Diagnostic accuracy of genetic markers and nucleic acid techniques for the detection of *Leptospira* in clinical samples: A meta-analysis

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Abstract

**Background**

Leptospirosis is often difficult to diagnose because of its nonspecific symptoms. The drawbacks of direct isolation and serological tests have led to the increased development of nucleic acid-based assays, which are more rapid and accurate. A meta-analysis was performed to evaluate the diagnostic accuracy of genetic markers for the detection of *Leptospira* in clinical samples.

**Methodology and principle findings**

A literature search was performed in Scopus, PubMed, MEDLINE and non-indexed citations (via Ovid) by using suitable keyword combinations. Studies evaluating the performance of nucleic acid assays targeting leptospire genes in human or animal clinical samples against a reference test were included. Of the 1645 articles identified, 42 eligible studies involving 7414 samples were included in the analysis. The diagnostic performance of nucleic acid assays targeting the *rrs*, *lipL32*, *secY* and *flaB* genes was pooled and analyzed. Among the genetic markers analyzed, the *secY* gene showed the highest diagnostic accuracy measures, with a pooled sensitivity of 0.56 (95% CI: 0.50–0.63), a specificity of 0.98 (95% CI: 0.97–0.98), a diagnostic odds ratio of 46.16 (95% CI: 6.20–343.49), and an area under the curve of summary receiver operating characteristics curves of 0.94. Nevertheless, a high degree of heterogeneity was observed in this meta-analysis. Therefore, the present findings here should be interpreted with caution.

**Conclusion**

The diagnostic accuracies of the studies examined for each genetic marker showed a significant heterogeneity. The *secY* gene exhibited higher diagnostic accuracy measures compared with other genetic markers, such as *lipL32*, *flaB*, and *rrs*, but the difference was not significant. Thus, these genetic markers had no significant difference in diagnostic accuracy for leptospirosis. Further research into these genetic markers is warranted.
Leptospirosis is a globally important zoonotic disease caused by *Leptospira* spp. This disease is often difficult to diagnose because its clinical manifestations resemble those of other diseases, such as dengue and malaria. Leptospirosis is often misdiagnosed, leading to improper medical management of patients. Accurate and timely diagnosis of the disease is important because antibiotic therapy is the most effective during the early stage. Nucleic acid assays are superior to most currently available diagnostics because they provide a definitive diagnosis during the acute stage of the disease even before antibodies are detectable. Nevertheless, the choice of genetic markers for these assays remains perplexing. Hence, this study evaluated the diagnostic accuracy of these genetic markers by pooling and analyzing them simultaneously. Results revealed that nucleic acid assays targeting the *secY* gene of leptospires had better diagnostic accuracy with the three other genetic markers coming close with good diagnostic performance. With the heterogeneity observed, there is no clear cut answer as to which of these markers is the best for diagnosing leptospirosis. Nevertheless, the analyses in this study suggested that all four markers exhibited good diagnostic measures and are promising targets for the future development of nucleic acid-based diagnostics.

Introduction

Leptospirosis is a worldwide zoonotic disease recognized as an important emerging infectious disease in the past few decades. This disease occurs in diverse epidemiological settings, especially in tropical or subtropical regions of the world but imparts the greatest burden on resource-limited populations [1]. Leptospirosis was estimated to cause a million cases and close to 60,000 deaths annually [2]. Leptospirosis affects risk groups that are exposed to animal reservoirs or contaminated environments but exerts a broader health impact on impoverished farmers from the tropical regions [3]. This disease has also emerged as a health threat in new settings due to the influence of globalization and climate change, where natural disasters and extreme weather events are now recognized to precipitate epidemics [4, 5].

This disease is caused by spirochetes belonging to the genus *Leptospira*, comprising of both saprophytic and pathogenic species. The clinical manifestations of human leptospirosis are diverse, ranging from mild, flu-like illness to a more severe form of the disease known as Weil’s syndrome, which is characterized by jaundice, acute renal and hepatic failure, pulmonary distress, and hemorrhage, which can lead to death. These symptoms are similar to those of other infectious diseases, such as dengue fever and malaria, often causing misdiagnosis. Early diagnosis of this disease is crucial because antibiotic therapy provides the greatest benefit and is the most efficacious when initiated early in the course of an illness [3].

Dark-field microscopy is a conventional method for leptospirosis diagnosis through direct microscopic observation of clinical specimens. However, the sensitivity of this method is low, and the result is affected by the timing of sample collection and the skill of laboratory personnel [1]. *Leptospira* can be isolated from clinical specimens through inoculation into an appropriate culture medium, but its application in the field is hampered by the long doubling time and the need for special media in addition to its low sensitivity [6]. Microscopic agglutination test (MAT) is the current reference standard serological diagnostic test in leptospirosis. However, MAT requires the maintenance of live leptospires. As a minimum, the panel of live leptospires should include all locally circulating serovars; otherwise, an incomplete panel could lead
to a false negative result [7]. Therefore, although MAT is considered as the gold standard test for leptospirosis diagnosis, it is laborious and its requirement for a large panel of live Leptospira culture hinders its standardization [8]. Other serological tests also have been developed with the likes of ELISA, complement fixation, indirect hemagglutination, latex bead agglutination, and indirect immunofluorescence [1, 3, 9], but all have been hampered by their low sensitivities for the initial management of acute leptospirosis [8].

