Meta-Analysis of Bovine Leptospirosis Prevalence in India

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Abstract

Leptospirosis is a globally significant and neglected zoonotic disease caused by pathogenic *Leptospira* spp., affecting a wide range of mammalian hosts including humans, cattle, and buffaloes. In livestock, it leads to considerable economic losses through abortions, stillbirths, reduced fertility, decreased milk production, especially in tropical and subtropical regions where environmental conditions favor bacterial persistence and transmission. Despite its severity, bovine leptospirosis remains underreported in endemic regions such as India. This meta-analysis synthesized data from 46 studies (2001–2021) to estimate the pooled prevalence, epidemiology, and diagnostic challenges of bovine leptospirosis in India. The pooled prevalence was 29% in cattle and 32% in buffaloes. Seropositivity ranged from 50–70% in animals with reproductive disorders to 15–20% in healthy bovines. Coastal states such as Gujarat, Andhra Pradesh, Maharashtra, Tamil Nadu, Kerala, and the Andaman Islands showed the highest prevalence, influenced by favourable ecological conditions. Twenty pathogenic *Leptospira* serogroups were identified, with dominant serogroups (Sejroe, Icterohaemorrhagiae, Hebdomadis,

33 Pomona etc.), and evidence of temporal shifts in the prevalent serogroups. Significant diagnostic

34 challenges included variability in sample sizes, heterogeneity among studies, and the limited sensitivity

35 of enzyme-linked immunosorbent assay (ELISA) compared to the gold-standard MAT (Microscopic

36 Agglutination Test). These findings underscore the urgent need for enhanced surveillance, incorporation

37 of diverse serogroups into diagnostic panels, and region-specific vaccination strategies. Strengthening

38 molecular diagnostic tools, improving seroepidemiological studies, and implementing targeted control

39 measures are essential to reduce the impact of leptospirosis on livestock productivity and public health

40 in India. This work offers critical insights to inform policy decisions and intervention strategies for

41 effective disease management.

43

1. Context

45 Leptospirosis, a rapidly re-emerging zoonotic disease, has gained global significance due to its

46 extensive impact on public health and animal productivity. This neglected disease, caused by

47 pathogenic *Leptospira* spp., is prevalent in tropical and subtropical regions, leading to significant

48 morbidity and mortality in both humans and animals (1). Bovine leptospirosis, in particular, imposes

49 substantial economic losses through reproductive failures, reduced milk yield, abortions, and calf

50 mortality (2). Livestock farming, especially in endemic regions, not only exacerbates disease

51 transmission but also increases occupational hazards for animal handlers. Bovines, acting as carriers of

52 pathogenic *Leptospira*, excrete large quantities of bacteria in their urine, perpetuating environmental

53 contamination and transmission to other animals and humans (2). Clinical outcomes in cattle range from

54 subclinical infections with serogroup Sejroe to acute presentations with serogroup Pomona, which cause

55 high fever, jaundice, hemoglobinuria, and death (2). Studies from India have reported leptospirosis

56 prevalence in coastal states such as Odisha, Maharashtra, Kerala, Tamil Nadu, Gujarat, and the

57 Andaman Islands, with varied serogroups dominating across regions and time periods (3-5).

58 Despite its significance, bovine leptospirosis remains under-researched in several aspects.

59 Although seroprevalence studies exist, they are geographically limited, and uniform data across India

60 are unavailable. Molecular studies have identified circulating serovars, but data on genomic diversity,

61 environmental persistence, and transmission dynamics are limited (6, 7). Buffaloes are

62 underrepresented, and longitudinal studies assessing risk factors and seasonal trends are lacking,

63 hindering effective control strategies (3, 4). Diagnostic challenges further complicate disease control.

64 Although the Microscopic Agglutination Test (MAT) is the gold standard, it is underutilized due to

65 infrastructure limitations. Previous studies also reveal conflicting findings regarding risk factors and

66 predominant serogroups, highlighting the need for updated and standardized data. For instance, the shift

67 in predominant serogroups, underscores dynamic transmission patterns influenced by environmental

68 and host factors (8-10). These gaps necessitate a systematic, evidence-based approach to consolidating

69 existing knowledge.

70 A meta-analysis addressing bovine leptospirosis is crucial for filling existing knowledge gaps

71 and providing a comprehensive overview of its prevalence, risk factors, and serogroups distribution. By

72 integrating data from diverse studies, it can offer robust estimates of disease burden, identify regional

73 patterns, and clarify conflicting results. This approach also highlights diagnostic and epidemiological

74 trends, paving the way for improved surveillance and control strategies. Given the economic and

75 zoonotic implications of leptospirosis, such insights are invaluable for policymakers, veterinarians, and

76 public health professionals (11). This meta-analysis aims to estimate the pooled prevalence of

77 leptospirosis in bovines in India, identify predominant serogroups and their regional distribution over

78 time, and evaluate diagnostic approaches used in prevalence studies. The findings are expected to

79 inform public health and veterinary policy by providing evidence-based prevalence data that can guide

80 the design of region-specific vaccination strategies, the development of comprehensive diagnostic

81 panels that reflect circulating serovars, and the prioritization of resource allocation for surveillance

82 programs (2). Furthermore, by identifying gaps in diagnostic sensitivity and study heterogeneity, this

83 study supports the need for upgrading diagnostic infrastructure, particularly through the inclusion of

84 molecular methods and intermediate serogroups in diagnostic assays.

85 **2. Evidence Acquisition**

86 **2.1. Meta-analysis, Literature search strategy**

87 The systematic review and meta-analysis followed the PRISMA standards established by the

88 Cochran Collaboration (12). A comprehensive literature review was conducted to gather relevant data

89 on leptospirosis prevalence in India. Published studies were retrieved from multiple databases,

90 including Google Scholar, Scopus, Science Direct, and PubMed, with additional publications sourced

91 through manual cross-referencing. A systematic search (January 2001–December 2021) identified

92 1,347 articles using predefined keyword combinations [(Prevalence OR Incidence OR Frequency OR

93 Detection OR Occurrence) AND (*Leptospira* OR leptospirosis) AND (Bovine OR Cattle OR Buffalo)

94 AND India]. Rayyan QCRI was used for systematic reviews, with two researchers independently

95 conducting blind screenings and resolving conflicts. References were managed using Endnote version

96 20.0, and QGIS software (version 3.22.1) was utilized to map bovine leptospirosis distribution across

97 states and regions.

