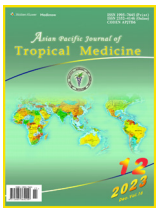

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Vaccine development for leptospirosis: A systematic review

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ABSTRACT

Objective: To assess the efficacy of various types of vaccines developed for leptospirosis.

Methods: A comprehensive search was conducted in three databases: PubMed, Scopus, and Cochrane Library. Two authors (YS and MN) selected the articles based on manual screening. The study eligibility criteria are all *Leptospira* species regardless of any cluster (pathogenic, intermediate and non-pathogenic). This study recorded articles with positive and negative results and showed a comparison among various membrane proteins as vaccine candidates. The studies on the effectiveness of outer membrane protein as vaccine candidates were also included. The articles obtained in the databases were imported into the WPS spreadsheet, and duplicate documents were removed manually.

Results: A total of 24 studies were included in the review, which evaluated various types of leptospirosis vaccines. Multiple vaccines were developed and tested; however, the heterogeneity of *Leptospira* species pose a challenge. As an effective approach, an epitope based vaccine shows quite a promising result. However, sufficient validation, testing and clinical trials are required.

Conclusions: Developing an effective vaccine for leptospirosis remains a global health priority. While significant progress has been made in recent years, there is a need for further research to optimize vaccine development and to ensure that vaccines are accessible and effective for high-risk populations.

KEYWORDS: *Leptospira*; Vaccine candidate; Outer membrane protein

1. Introduction

Leptospira is a Gram-negative and zoonotic bacterium widely spread to humans and animals[1]. *Leptospira* species have been classified into three clusters: pathogenic, intermediate, and non-pathogenic. The number of *Leptospira* species has increased compared to previous years. The cluster of *Leptospira* differs according to their lipopolysaccharides (LPS)[2,3]. In addition, exposure to contaminated water sources is the most significant risk for this infectious disease[4].

Significance

Leptospirosis is a bacterial infection that affects humans and animals and is spread through contact with contaminated water, soil, or animal urine. Epitope vaccines which target particular regions of the *Leptospira* bacteria, lowering the possibility of side effects and boosting vaccine effectiveness, are a promising method for developing a vaccine against leptospirosis. They are desirable for mass vaccination programs because they are affordable and straightforward to store and transport. Overall, using epitope vaccines is a promising approach for creating a leptospirosis vaccine that is both safe and effective. Further investigation is required to improve epitope delivery and selection strategies and assess the effectiveness of epitope vaccines in clinical trials.

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The symptoms of leptospirosis are similar to those of other febrile illnesses, such as dengue, malaria, chickenpox, and typhoid. Hence, this contributes to misdiagnosis[5]. The signs and symptoms in humans differ according to one's immunity, with the range of none, mild, and severe. The mild range of symptoms is known as anicteric. During this stage, the patient would not seek medical check-ups as the symptoms are headache, abdominal pain, skin rash, and chills. Conversely, the severe stage is known as the icteric stage, which would show myocarditis (heart-related problems), pulmonary haemorrhage syndrome, kidney failure, and jaundice. Eventually, this stage would lead to fatality[6,7].

Referring to the World Health Organization (WHO), more than 500 000 cases of leptospirosis occur worldwide each year, with a mortality rate of 5%-25% in severe cases[8]. However, the actual number of cases is likely much higher, as many could be undiagnosed or unreported due to the symptoms shown[9]. Based on Wilkinson *et al.*, approximately 59 000 deaths are recorded annually out of one million infected instances worldwide[2].

In prevention, many studies have been conducted on developing an effective vaccine for leptospirosis. However, developing vaccines against leptospirosis remains challenging due to the numerous *Leptospira* species and the evolved pathogenic mechanisms[6]. Therefore, this systematic review furnishes a summary of the developed vaccines for leptospirosis. In addition, this review also aims to reconstruct current knowledge on vaccine candidate selection, types of vaccines designed, methodologies and outcomes.

2. Materials and methods

This systematic study was conducted as per the PRISMA (Preferred Reporting Item for Systemic Review and Meta-Analyses), 2020 guidelines. It was registered under PROSPERO (Registration Number: CRD42021257531).

2.1. Analysis of article content-inclusion and exclusion criteria

Two researchers (YS and MN) read the selected article and the collected data was compared and analyzed. The analyzed data was saved in a WPS spreadsheet file to be compared. The inclusion and exclusion criteria were categorized according to the PICO element as tabulated in Table 1. PICO is an acronym which stands for population, intervention, comparison and outcome. This method is used in evidence-based medicine and healthcare research to formulate straightforward and focused research questions. Thus, this allows for reducing the level of biases or limitations.

2.2. Literature search

Articles were sought in November 2022 from three databases; PubMed, Scopus, and Cochrane Library. We use the following search terms: [Leptospir* AND (Outer membrane protein OR Vaccination OR Immunization)] in combination. The literature review screening and data extraction were done independently by two researchers. Two researchers discussed and dealt with differences in the summary of the results. All the results obtained in the database were imported into the WPS spreadsheet, and duplicate documents were removed manually. The initial screening was completed by reading the title and abstract, and the secondary screening by reading the full text. The extracted data included *Leptospira* strains, vaccine candidate, vaccine type, adjuvant, animal model, route of administration, and a vital outcome of the development.