These drawbacks have led to the increased development and use of nucleic acid-based diagnostics, such as conventional and real-time polymerase chain reaction (PCR) and isothermal amplification methods, which feature high sensitivity [10]. The advantage of nucleic acid-based diagnostics lies in their ability to obtain a definitive diagnosis during the acute stage of the disease even before antibodies are detectable [8]. Hence, these methods tend to replace the serological methods in endemic zones. They are normally based on the detection of a certain gene present in Leptospira. Genes such as rrs, secY, lipL32, flaB, lfb1, ligA, and ligB2 have all been used as targets of nucleic acid-based diagnosis [11–14] and have been detected from blood, urine, cerebrospinal fluid, and tissue samples [15].

However, little is known about the diagnostic accuracy of each genetic marker. In addition, most studies had a low number of samples, which limited the statistical power and scientific reliability of the results. This meta-analysis was conducted to pool and analyze simultaneously all studies that used nucleic acid techniques to detect Leptospira in clinical samples of humans and animals. This pooled analysis aimed to provide a precise estimation of the diagnostic accuracy of nucleic acid techniques to detect Leptospira.

Methods

Literature search

A systematic review of nucleic acid techniques in detecting Leptospira was conducted based on the principles recommended in the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statements (S1 Checklist).

Data sources. Relevant studies were identified by systematic search of electronic databases Scopus, PubMed, MEDLINE (from 1946 until present; via Ovid), and non-indexed citations (via Ovid).

Search strategy. The search of relevant studies was carried out up to December 2018 by using subject headings and free text terms. The search was carried out with the keywords “(leptospirosis OR leptospira”) AND (human OR patient OR animal OR clinical) AND (sensitivity OR specificity OR “true positive” OR “true negative” OR “false positive” OR “false negative”).

Inclusion criteria

Cross-sectional and cohort studies that assessed nucleic acid techniques for the detection of Leptospira in human or animal clinical samples against at least one reference test were included, regardless of publication year. Laboratory diagnoses of leptospirosis are usually based on several methods or a combination of these methods due to the temporal nature of the disease progression and the absence of a satisfactory universal reference test [8]. Studies with reference tests such as MAT, any PCR-based tests, isolation of leptospires through culture, or the detection of antibodies to the bacteria, were considered for inclusion. Studies must directly or indirectly provide at least four values, which are number of true positives (TPs), false positives (FPs), true negatives (TNs), and false negatives (FNs), to construct or reconstruct a two-by-two table. Only articles published in English were evaluated.
Exclusion criteria

The relevance of each study was determined based on their types. Reviews that do not contain original data and proceedings that did not employ any peer-review process were excluded. In addition, letters, editorials, and case reports were excluded. The objectives and methods were assessed, and studies were excluded if (1) samples were not tested by at least one reference test; (2) they involved spiked samples; (3) they involved experimentally infected animals; (4) data to derive a two-by-two table were insufficient; and (5) multiple genes were targeted in the index test.

Data abstraction

Study selection. The titles and abstracts of potentially relevant studies from the literature search were screened by one reviewer in accordance with the eligibility criteria and further confirmed by a second reviewer. After the exclusion of duplicated records, studies without abstract, and apparently irrelevant studies, the full-text articles of remaining studies were screened by two reviewers. Disagreements about study inclusion and exclusion were resolved between the reviewers by consensus.

Data extraction and quality assessment. Data were extracted primarily by one reviewer and cross-checked by a second reviewer. Data collected from eligible studies included the first author name, publication year, characteristics of study population, number of samples, type of samples, type of method used as index test and reference test, and number of TPs, FPs, TNs, and FNs. Any disagreements between the two reviewers were documented and resolved through discussion with a third reviewer.

Data analysis

The extracted data were compiled in a summary table, and the numbers of TPs, FPs, TNs, and FNs were used to calculate the sensitivity and specificity in each study. The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) were determined for each group. For each statistic, the corresponding 95% confidence intervals (95% CI) were also calculated. The DOR is a single measure of diagnostic test performance that describes the odds of having a positive result in participants with a positive reference test compared against the odds in those with a negative reference test [16]. The DORs were evaluated using the DerSimonian-Laird method (random effects model) [17]. Summary receiver operating characteristic (SROC) curves that show the relationship between sensitivity and false positives rate (1 − specificity) were constructed to summarize the results. The area under the curve (AUC) of the SROC was calculated and proposed as a means to assess diagnostic data in the context of meta-analysis [18].

Heterogeneity was assessed by using $I^2$ statistics and was interpreted as follows: an $I^2$ value of less than 50% indicates homogeneity among the studies in the analysis, whereas an $I^2$ value of more than 50% represents substantial heterogeneity among the studies [19]. One of the causes for heterogeneity observed in the meta-analysis of diagnostics is the threshold effect, which occurs if the studies use different thresholds to define a positive test result. In the present analysis, the presence of the threshold effect was determined by calculating the Spearman correlation coefficient between the sensitivity and specificity of the included studies [20]. In the absence of the threshold effect, meta-regression and subgroup analyses were performed to explore the contribution of individual factors on the heterogeneity observed, where a $p$ value of less than 0.05 indicates a contribution to heterogeneity. All statistical analyses were carried out using Meta-DiSc software (version 1.4) [21].
Results

Literature search and study characteristics

The study selection process is presented in Fig 1. The search for literature was completed in December 2018 and identified 1645 records, of which 128 full-text articles were retrieved to assess for eligibility. Of these full-text articles, 86 were excluded after further scrutiny. Forty-two studies involving 7414 samples met the inclusion criteria and were included in the meta-analysis. Detail and characteristics of each included study are presented in Table 1. The studies included were conducted in different countries and were published from 1992 to 2018. Some of the included studies used different methods; thus, the data were reported as separate independent studies [12, 22–32].

