújo HL, Lilenbaum W, Machado G, Tonin AA, Baldissera MD, et al. Bovine leptospirosis: Prevalence, associated risk factors for infection and their cause-effect relation. Microb Pathog [Internet]. 2017 Jun;107:149–54. Available from: https://doi.org/10.1016/j.micpath.2017.03.032

89. Desa G, Deneke Y, Begna F, Tolosa T. Seroprevalence and associated risk factors of Leptospira interrogans serogroup Sejroe serovar Hardjo in dairy farms in and around Jimma Town, Southwestern Ethiopia. Vet Med Int [Internet]. 2021 Sep 17;2021:1–10. Available from: https://doi.org/10.1155/2021/6061685

90. Campos ÂP, Miranda DFH, Rodrigues HWS, da Silva Carneiro Lustosa M, Martins GHC, Mineiro ALBB, et al. Seroprevalence and risk factors for leptospirosis in cattle, sheep, and goats at consorted rearing from the state of Piauí, northeastern Brazil. Trop Anim Health Prod [Internet]. 2017 Jun 1;49(5):899–907. Available from: https://doi.org/10.1007/s11250-017-1255-2

91. Olmo L, Reichel MP, Nampanya S, Khounsy S, Wahl LC, Clark BA, et al. Risk factors for Neospora caninum, bovine viral diarrhoea virus, and Leptospira interrogans serovar Hardjo infection in smallholder cattle and buffalo in Lao PDR. PLoS One [Internet]. 2019 Aug 8;14(8):e0220335. Available from: https://doi.org/10.1371/journal.pone.0220335

92. Montes V, Monti G. Pathogenic Leptospira spp. seroprevalence and herd-level risk factors associated with chilean dairy cattle. Animals [Internet]. 2021 Nov 4;11(11):3148. Available from: https://doi.org/10.3390/ani11113148

93. Givens MD. Review: Risks of disease transmission through semen in cattle. Animal [Internet]. 2018;12:s165–71. Available from: https://doi.org/10.1017/S1751731118000708

94. Eaglesome MD, Garcia Animal Diseases Research Institute NEPEAN 3851 Fallowfield Road P.O. Box 11300 Station H Nepean Ontario K2H 8PH (Canada)) MM (Agriculture C. Microbial agents associated with bovine genital tract infections and semen. Part I. Brucella abortus, Leptospira, Campylobacter fetus and Tritrichomonas foetus. Vol. v. 62, Veterinary Bulletin (United Kingdom). 1992.

95. Barragan VA, Mejia ME, Trávez A, Zapata S, Hartskeerl RA, Haake DA, et al. Interactions of Leptospira with environmental bacteria from surface water. Curr Microbiol [Internet]. 2011 Jun 10;62(6):1802–6. Available from: https://doi.org/10.1007/s00284-011-9931-3

96. Smith CE, Turner LH. The effect of pH on the survival of leptospires in water. Bull World Health Organ. 1961;24(1):35–43.

97. Barragan V, Olivas S, Keim P, Pearson T. Critical knowledge gaps in our understanding of environmental cycling and transmission of Leptospira spp. Appl Environ Microbiol [Internet]. 2017 Oct;83(19). Available from: https://doi.org/10.1128/AEM.01190-17

98. Main climates of Europe — European Environment Agency [Internet]. [cited 2023 Feb 9]. Available from: https://www.eea.europa.eu/data-and-maps/figures/climate

99. Chadsuthi S, Chalvet-Monfray K, Wiratsudakul A, Modchang C. The effects of flooding and weather conditions on leptospirosis transmission in Thailand. Scientific Reports 2021 11:1 [Internet]. 2021 Jan 15;11(1):1–12. Available from: https://doi.org/10.1038/s41598-020-79546-x

100. Joshi YP, Kim EH, Cheong HK. The influence of climatic factors on the development of hemorrhagic fever with renal syndrome and leptospirosis during the peak season in Korea: An ecologic study. BMC Infect Dis [Internet]. 2017 Jun 7;17(1):1–11. Available from: https://doi.org/10.1186/s12879-017-2506-6