2.3. Methodology quality evaluation

Assessing the risk of bias is according to Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) based on guideline 2.0[10].

Table 1. The patients, intervention, comparison, outcome strategy, and type of study (PICO) element table.

PICO element	Inclusion	Exclusion
Population	Studies of *Leptospira* species regardless of the clusters (pathogenic, intermediate, and non-pathogenic)	Studies that are not related to *Leptospira* species
Intervention	Studies that include membrane protein as its vaccine candidate	Studies that include membrane protein as their vaccine candidate but did not explain the methods used in detail
Comparison	Studies that developed a vaccine based on transmembrane and peripheral membrane protein	Studies that do not include outer membrane protein as its vaccine candidate
Outcome	*In vivo* studies that have recorded positive or negative outcomes Studies that have recorded the effectiveness of outer membrane protein as a vaccine candidate	Studies that do not include *in vivo* animal models
Study design	Research articles from the recent nine years (2014-2022)	Inaccessible full-text articles Duplicate articles Other languages

3. Results

3.1. Data collection and analysis

The initial search retrieved 5 302 articles using PubMed, Scopus and Cochrane Library databases after removing 1 203 duplicates and 1 757 other language articles. Therefore, only English-based articles were submitted to the inclusion criteria. Finally, 24 articles selected based on their abstract and full text were included for the analysis as summarized in Figure 1.

The ARRIVE 2.0 criteria were used to assess bias in this investigation, ensuring transparency in evidence synthesis and findings. While Figure 2 showed shortcomings in critical areas such as experimental animal reporting, housing, generalizability, and protocol registration numbers, there is comprehensive information in areas such as study design, statistical methods, results, abstract, objective, and ethical statements. Authors should resolve the observed deficiencies to strengthen the manuscript's completeness

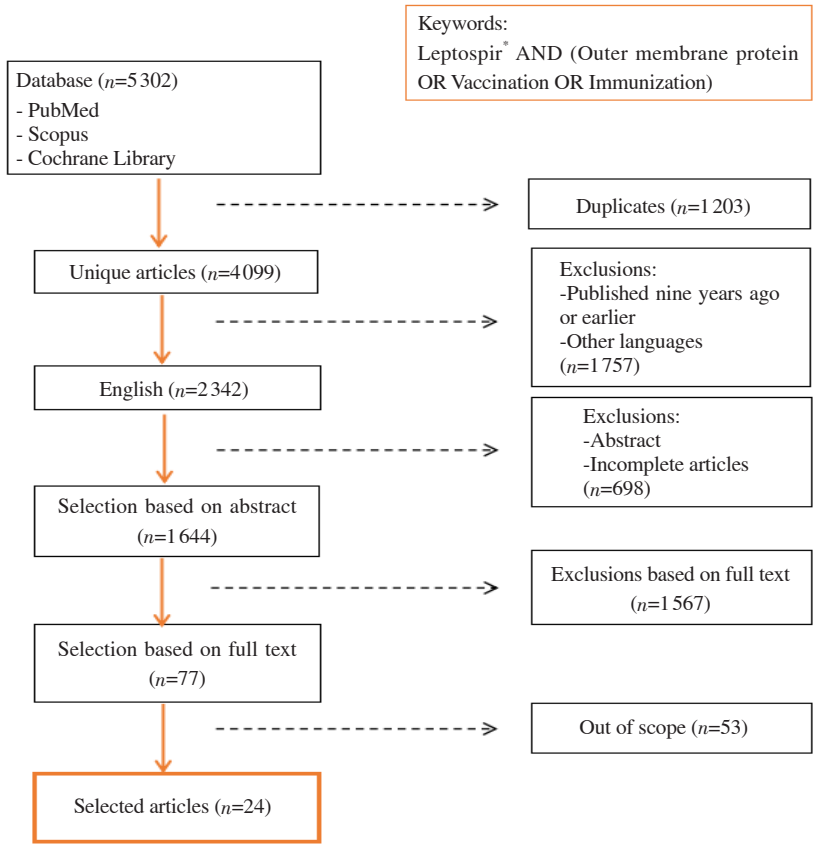

Figure 1. Literature search analysis according to the PRISMA guidelines.

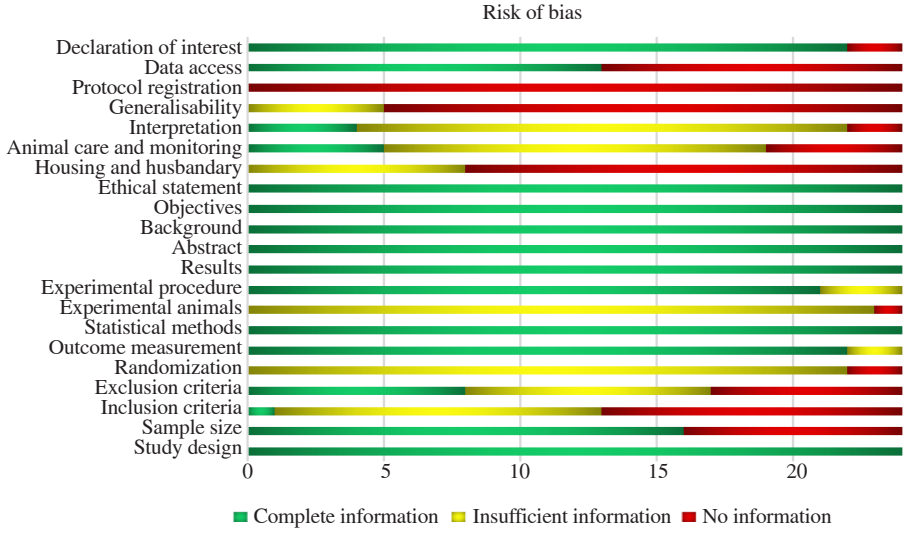

Figure 2. The quality evaluation of the selected literature reviews is analyzed based on 21 categories obtained by ARRIVE guidelines.