Accuracy of nucleic acid techniques targeting the *rrs* gene

The pooled sensitivity of techniques targeting the *rrs* gene was 0.51 (95% CI: 0.48–0.54), whereas the pooled specificity was 0.90 (95% CI: 0.89–0.91). Fig 2 shows the detailed forest plot of the sensitivities and specificities of the included studies. The pooled DOR was 13.58 (95% CI: 6.66–27.67), as shown in Fig 3. Fig 4 shows the SROC curve; the AUC and the pooled diagnostic accuracy (Q̃) were 0.88 and 0.81, respectively. Significant heterogeneity was observed among the studies (sensitivity, $I^2 = 95.3\%$; specificity, $I^2 = 96.6\%$; DOR, $I^2 = 84.0\%$).
| Study | Country | Study population | Number of samples | Sample type | Index test | Gene target | Reference test | TP | FP | FN | TN |
|-------|---------|-----------------|------------------|-------------|------------|-------------|----------------|----|----|----|----|
| [10]  | Brazil  | Suspected leptospirosis patients | 478 | Human—DNA from serum | Taqman qRT-PCR | rrs | MAT | 3 | 32 | 30 | 413 |
| [11]  | Netherlands | Suspected leptospirosis patients | 133 | Human—DNA from serum or blood | SYBR Green qPCR | secY | Culture | 15 | 8 | 0 | 110 |
| [12]  | Pacific Islands | Suspected leptospirosis patients | 51 | Human—DNA from serum | SYBR Green qPCR | lfb1 | MAT | 12 | 13 | 6 | 20 |
| [12]  | Pacific Islands | Suspected leptospirosis patients | 51 | Human—DNA from serum | Nested PCR | rrs | MAT | 12 | 13 | 6 | 20 |
| [22]  | Sri Lanka | Febrile patients | 105 | Human—DNA from serum | Taqman qPCR | rrs | MAT | 9 | 1 | 40 | 55 |
| [22]  | Sri Lanka | Febrile patients | 105 | Human—DNA from serum | Taqman qPCR | rrs | MAT | 25 | 1 | 24 | 55 |
| [23]  | Brazil | Suspected leptospirosis patients | 521 | Human—DNA from serum | Conventional PCR | rrs | MAT | 4 | 0 | 24 | 493 |
| [23]  | Brazil | Suspected leptospirosis patients | 521 | Human—DNA from serum | Nested PCR | rrs | MAT | 24 | 0 | 4 | 493 |
| [24]  | Brazil | Clinically confirmed leptospirosis patients, patients of other febrile diseases and healthy individuals | 77 | Human—DNA from blood or urine | Conventional PCR | LP1 | MAT | 11 | 0 | 22 | 44 |
| [24]  | Brazil | Clinically confirmed leptospirosis patients, patients of other febrile diseases and healthy individuals | 77 | Human—DNA from blood or urine | Conventional PCR | secY | MAT | 19 | 0 | 14 | 44 |
| [25]  | NR | Stray and household cats (healthy, non-vaccinated) | 63 | Animal—DNA from serum or urine | LAMP | lipL32 | Taqman qPCR (lipL32) | 22 | 0 | 2 | 39 |
| [25]  | NR | Stray and household cats (healthy, non-vaccinated) | 63 | Animal—DNA from serum or urine | Nested PCR | lipL32 | Taqman qPCR (lipL32) | 17 | 3 | 7 | 36 |
| [25]  | NR | Stray and household cats (healthy, non-vaccinated) | 63 | Animal—DNA from serum or urine | Conventional PCR | rrs | Taqman qPCR (lipL32) | 1 | 0 | 23 | 39 |
| [25]  | NR | Stray and household cats (healthy, non-vaccinated) | 63 | Animal—DNA from serum or urine | Conventional PCR | secY | Taqman qPCR (lipL32) | 3 | 0 | 21 | 39 |
| [26]  | Philippines | Clinically confirmed leptospirosis patients | 113 | Human—DNA from urine pellet | SYBR Green qPCR | flaB | MAT | 3 | 4 | 74 | 32 |
| [26]  | Philippines | Clinically confirmed leptospirosis patients | 113 | Human—DNA from plasma or urine pellet | LAMP | rrs | MAT | 2 | 3 | 75 | 33 |
| [27]  | Japan | Stray rats | 18 | Animal—Boiled urine sample | Nested PCR | flaB | Culture | 6 | 0 | 6 | 6 |
| [27]  | Japan | Stray rats | 16 | Animal—Urine pellet sample | Nested PCR | flaB | Culture | 9 | 1 | 2 | 4 |
| [27]  | Japan | Stray rats | 18 | Animal—Boiled urine sample | LAMP | rrs | Culture | 11 | 2 | 1 | 4 |
| [27]  | Japan | Stray rats | 16 | Animal—Urine pellet sample | LAMP | rrs | Culture | 10 | 2 | 1 | 3 |
| [28]  | Thailand | Clinically confirmed leptospirosis patients | 266 | Human—DNA from blood | LAMP | lipL41 | Culture, MAT | 50 | 13 | 83 | 120 |
| [28]  | Thailand | Clinically confirmed leptospirosis patients | 266 | Human—DNA from blood | LAMP | rrs | Culture, MAT | 58 | 22 | 75 | 111 |