98 **2.2. Study selection and Data collection**

99 A systematic search (January 2001–December 2021) identified 1,347 articles using predefined

100 keyword combinations," as recommended, to improve clarity and precision. The systematic review

101 process is summarized in Fig. 1. Of the 1,347 records identified, 1,199 were excluded as duplicates or

102 irrelevant. Studies were selected based on predefined PRISMA-aligned criteria: (i) original research on

103 bovine leptospirosis in India (2001–2021), (ii) use of serological diagnostics (MAT or ELISA), and (iii)

availability of data on sample size, number of positives, species (cattle and/or buffalo), and location. Exclusion criteria included non-relevant topics, non-bovine or non-Indian studies, reviews, experimental trials, non-English articles, and missing essential data. After title and abstract screening, 96 full-text articles were assessed for eligibility. Thirty-four studies were excluded due to missing key information (e.g., sample size, diagnostic method, or species), and 14 were excluded after quality assessment due to interrater disagreement or outbreak-only data. Additionally, three relevant studies were included from the authors' personal database. Of the 51 studies eligible for synthesis, five were excluded during subgroup analysis due to high heterogeneity and methodological inconsistencies, resulting in a final total of 46 studies included in the meta-analysis. Extracted variables included author, year, region, host species, sample size, number of positives, and diagnostic method.
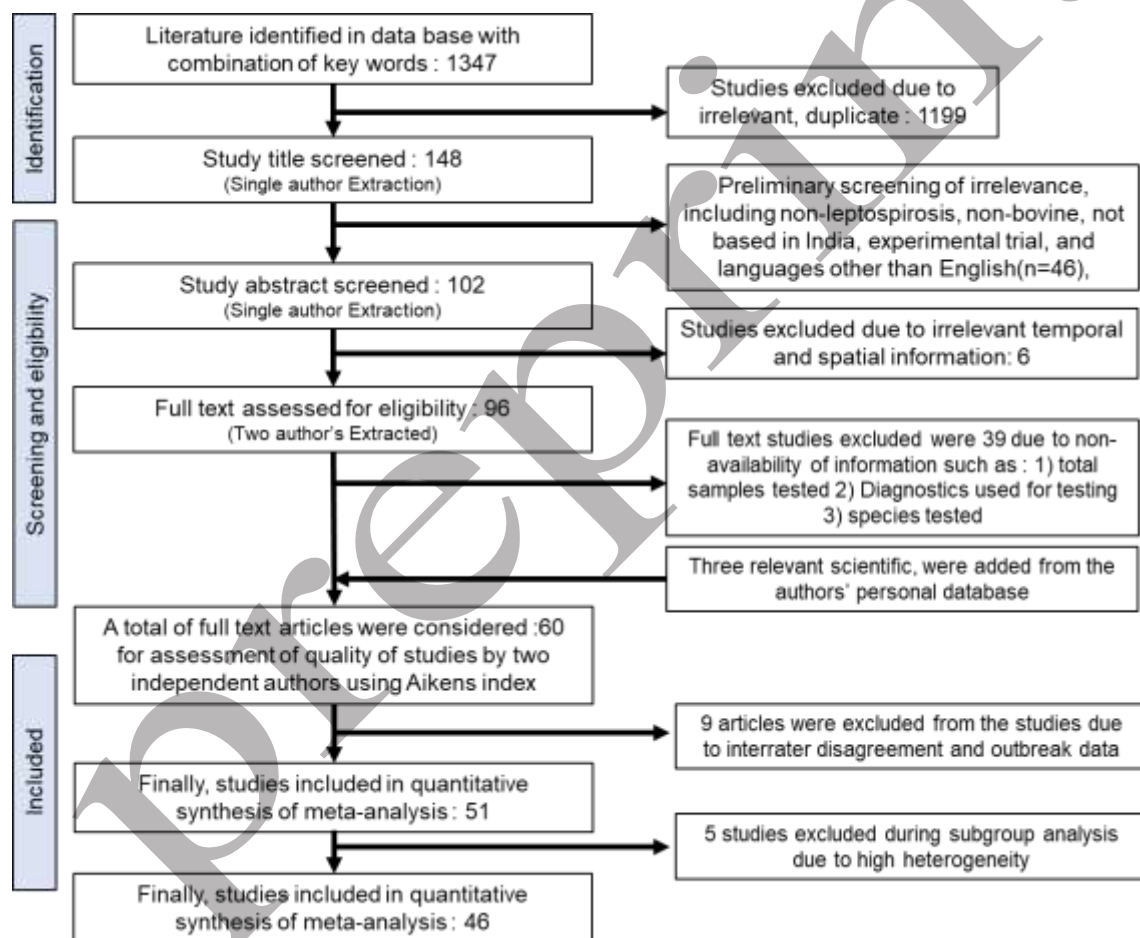

Figure 1. *PRISMA flow diagram showing the selection of studies for meta-analysis on bovine leptospirosis in India (2001–2021). Of 1,347 records identified, 46 studies were included after screening, quality assessment, and exclusion based on relevance, data completeness, and interrater agreement.*

2.3. Quality assessment

Quality control was independently performed by two researchers using a 7-item Likert scale (1–5) to assess the quality of each article, with higher scores indicating more reliable studies. The ratings were used to calculate the Aiken validity coefficient (13). Meta-analysis was conducted with R (version 3.2.5) using the "meta" package (14). Forest plots were used to graphically represent effect sizes, employing fixed-effect or random-effect models based on heterogeneity (I^2). The random-effect model was applied when significant heterogeneity was observed, calculated using Cochran's Q statistic, τ^2, H-value, and P-values (13).

Meta-regression analysis examined estimates and study variation through weighted linear regression, where effect size was regressed onto moderators like geographic region, years, species, and sample size (13, 15). Subgroup analyses were performed for variables with $p<0.05$ in univariate meta-regression, retaining only those significant at $p\leq0.05$ in the final model. This approach improved analytical power and minimized false-positive results. Sensitivity analyses tested the robustness of results and identified articles influencing heterogeneity. Publication bias was assessed using funnel plots with Arcsine-transformed proportions and standard errors (15).