and trustworthiness, emphasizing the need of giving extensive details in experimental publications for a strong study presentation[10].

3.3. Quality assessment

This systematic paper was analyzed according to the ARRIVE 2.0 guidelines. All 24 selected papers produced complete information for their study design, statistical methods, results, abstract, background, objectives and ethical statements (Figure 2). It is a graphical representation of the risk of bias. However, there were 23 studies that lacked details about the provenance of animals, their health status, genetic modifications, and the genotype of the animal model. Similarly, there were eight articles with incomplete information and 16 articles with no information on housing and husbandry settings for animal study. Therefore, this would lead to a lack of experiment transparency. Lack of transparency can undermine the paper's credibility. In that case, it can be difficult or impossible for other researchers to replicate the study, which is essential for validating the results and advancing scientific knowledge. Thus, including detailed information on the experimental animal procedure in a review paper is necessary to ensure transparency and facilitate replication. Failure to do so can negatively impact the validity and reliability of the study results and the credibility of the paper[35,36].

3.4. Leptospira species

Leptospira (L.) interrogans is one of the most important species of the genus *Leptospira* and is responsible for most leptospirosis cases worldwide. Due to the disease's significant impact on animal health and production, extensive research has been conducted on *L. interrogans*[37]. Several factors, including animal activism, may influence the incidence and prevalence of the disease, the environment's suitability for the organism's survival, and human behaviour and work habits[38]. Based on Table 2, most researchers conducted their study referring to *L. interrogans*. This could be due to a large number of related cases reported in both humans and animals. This has prompted intensive research into the epidemiology, pathogenesis, and immunology, as well as the development of an effective method for diagnosing, treating, and preventing *Leptospira*[6].

Conversely, the researchers could include intermediate *Leptospira* species in their vaccine development. Intermediate *Leptospira* species were reported to cause mild to severe forms of leptospirosis in humans; however, the findings about its pathogenicity status are still unclear[39]. Intermediate *Leptospira* species can cause significant health problems and should not be underestimated. Early diagnosis, proper treatment and vaccination are essential measures to manage and prevent leptospirosis in both humans and animals. More research is needed to analyze, identify, and comprehend the virulence level of intermediate species[40]. Identification of suitable vaccine candidates is based on the pathogenic, regionally prevalent species.

3.5. Candidate selection

In the quest for an effective *Leptospira* vaccine, scientists have turned their attention to the outer membrane proteins (OMPs), which serve as ideal candidates due to their accessibility to the immune system. This accessibility enhances the chances of a robust immune response[41]. For instance, a study by Vijayachari and the team demonstrated that immunizing hamsters with LipL45 provided significant protection against virulent *Leptospira* strains, showcasing the potential of OMPs[15]. Likewise, vaccines containing LigA and LigB proteins have proven effective against multiple *Leptospira* serovars, reducing bacterial burden and tissue damage while inducing a broad, cross-reactive immune response in animal models[31].

Conversely, transmembrane and peripheral membrane proteins are rarely chosen as vaccine candidates[42]. These proteins are nestled within cell membranes, making them less accessible to immune cells and potentially hindering recognition and response. OmpL1, a transmembrane protein, has shown promise in eliciting a robust immune response in animal models, making it an attractive vaccine target for leptospirosis[13]. However, further research is required to assess its efficacy and safety fully. LipL32, another commonly selected vaccine candidate, has demonstrated the ability to induce high levels of antibody production. While LipL32 holds diagnostic potential, it has fallen short in conferring animals protection, highlighting the multifaceted challenges of *Leptospira* vaccine development[43].

3.6. Type of vaccines

In the quest to develop a robust vaccine against leptospirosis, researchers have turned to recombinant-based vaccines, a cutting-edge approach that merges specific genes with empty plasmids to create a broader shield against leptospiral infections, as shown in Figure 3[44]. While promising results have emerged from preclinical and clinical studies, challenges persist, notably the bacterium's crafty antigenic variation. Leptospires boast high genetic diversity and numerous serovars, each potentially expressing distinct surface proteins, making it a formidable task to pinpoint a universal antigen capable of safeguarding against all variations[45,46].

Antigenic variation is a fascinating adaptive strategy that pathogens like *Treponema* and *Borrelia* employ to evade host immune responses. *Treponema pallidum*, the bacterium responsible for syphilis, uses extensive antigenic variation through its *Treponema pallidum* repeat protein K (TprK) to switch its surface antigens and

Table 2. Characteristics of the included studies.