(Continued)
Table 1. (Continued)

| Study | Country         | Study population                          | Number of samples | Sample type                  | Index test | Gene target | Reference test | TP | FP | FN | TN |
|-------|-----------------|-------------------------------------------|-------------------|------------------------------|------------|-------------|----------------|----|----|----|----|
| [29]  | Brazil          | Suspected leptospirosis patients          | 332               | Human—DNA from serum         | Taqman qPCR| lipL32      | Culture, MAT   | 37 | 3  | 90 | 202|
|       | Brazil          | Suspected leptospirosis patients          | 332               | Human—DNA from whole blood   | Taqman qPCR| lipL32      | Culture, MAT   | 77 | 12| 50 | 193|
| [30]  | Thailand        | Febrile patients                          | 266               | Human—DNA from blood         | Taqman qPCR| lipL32      | Culture, MAT   | 57 | 9  | 76 | 124|
|       | Thailand        | Febrile patients                          | 266               | Human—DNA from blood         | Taqman qPCR| rrs         | Culture, MAT   | 74 | 14| 59 | 119|
| [31]  | Argentina       | Clinically confirmed leptospirosis patients and non-cases | 234             | Human—DNA from serum or blood| Conventional PCR| lipL32    | Culture, MAT   | 26 | 2  | 81 | 125|
|       | Argentina       | Clinically confirmed leptospirosis patients and non-cases | 234             | Human—DNA from serum or blood| Taqman qPCR| lipL32      | Culture, MAT   | 47 | 9  | 60 | 118|
| [32]  | Denmark         | Suspected leptospirosis patients          | 51                | Human—DNA from urine         | Taqman qPCR| lipL32      | MAT            | 3  | 1  | 0  | 47 |
|       | Denmark         | Suspected leptospirosis patients          | 51                | Human—DNA from urine         | Taqman qPCR| rrs         | MAT            | 3  | 1  | 0  | 47 |
| [33]  | Sri Lanka       | Suspected leptospirosis patients          | 40                | Human—DNA from serum         | Taqman qPCR| rrs         | MAT            | 5  | 5  | 11 | 19 |
| [34]  | NR              | Suspected leptospirosis patients          | 63                | Human—DNA from serum or blood| Recombinase polymerase amplification| lipL32 | Culture | 18 | 1  | 1  | 43 |
| [35]  | Brazil          | Suspected leptospirosis patients          | 46                | Human—RNA from blood         | Taqman qRT-PCR| rrs       | Culture, MAT, qPCR | 14 | 0  | 8  | 24 |
| [36]  | Sri Lanka       | Suspected leptospirosis patients          | 170               | Human—DNA from blood         | Nested PCR | rrs        | MAT            | 7  | 7  | 54 | 102|
| [37]  | Thailand        | Febrile patients                          | 418               | Human—DNA from blood         | Nested PCR | rrs        | Culture        | 37 | 81 | 2  | 298|
| [38]  | Barbados        | Post-mortem samples                      | 13                | Human—DNA from organ         | Conventional PCR| secY      | Culture, MAT   | 2  | 0  | 6  | 5  |
| [39]  | USA             | Random                                    | 34                | Animal— Urine pellet sample  | Conventional PCR| IS1500  | MAT            | 23 | 3  | 7  | 1  |
| [40]  | Czech Republic  | Suspected leptospirosis patients          | 852               | Human—DNA from plasma, urine or CSF | Conventional PCR| secY    | MAT            | 14 | 1  | 21 | 816|
| [41]  | Sri Lanka       | Suspected leptospirosis patients          | 95                | Human—DNA from blood         | SYBR Green qPCR| secY   | MAT            | 44 | 3  | 21 | 27 |
| [42]  | Uruguay         | Suspected leptospirosis patients          | 183               | Human—DNA from serum         | SYBR Green qPCR| lipL32  | MAT            | 26 | 0  | 59 | 98 |
| [43]  | Turkey          | Suspected leptospirosis patients and animals | 133              | Human and animal—DNA from serum| Nested PCR| rrs       | MAT            | 90 | 2  | 0  | 41 |
| [44]  | NR              | Suspected leptospirosis dogs              | 135               | Animal—DNA from serum        | Nested PCR | rrs       | MAT            | 47 | 23 | 4  | 61 |
| [45]  | Thailand        | Wild rodents                              | 36                | Animal—DNA from kidney       | Taqman qPCR| lipL32    | Conventional PCR (gyrB) | 4  | 0  | 0  | 32 |
| [46]  | NR              | Suspected leptospirosis patients and healthy controls | 28               | Human—DNA from urine, CSF or blood| Conventional PCR| rrs    | MAT            | 4  | 0  | 2  | 22 |
| [47]  | Malaysia        | Suspected leptospirosis patients          | 65                | Human—DNA from blood         | Taqman qPCR| rrs       | Commercial GenoAmp qPCR leptospirosis kit | 10 | 1  | 0  | 54 |

(Continued)
The Spearman correlation coefficient was calculated to be 0.273 with $p = 0.160$ ($>0.05$), indicating that the significant heterogeneity was not due to the threshold effect. Hence meta-regression and subgroup analyses were performed to explore the overall heterogeneity and identify the source of heterogeneity, including the type of index test and type of sample (human or animal). The meta-regression analysis did not demonstrate that these two covariates contributed to the heterogeneity ($p > 0.05$ for both covariates). Subgroup analyses were performed on the basis of these covariates, and the results are shown in Table 2. Only one study dealt with human and animal samples; thus, analysis was not performed for this sample category.