3. Results

3.1. Studies and Quality of bias assessment

Out of the total of 1,347 studies, sixty studies were subjected to full-text reviews and further scrutiny for bias quality based on inter-rater consensus and agreement calculated using Aiken's V-value index as described by SowjanyaKumari et al. (16). Overall, forty-six publications were ultimately chosen for meta-analysis with the details presented in the PRISMA flow chart (Fig. 1). The prevalence of leptospirosis was calculated using a total sample size of 18354 out of which cattle alone contributed to 16202 cases followed by buffalo with 2152. Further, for visual inspection of publication bias, a funnel plot-based technique was employed by plotting the Arcsine transformation proportion on the X-axis, and standard error on the Y-axis, creating a funnel plot and interpreting as described by SowjanyaKumari et al. (16). The figure illustrates the asymmetry observed suggests possible publication bias and substantial heterogeneity among the included studies (Fig. 2).
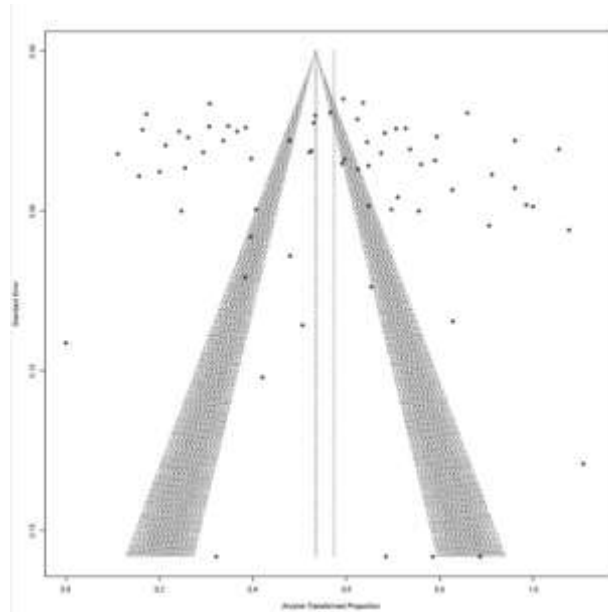
Figure 2. Funnel plot for examination of publication bias of the prevalence estimates of leptospirosis in bovine from India during the year 2001-2021.

3.2. Meta-regression

Meta-regression was performed using a univariate approach to identify factors influencing the magnitude and direction of heterogeneity. The results (Table 1) indicated that detection methods and study regions significantly affected overall heterogeneity at a 5% significance level. These findings highlighted the need for subgroup and sensitivity analyses to refine the prevalence estimates of bovine leptospirosis. Subgroup analysis was conducted for covariates such as detection methods, sample size, study region, and animal groups to account for their impact on heterogeneity (Table 2). The forest plot (Fig. 3) illustrates pooled prevalence estimates and 95% confidence intervals across subgroups, showing substantial heterogeneity ($I^2 > 98\%$), with variation based on species, region, and diagnostic method.

Table 1. The univariate meta-regression analysis of bovine leptospirosis.

Predictors	Estimate	SE	z value	τ^2	I^2 (%)	H^2	R^2 (%)	Qm	p Value
Region	0.7573	0.1643	4.6082	0.0657	98.44	64.05	9.01	13.6528	0.0338*
Test	0.2937	0.0825	3.5615	0.0579	98.28	58.13	19.80	17.5436	0.0002***
Species	0.5793	0.0640	9.0498	0.0729	98.65	73.86	0.00	0.2731	0.6012

Where* Indicate the 5 % level of significance, *** 0.1 % level of significance.
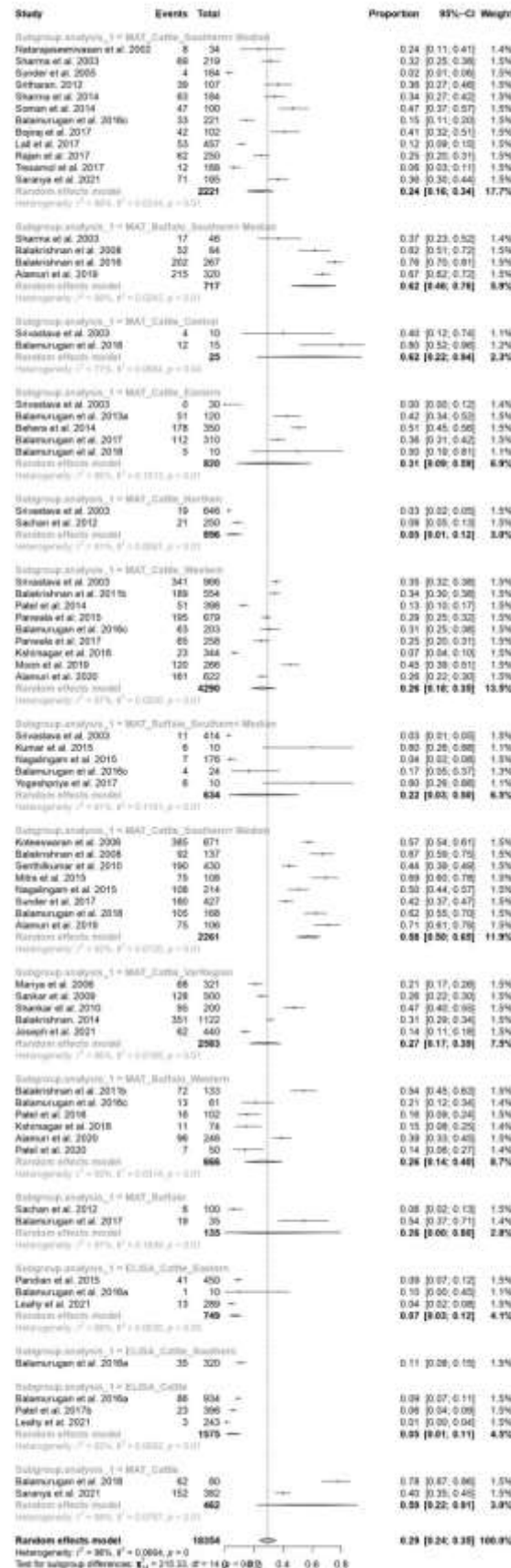
162
163 **Figure 3.** Forest plot of subgroup and sensitivity analyses of bovine leptospirosis prevalence in India.
164   The plot shows pooled prevalence estimates with 95% confidence intervals across different
165   subgroups, including species (cattle, buffalo), regions, and diagnostic methods (MAT, ELISA).

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Table 2. The estimated pooled prevalence of bovine leptospirosis.

