CLINICAL PRACTICE GUIDELINES
ON
LEPTOSPIROSIS IN CHILDREN
2019

MEMBERS OF THE LEPTOSPIROSIS CLINICAL PRACTICE GUIDELINES

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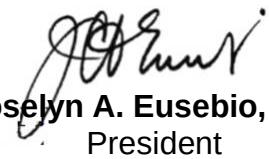
MESSAGE

Leptospirosis in our country remains to be of public health concern as it affects all age groups. Its clinical presentation is nonspecific and it is commonly considered alongside other endemic illnesses, such as Dengue Fever. Prompt recognition is important as it impacts on management, preventing further morbidity and mortality.

The Clinical Practice Guidelines on Leptospirosis is a collaborative effort of the Philippine Pediatric Society and the Pediatric infectious Disease Society of the Philippines.

In providing a template of evidence based information, it is hoped for, that the answers to the more common concerns are met with the ultimate objective of providing the best care to our patients.



Joselyn A. Eusebio, M.D.
President
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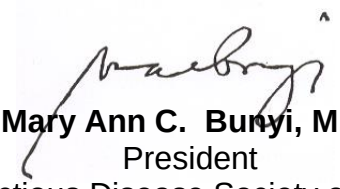
MESSAGE

The Pediatric Infectious Disease Society of the Philippines is once again fortunate to have the opportunity to engage in knowledge sharing with the medical community thru the Clinical Practice Guidelines on Leptospirosis.

The Committee collectively chose to concentrates work on the more common clinical questions in the following areas: clinical presentation, diagnostics, the use of antibiotics, and pre- and post-exposure prophylaxis. Subsequent recommendations made have been based on available current scientific evidence, and have been presented to essential stakeholders.

This guideline is our modest contribution as a subspecialty organization aligned and committed in helping lead the fight against childhood infectious diseases.



Mary Ann C. Bunyi, M.D.
President
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FOREWORD

Leptospirosis, long recognized as a zoonosis causing significant disease in humans, remain to be one of the major public health issues in the Philippines.

Available guidelines mostly have catered to the adult population and management has been largely dependent on studies done on adults as well. The Pediatric Infectious Disease Society of the Philippines, cognizant of the gaps in knowledge, initiated the development of a guideline involving the more frequent concerns that beset the medical practitioner in managing leptospirosis in children.

It is with a sense of purpose and hope that this guideline would enable our colleagues to better recognize and manage leptospirosis in clinical practice.

Maria Anna P. Bañez, M.D.
Over-All Chair
Leptospirosis Clinical Practice Guidelines

EXECUTIVE SUMMARY

2019 PPS-PIDSP CLINICAL PRACTICE GUIDELINES ON LEPTOSPIROSIS

Leptospirosis is a disease prevalent mostly in tropical and subtropical countries. Its potential to be a concerning problem emerges with the onset of the rainy season, as flooding and heavy rainfall facilitate disease epidemics. Among those at risk of contracting the disease are field workers, veterinarians, sewer workers, military personnel and those who swim or wade in contaminated waters.

In the absence of an existing evidence-based guideline for the pediatric age group, this first edition hopes to standardize approach to diagnosis, antibiotic management, and prevention of leptospirosis. The intended users are primary care physicians, family medicine physicians, pediatricians, and other healthcare workers involved in the management of leptospirosis in children.

Ten priority questions were identified by a group of experts composed of an oversight committee, a guideline writing panel, and a technical review committee. The GRADE methodology was used to determine the quality of evidence of each recommendation. The draft recommendations (summarized below) were finalized after these were presented to and voted on by a panel of stakeholders.