101. Suk JE, Vaughan EC, Cook RG, Semenza JC. Natural disasters and infectious disease in Europe: A literature review to identify cascading risk pathways. Eur J Public Health [Internet]. 2020 Oct 1;30(5):928–35. Available from: https://doi.org/10.1093/eurpub/ckz111

102. Semenza JC, Menne B. Climate change and infectious diseases in Europe. Lancet Infect Dis [Internet]. 2009 Jun;9(6):365–75. Available from: https://doi.org/10.1016/S1473-3099(09)70104-5

103. Bezirtzoglou C, Dekas K, Charvalos E. Climate changes, environment and infection: Facts, scenarios and growing awareness from the public health community within Europe. Anaerobe [Internet]. 2011 Dec;17(6):337–40. Available from: https://doi.org/10.1016/j.anaerobe.2011.05.016

104. Mora C, McKenzie T, Gaw IM, Dean JM, von Hammerstein H, Knudson TA, et al. Over half of known human pathogenic diseases can be aggravated by climate change. Nat Clim Chang [Internet]. 2022 Sep 8;12(9):869–75. Available from: https://doi.org/10.1038/s41558-022-01426-1

105. Rossati A. Global warming and its health impact. Int J Occup Environ Med [Internet]. 2017 Jan 1;8(1):7–20. Available from: https://doi.org/10.15171/IJOEM.2017.963

106. Picardeau M. Diagnosis and epidemiology of leptospirosis. Med Mal Infect [Internet]. 2013 Jan;43(1):1–9. Available from: https://doi.org/10.1016/J.MEDMAL.2012.11.005

107. Grüner Bericht 2022: Die Situation der österreichischen Land- und Forstwirtschaft [Internet]. 2022 [cited 2023 Feb 9]. Available from: https://gruenerbericht.at/cm4/jdownload/send/2-gr-bericht-terreich/2398-gb2022

108. Bijttebier J, Coopmans I, Appel F, Unay Gailhard I, Wauters E. Project acronym: SURE-Farm D3.1 Report on current farm demographics and trends work performed by P3 (OCILVO), in cooperation with P12 (IAMO).

109. Farms and farmland in the European Union - statistics - statistics explained [Internet]. [cited 2023 Feb 11]. Available from: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Farms_and_farmland_in_the_European_Union_-_statistics#The_evolution_of_farms_and_farmland_between_2005_and_2020

110. Hernández-Rodríguez P, Gómez AP, Villamil LC. Implications of urban and rural agricultural practices on the transmission of leptospirosis. Agrociencia. 2017 Jan;51:725–41.

111. Bianchi MC, Bava L, Sandrucci A, Tangorra FM, Tamburini A, Gislon G, et al. Diffusion of precision livestock farming technologies in dairy cattle farms. animal [Internet]. 2022 Nov;16(11):100650. Available from: https://doi.org/10.1016/j.animal.2022.100650

112. Haidich AB. Meta-analysis in medical research. Hippokratia. 2010;14(Suppl 1):29.

113. Miller E, Barragan V, Chiriboga J, Weddell C, Luna L, Jiménez DJ, et al. Leptospira in river and soil in a highly endemic area of Ecuador. BMC Microbiol [Internet]. 2021 Dec 7;21(1):17. Available from: https://doi.org/doi.org/10.1186/s12866-020-02069-y

6. Annex

Table 7. Data extraction table of risk factors, 2001-2021; Assoc.: association; B: beef herd; D: dairy herd; Epi. unit: epidemiological unit; NA: Not available; Neg.: Negative; NS: Non significant; Not evid.: Not evidenced; Obs.: observational/hypothetical; OR: odds ratio; Pos.: positive; Signif.: Significant; Stat.: Statistics used in the study; Stat. value: value of the statistics; UK: United Kingdom; 95 % CI: 95 % confidence interval;