Group	Variables	No. of study	No. of animals sampled	No. of positive animal	Pooled estimate %	95% Confidence Interval (CI)	Tau-squared (τ^2)	I^2%	p-value
Species	Cattle	52	16202	4443	29	23-35	0.0628	98	<0.01
	Buffalo	17	2152	760	32	20-46	0.0817	98	<0.01
Region	Southern	30	6153	2268	36	27-46	0.071	98	<0.01
	Central	2	25	14	62	22-94	0.0684	77	<0.01
	Eastern	8	1604	420	24	9-42	0.0854	98	<0.01
	Northern	3	996	46	5	2-09	0.0036	82	<0.01
	Western	17	6288	1532	23	17-30	0.0274	97	<0.01
	North Eastern	3	705	217	34	1-85	0.2333	99	<0.01
Test	MAT	62	15710	5001	33	27-39	0.0613	98	<0.01
	ELISA	7	2644	202	6	4-10	0.0056	86	<0.01
Period	2001-2010	16	4872	1483	27	16-41	0.0812	99	<0.01
	2010-2021	53	13482	3720	30	24-37	0.0633	98	<0.01
States	Tamil Nadu	12	2414	1415	43	25-62	0.1085	98	<0.01
	A & N Islands	6	1605	461	29	11-51	0.0809	98	<0.01
	Andhra Pradesh	6	1938	607	53	25-80	0.1267	99	<0.01
	Uttar Pradesh	2	934	2	5	2-10	0.0044	89	<0.01
	Haryana	3	167	11	15	0-72	0.2753	93	<0.01
	Maharashtra	5	578	300	62	34-86	0.1008	98	<0.01
	Madhya Pradesh	1	10	4	40	12-74	-	-	-
	Himachal Pradesh	2	20	5	49	0-100	1.2004	97	<0.01
	West Bengal	2	380	178	15	0-85	0.3107	99	<0.01
	Karnataka	3	189	34	18	1-47	0.045	96	<0.01
	Gujarat	13	5304	1226	21	14-28	0.0251	97	<0.01
	Tripura	1	40	0	0	0-9	-	-	-
	Odisha	2	465	182	39	35-44	0	0	0.38
	Kerala	3	423	85	22	4-49	0.0613	97	<0.01
	Bihar	2	739	54	7	3-12	0.0036	84	0.01
	Punjab	2	494	10	17	0-17	0.2003	90	<0.01
	Telangana	3	387	70	17	3-76	0.1521	98	<0.01
	Jharkhand	2	20	6	33	1-72	0.0825	77	0.04
	Chhattisgarh	2	53	21	51	4-97	0.1677	94	<0.01
	Puducherry	1	250	62	25	20-31	-	-	-
	Sikkim	1	80	62	78	67-86	-	-	-
	Uttarakhand	1	22	22	100	85-100	-	-	-
	Assam	2	625	155	15	25-38	0.1623	99	<0.01
	Overall	**46**	**18354**	**5303**	**29**	**24-35**	**0.0664**	**98**	**<0.01**

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3.3. Prevalence estimates

The overall pooled prevalence of bovine leptospirosis was estimated at 29% (95% CI: 24–35) using a random-effects meta-analysis. The analysis showed significant heterogeneity, with I^2 = 98%, τ^2 = 0.0664, and p < 0.01 (Table 2). Subgroup analysis revealed a 33% prevalence for MAT (95% CI: 27–39, I^2 = 98%, τ^2 = 0.0613) and 6% for ELISA (95% CI: 4–10, I^2 = 86%, τ^2 = 0.0056). Regional analysis showed the highest prevalence in the Central (62%, 95% CI: 22–94) Southern (36%, 95% CI: 27-46), followed by the Southern (36%, 95% CI: 27-46), North Eastern (34%, 95% CI: 1–85), Eastern (24%,

176 95% CI: 09–42), Western (23%, 95% CI: 17–30), and Northern regions (5%, 95% CI: 2–9). Species-

177 wise prevalence was 29% in cattle (95% CI: 23–35) and 32% in buffaloes (95% CI: 20–46).

178 The studies were categorized into two periods, 2001–2010 and 2011–2021. Despite 60 studies

179 qualifying for review, inter-rater disagreement led to 46 studies being included in the meta-analysis

180 (Table 3). Earlier research by Zaki et al. (17) reported a 24.9% pooled prevalence in Southeast Asian

181 livestock, lower than the present estimates of 29% in cattle and 32% in buffaloes. The discrepancy may

182 reflect differences in sample sizes, diagnostic methods used (e.g., MAT and ELISA), and regional

183 variations in study design and coverage. As India's livestock census data (2019) indicates a stable cattle-

184 to-buffalo ratio of 1:1.9, yet buffalo sample sizes (n=2152) were significantly lower than cattle

185 (n=16202), possibly due to fewer studies on buffaloes and underreporting of outbreaks. Over the past

186 two decades, a noticeable increase in bovine leptospirosis reports was observed, with a pooled

187 prevalence of 27% (10 studies) in 2001–2010 and 30% (30 studies) in 2011–2021. This increase

188 highlights the growing recognition of leptospirosis as a significant veterinary and public health issue.

189 **Table 3. Evidence of leptospirosis prevalence in bovine study published during 2001-2021**

190

Study	Year	Study year	Animal condition	Species	State
Natarajaseenivasan et al. (18)*	2002	2000	Healthy	Cattle	Tamil Nadu
Sharma et al. (19)*	2003	2003	Healthy	Cattle/ Buffalo	Andaman & Nicobar Islands
Srivastava and Kumar (20)*	2003	1990-2003	Abortion, Repeat breeding, Reproductive disorders, Sterility etc.	Cattle/ Buffalo	Various states in India
Sunder et al. (21)*	2005	-	-	Cattle	Andaman & Nicobar Islands
Mariya et al. (22)*	2006	2006	Abortion, Mastitis, Repeat breeding, Reproductive problems, Weak calves	Cattle	Various states in India
Koteeswaran (23)*	2006	2004-2006	Healthy	Cattle/ Buffalo	Tamil Nadu
Balakrishnan et al. (24)*	2008	2006	Abortion, Anorexia, Apparently healthy, History, Pyrexia, Repeat breeding	Cattle/ Buffalo	Andhra Pradesh
Sankar et al. (25)*	2009	2009	-	Cattle	Various states in India
Balakrishnan et al. (26)	2009	-	Hemorrhagic mastitis	Cattle	Tamil Nadu
Sankar et al. (27)*	2010	-	Abortion spontaneous, Birth of weak calves, Clinical suspicion, Mastitis, Mummified fetuses, Repeat breeding after artificial insemination or natural breeding, Reproductive problems	Cattle	Various states in India
Senthilkumar et al. (28)*	2010	-	Abortion, history of illness, apparently healthy	Cattle	Tamil Nadu
Natarajaseenivasan et al. (7)	2011	-	Abortion, apparently healthy	Cattle	Tamil Nadu
Balakrishnan et al. (29)*	2011	-	Healthy, Haemorrhagic Mastitis, Jaundice, Repeat breeding, Abortion	Cattle/ Buffalo	Gujarat
Joseph et al. (30)*	2012	2010	Clinically suspected	Cattle	Various states in India
Sritharan (31)*	2012	-	Healthy	Cattle	Telangana
Prameela et al. (32)	2013	2006- 2010	Abortion, apparently Healthy, clinically ailing, pyrexia	Cattle	Andhra Pradesh