No.	Recommendation	Strength of Recommendation	Quality of Evidence
1	***Clinical manifestations suggestive of leptospirosis in children with acute fever and possible exposure***		
	Recommendation 1: Among children with acute fever and possible exposure, the presence of any or all of the following clinical manifestations should make one highly suspect leptospirosis: • Renal syndrome (defined as any sign or symptom pointing to a possible kidney damage) • Chest pain • Cardiac syndrome (defined as any sign or symptom pointing to a possible cardiac involvement) AND/OR • Conjunctival suffusion/red eye	Strong	Very low
	Recommendation 2: Among children with acute fever and possible exposure, the presence of any or all of the following clinical manifestations may make one highly suspect leptospirosis: • Arthralgia • Myalgia • Muscle tenderness	Strong	Very low
2	***Clinical findings associated with increased risk of mortality***		
	Recommendation 1: In children with leptospirosis, the presence of any one of the following signs and symptoms increases the risk of mortality: • Pallor • Loss of consciousness • Murmur • Meningism • Irregular rhythm • Dyspnea • Pulmonary hemorrhage • Convulsions/seizure • Crackles/rales on lung auscultation • Hemoptysis • Anuria • Disorientation • Jaundice • Tachycardia	Strong	Very low

3	***Laboratory findings associated with severe leptospirosis*** ***Recommendation 1***: The following laboratory parameters are ~~a~~ssociated with severe leptospirosis: • Deranged prothrombin time (prothrombin time greater than or equal to 15 seconds; prothrombin time less than 68%) • Elevated AST/ALT ratio (greater than or equal to 2) • Elevated LDH (greater than or equal to 390 IU/L) • Elevated CRP (greater than 282 mg/L) • Elevated creatine phosphokinase (greater than 443 U/L) ***Recommendation 2***: There is insufficient evidence to suggest that the following laboratory tests are associated with severe leptospirosis: • Elevated blirubin (greater than 49 µmol/L; total bilirubin greater than or equal to 35 µmol/L) • Thrombocytopenia (less than 92 x 10^9/L) • Elevated creatinine (greater than 154 µmol/L) • Elevated BUN (greater than 9.3 mmol/L) • Hematuria • Decrease in hemoglobin (less than 12.2 g/dL)	Strong Strong	Very low Very low
4	***Use of IgM Immunochromatography Test (ICT) as a rapid test in the diagnosis of leptospirosis in children*** ***Recommendation 1***: IgM ICT may be used as a rapid test in the diagnosis of leptospirosis in children.	Strong	Moderate
5	***Use of IgM ELISA as a rapid test in the diagnosis of leptospirosis in children*** ***Recommendation 1***: IgM ELISA may be used as a rapid test in the diagnosis of leptospirosis in children.	Weak	Low
6	***Use of PCR in the diagnosis of leptospirosis in children*** ***Recommendation 1***: PCR may be used in the diagnosis of leptospirosis in children.	Strong	Low

7	***Effectiveness of antibiotics in the treatment of children with leptospirosis*** ***Recommendation 1***: The use of antibiotics may be considered in the treatment of children with leptospirosis, but there is no evidence to suggest that this may decrease mortality, duration of fever, renal complications, and the need for dialysis.	Strong	Very low
8	***Doxycycline as pre-exposure prophylaxis in the prevention of leptospirosis in children*** ***Recommendation 1***: Doxycycline as pre-exposure prophylaxis may be used to prevent both asymptomatic laboratory-identified leptospiral infection and symptomatic leptospirosis in those who live in and intend to visit highly endemic areas.	Strong	Very low
9	***Doxycycline as post-exposure prophylaxis in the prevention of leptospirosis in children*** ***Recommendation 1***: The use of doxycycline may be considered as post-exposure prophylaxis but there is no evidence in children to suggest that it can prevent symptomatic leptospirosis.	Strong	Very low
10	***Use of antibiotics other than doxycycline as post-exposure prophylaxis for leptospirosis in children*** ***Recommendation 1***: Oral penicillin may be used for post-exposure prophylaxis to prevent symptomatic leptospirosis in high transmission areas but there are no studies in children.	Strong	Very low