Citation short	Study design	Coun-try	Epi. unit	Sample size	Herd type	Dependent variable	Risk category	Risk factor	Assoc.	Model	Stat.	Stat. value	95% CI	P-value
Alonso-Andicoberry et al., 2001	Cross-sectional	Spain	Animal / herd	81 dairy herds, 134 beef herds (total 1993 animals)	D & B	Within-herd seroprevalence	Herd management practices	Herd size	Not evid.	Chi-squared test or Fisher's exact test	NA	NA	NA	NS
						Within-herd seroprevalence (serovar Copenhageni)	Animal	Type of production	Signif.	Chi-squared test or Fisher's exact test	NA	NA	NA	<0.05
						Within-herd seroprevalence (serovar Grippotyphosa)	Animal	Type of production	Signif.	Chi-squared test or Fisher's exact test	NA	NA	NA	<0.001
						Within-herd seroprevalence (serovar Tarassovi)	Animal	Type of production	Signif.	Chi-squared test or Fisher's exact test	NA	NA	NA	<0.05
No author listed, 2015	Clinical case investigation	UK	Animal	12 cattle	D	Animal (sero)positivity to Leptospira	Environmental factors	Access to natural water source	Pos.	NA	NA	NA	NA	Obs.
Atxaerandio et al., 2005	Case control	Spain	Animal / herd	Seroprevalence: 697 cattle; 32 herds for adult cows, 29 herds for pregnant heifers. Case-control: 144 cattle as case, 380 as control.	D	Animal (sero)positivity to Leptospira (serovar Bratislava)	Animal	Age	Neg.	Chi-squared test	Chi-square	NA	NA	0.021
						Animal (sero)positivity to Leptospira (serovar Bratislava)	Clinical signs	Abortion year before	Pos.	Logistic regression	OR	3.23	1.3 – 8.1	0.05
Barrett et al., 2018	Cross-sectional	Rep. of Ireland	Herd	161 cattle herds (6049 cattle)	B	Within-herd seroprevalence	Herd management practices	Herd size	Pos.	Linear regression	linear regression coefficient	0.093	0.035 – 0.15	0.002
						Within-herd seroprevalence	Biosecurity	Animal introduction: purchase	Pos.	Linear regression	linear regression coefficient	0.247	0.084 – 0.41	0.003
						Within-herd seroprevalence	Biosecurity	BVD vaccination	Pos.	Linear regression	linear regression coefficient	27.979	3.293 – 52.66	0.027

Bishop et al., 2010	Cross-sectional	UK	Herd	57 herds	D	Herd (sero)positivity to Leptospira	Herd management practices	Herd size	Pos.	Wilcoxon rank-sum test	NA	NA	NA	<0.01
						Herd (sero)positivity to Leptospira	Biosecurity	Animal introduction: purchase	Not evid	NA	OR	16.3	0.33 – 791.3	NS
						Within-herd seroprevalence	Biosecurity	Bull management practice: hiring bulls	Not evid	NA	OR	3.2	NA	NS
						Herd (sero)positivity to Leptospira	Infectious diseases	Testing positive for BVD	Pos.	NA	OR	42	3.7 - 481	<0.05
						Herd (sero)positivity to Leptospira	Infectious diseases	Testing positive for BHV-1	Pos.	NA	OR	7.5	1.2 – 45.8	<0.05
Delooz et al., 2015	Clinical case investigation	Belgium	Animal	19 cattle that showed icteric abortions. 22 control cattle (= non-icteric presentation of abortions at time period as the previous group).	NA	Animal (sero)positivity to Leptospira	Clinical signs	Symptom=icteric abortion	Pos.	NA	OR	48	5 - 442	NA
Williams et al., 2014	Cross-sectional	UK	Herd	1088 herds	D	Herd (sero)positivity to Leptospira	Herd management practices	Herd size	Pos.	One-way ANOVA	NA	NA	NA	<0.001
						Herd (sero)positivity to Leptospira	Biosecurity	Animal introduction: purchase	Pos.	Binary logistical regression	OR	2.57	1.95 – 3.78	<0.001
						Herd (sero)positivity to Leptospira	Biosecurity	Time since last purchase of cattle	Neg.	Kruskal-Wallis ANOVA	NA	NA	NA	<0.001
						Within-herd seroprevalence	Herd management practices	Calving season	Not evid	Binary logistical regression	NA	NA	NA	NS
						Herd (sero)positivity to Leptospira	Biosecurity	Bull management practice	Not evid	Binary logistical regression	NA	NA	NA	NS
						Herd (sero)positivity to Leptospira	Infectious diseases	Testing positive for BHV-1	Pos.	Chi-squared test	Chi-square	3.43	NA	<0.001
						Herd (sero)positivity to Leptospira	Infectious diseases	Testing positive for BVD	Pos.	Chi-squared test	Chi-square	2.16	NA	<0.001
Wasiński et al., 2012	Case control	Poland	Animal	Community A: 41 cattle; Community B: 40 cattle	NA	Herd antibody titer level	Environmental factors	Exposure to flooding	Pos.	Mann-Whitney test	NA	NA	NA	0.0128
						Herd (sero)positivity to Leptospira	Environmental factors	Exposure to flooding	Not evid	Student's t-test	NA	NA	NA	NS