Study	Year	Study year	Animal condition	Species	State
Balamurugan et al. (33)*	2013	2013	Abortion, history of illness, Other reproductive problems/disorders	Cattle	Odisha
Sachan et al. (34)*	2012	2008-2010	Abortion, Fever, Jaundice, Repeat breeding, etc	Cattle/ Buffalo	Uttar Pradesh
Deneke et al. (35)	2014	-	Clinically suspected	Cattle	Various states in India
Patel et al. (36)*	2014	-	Healthy	Cattle	Gujarat
Sharma et al. (37)*	2014	2003-2005	Healthy	Cattle	Andaman & Nicobar Islands
Behera et al. (38)*	2014	2011-2012	Abortion, Haemogalactia, etc., History of illness, Infertility	Cattle	Odisha & West Bengal
Balakrishnan (39)*	2014	-	Abortion, Different clinical conditions, Haemorrhagic mastitis, Jaundice, Repeat breeding, Suspected of leptospirosis	Cattle	Various states in India
Soman et al. (40)*	2014	-	Healthy	Cattle	Kerala
Kumar et al. (41)*	2015	-	Abortions sporadic, Fever, Hemolactia, Reduced milk yield	Buffalo	Andhra Pradesh
Pandian et al. (42)*	2015	2008-2010	-	cattle	Bihar
Mitra et al. (43)*	2015	-	-	Cattle	Andaman & Nicobar Islands
Nagalingam et al. (44)*	2015	-	Abortion, Apparently Healthy, Retention of fetal membranes, Reproductive problems	Cattle/ Buffalo	Southern India
Panwala and Mulla (45)*	2015	2012-2013	-	Cattle	Gujarat
Balakrishnan et al. (46)*	2015	-	Anorexia, pyrexia, mastitis, abortion, premature calving and infertility and apparently healthy animals.	Buffalo	Tamil Nadu
Balamurugan et al. (4)*	2016	2015	Abortion, Apparently Healthy, History of illness, Repeat breeding, Reproductive disorders, etc.	Cattle	Various states in India
Balamurugan et al. (47)*	2016	2011-2016	Healthy	Cattle/ Buffalo	Maharashtra
Balamurugan et al. (48)	2016	2011-2012	Healthy	Cattle/ Buffalo	Various states in India
Patel et al. (49)*	2016	-	Abortion, Anorexia, Apparently healthy, Fever, Mastitis, Repeat breeding, Suspected for leptospirosis	Buffalo	Gujarat
Balamurugan et al. (11)*	2017	2011-2014	-	Cattle/ Buffalo	Odisha
Bojiraj et al. (50)*	2017	-	Healthy, Clinically suspected	Bovine	Tamil Nadu
Yogeshpriya et al. (51)*	2017	-	Abortion Sporadic, Reduced milk yield	Buffalo	Tamil Nadu
Lall et al. (5)*	2017	2013-2014	Healthy	Cattle	Andaman & Nicobar Islands
Patel et al. (52)*	2017	-	Abortion, Agalactia, Apparently healthy, Clinically ailing, Fever, Mastitis, Oligolactia, Repeat breeder	Cattle	Gujarat
Panwala (53)*	2017	2008	Healthy	Cattle	Gujarat
Rajan et al. (54)*	2017	-	-	Cattle	Puducherry
Tresamol et al. (55)*	2017	2013- 2014	Healthy	Cattle	Kerala
Kshirsagar et al. (56)*	2018	-	-	Cattle/ Buffalo	Gujarat
Sunder et al. (57)*	2017	2015	Healthy	Cattle	Andaman & Nicobar Islands
Balamurugan et al. (3)*	2018	2015- 2016	Abortion, Anoestrus, Apparently healthy, Endometritis, History of	Cattle	Various states of India

Study	Year	Study year	Animal condition	Species	State
			illness, Repeat breeding, Reproductive disorders		
Alamuri et al. (58)*	2019	2016- 2017	Abortion, Agalactia, Apparently Healthy, Infertility, Stillbirth	Cattle/ Buffalo	Andhra Pradesh
Moon et al. (59)*	2019	2017-2018	Abortion, Apparently Healthy, History of illness, Repeat breeding, Reproductive disorders, etc.	Cattle	Maharashtra
Patel et al. (60)*	2020	NA	Healthy, Clinically suspected buffaloes	Buffalo	Gujarat
Alamuri et al. (8)*	2020	2015 -2016	Healthy	Cattle/ Buffalo	Gujarat
Leahy et al. (61)*	2021	2015–2016	NM	Cattle	Assam & Bihar
Saranya et al. (62)*	2021	2017-2019	Healthy	Cattle/ Buffalo	Assam and Tamil Nadu

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192 *Study and animal included in the meta-analysis after the exclusion of studies due to interrater disagreement.

193

194 In India, bovine leptospirosis has been reported across 23 states, with the highest prevalence in coastal

195 and endemic regions like Gujarat, Andhra Pradesh, Maharashtra, Tamil Nadu, Kerala, and the Andaman

196 Islands. Sample sizes, serovar panels, and diagnostic criteria varied significantly among studies. MAT,

197 with higher sensitivity, showed a pooled prevalence of 33%, compared to 6% for ELISA, which

198 primarily targeted the Sejroe serogroup and had limited sensitivity. To improve diagnostic accuracy, the

199 use of ELISA kits with broader serogroup coverage is recommended. Additionally, integrating

200 molecular tools such as PCR especially for detecting carrier animals—can enhance surveillance and

201 detection strategies. Seroprevalence data indicate the circulation of ~20 serogroups, including Sejroe,

202 Icterohaemorrhagiae, Hebdomadis, Pomona, Autumnalis, Canicola, Hurstbridge, Javanica, Tarassovi,

203 etc. A temporal shift in dominant serogroups was observed in frequently monitored states. Notably,

204 previously underreported states like Sikkim, Uttarakhand, and Chhattisgarh showed higher prevalence

205 in recent studies, suggesting possible disease spread.

206 **3.4. Region-wise prevalence estimates**

207 For the meta-analysis, regional subgrouping was done for Southern, Western, Eastern, Northern,

208 Central, and North-Eastern regions. Studies with state/UT-specific data excluded due to publication bias

209 were analyzed separately to estimate state-wise bovine leptospirosis prevalence. The pooled prevalence

210 and cumulative reactive serogroup patterns are shown in Fig. 4A, 4B. The high prevalence observed in

211 coastal states such as Gujarat, Andhra Pradesh, Maharashtra, Tamil Nadu, Kerala, and the Andaman

212 Islands can be attributed to specific environmental factors that favor the survival and transmission of

213 *Leptospira* spp. These include high annual rainfall, recurrent flooding, warm and humid climatic

214 conditions, and water stagnation, all of which support the environmental persistence of leptospires.