Study	*Leptospira (L.)* species	Vaccine candidate	Adjuvant	Type of vaccine	Animal model	Route of immunization	Base outline	Outcome
Bacelo *et al*, 2014[11]	*L. interrogans*	rLigANI	Xanthan gum	Subunit	Hamsters	SC	Promising outcome	This paper demonstrated that the xanthan gum enhanced the immune response and the immune protection induced by rLigANI, a poorly immunogenic antigen, in the subunit vaccine preparation against leptospirosis.
Monaris *et al*, 2015[12]	*L. interrogans*	LigA	Aluminum hydroxide	Subunit	Hamsters	SC	Promising outcome	The study presents promising outcomes as vaccine formulations containing recombinant *Leptospira interrogans* proteins elicited robust antibody responses and demonstrated substantial immunoprotection in hamsters.
Oliveira *et al*, 2015[13]	*L. interrogans*	OmpL37	Not stated	Subunit & DNA vaccine	Hamster	IP	Mixed outcome due to multiple groups	Different vaccination groups exhibited varying levels of mRNA expression for various cytokines, suggesting a complex and multifaceted immune response to the vaccine.
Forster *et al*, 2015[14]	*L. interrogans*	LigA LigB	Alhydrogel	DNA prime-protein boost based vaccine	Hamsters	IP	Promising outcome	It indicates that vaccine build around the LigBrep protein can trigger significant antibody.
Vijayachari *et al*, 2015[15]	*L. interrogans*	LipL45	Not stated	DNA vaccine	Hamsters	IM	Promising outcome	It indicates that the use of the pLipL45 DNA vaccine in mice enhances the production of immune response specifically IFN-γ and IL-12 associated Th1 response.
Lin 2016[16]	*L. interrogans*	OmpL1 LipL32 LipL21	Aluminum hydroxide	Chimeric epitope vaccine	Guinea pigs	SC	Lack of information	It is mentioned that an antigen-specific humoral immune response was induced by the vaccine. Additionally, an increase in specific types of immunoglobulin (IgG1 and IgG2a) was noted in the animals that were vaccinated when compared to those not treated with the vaccine. There is also mention of IgG2a being higher than IgG1, which indicates a tendency towards a Th1 polarized immune response in the vaccinated guinea pigs.
Fernandes *et al*, 2017[17]	*L. interrogans*	LigA Mce Lsa45 OmpL1 LipL42	DDA	Multiepitope chimeric protein	Hamsters	IP	Promising outcome	A new chimeric protein was constructed when evaluated using various adjuvant shows to confer protection against lethal infection in hamsters' model of leptospiral.
Conrad *et al*, 2017[18]	*L. interrogans*	LigB	Aluminum hydroxide	Subunit	Hamsters	IM	Promising outcome	The LigB subunit vaccine was shown to confer sterile immunity against leptospirosis in hamsters. This suggests that the vaccine could be a vital tool in preventing and controlling the disease. However, a few instances in which the vaccine did not work highlights the need for further study to understand and improve the vaccine's effectiveness.
Evangelista *et al*, 2017[19]	*L. interrogans*	LigA LigB	Freund's	Recombinant	Hamsters	SC	Promising outcome	It demonstrates that all animals in the study, which were immunized with recombinant LigA7'-13 protein alone, or in combination with LigB0-7, survived the challenge. This evidence suggests potential immunoprotective properties of these recombinant proteins against acute leptospirosis, which is a positive outcome for the development of vaccines for this disease.
Raja *et al*, 2018[20]	*L. interrogans*	RecA FliD	Alhydrogel	DNA based	Hamsters	IM	Promising outcome	The researchers found that a prime-boost vaccination strategy using RecA and FilD protein provided a significant level of protection against both homologous and cross-clade heterologous leptospiral infection.
Teixeira *et al*, 2018[21]	*L. interrogans* *L. borgpetersenii* *L. kirshneri* *L. santarosai* *L. noguchii* *L. biflexa*	Lsa 46 Lsa 77	Alhydrogel	Recombinant	Hamsters	SC	Mixed outcome	This combined use resulted in an increased survival rate and decreased bacterial burden in hamsters. However, it is important to note that the data were not statistically significant.

Table 2. Continued.