Velasova et al., 2017	Cross-sectional	UK	Herd	225 herds	D	Within-herd seroprevalence	Herd management practices	Herd size	Not evid	Logistic regression	OR	1	0.4 – 2.8	NS
						Within-herd seroprevalence	Environmental factors	Geographical location	Signif.	Logistic regression	NA	NA	NA	<0.001
						Herd (sero)positivity to Leptospira	Infectious diseases	Testing positive for BVD	Pos.	Correlation	Phi-correlation coefficient	0.41	NA	NA
						Herd (sero)positivity to Leptospira	Infectious diseases	Testing positive for BHV-1	Pos.	Correlation	Phi-correlation coefficient	0.59	NA	NA
Van Schaik et al., 2002	Longitudinal	Netherlands	Herd	95 herds	D	Incidence of Leptospira	Biosecurity	Animal introduction: purchase	Pos.	NA	NA	NA	NA	Obs.
Rypuła et al., 2014	Cross-sectional	Poland	Herd	309 herds	D	Within-herd seroprevalence	Herd management practices	Herd size	Not evid	Chi-squared test	Chi-square	NA	NA	NS
						Within-herd seroprevalence	Environmental factors	Geographical location	Observed	NA	NA	NA	NA	Obs.
Schmid et al., 2005	Cross-sectional	Germany	Animal / herd	3463 cattle (1213 herds)	D & B	Herd (sero)positivity to Leptospira	Environmental factors	Geographical location	Observed	NA	NA	NA	NA	Obs.
Guitián et al., 2001	Cross-sectional	Spain	Animal	442 cattle (15 herds)	D	Incidence of Leptospira serogroup Australis (Bratislava)	Environmental factors	Season spring	Not evid	Proportional hazards regression method	OR	0.38	0.05 – 3.29	0.57
	Cohort study		Animal	219 cattle (9 herds) - several samples per animal (1060 samples; average 4.8 samples per cattle)	D	Incidence of Leptospira serogroup Australis (Bratislava)	Environmental factors	Season summer	Not evid	Proportional hazards regression method	OR	1.16	0.38 – 3.6	0.38
						Incidence of Leptospira serogroup Australis (Bratislava)	Environmental factors	Season fall	Not evid	Proportional hazards regression method	OR	0.63	0.2 - 2	0.44
						Incidence of Leptospira Grippotyphosa	Environmental factors	Season spring	Pos.	Proportional hazards regression method	OR	3.74	1.04 – 13.45	0.04
						Incidence of Leptospira Grippotyphosa	Environmental factors	Season summer	Not evid	Proportional hazards regression method	OR	1.36	0.38 – 4.95	0.64
						Incidence of Leptospira Grippotyphosa	Environmental factors	Season fall	Not evid	Proportional hazards regression method	OR	0.33	0.06 – 1.84	0.21
Habus et al., 2017	Longitudinal	Croatia	Animal	22669 cattle	NA	Seroprevalence	Environmental factors	Extreme weather event	Observed	NA	NA	NA	NA	Obs.