215 Additionally, poor drainage, close proximity between livestock and contaminated water sources, and

216 high rodent population densities in these regions further enhance the risk of transmission to animals and
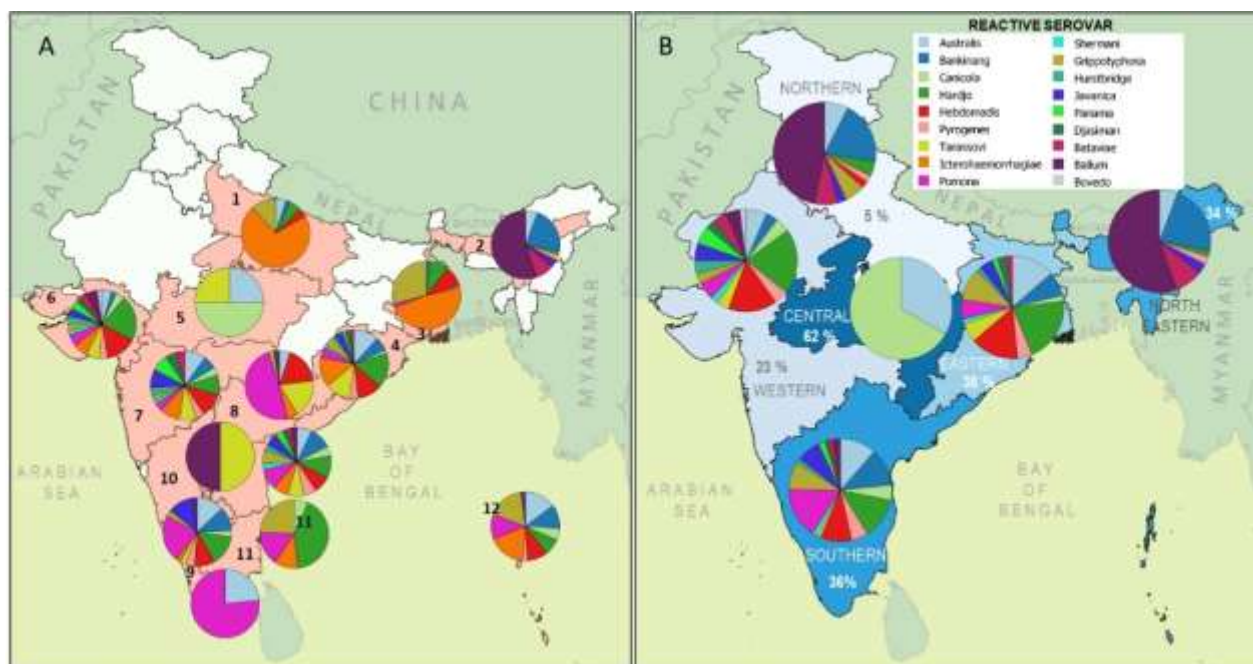
217 humans (10).

Figure 4. Distribution of reactive *Leptospira* serogroups in bovines across India (2001–2021). (A) State-wise cumulative prevalence of antibodies against different serogroups. (B) Region-wise cumulative serogroup prevalence with corresponding pooled prevalence percentages. Pie charts represent the proportion of reactive serogroups identified in each state or region, with a consistent color code used across both panels.

3.4.1. Southern region

The southern region had the highest number of reports (25), covering seven states/UTs, six with a large coastal area, except for Telangana. All the states/UTs in the southern region are endemic to leptospirosis, with reports from both humans and animals. In the present study, an estimated pooled prevalence of 36% (95% CI: 27-46%) was observed for bovine leptospirosis in the southern region.

Andaman & Nicobar Islands (A&N): The A&N Islands, a union territory of India, is a highly endemic region for leptospirosis. Early cases resembling Weil's disease were reported in the 1880s and later confirmed as leptospirosis in the 1930s (63). Leptospirosis re-emerged in the 1980s, with follow-up seroepidemiological studies confirming its endemicity in humans and animals (5, 57). The A&N Islands are a well-studied region for leptospirosis, noted for implementing a "one-health" program for its control and prevention (10), which has successfully reduced human leptospirosis cases (5, 10). However, studies in livestock over the last two decades show varying seroprevalence trends (10). In the present analysis, A&N Islands showed an estimated pooled prevalence of 29% (95% CI: 11–51%) (5, 19, 21, 37, 43, 57). Most studies focused on cattle, as buffaloes are scarce in these islands (20th Livestock Census). Overall seropositivity in cattle declined from 34.4% during 2003–2005 to 11.6% during 2013–2014, with predominant serogroups Icterohemorrhagiae, Sejroe, and Pomona. A shift in predominant serogroups was noted from Grippotyphosa to Icterohemorrhagiae (5). Moreover, studies conducted after that also showed an increased prevalence in 2014-2015, with Autumnalis and Sejroe as the

242   predominant serogroup (43), whereas during 2015-2016, the predominant serogroup Hebdomadis,
243   Icterohaemorrhagiae, and Australis (57). The high endemicity in the A&N Islands is attributed to the
244   presence of susceptible hosts (carriers, reservoirs, and accidental hosts), and favorable geographical and
245   climatic conditions. A recent study in these islands reported serogroups Tarassovi and Djasiman
246   (pathogenic) and Hurstbridge (intermediate), which were not previously documented (9).

247   *Andhra Pradesh:* Andhra Pradesh, a coastal state along the Bay of Bengal, is highly endemic and
248   enzootic for leptospirosis, with increasing reports over the past two decades. The present meta-analysis
249   revealed a higher prevalence of 53% (95% CI: 25–80%) in Andhra Pradesh (3, 24, 41, 48, 58).
250   Chronologically, (39) reported 50.21% prevalence in cattle and 68.64% in buffaloes, with Hebdomadis,
251   Pomona, Ballum, and Sejroe as predominant serogroups. Subsequent studies showed variable
252   prevalence, including 19.65% in 2013 (32) and 4% by ELISA in 2015, but 70% by MAT with
253   serogroups Javanica, Panama, and Hebdomadis (3, 48). A 2016–2017 study reported 68.08%
254   prevalence in Prakasam, Kurnool, and other districts, with predominant serogroups Hebdomadis,
255   Pomona, Sejroe, and Tarassovi (58). Telangana is an understudied endemic neighboring state of Andhra
256   Pradesh, Karnataka and Maharashtra had a pooled prevalence of 17% (95% CI: 03-76%) in the (3, 31,
257   47, 48). A recent study conducted in Telangana at the animal level and farm level seroprevalence found
258   more than 40 and 70%, respectively, with an increased threat to other livestock and public health and
259   needs appropriate control measures (64).

260   *Karnataka:* Karnataka, located in southwest peninsular India, has two coastal districts along the Arabian
261   Sea. While reports on human leptospirosis are available, data on bovine leptospirosis in the state are
262   limited. Between 2001 and 2021, only three studies reported an estimated prevalence of 18% (95% CI:
263   01–47%) based on a small sample size using the Linnodee ELISA Hardjo kit and MAT (3, 4, 20, 48).
264   Systematic serosurveillance is required to accurately estimate bovine leptospirosis prevalence. In a
265   separate study, serum samples from 582 animals with reproductive disorders (314 cattle and 268
266   buffaloes) across Bengaluru, Belgaum, Gulbarga, and Mysuru divisions showed an overall
267   seroprevalence of 28% (29% in buffaloes and 27% in cattle). Higher seropositivity was observed in
268   Bengaluru, Mysuru, and Belgaum compared to Gulbarga, likely due to greater risk factors in these
269   divisions. Antibodies against six serogroups were identified, with Sejroe (34.35%), Pomona (16.56%),
270   and Canicola (11.66%) being predominant, followed by Icterohaemorrhagiae (10.43%), Hebdomadis
271   (9.81%), and Autumnalis (6.74%). Among the 163 MAT-positive samples, 62.58% had a history of
272   abortion, 28.22% showed repeat breeding, and 9.2% exhibited other clinical signs.