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CHAPTER 1

INTRODUCTION

Leptospirosis, caused by a bacteria belonging to the genus *Leptospira sp.*, is a zoonotic disease that is transmissible to humans commonly thru exposure to vehicles (water, food, or soil) contaminated by urine from infected animals. Main reservoirs of the causative agent are rodents, livestock and dogs. Although leptospirosis occurs worldwide, it is most prevalent in the tropical and subtropical areas. The disease is also common in urban slum areas with inadequate water treatment and improper waste disposal. Leptospirosis can be both an occupational and recreational hazard. Among the groups at risk for the disease are field workers such as farmers and sugar cane workers, veterinarians, sewer workers, military personnel, and those who wade or swim in contaminated waters. Flooding after typhoons, excessive rainfall and other effects of extreme weather conditions propagate disease epidemics (WHO, 2010; WHO, 2017).

A systematic review on the global burden of leptospirosis that utilized morbidity and mortality studies and databases determined an overall estimate of 1.03 million cases of disease occurring annually worldwide. This resulted to about 2.9 million Disability Adjusted Life Years (DALYs). Countries in South and Southeast Asia are among the areas identified to have high disease morbidity (Torgerson, 2015).

Data from the Epidemiology Bureau of the Department of Health (DOH) show that from January 1, 2017 to December 2, 2017, there were a total of 2,495 leptospirosis cases nationwide. This is 49.1% higher than the reported cases from the previous year. Majority of the reported cases belonged to the 15 to 19 year old age group. There were 261 deaths, giving a case fatality rate (CFR) of 10.46%, and the age group with the highest CFR was the 45 to 49 year old age group (DOH, 2017). The year 2018 saw an even greater number of affected individuals, with 5,232 leptospirosis cases reported from January to December 31, 2018. This figure is 71% higher than in 2017. The 20 to 24 year old age group had the highest number of cases. There were 505 deaths (CFR 9.65%) (DOH, 2018). In July 2018, the DOH declared a leptospirosis outbreak in the National Capital Region (Philippine News Agency, 2018).

I. RATIONALE FOR THE GUIDELINE

The CPG, in the absence of an existing evidence-based guideline for the pediatric age group, hopes to standardize approach to diagnosis and antibiotic management of leptospirosis and answer concerns on the use of agents for the prevention of leptospirosis in exposed populations.

II. BACKGROUND

Typhoon “Ondoy” was one of the most destructive calamities that ravaged the country in September 2009, submerging many cities in NCR after its wake. An outbreak of leptospirosis occurred soon after. A report from the National Disaster Coordinating Council (NDCC) showed that there were 2,299 hospital admissions from October 1 to November 19, 2009 in 15 Sentinel Hospitals in Metro Manila due to leptospirosis, with 178 deaths recorded (NDCC, 2009). At this time, the Philippine Society for Microbiology and Infectious Diseases (PSMID), the Philippine Society of Nephrology (PSN) and the Council for Critical Care and Vascular Pulmonary Diseases of the Philippine College of Chest Physicians (PCCP) drafted interim guidelines on the diagnosis, management and prevention of leptospirosis to guide health workers handling diseased patients in affected areas. The interim guidelines were later finalized and updated as

"Philippine Clinical Practice Guidelines (CPG) on the Diagnosis, Management and Prevention of Leptospirosis in Adults 2010" by the Leptospirosis Task Force composed of members of the PSMID, PSN and PCCP (PSMID, 2010).

In August 2012, the Pediatric Infectious Disease Society of the Philippines (PIDSP) released a "Post Disaster Interim Advice on the Prevention of Leptospirosis in Children" to guide physicians and parents on the prevention of leptospirosis (PIDSP, 2012).

In 2014, under the leadership of Dr. Salvacion Gatchalian, PIDSP formed CPG committees. Leptospirosis was one of the priority diseases identified that needed a guideline. Dr. Gyneth Bibera headed the initial Leptospirosis CPG group. There was an initial draft developed, but it did not utilize the GRADE method. There was likewise an initial attempt to incorporate the management of renal complications in children, with the help of then president of the Philippine Nephrology Society of the Philippines (PNSP), Dr. Norma Zamora. It was subsequently decided that a separate working group will be formed to address renal issues in leptospirosis.