### Accuracy of nucleic acid techniques targeting the lipL32 gene

The pooled sensitivity and specificity of techniques using the lipL32 gene as the target of detection were 0.42 (95% CI: 0.39–0.46) and 0.95 (95% CI: 0.94–0.97), respectively. The detailed forest plots of the sensitivities and specificities of the included studies are shown in Fig 5. The pooled DOR was 19.71 (95% CI: 10.15–38.29), as shown in Fig 6. The SROC curve is presented in Fig 7 with an AUC of 0.92 and $Q^*$ value of 0.85. Significant heterogeneity was observed among the studies (sensitivity, $I^2 = 91.1%$; specificity, $I^2 = 75.9%$; DOR, $I^2 = 72.4%$).

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**Table 1.** (Continued)

| Study | Country | Study population | Number of samples | Sample type | Index test | Gene target | Reference test | TP   | FP   | FN   | TN   |
|-------|---------|------------------|-------------------|-------------|------------|-------------|----------------|------|------|------|------|
| [48]  | India   | Suspected leptospirosis patients | 207               | Human—DNA from serum | Taqman qPCR | lipL32 | MAT | 84   | 10   | 77   | 36   |
| [49]  | India   | Suspected leptospirosis patients | 134               | Human—NR | Conventional PCR | secY | MAT | 34   | 4    | 1    | 95   |
| [50]  | NR      | Suspected leptospirosis patients | 42                | Human—DNA from blood or urine | Conventional PCR | flaB | MAT | 39   | 0    | 0    | 3    |
| [51]  | Brazil  | Suspected leptospirosis patients | 92                | Human—DNA from serum | Conventional PCR | secY | MAT | 17   | 13   | 30   | 32   |
| [52]  | India   | Suspected leptospirosis patients | 207               | Human—DNA from blood | Nested PCR | lipL32 | Culture | 21   | 3    | 79   | 104  |
| [53]  | Nicaragua | Febrile patients | 85                | Human—DNA from blood | Taqman qPCR | lipL32 | MAT | 11   | 6    | 27   | 41   |
| [54]  | Brazil  | Patients with meningeal abnormalities | 39               | Human—DNA from CSF | Conventional PCR | rrs | MAT | 10   | 13   | 2    | 14   |
| [55]  | India   | Suspected leptospirosis patients | 100               | Human—DNA from serum | Conventional PCR | rrs | MAT | 2    | 4    | 16   | 78   |
| [56]  | India   | Asymptomatic participants | 196              | Human—DNA from urine | Taqman qPCR | rrs | MAT | 37   | 67   | 22   | 70   |
| [57]  | NR      | Suspected leptospirosis patients | 231              | Human—DNA from serum or blood | Taqman qPCR | rrs | Culture | 27   | 1    | 1    | 202  |
| [58]  | NR      | Wild animals | 220             | Animal—DNA from serum | Taqman qPCR | rrs | MAT | 0    | 1    | 14   | 205  |
| [59]  | Brazil  | Suspected leptospirosis patients | 55               | Human—DNA from serum or plasma | Taqman qRT-PCR | rrs | MAT | 6    | 47   | 0    | 2    |
| [60]  | Laos    | Febrile patients | 787             | Human—DNA from blood or urine | Taqman qPCR | rrs | Culture, MAT | 7   | 69   | 26   | 685  |

NR represents information not reported

https://doi.org/10.1371/journal.pntd.0008074.t001
The Spearman correlation coefficient was -0.077 with \( p = 0.794 \) (\( > 0.05 \)), indicating that significant heterogeneity was not due to the threshold effect. Meta-regression and subgroup analyses were then performed to explore the heterogeneity and identify the source of heterogeneity. The meta-regression analysis did not demonstrate that the two covariates (type of index test and type of sample) contributed to the heterogeneity (\( p > 0.05 \) for both). Subgroup analyses were performed on the basis of these covariates, and the results are shown in Table 3. The AUC for the subgroup of index test (nested PCR) was not computed because of the low number of studies (\( n = 2 \)). Analysis for the following index tests could not be performed because only one study was present in each category: conventional PCR, LAMP, and recombinase polymerase amplification.

Accuracy of nucleic acid techniques targeting the \( secY \) gene

Fig 8 shows the forest plots of the sensitivities and specificities of techniques targeting the \( secY \) gene. The pooled sensitivity and specificity were 0.56 (95% CI: 0.50–0.63) and 0.98 (95% CI: 0.97–0.98), respectively. The pooled DOR was valued at 46.16 (95% CI: 6.20–343.49) and is presented in Fig 9. Fig 10 shows the SROC curve with the AUC at 0.94 and the pooled diagnostic accuracy (Q) at 0.88. Among these studies, significant heterogeneity was observed (sensitivity, \( I^2 = 92.0\% \); specificity, \( I^2 = 92.4\% \); DOR, \( I^2 = 88.3\% \)).