Leonard et al., 2004	Cross-sectional	Rep. of Ireland	Herd	347 herds	D	Within-herd antibody titer level	Herd management practices	Herd size	Pos.	Correlation	Spearman correlation coefficient	0.27	NA	<0.0001
						Herd (sero)positivity to Leptospira	Environmental factors	Geographical location	Signif.	Logistic regression	F-value	4.73	NA	<0.05
						Within-herd antibody titer level	Environmental factors	Geographical location	Signif.	Logistic regression	F-value	4.73	NA	<0.01
Lindahl et al., 2011	Cross-sectional	Sweden	Animal	610 cattle (20 herds)	D	Seroprevalence	Environmental factors	Geographical location	Not evid	Fisher's exact test	NA	NA	NA	NS
Lindahl et al., 2011	Cross-sectional	Sweden	Animal	610 cattle (20 herds)	D	Seroprevalence	Animal	Age	Pos.	NA	NA	NA	NA	Obs.
Marquez et al., 2019	Cross-sectional	France	Animal	79 cattle	B	Animal (sero)positivity to Leptospira	Biosecurity	Presence of rodents	Pos.	NA	NA	NA	NA	Obs.
Mori et al., 2017	Clinical case investigation	Belgium	Animal	26 cattle	D	Animal (sero)positivity to Leptospira	Environmental factors	Access to natural water source	Pos.	NA	NA	NA	NA	Obs.
						Animal (sero)positivity to Leptospira	Biosecurity	Use of swine manure	Pos.	NA	NA	NA	NA	Obs.
						Animal (sero)positivity to Leptospira	Animal	Age	Pos.	NA	NA	NA	NA	Obs.
O' Doherty et al., 2014	Cross-sectional	Rep. of Ireland	Herd	309 herds	D	Herd (sero)positivity to Leptospira	Biosecurity	Movement of cattle onto and off the farm	Pos.	Multivariable analysis	OR	15.15	1.35 – 170.27	0.03
						Herd (sero)positivity to Leptospira	Herd management practices	Calves grazing on cows pasture	Pos.	Multivariable analysis	OR	13.69	1.21 – 154.54	0.03
						Herd (sero)positivity to Leptospira	Herd management practices	Herd size	Pos.	Multivariable analysis	OR	0.02	0.0005 – 0.62	0.03
						Herd (sero)positivity to Leptospira	Biosecurity	Minimizing numbers of visitors to the farm	Pos.	Univariate analysis	NA	NA	NA	0.15
						Herd (sero)positivity to Leptospira	Biosecurity	Using agricultural contractors without insisting that their equipment was clean and disinfected	Pos.	Univariate analysis	NA	NA	NA	0.10