Study	*Leptospira (L.)* species	Vaccine candidate	Adjuvant	Type of vaccine	Animal model	Route of immunization	Base outline	Outcome
Larre *et al*, 2018[22]	*L. interrogans*	LemA Erp Y	AddaVax	Recombinant	Hamsters	IM	Promising outcome	The study demonstrates a potential for leptospirosis vaccines using recombinant proteins LemA and Erp Y-like from *Leptospira interrogans*. The vaccine provided protection and exhibited organ lesions in certain hamsters.
Techwiwattanaboon *et al*, 2019[23]	*L. interrogans*	LigAc LenA LcpA Lsa 23	LMQ	Multisubunit	Hamsters	IP	Promising outcome	Multisubunit vaccine for leptospirosis, utilizing four recombinant factor H binding proteins (FHBPs) with an adjuvant. This vaccine demonstrated partial protection against a lethal challenge, particularly when combined with the LigAc vaccine, suggesting potential advancements in leptospirosis vaccine development.
Costa *et al*, 2019[24]	*L. interrogans*	LipL32 LigAni LigBrep	Aluminum hydroxide & Montanide	Recombinant	Sheeps	IM	Promising outcome	Recombinant proteins LigAni, LigBrep, and LipL32 induced a robust humoral immune response in sheep, resulting in high serum immunoglobulin titers.
Larre *et al*, 2019[25]	*L. interrogans*	LigAni LigBrep LipL32 LemA	*Mycobacterium bovis* BCG	Recombinant	Hamsters	SC	Promising outcome	The study demonstrates the efficacy of chimeric antigens delivered through recombinant BCG strains in protecting against leptospirosis, with 100% protection observed for rBCG strains expressing the chimeric antigen 1 in hamsters. This breakthrough suggests the potential development of effective leptospirosis vaccines.
Techawiwattanaboon *et al*, 2020[26]	*L. interrogans*	LigAc	LMQ	Recombinant	Syrian hamster	IM & SC	Promising outcome	The study demonstrates the development of a leptospirosis subunit vaccine using LigAc antigen. Both IM and SC administration methods were effective, with two IM vaccinations produce significantly higher antibody responses. LigAc, a highly promising antigen, showed potential for use in subunit vaccines against leptospirosis and contributed to the promising findings in animal models.
Larre *et al*, 2020[27]	*L. interrogans*	Lem A	Freund	DNA Nanovaccine	Hamsters	IM	Promising outcome	The study explains nanovaccines utilizing NPs and rLemA DNA demonstrated enhanced protection in hamsters against lethal *Leptospira* infection compared to vaccines without nanocarriers. These findings suggest a potentially valuable approach to combatting leptospirosis and underscore the effectiveness of the nanovaccine strategy.
Teixeira *et al*, 2020[28]	*L. interrogans*	Lsa25.6 Lsa16 LipL46 Lsa14 Lsa24.9 rLIC11711 rLIC13259	Alum	Recombinant	Hamsters	IP	Promising outcome	The study shows developing a vaccine against leptospirosis, with certain outer membrane proteins from *Leptospira interrogans* demonstrating the ability to induce renal clearance in immunized animals. However, it emphasizes the need for a targeted immune response against specific key antigens and acknowledges that achieving complete sterilizing immunity remains a challenge.
Lauretti-Ferreira *et al*, 2020[29]	*L. interrogans*	LPS	Not stated	Whole cell vaccine serovar independent	Hamsters	Not stated	Promising outcome	The paper suggests that bivalent vaccines with reduced LPS content could be a viable strategy for protection against various *Leptospira* serovars. The experiments demonstrated significantly high survival rates in immunized hamsters, and the anticipated lower reactogenicity of low LPS vaccines adds to their potential.

Table 2. Ccontinued.

Study	*Leptospira (L.)* species	Vaccine candidate	Adjuvant	Type of vaccine	Animal model	Route of immunization	Base outline	Outcome
Schuler *et al*, 2021[30]	*L. noguchii* *L. kirschneri* *L. alstonii* *L. borgpetersenii* *L. weilii* *L. wolffi* *L. licerasiae* *L. meyeri* *L. yanagawae* *L. biflexa* *L. illini*	GspD	Freund	Recombinant	Rat	IP	Not significant outcome	The excerpts provided describe experimental procedures involving preparations of antigen and bactericidal assays among others. However, the specific results of these experiments are not reported here.
Wunder *et al*, 2021[31]	*L. interrogans*	FcpA	AddaVax	Live attenuated	Hamsters	SC	Promising outcome	Live-attenuated vaccine model for *Leptospira*, demonstrating cross-protective immunity against different species of the same organism. The vaccine shows effectiveness in terms of reduced mortality and colonization rates, supported by proteome array analysis and immunogenicity, though additional research is needed for more comprehensive conclusions.
Chaurasia *et al*, 2022[32]	*L.interrogans*	PF07598	Glucioyranosyl lipid A/ squalene oil-in-water	Serovar-independent pan-leptospirosis	Mice	IM	Promising outcome	The paper demonstrates that vaccination with proteins encoded by the *Leptospira interrogans* PF07598. These findings support the potential of VM proteins as vaccine candidates for leptospirosis.
Bettin *et al*, 2022[33]	*L. interrogans*	LIC10896 LIC10964 LIC12374	Freund	Recombinant	Syrian Hamster	IM	Promising outcome	The paper identifies conserved surface-exposed regions in leptospiral Ton B-dependent receptors and uses them to develop a multi-epitope fusion protein. When tested with Bacille Calmette-Guérin as a vaccine carrier, this protein induces a protective immune response in hamsters, including the production of specific antibodies and sterilizing immunity, suggesting its potential as an effective vaccine against leptospirosis.
Martins *et al*, 2022[34]	*L. santarosai*	LipL32	Aluminium Potassium Sulfate	Inactivated	Sheeps	IM	Not significant outcome	The paper's results suggest that vaccination effectively prevents renal and genital leptospirosis in sheep, with a substantial seroconversion observed by day 14 post-vaccination. However, no significant difference between vaccine groups was observed after day 60 post-infection

Promising outcome refers to a positive result. Mixed outcome refers to results which are positive and negative. This could be due to the various concentrations of vaccines immunized to the animal model. Not significant outcome could be due to the statistical analysis performed by the authors. Lack of information refers to authors who do not provide sufficient information. BCG: bacillus Calmetter-Guerin. IM: intramuscular injection; SC: subcutaneous injection; IP: intraperitoneal injection.