Using the GRADE approach, this current guideline was created to address issues on recognition, diagnosis, antibiotic management and prevention of leptospirosis in children.

III. GUIDELINE OBJECTIVES:

1. To provide an evidence-based guideline in the diagnosis, antibiotic management, and prevention of leptospirosis in children.
2. To improve patient outcome through early identification of disease and timely intervention of cases for the prevention of complications.
3. To provide recommendations on pre- and post-exposure prophylaxis of leptospirosis in children.

IV. TARGET USERS

These guidelines are intended for primary care physicians, family medicine physicians, pediatricians, and other healthcare workers involved in caring for children with leptospirosis.

V. ORGANIZATION OF THE CLINICAL PRACTICE GUIDELINE ON LEPTOSPIROSIS:

A. Oversight (Steering) Committee (OC)

The Oversight Committee is composed of PIDSP members responsible for formulating the CPG's objectives and determining the intended users of the guideline.

The OC was tasked to schedule activities, coordinate with members of the Technical Review Committee (TRC) and organize the multisectoral stakeholders panel in charge of the final recommendations.

B. Guideline Writing Panel (GWP)

The GWP is composed of specialists in the field of infectious disease and epidemiology. They are responsible for the content of the summary of evidence and the draft recommendations.

C. Technical Review Committee (TRC)

Literature search, tracking and retrieving the journals, appraisal and summary of evidence were done by epidemiologists from the University of the East Ramon Magsaysay Memorial Medical Center and St. Luke's Medical Center.

D. Stakeholders Panel (Voting Consensus Panel)

This panel is composed of stakeholders including heads of societies, representatives from academic institutions, and representatives from government and non-government health agencies. The members are responsible for reviewing the draft recommendation statements and evidence, and will participate in panel deliberation through discussion and voting.

VI. DECLARATION OF CONFLICTS OF INTEREST

Members of the oversight committee, guideline writing panel, and the technical review committee declared potential conflicts of interest prior to the start of activities pertinent to the development of this guideline (see Appendix B).

VII. METHODOLOGY

A. Identifying the Guideline Questions

Ten (10) questions were chosen by the Oversight Committee (OC) and the Guideline Panel (GWP) based on the following: (1) relevance, (2) priority and perceived urgency, (3) inconsistency of evidence, and (4) controversies.

The following are the clinical questions contained in this guideline:

Question 1: Among children with acute fever and possible exposure, what clinical manifestations should make one suspect leptospirosis?
Question 2: Among children with leptospirosis, what are the signs and symptoms associated with an increased risk of mortality?
Question 3: What laboratory findings are associated with severe leptospirosis?
Question 4: Can IgM Immunochromatography Test (ICT) be used as a rapid test in the diagnosis of leptospirosis in children?
Question 5: Can IgM Enzyme-linked Immunosorbent Assay (ELISA) be used as a rapid test in the diagnosis of leptospirosis in children?
Question 6: Can Polymerase Chain Reaction (PCR) be used in the diagnosis of leptospirosis in children?
Question 7: How effective is the use of antibiotics in the treatment of children with leptospirosis?
Question 8: How effective is doxycycline as pre-exposure prophylaxis in the prevention of leptospirosis in children?
Question 9: How effective is doxycycline as post-exposure prophylaxis in the prevention of leptospirosis in children?
Question 10: Is there evidence to recommend the use of antibiotics other than doxycycline as post-exposure prophylaxis for leptospirosis in children?

The issues on the management of renal complications, such as IV hydration and the need for dialysis, were not included as it was agreed upon with the Pediatric Nephrology Society of the Philippines (PNSP) that a separate guideline on these will be developed.