						Herd (sero)positivity to Leptospira	Herd management practices	Percentage of first lactation animals	Pos.	Univariate analysis	NA	NA	NA	0.13
						Herd (sero)positivity to Leptospira	Herd management practices	Rearing of calves on out farms	Pos.	Univariate analysis	NA	NA	NA	0.13
						Herd (sero)positivity to Leptospira	Herd management practices	Housing of calves later in the year	Pos.	Univariate analysis	NA	NA	NA	0.06
						Herd (sero)positivity to Leptospira	Biosecurity	Oral drenching equipment was regularly cleaned	Pos.	Multivariable analysis	OR	0.02	0.0005 – 0.74	0.03
O' Doherty et al., 2013	Cross-sectional	Rep. of Ireland	Herd	309 herds	D	Herd (sero)positivity to Leptospira	Study protocol	Sample date	Not evid	Generalised estimating equations	NA	NA	NA	0.39
						Incidence of Leptospira	Infectious diseases	Incidence of Neospora caninum	Not evid	Generalised estimating equations	NA	NA	NA	0.53
						Incidence of Leptospira	Infectious diseases	Incidence of Salmonella	Pos.	Generalised estimating equations	NA	NA	NA	0.002
Pyskun et al., 2019	Cross-sectional	Ukraine	Animal	573 cattle	NA	Seroprevalence	Environmental factors	Geographical location	Observed	NA	NA	NA	NA	Obs.
Rifatbegović & Maksimović, 2011	Longitudinal	Bosnia and Herzegovina	Animal	75206 cattle: dairy cows (64716) and quarantined dairy heifers imported into the country (10490).	D	Seroprevalence	Environmental factors	Geographical location	Signif.	Chi-squared test	Chi-square	NA	NA	<0.001
						Seroprevalence	Study protocol	Year of sampling	Signif.	Chi-squared test	Chi-square	NA	NA	<0.05
Ryan et al., 2012a	Cross-sectional	Rep. of Ireland	Herd	288 herds	B	Herd seroprevalence	Environmental factors	Geographical location	Signif.	NA	95%CI	NA	NA	<0.05
			Animal	5366 cattle		Seroprevalence	Environmental factors	Geographical location	Signif.	NA	95%CI	NA	NA	<0.05
						Within-herd seroprevalence	Herd management practices	Herd size	Pos.	One-way ANOVA test	F-value	NA	NA	<0.001
						Seroprevalence	Animal	Breed	Signif.	NA	95%CI	NA	NA	P<0.05
						Seroprevalence	Animal	Age	Not evid	NA	95%CI	NA	NA	NA
						Seroprevalence	Animal	Sex	Not evid	NA	95%CI	NA	NA	NA
Leboeuf et al., 2004	Clinical case	France	Animal	10 cattle	D	Severity of clinical symptoms	Infectious diseases	Testing positive for BVD	Pos.	NA	NA	NA	NA	Obs.

	investigation					Severity of clinical symptoms	Infectious diseases	Testing positive for Anaplasma phagocytophilum	Pos.	NA	NA	NA	NA	Obs.
Ventejou et al., 2010	Clinical case investigation	France	Animal	5 cattle	D	Animal (sero)positivity to Leptospira	Environmental factors	Access to pasture	Pos.	NA	NA	NA	NA	Obs.
						Animal (sero)positivity to Leptospira	Biosecurity	Presence of rodents	Pos.	NA	NA	NA	NA	Obs.
Ryan et al., 2012b	Cross-sectional	Republic of Ireland	Herd	Seroprevalence study: 288 herds; risk factor analysis: 128 herds	B	Herd (sero)positivity to Leptospira	Herd management practices	Herd size	Pos.	Multivariable logistic regression	OR	2.039	1.252 – 3.322	0.004
						Herd (sero)positivity to Leptospira	Environmental factors	Geographical location	Not evid	Multivariable logistic regression	NA	NA	NA	NS
						Herd (sero)positivity to Leptospira	Environmental factors	Geographical location	Signif.	Univariate analysis	OR	1.378	1.063 – 1.786	0.016
						Herd (sero)positivity to Leptospira	Herd management practices	Herd size	Pos.	Multivariable logistic regression	OR	2.039	1.252 – 3.32	0.004
						Herd (sero)positivity to Leptospira	Infectious diseases	History of leptospirosis	Not evid	Multivariable logistic regression	NA	NA	NA	0.065
						Herd (sero)positivity to Leptospira	Clinical signs	Weak calves the previous year	Not evid	Multivariable logistic regression	NA	NA	NA	0.168
						Herd (sero)positivity to Leptospira	Biosecurity	Bull management practice: use stock bull	Pos.	Multivariable logistic regression	OR	3.056	1.150 – 8.120	0.025
						Herd (sero)positivity to Leptospira	Biosecurity	Animal introduction: purchase	Not evid	Multivariable logistic regression	NA	NA	NA	0.093
						Herd (sero)positivity to Leptospira	Environmental factors	Grazing acres	Pos.	Multivariable logistic regression	OR	1.607	1.013 – 2.548	0.044
						Herd (sero)positivity to Leptospira	Environmental factors	Surface of the grazing acres	Not evid	Multivariable logistic regression	NA	NA	NA	0.144
						Herd (sero)positivity to Leptospira	Environmental factors	Percentage wet land grazed	Neg.	Multivariable logistic regression	OR	0.588	0.368 – 0.940	0.026
						Herd (sero)positivity to Leptospira	Environmental factors	Straw-bed shed	Pos.	Multivariable logistic regression	OR	4.304	0.897 – 20.66	0.068
						Herd (sero)positivity to Leptospira	Herd management practices	Cows and heifers separate at calving	Signif.	Multivariable logistic regression	NA	NA	NA	0.003