The Spearman correlation coefficient was 0.000 with \( p = 1.000 \) (\( > 0.05 \)), indicating that the heterogeneity was not due to the threshold effect. Thus, meta-regression and subgroup analyses were performed to explore the source of heterogeneity. The meta-regression analysis did not demonstrate that the two covariates contributed to the heterogeneity (\( p > 0.05 \) for both covariates). Subgroup analyses based on these covariates were performed, and the results are shown in Table 4. The AUC for the subgroup of index test (qPCR) was not determined because of the low number of studies (\( n = 2 \)). Analysis for animal samples was not performed because only one study was present in this category.
For techniques targeting the \textit{flaB} gene, the pooled sensitivity and specificity of the included studies were 0.41 (95% CI: 0.33–0.50) and 0.90 (95% CI: 0.78–0.97), respectively. The forest plots of the sensitivities and specificities are shown in Fig 11. The pooled DOR was 10.42 (95% CI: 0.44–244.84), as shown in Fig 12. The SROC curve is presented in Fig 13, with the AUC at 0.92 and pooled diagnostic accuracy (Q) at 0.86. Significant heterogeneity was observed when computing the pooled sensitivity ($I^2 = 97.8\%$) and DOR ($I^2 = 82.1\%$).

The Spearman correlation coefficient was 0.600 with $p = 0.400$ ($> 0.05$), eliminating the possibility of the threshold effect. The meta-regression analysis did not demonstrate that the two covariates contributed to the heterogeneity ($p > 0.05$ for both covariates). Subgroup analyses were not performed because of the limited number of studies in each subgroup.

**Accuracy of nucleic acid techniques targeting the \textit{flaB} gene**

For techniques targeting the \textit{flaB} gene, the pooled sensitivity and specificity of the included studies were 0.41 (95% CI: 0.33–0.50) and 0.90 (95% CI: 0.78–0.97), respectively. The forest plots of the sensitivities and specificities are shown in Fig 11. The pooled DOR was 10.42 (95% CI: 0.44–244.84), as shown in Fig 12. The SROC curve is presented in Fig 13, with the AUC at 0.92 and pooled diagnostic accuracy (Q) at 0.86. Significant heterogeneity was observed when computing the pooled sensitivity ($I^2 = 97.8\%$) and DOR ($I^2 = 82.1\%$).

The Spearman correlation coefficient was 0.600 with $p = 0.400$ ($> 0.05$), eliminating the possibility of the threshold effect. The meta-regression analysis did not demonstrate that the two covariates contributed to the heterogeneity ($p > 0.05$ for both covariates). Subgroup analyses were not performed because of the limited number of studies in each subgroup.
Fig 4. SROC curve of targeting the rrs gene in the detection of Leptospira.

https://doi.org/10.1371/journal.pntd.0008074.g004

Table 2. Meta-analysis results of studies targeting the rrs gene for the detection of Leptospira.

|               | Sensitivity (95% CI) | Specificity (95% CI) | PLR (95% CI)   | NLR (95% CI)   | DOR (95% CI)   | AUC     |
|---------------|----------------------|----------------------|----------------|----------------|----------------|---------|
| All studies   | 0.51 (0.48–0.54)     | 0.90 (0.89–0.91)     | 4.36 (2.80–6.79) | 0.56 (0.44–0.70) | 13.58 (6.66–27.67) | 0.88    |
| Subgroup (Type of index test) |                      |                      |                |                |                |         |
| Conventional PCR | 0.24 (0.15–0.34)     | 0.97 (0.96–0.98)     | 7.09 (1.27–39.72) | 0.86 (0.70–1.05) | 11.61 (2.43–55.45) | 0.78    |
| Nested PCR    | 0.76 (0.70–0.80)     | 0.89 (0.87–0.91)     | 4.62 (2.32–9.21)  | 0.13 (0.01–2.20) | 53.18 (6.83–413.92) | 0.95    |
| qPCR          | 0.50 (0.45–0.55)     | 0.90 (0.89–0.92)     | 8.14 (3.00–22.09) | 0.62 (0.42–0.90) | 17.21 (5.19–57.05) | 0.92    |
| qRT-PCR       | 0.38 (0.26–0.51)     | 0.85 (0.81–0.88)     | 2.82 (0.15–53.67) | 0.68 (0.23–2.04) | 3.80 (0.21–67.78) | 0.68    |
| LAMP          | 0.35 (0.29–0.41)     | 0.84 (0.78–0.89)     | 2.01 (1.04–3.91)  | 0.64 (0.34–1.16) | 3.57 (0.70–18.23) | 0.83    |
| Subgroup (Type of sample) |                      |                      |                |                |                |         |
| Human samples | 0.44 (0.41–0.48)     | 0.90 (0.89–0.91)     | 4.45 (2.67–7.40)  | 0.61 (0.49–0.77) | 11.09 (5.17–23.79) | 0.83    |
| Animal samples| 0.62 (0.52–0.71)     | 0.92 (0.88–0.94)     | 3.22 (2.34–4.45)  | 0.39 (0.17–0.92) | 20.90 (8.55–51.08) | 0.89    |

https://doi.org/10.1371/journal.pntd.0008074.t002
Discussion

In this meta-analysis, 42 studies involving 7414 samples were included to investigate the diagnostic accuracy of various nucleic acid techniques. Many nucleic acid diagnostics for leptospirosis have been developed and designed to either target housekeeping genes that are common to all species of *Leptospira* or pathogenic species-specific genes. Here, from the literature searched, we pooled and analyzed the diagnostic performance of nucleic acid assays targeting

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**Fig 5. Forest plots of sensitivity and specificity of studies using *lipL32* as the target gene for the detection of *Leptospira*.

https://doi.org/10.1371/journal.pntd.0008074.g005**

**Fig 6. Forest plot of DOR of targeting the *lipL32* gene in the detection of *Leptospira*.

https://doi.org/10.1371/journal.pntd.0008074.g006**
the rrs, lipL32, secY and flaB genes. The IS1500, LP1, lfb1, and lipL41 genes were not pooled and analyzed because only one study included each of these genes [12, 24, 28, 39].