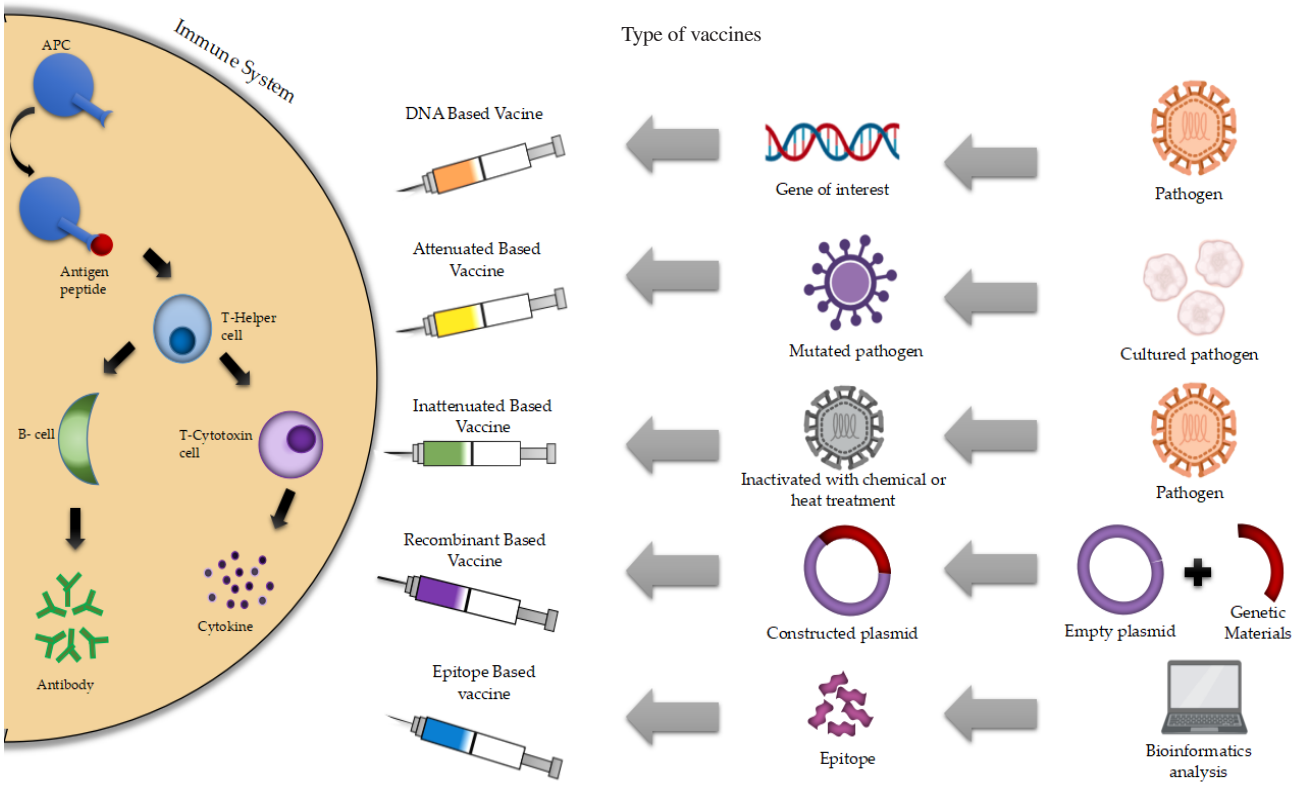

Figure 3. The flow of designing various types of vaccines.

avoid detection by the host immune system. TprK which has 12 orthologs is highly heterogeneous in inter and intra strain levels[47]. Similarly, *Borrelia burgdorferi*, the causative agent of Lyme disease, utilizes a diverse array of outer surface proteins S (Osps) that can undergo recombination and switching during infection, enabling it to evade immune surveillance and establish persistent infections. These antigenic variations challenge the host immune response and pose significant obstacles in vaccine development and therapeutic strategies against these pathogens[48].

Contrastingly, less studied and developed for epitope-based vaccines offer a streamlined approach targeting the most critical pathogen epitopes. These vaccines minimize the risk of side effects and adverse reactions, presenting only the necessary epitopes to the immune system. They also hold the potential to induce a potent and specific immune response, offering a finely tuned defence mechanism[49]. Notably, a study in 2016 unveiled a multiepitope vaccine that triggered a significant humoral immune response in mice, providing partial protection against lethal challenges from *L. interrogans*. In the ongoing battle against these wily bacteria, these diverse vaccine strategies offer hope in conquering leptospirosis and other infectious foes[16].

3.7. Routes of immunization

Based on Table 2, the vaccines developed for leptospirosis are based on intramuscular injection (IM), subcutaneous injection (SC) and intraperitoneal injection (IP)-various immunization methods with multiple functions and benefits. Most leptospiral vaccines are injected intramuscularly. Based on Techawiwattanaboon *et al.*, the immune response produced by IM and SC immunization methods has some significant differences. IM immunization generates a localized immune response at the site of injection. This can result in a strong antibody response to the antigen. In contrast, SC immunization typically induces a slower and milder immune response, as the antigen is released slowly from the tissue layer[26]. On the other side, the IP immunization method is commonly used in animals, particularly in laboratory settings. Thus, IP immunization typically generates a systemic immune response which can result in a broader range of immune responses against the antigen[50]. Figure 4 explains the benefits of three immunization methods. Thus far, the route of immunization depends on the type of vaccine, the age and health of the organisms, and the availability of resources and expertise.