273   *Kerala:* An estimated pooled prevalence of 22% (95% CI: 04–49%) was observed in Kerala, a highly
274   endemic region for leptospirosis in southern India, affecting humans and animals with high morbidity
275   and mortality rates (40, 48, 55). Animal studies are limited compared to human data. In 2014, Soman

et al. reported a seroprevalence of 47% using the ELISA kit. Conversely, in 2015, Balamurugan et al. observed a lower prevalence of 19.25%, with Sejroe, Autumnalis, Tarassovi, and Icterohaemorrhagiae as predominant serogroups. In Thrissur district (2013–2014), a 6.38% prevalence was reported using rLipL32-based ELISA (55).

Tamil Nadu and Puducherry: Leptospirosis poses a significant challenge to dairy farms in Tamil Nadu, causing abortions, stillbirths, and infertility, with frequent outbreaks. The estimated pooled prevalence in Tamil Nadu was 43% (95% CI: 25–62%) (7, 48, 62). In 2010, MAT reported 44.7% seropositivity while evaluating rLipL41-based ELISA and LAT (35). In 2011, Tiruchirappalli farms reported a prevalence of 87.18% with Javanica, Autumnalis, and Sejroe as predominant serogroups (7). Studies also found 50% seropositivity by ELISA and 66.10% by MAT, with Sejroe, Hurstbridge, and Shermani as dominant serogroups (48). Puducherry reported a prevalence of 25% (95% CI: 20–31%) with Sejroe, Grippotyphosa, and Pomona as major serogroups (54).

3.4.2. Western region

Western region: The western region, comprising two states/UTs, had 13 reports with an estimated pooled prevalence of 23% (95% CI: 17–30%). Predominant serogroups included Sejroe, Hebdomadis, Tarassovi, Australis, Icterohaemorrhagiae, Pomona, Javanica, Canicola, Grippotyphosa, and others.

Maharashtra: In the western region, Maharashtra exhibited a high prevalence of 62% (95% CI: 34–86%) (20, 48, 59). The state has reported significant human mortality due to leptospirosis in the past decade. In 2015, a study in the Konkan region reported a seroprevalence of 69.44%, with Australis, Sejroe, Hebdomadis, Autumnalis, Icterohaemorrhagiae, Tarassovi (Kaup), and Tarassovi as predominant serogroups (47). Another study reported a 30.3% prevalence in an organized dairy farm using an ELISA kit (4). Additionally, a 39.3% seropositivity rate was observed with serogroups Hurstbridge, Tarassovi (Kaup), Sejroe, and Tarassovi (48). Furthermore, cattle associated with reproductive disorders exhibited 62.50% seropositivity, with Icterohaemorrhagiae, Tarassovi, and Panama as predominant serogroups (3).

Gujarat: Located in the western region, Gujarat is highly endemic for leptospirosis, particularly in Surat and Navsari districts. Similar to the A & N Islands, Gujarat has adopted a one-health approach over the past decade, significantly reducing human leptospirosis mortality through rodent control measures (65). However, bovine leptospirosis remains a critical issue. An estimated pooled prevalence of 21% (95% CI: 14–28%) was observed in bovines based on multiple studies (8, 48, 52). Srivastava and Kumar (20) reported a 28.6% seroprevalence using MAT in cattle with reproductive disorders during 1990–2003. In 2011, a seroprevalence of 38.55% was reported among cattle and buffaloes in organized farms, with Sejroe, Hebdomadis, and Ballum as predominant serogroups (29). A study in endemic districts

(Navsari, Surat, Tapi, and Valsad) found a 12.81% prevalence, with Pomona, Sejroe, and Icterohaemorrhagiae as major serogroups (36). Further, 13.51% prevalence of Sejroe using a Bovine Hardjo ELISA kit and 23.7% by MAT, with reactive serogroups including Sejroe, Tarassovi (Kaup), and Pomona (4, 48). Patel et al. (52) found a 5.77% seroprevalence using ELISA in clinically ailing and healthy cattle, while Balamurugan et al. (3) reported 62.5% prevalence in healthy cattle with reproductive histories, identifying serogroups Pyrogenes, Javanica, Icterohaemorrhagiae, and others.

3.4.3. Eastern region

The Eastern region included eight reports from four states/UTs, with an estimated pooled prevalence of 24% (95% CI: 09–42%). Predominant serogroups in the region were Icterohaemorrhagiae, Sejroe, Hebdomadis, Tarassovi, Australis, Grippotyphosa, Autumnalis, Pomona, Pyrogenes, Javanica, Djasiman, Bataviae, Canicola, Panama, Shermani, and Hurstbridge. The state-wise findings are summarized below:

Odisha: A known endemic state, Odisha frequently experiences leptospirosis outbreaks in humans and animals following cyclones and floods. Over the last two decades, an estimated pooled prevalence of 39% (95% CI: 35–44%) was observed (33, 66). Between 2011 and 2014, prevalence was reported at 38%, with predominant serogroups Australis, Autumnalis, Canicola, Sejroe, Hebdomadis, and Icterohaemorrhagiae. In 2013, seroprevalence reached 42.5%, primarily against Australis and Sejroe serogroups (33). Behera et al. (38) reported 55.5% prevalence, with predominant antibodies against Icterohaemorrhagiae, Hebdomadis, Grippotyphosa, and Sejroe.

Bihar: Two studies estimated a pooled prevalence of 7% (95% CI: 03–12%) using the Hardjo ELISA test (42, 61). *West Bengal:* The estimated pooled prevalence was 15% (95% CI: 0–85%) with predominant serogroups Icterohaemorrhagiae, Grippotyphosa, Hebdomadis, and Sejroe (20, 38). *Jharkhand:* Under-reported with limited data, Jharkhand showed an estimated pooled prevalence of 33% (95% CI: 01–72%) with predominant reactive serogroups Hebdomadis, Pyrogenes, Tarassovi, Pomona, Icterohaemorrhagiae, and Djasiman (3, 48).