The rrs gene is a housekeeping gene found ubiquitously among leptospires. The present meta-analysis showed that assays targeting the rrs gene have been well-established and largely

Table 3. Meta-analysis results of studies targeting the lipL32 gene for the detection of Leptospira.

| Subgroup (Type of index test) | Sensitivity (95% CI) | Specificity (95% CI) | PLR (95% CI) | NLR (95% CI) | DOR (95% CI) | AUC  |
|-------------------------------|----------------------|----------------------|--------------|--------------|--------------|------|
| All studies                   | 0.42 (0.39–0.46)     | 0.95 (0.94–0.97)     | 9.42 (5.64–15.75) | 0.61 (0.51–0.71) | 19.71 (10.15–38.29) | 0.92 |
| Subgroup (Type of sample)     |                      |                      |              |              |              |      |
| Nested PCR                    | 0.31 (0.23–0.40)     | 0.96 (0.91–0.98)     | 8.35 (3.71–18.79) | 0.52 (0.17–1.65) | 15.31 (4.93–47.52) | NA   |
| qPCR                          | 0.44 (0.41–0.48)     | 0.95 (0.93–0.96)     | 7.83 (4.21–14.56) | 0.62 (0.54–0.72) | 13.64 (6.52–28.54) | 0.75 |
| Human samples                 | 0.40 (0.37–0.44)     | 0.95 (0.94–0.96)     | 8.33 (4.82–14.37) | 0.65 (0.57–0.75) | 14.65 (7.57–28.32) | 0.86 |

Fig 7. SROC curve of targeting the lipL32 gene in the detection of Leptospira.

https://doi.org/10.1371/journal.pntd.0008074.g007

https://doi.org/10.1371/journal.pntd.0008074.t003
used for diagnostics. Targeting the rrs gene can allow the detection of pathogenic or saprophytic Leptospira species [59]. The rrs gene is also present in two copies per Leptospira, which could consequently increase its chance of being amplified [61, 62]. Meanwhile, the secY gene encodes for preprotein translocase for Leptospira and is located within the S10-spc-α locus containing genes for ribosomal proteins [63]. Similar to the rrs gene, it is a housekeeping gene that is also common to all leptospires [11]. The secY gene consists of alternating conserved and variable regions, making it suitable to design primers that can generate amplicons across the Leptospira genus and enable phylogenetic interpretation through the variable regions [64].

The lipL32 gene encodes a major lipoprotein located in the outer membrane of leptospires. The lipL32 gene is present in all species from both pathogenic and intermediate strains but absent in saprophytic strains, suggesting its critical role in infection [65, 66]. The sequence of the lipL32 gene is highly conserved across the pathogenic species of Leptospira, with more than 94% amino acid sequence identities [67]. Thus, the absence of lipL32 in saprophytic Leptospira makes it a specific and appropriate gene target for diagnosing leptospirosis [25]. Another gene that can be used to differentiate between pathogenic and saprophytic leptospires is the flaB gene. This gene encodes for flagellin, a class B polypeptide subunit of the periplasmic flagella. The sequence of flaB is also highly conserved among pathogenic serovars of Leptospira [68].

Fig 8. Forest plots of sensitivity and specificity of studies using secY as the target gene for the detection of Leptospira.

https://doi.org/10.1371/journal.pntd.0008074.g008

Fig 9. Forest plot of DOR of targeting the secY gene in the detection of Leptospira.

https://doi.org/10.1371/journal.pntd.0008074.g009
Similar to the *lipL32* gene, the absence of *flaB* in the saprophytic strains allows this gene to be a good target for detecting pathogenic leptospires [50, 69].

As shown in Table 5, nucleic acid techniques targeting the *secY* gene exhibited better pooled sensitivity and specificity when compared against assays targeting the *rrs*, *lipL32*, or *flaB* gene.

![SROC curve](https://doi.org/10.1371/journal.pntd.0008074.g010)

**Table 4. Meta-analysis results of studies targeting the *secY* gene for the detection of *Leptospira*.**

|                      | Sensitivity (95% CI) | Specificity (95% CI) | PLR (95% CI)   | NLR (95% CI)   | DOR (95% CI)   | AUC     |
|----------------------|----------------------|----------------------|----------------|----------------|----------------|---------|
| All studies          | 0.56 (0.50–0.63)     | 0.98 (0.97–0.98)     | 12.94 (3.74–44.72) | 0.49 (0.30–0.82) | 46.16 (6.20–343.49) | 0.94    |
| Subgroup (Type of index test) |                      |                      |                |                |                |         |
| Conventional PCR     | 0.49 (0.41–0.56)     | 0.98 (0.97–0.99)     | 15.63 (2.04–119.78) | 0.60 (0.37–0.97) | 39.57 (2.44–642.05) | 0.91    |
| qPCR                 | 0.74 (0.63–0.83)     | 0.93 (0.87–0.96)     | 10.50 (4.65–23.71) | 0.13 (0.00–6.15) | 65.78 (3.37–1284.84) | NA      |
| Subgroup (Type of index test) |                      |                      |                |                |                |         |
| Human samples        | 0.61 (0.54–0.67)     | 0.97 (0.96–0.98)     | 13.17 (3.52–49.33) | 0.45 (0.27–0.77) | 54.70 (5.97–501.49) | 0.94    |