4. Discussion

Vaccines are developed to help prevent leptospiral spread. This systematic review is based on a rigorous process to identify articles on leptospiral vaccine development. Thus, this systematic review has involved 24 selected articles from three databases. In this discussion, we will review some critical challenges that need to be addressed to

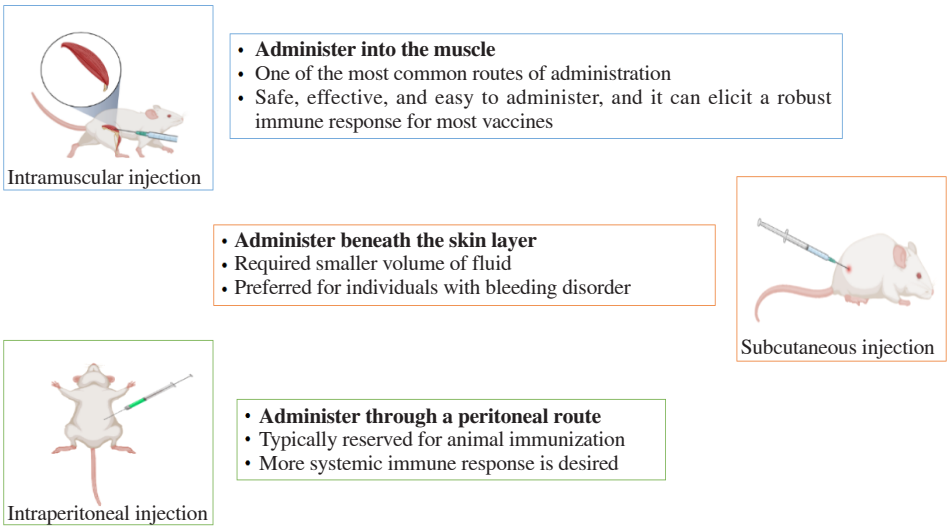

Figure 4. Various routes of immunization tested on animal models.

prevent and control the spread of leptospirosis. Leptospirosis poses significant risks to various groups, including agricultural workers, veterinarians and wildlife personnel due to their frequent exposure to the bacteria[6]. However, the disease's impact extends beyond these groups to individuals with outdoor activities, rural residents, and even urban populations in some areas. Zoonotic nature and effective vaccines are crucial to protect high risk occupational groups and to reduce disease spread in communities, playing a pivotal role in public health efforts to control leptospirosis and safeguard populations at risk[51].

There are several leptospiral vaccines designed for animal use, particularly in dogs and cattle, which have been in use for more than two decades. These vaccines serve dual purposes: safeguarding kidney tissues from *Leptospira*-induced harm and mitigating the bacterium's transmission *via* urine. Notably, canine vaccines like Nobivac have been instrumental in preventing severe kidney damage in dogs affected by leptospirosis, particularly in endemic regions[52]. Similarly, vaccines such as Leptoferm and Spirovac have been employed in cattle for extended periods to reduce the impact of leptospirosis on kidney health and minimize urinary shedding of the pathogen[53]. These enduring vaccine interventions have preserved animal health and contributed to the broader public health goal of limiting the environmental dissemination of *Leptospires*, benefiting both animal and human populations[6]. Nonetheless, developing effective leptospiral vaccines for at-risk individuals continues to face various challenges[44].

As mentioned earlier, developing vaccines for high-risk individual is crucial to safeguard their health and prevent disease outbreaks. However, identifying the suitable vaccine candidate is a pivotal step in the vaccine development process. Kashyap and their team have highlighted that exposed-surface OMPs, which showed promise as vaccine candidates due to their ability to stimulate the immune system effectively and their ease of large-scale purification and production[54]. OMPs are recognized for their high immunogenicity because they are readily detected by the immune system, being exposed to the bacterial cell surface. Additionally, OMPs often play a role in bacterial virulence, making them attractive targets to reduce infection severity or prevent it altogether[55]. A study by Awanye *et al.*, had proven that OMPs (PorA, PorB, OpcA, PilQ) from *Neisseria meningitidis* can exhibit a significant level of immune response in humans and it is a promising vaccine candidate[56]. Similarly, the Omp16 and Omp19 from *Brucella* has been proven to be a protective antigen[57].

The potential of OMPs as vaccine candidates in the context of leptospirosis is promising but marred by concerns regarding their purity and concentration. Large surface-exposed OMPs such as LigA, LigB, Lsa46, and Lsa77 are considered valuable for diagnostic and vaccine purposes due to their immunogenicity and roles in host-pathogen interactions. However, their production is challenged by the complexity and difficulty of obtaining pure and stable forms of these proteins[43,54,58]. Additionally, well-known OMPs like LipL32 and LemA, which also have diagnostic and vaccine potential, suffer from production obstacles, primarily stemming from low concentration and impurities in *Leptospira* cultures. These challenges hinder their practical utility in diagnostic assays and vaccine development efforts[27,43,59,60].