						Herd (sero)positivity to Leptospira	Environmental factors	Out-winter fed in fields	Not evid	Multivariable logistic regression	NA	NA	NA	0.142
Ukhovskyi et al., 2014	Clinical case investigation	Ukraine	Animal	1834316 cattle	NA	Seroprevalence	Environmental factors	Geographical location	Observed	NA	NA	NA	NA	Obs.
Grégoire et al., 2020	Longitudinal	Belgium	Animal	116 foetuses; 88 dams. 20 coupled dams' sera and foetuses' pleural fluids, and six individual foetuses' pleural fluids.	NA	Seropositivity to Leptospira in aborted dam (titer 1:100)	Clinical signs	Necropsy aborted foetus: icterus	Pos.	OR, Chi-squared test or Fisher's exact test	OR	13.55	4.56 – 40.24	<0.001
						Seropositivity to Leptospira in aborted dam (titer 1:100)	Clinical signs	Necropsy aborted foetus: splenomegaly	Pos.	OR, Chi-squared test or Fisher's exact test	OR	5.69	2.33 – 13.9	<0.001
						Seropositivity to Leptospira in aborted dam (titer 1:100)	Clinical signs	Necropsy aborted foetus: coppery liver	Pos.	OR, Chi-squared test or Fisher's exact test	OR	4.43	1.68 – 11.66	0.004
						Seropositivity to Leptospira in aborted dam (titer 1:100)	Clinical signs	Necropsy aborted foetus: icterus + splenomegaly + coppery liver	Pos.	OR, Chi-squared test or Fisher's exact test	OR	6.44	2.02 – 20.52	0.002
						Seropositivity to Leptospira in aborted dam (titer 1:100)	Clinical signs	Necropsy aborted foetus: peri-renal haemorrhages	Not evid	OR, Chi-squared test or Fisher's exact test	OR	0.75	0.33 – 1.72	NS
						Seropositivity to Leptospira in aborted dam (titer 1:100)	Clinical signs	Necropsy aborted foetus: extended haemorrhagic pattern	Not evid	OR, Chi-squared test or Fisher's exact test	OR	3.49	0.39 – 31.12	NS
						PCR positive in aborted foetus	Clinical signs	Necropsy aborted foetus: icterus	Pos.	OR, Chi-squared test or Fisher's exact test	OR	70.91	8.57 – 586.89	<0.001
						PCR positive in aborted foetus	Clinical signs	Necropsy aborted foetus: splenomegaly	Pos.	OR, Chi-squared test or Fisher's exact test	OR	19.03	3.95 – 91.81	<0.001
						PCR positive in aborted foetus	Clinical signs	Necropsy aborted foetus: coppery liver	Pos.	OR, Chi-squared test or Fisher's exact test	OR	7.6	2.58 – 22.38	<0.001

						PCR positive in aborted foetus	Clinical signs	Necropsy aborted foetus: icterus + splenomegaly + coppery liver	Pos.	OR, Chi-squared test or Fisher's exact test	OR	9	2.95 – 27.45	<0.001
						PCR positive in aborted foetus	Clinical signs	Necropsy aborted foetus: peri-renal haemorrhages	Not evid	OR, Chi-squared test or Fisher's exact test	OR	1.22	0.42 – 3.54	NS
						PCR positive in aborted foetus	Clinical signs	Necropsy aborted foetus: extended haemorrhagic pattern	Not evid	OR, Chi-squared test or Fisher's exact test	OR	0.58	0.05 – 6.76	NS

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