https://doi.org/10.1371/journal.pntd.0008074.t004
Sensitivity and specificity are true performance statistics of the test, where sensitivity measures for the proportion of samples tested positive among those tested positive using a reference test while specificity measures the proportion of samples tested negative among those tested negative in a reference test [70]. The PLR of assays targeting secY is also the highest among others and is the only one with a value more than 10. This result indicates that the positive results obtained from assays targeting secY are useful for the confirmation of leptospirosis [71]. Sensitivity and specificity play important roles in determining the DOR of a test. For example, tests with high sensitivity and specificity with low FPs and FNs result in a high DOR [16]. In our analysis, the pooled DOR of assays targeting secY was the highest among other genes. Collectively, the pooled sensitivity, specificity, DOR, likelihood ratio, and AUC data all support that assays targeting the secY gene are highly discriminatory for the detection of Leptospira.

Stratified analyses were performed for each gene analyzed according to the type of index test. For the rrs gene, subgroup analysis revealed that nested PCR assays targeting the gene are superior over other tests and slightly better than qPCR, as exhibited by the higher DOR and AUC of the SROC curve. In a previous study, qRT-PCR assay targeting the rrs gene was compared against nested PCR assay of the same gene, and the diagnostic performance was comparable between the two [72]. Another study on the detection of Strongyloides stercoralis also found that nested PCR shows better diagnostic sensitivity than real-time PCR [73]. However superior nested PCR is, real-time PCR methods are usually preferred over the former because they provide an accurate diagnosis faster than nested PCR assays [74]. By contrast, qPCR assay targeting the lipL32 gene showed slightly better performance than nested PCR assay, but this finding is inconclusive because AUC data for nested PCR assay was not computed due to the lack of studies. As for the secY gene, although AUC data for qPCR were not determined, the sensitivity and DOR of qPCR were significantly higher than those of conventional PCR. When
the data were stratified according to the type of samples, assays targeting the \textit{rrs} gene showed slightly better diagnostic performance on animal than human samples. Meanwhile, the diagnostic performance of the other genes on animal samples was not computed because of the lack of studies. As for human samples, assays targeting the \textit{secY} gene showed the best diagnostic performance in terms of DOR, followed by those targeting \textit{lipL32}, \textit{rrs}, and \textit{flaB}.

Table 5. Summary of diagnostic accuracy measures of genetic markers for the detection of \textit{Leptospira} in clinical samples.

| Gene | Sensitivity (95% CI) | Specificity (95% CI) | PLR (95% CI) | NLR (95% CI) | DOR (95% CI) | AUC  |
|------|----------------------|----------------------|--------------|--------------|--------------|------|
| \textit{rrs}   | 0.51 (0.48–0.54)     | 0.90 (0.89–0.91)     | 4.36 (2.80–6.79) | 0.56 (0.44–0.70) | 13.58 (6.66–27.67) | 0.88 |
| \textit{lipL32} | 0.42 (0.39–0.46)     | 0.95 (0.94–0.97)     | 9.42 (5.64–15.75) | 0.61 (0.51–0.71) | 19.71 (10.15–38.29) | 0.92 |
| \textit{secY}   | 0.56 (0.50–0.63)     | 0.98 (0.97–0.98)     | 12.94 (3.74–44.72) | 0.49 (0.30–0.82) | 46.16 (6.20–343.49) | 0.94 |
| \textit{flaB}   | 0.41 (0.33–0.50)     | 0.90 (0.78–0.97)     | 2.43 (0.44–13.52) | 0.36 (0.11–1.18) | 10.42 (0.44–244.84) | 0.92 |

https://doi.org/10.1371/journal.pntd.0008074.t005
However, the findings of this meta-analysis should be interpreted with caution, considering the significant unexplained heterogeneity. The heterogeneity of the analyzed studies could be due to the vastly different sample sizes among the included studies. Moreover, the low number of studies in assays targeting the *flaB* gene and in some subgroups might have also biased the results. Another possible contribution to the heterogeneity of the studies was the variability in the DNA sample extraction approach, reference test, and the stage of the disease when samples were collected from the patients and animals. The timing of sample collection is crucial for the detection of *Leptospira* DNA because it is present in the blood of the patient in the first 5 to 10 days after the onset of the disease [11]. As mentioned previously, although MAT has been the gold standard for the diagnosis of leptospirosis, its application is limited by its difficulty to be standardized [8], suggesting that this limitation also contributed to the heterogeneity observed. In these nucleic acid assays, the different targeting regions within each gene would represent a major factor influencing the sensitivity and specificity of a diagnostic test. These limitations could have negatively influenced the overall results of this work.

In short, current evidence suggests that the *secY* gene has better diagnostic accuracy measures with *lipL32*, *flaB*, and *rrs* coming close as promising genetic markers for leptospirosis diagnosis. However, the high degree of heterogeneity observed in this meta-analysis mitigates any conclusions drawn from the combined data. Nevertheless, future studies evaluating the nucleic acid-based diagnostic assays should consider the timing and stage of the disease for sample collection, the choice of reference test to be compared with, or the statistical methods to optimize the imperfect reference tests, in an effort to reduce the heterogeneity between the studies while increasing the comparability of results.

**Supporting information**

S1 Checklist. PRISMA checklist.

(DOC)

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