Transmembrane and peripheral membrane proteins are less suitable as vaccine candidates due to their inaccessibility. Transmembrane proteins, embedded in lipid bilayers, are highly insoluble and challenging to purify, and their native expression is difficult[61]. Conversely, peripheral proteins, while attached to the membrane's surface, can be shielded from the immune system, making it harder

to trigger a robust immune response against them. Additionally, targeting certain transmembrane and peripheral proteins in vaccines can pose toxicity risks to the host, potentially leading to undesirable side effects or severe adverse reactions[62].

Based on Table 2, recombinant vaccines for *Leptospira* have been developed and tested in preclinical and clinical studies[62]. Therefore, there are some challenges in the development of the recombinant vaccine. Leptospiral bacteria have a high genetic diversity and different serovars that may express other surface proteins. Hence, it would be difficult to identify a universal antigen that can provide broad protection against all serovars. Several surface proteins of *Leptospira* have been identified as potential vaccine candidates[12]. Nevertheless, the protection mechanism remains unknown. In addition, recombinant vaccines require additional regulatory approval and safety testing compared to other types of vaccines. This will lead to an increase in the time and cost of development[44].

Epitope-based vaccines can be precisely designed to target highly conserved epitopes shared by multiple pathogen strains, resulting in a focused and efficient immune response[63]. Unlike traditional vaccines containing numerous antigens, epitope-based vaccines consist of specific epitopes recognized by the immune system, reducing the risk of adverse reactions. Moreover, they can elicit a stronger immune response by targeting multiple epitopes. Computational tools enable the efficient and cost-effective design of epitope-based vaccines. In the case of leptospirosis, researchers have successfully identified immunogenic epitopes using bioinformatics and tested a vaccine candidate that induced both humoral and cellular immune responses in mice[64,65]. Through creative tactics and worldwide collaboration, this strategy addresses the challenges of vaccine safety and accessibility.

The future of *Leptospira* vaccination necessitates a diversified strategy to comprehensively address the challenges of preventing and controlling leptospirosis. This multifaceted approach includes broadening serovars coverage, seeking cross-protection by identifying conserved antigens, and extending immunity to reduce the need for regular booster shots[66]. Ensuring vaccine safety and efficacy through rigorous clinical trials, improving affordability and accessibility in resource-limited areas, and adopting a One Health approach that includes animal vaccination to minimize human exposure are all pivotal components[67]. Harnessing genetic technology, fostering global collaboration, and adapting to the effects of climate change are also of paramount importance[68]. Therefore, this context supports epitope-based vaccination, which has emerged as a promising strategy due to its potential to overcome antigenic variation.

Consequently, numerous practical examples of epitope-based vaccines are created for different diseases. Development of an effective epitope-based vaccine against *Treponema*, the causative agent of syphilis, is an ongoing area of research with variable success depending on specific epitopes and vaccine candidates[64]. Proteins like TpF1, which contain immunogenic epitopes accessible to the immune system, have been explored for their potential as vaccine candidates[69]. The success of epitope-based vaccines hinges on epitope selection, vaccine design, and the ability to induce a strong and lasting immune response. Researchers typically conduct preclinical studies including animal experiments to assess vaccine effectiveness. However, developing vaccines for complex pathogens like *Treponema* are challenging, and ongoing research aims to enhance the effectiveness of epitope-based vaccines[70].

In the context of *Borrelia*, the pathogen responsible for Lyme disease, researchers have also delved into epitope-based strategies[48]. They have pinpointed specific epitopes within *Borrelia burgdorferi* outer surface proteins C (OspC) that play crucial roles in host-pathogen interactions[71]. These epitopes have become subjects of extensive investigation for their potential in crafting vaccines with enhanced specificity and reduced side effects. While epitope-based approaches hold promise, it's important to note that the success of these vaccines can vary, and their effectiveness in clinical settings may require further validation through rigorous testing and clinical trials[49].

However, this manuscript only refers to articles obtained from three databases over a nine-year period. This limited selection could be a disadvantage because it may have excluded more recent studies, thereby affecting the research's comprehensiveness. As a result, the risk of bias assessment revealed inadequate information, thus limiting the depth of research and adding ambiguity to the conclusions. Transparently highlighting these limits within the publication would demonstrate the authors' recognition of potential biases, bolstering trust and offering recommendations for future studies to fill these gaps[72].

5. Conclusions

It is necessary to develop a vaccine that is affordable, secure, and capable of offering cross-protection and long-lasting immunity. With the availability of leptospiral genome sequences, potential vaccine sequences could be *in silico* analyzed. The development of vaccines is interrelated with the selection of vaccine candidates and the formulation of vaccines. The conserved regions found in the pathogenic and intermediate *Leptospira* species should be considered when identifying vaccine candidates. Additionally, an epitope-based vaccine could be developed in place of the current one, producing better outcomes and minizing allergic and autoimmune reactions. Overall, despite the challenges, the development of a vaccine for leptospirosis would be a significant advancement in public health and disease prevention.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability

The published paper includes the data used to support the study's findings.

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Authors' contributions

The manuscript conceptualization and project supervision were led by NM. Authors YS and MN contributed to the original draft, conducted investigation and shaped the methodology of the manuscript. SAN, MRMA and SKD were pivotal in meticulously reviewing and editing the manuscript.

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