3.4.4. Northern region

There are very few studies in the Northern region of India, with an estimated pooled prevalence of 5% (95% CI: 02-09%) in bovines. The overall seroreactivity included Ballum, Icterohaemorrhagiae, Autumnalis, Australis, Bataviae, Grippotyphosa, Sejroe, Javanica, Hebdomadis, and Pyrogenes. The state-wise analysis shows Haryana with a prevalence of 15% (95% CI: 0-72%) (20) and Uttar Pradesh with 5% (95% CI: 02-10%), with reactivity to the Sejroe, Hebdomadis, Autumnalis, Australis, Icterohaemorrhagiae serogroup (20, 34). Other states with very low sample sizes are estimated to have 49% seropositivity in Himachal Pradesh (3, 20) and 29% in Punjab (3, 48). The serogroup predominance in these states was Hurstbridge, Panama, Sejroe, Hebdomadis in Haryana; Bataviae,

345 Shermani, Pyrogenes, Canicola in Himachal Pradesh; Icterohaemorrhagiae, Canicola, Hurstbridge,
346 Shermani, Australis in Uttarakhand; and Australis, Autumnalis, Sejroe, Icterohaemorrhagiae,
347 Hurstbridge in Punjab (3).

348

349 3.4.5. Central region

350 The Central region covered two states with only two reports, which showed the estimated pooled
351 prevalence of 62% (95% CI: 22-94%), with the overall prevalence of Canicola, Australis, and Tarassovi.
352 The prevalence for Chhattisgarh state was 51% (95% CI: 04-97%) (3), with the predominance of Sejroe,
353 Icterohaemorrhagiae, Canicola, Hurstbridge, Shermani, Australis, Tarassovi (Kaup), etc., whereas for
354 Madhya Pradesh, the estimated prevalence was 40% (95% CI: 12-74%) (20), with the predominance of
355 Tarassovi, Canicola, and Australis.

356

357 3.4.6. North-Eastern region

358 The North-Eastern region has been largely neglected in leptospirosis research, despite the disease being
359 first reported in Assam in the 1940s, where eight cases of jaundice resulted in four deaths on a tea estate.
360 Subsequent studies focused primarily on humans, with limited seroprevalence studies on animal hosts,
361 including reservoir rodent populations (61, 62, 67). Between 2001 and 2021, the region had three reports
362 from three states/UTs, showing an estimated pooled prevalence of 34% (95% CI: 01–85%) with
363 predominant serogroups including Ballum, Autumnalis, Bataviae, Australis, Javanica, Pyrogenes,
364 Sejroe, and Icterohaemorrhagiae. A study in Tripura reported no antibody prevalence in cattle (20).
365 Recent studies from 2018–2021 reported a pooled prevalence of 15% (95% CI: 25–38%) in Assam (61,
366 62) and 78% (95% CI: 67–86%) in Sikkim, though based on limited samples (3). This study investigated
367 the seroprevalence and serogroup distribution of leptospirosis in livestock across Assam, India,
368 revealing an overall seroprevalence of cattle (26.2 %) and buffalo (25 %), with uncommon serogroups
369 such as Mini, Manhao, and Cynopteri indicating potential transboundary transmission (68).

370

371 **3.5. Limitations of the Meta-Analysis Study**

372 The current meta-analysis encountered several limitations that may influence its outcomes. Small
373 sample sizes in many studies likely overestimated seropositivity rates, particularly in endemic areas.
374 State-wise pooled prevalence was analyzed separately due to inconsistent demographic data (age, sex)
375 and risk factor reporting. The high prevalence observed in the Central (62%) and North-Eastern (34%)
376 regions should be interpreted cautiously, as these estimates are based on a limited number of studies
377 with small sample sizes. This may have led to overestimation, underscoring the need for larger, well-
378 designed studies to better assess the true burden of leptospirosis in these areas. Significant heterogeneity
379 across studies suggests unaccounted factors, such as environmental influences and management
380 practices, affecting prevalence estimates. These limitations highlight the need for large-scale studies
381 with standardized methodologies to accurately assess bovine leptospirosis prevalence and risk factors.

4. Conclusions

Bovine leptospirosis in India remains underreported, with abortion cases in cattle and buffaloes often overlooked. This review reveals high seropositivity rates of 50–70% in bovines with reproductive disorders and 15–20% in healthy animals. Coastal and non-coastal states like Gujarat, Andhra Pradesh, Maharashtra, Tamil Nadu, Kerala, and South Andaman are highly endemic due to favorable environmental conditions. Predominant serogroups include Sejroe, Icterohaemorrhagiae, Hebdomadis, Pomona, Autumnalis, Canicola, Hurstbridge, Javanica, and Tarassovi, with temporal shifts in monitored regions. Geographically tailored diagnostic panels are crucial for identifying diverse serogroups and addressing diagnostic gaps. Mitigation measures, including targeted vaccination and robust surveillance, are essential to reduce the disease burden. Regular seroepidemiological studies in endemic areas are critical to monitor trends and guide interventions. Detailed studies using opportunistic sampling in reservoir hosts and humans can aid early diagnosis and treatment. Key challenges include limited diagnostics, lack of nationwide surveys, and inconsistent sampling strategies. Expanding molecular techniques and incorporating *Leptospira* intermediate species serovars into MAT panels will enhance diagnostic accuracy. While MAT remains the standard for seroprevalence studies, its limited sensitivity in detecting carriers or early infections underscores the need for molecular tools. PCR-based diagnostics can enhance detection of leptospiral DNA in asymptomatic animals and should be integrated with serology to improve diagnostic accuracy and surveillance. Comprehensive preventive strategies, including vaccination, biosecurity, and environmental management, are vital. Research into transmission dynamics and local serovar pathogenicity will facilitate effective early intervention and control, reducing leptospirosis's impact on animal and human health.

Author Contributions

K.V.K. Writing – original draft, review & editing, conceptualisation, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization; P.P.B., A.P., S.M. Writing – review & editing, Investigation, Methodology; R.L. and O.D Language and copy editing and re-writing of the

manuscript. S.K.S. Writing – review & editing, data curation, formal analysis, Investigation, Methodology; A.N. Writing – review & editing, data curation, formal analysis, Investigation, Methodology, software, validation; K.P.S. Writing – review & editing, data curation, formal analysis, Investigation, Methodology, software, supervision, validation, visualization; C.D. Writing – review & editing, Methodology; YPA. Writing – review & editing; S.K.P. Writing – review & editing, formal analysis, validation; S.P.K. Writing – review & editing, formal analysis, resources; N.S.S Writing – review & editing, Funding acquisition, resources; C.S. Writing – review & editing, conceptualisation, Formal analysis, Funding acquisition, supervision, validation; V.B. Writing – original draft, review & editing, conceptualisation, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization. All authors read and approved the final edited manuscript.

Ethics

Not applicable

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Data Availability Statement

The data used to support the findings of this study are included within the article or uploaded in journal as a supplementary documents.

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