B. Search and Retrieval of Relevant Articles

A systematic search of literature was conducted by the TRC using electronic databases and other conventional methods. Medline was searched for relevant articles indexed from 1966 to 2017 using the terms derived from each of the questions. MeSH terms were often used because of their ability to explode. In addition, a local database called Herdin was searched, but since the search engine was not as sophisticated, manual searching was conducted upon obtaining abstracts from a broad topic search. There were no restrictions placed on language, age, or year of publication. Meta-analyses or systematic reviews were retrieved and used when available.

Aside from searching electronic databases, local experts from the Philippine Pediatric Society and the Pediatric Infectious Disease Society of the Philippines were asked for articles that they were aware of, whether published or unpublished. Manual searching of bibliographies from eligible articles was also conducted to identify references missed during the initial search.

C. Grading the Quality of Evidence and Preparation of Evidence Summaries

The quality of evidence and strength of recommendation was rated using the GRADE methodology (GRADE Working Group, 2004) by the TRC (see Table 1).

The quality of evidence is defined as the confidence that the reported estimates of effect are adequate to support a specific recommendation. The GRADE system classifies the quality of evidence as high, moderate, low, and very low. Randomized controlled trials are initially rated as high-quality evidence but may be downgraded for several reasons, including risk of bias, inconsistency of results, indirectness of evidence, imprecision, and publication bias. Observational studies are initially rated as low-quality evidence but may be upgraded if the magnitude of the treatment effect is very large, if evidence indicates a dose-response relationship, or if all plausible biases would underestimate the effect.

Table 1. Quality of evidence rating using the GRADE methodology

Quality	Definition
High	Further research is unlikely to change confidence in the estimates of the effect.
Moderate	Further research is likely to have an important impact on confidence in the estimate of the effect and may change the estimate.
Low	Further research is very likely to have an important impact on the confidence of the effect and is likely to change the estimate.
Very Low	Any estimate of effect is very uncertain.

Additional categories considered when grading quality of evidence: (1) risk of bias (study limitations); (2) indirectness; (3) inconsistency; (4) imprecision; and (5) publication bias.

Deciding whether an outcome is critical, important but not critical, or not important, is a value judgment that should take into account the value of those who will be affected by adherence to subsequent recommendations. The outcome is considered as critical for a judgment if the risk of the adverse effect is serious and could result in mortality or a life-threatening condition. Other outcomes that are important but not critical are those that are significant but may not necessarily increase the risk for mortality.

D. Preparation of the Draft Recommendations

The GWP was tasked with reviewing and evaluating the quality of evidence and the draft recommendations submitted by the TRC. They were also responsible for revising and finalizing the guideline recommendations.

E. Consensus Development Process

1. Panel's Declaration of Conflict of Interest (COI) and Management of the Identified COI

Members of the panel were made to accomplish a Declaration of Conflict of Interest Form prior to the presentation of the evidence-based draft. There were two members identified with connections to a pharmaceutical company manufacturing antibiotics. One of them is the spouse of a company executive and the other is the head of the CME arm of the company. These panel members were excluded from the voting process on the clinical questions that addressed antibiotic and prophylactic management of leptospirosis

2. Panel of Stakeholders

The first evidence-based draft was circulated to the panelists one week prior to the scheduled en-banc meeting to allow review of the recommendation statements. During the meeting, the members of the GWP presented each recommendation with the supporting evidence. Using the nominal group technique, each recommendation was discussed, taking into account not only supporting evidence but also consideration of other criteria.

Table 2. Criteria for consideration in recommendation development

Domain	Rationale
Quality of evidence	Assessment of the degree of confidence in the estimate of the effect
Benefits and harms (Risks)	Desirable effects (benefits) need to be weighed against harmful or undesirable effects (risks), considering any previous recommendation or another alternative. The larger the gap or gradient in favor of the benefits over the risks, the more likely that a strong recommendation will be made.
Values and preferences	Judgment of how much the people affected by the intervention or option value each of the outcomes.
Acceptability	How much an intervention or recommendation is accepted by the people who are affected by it or by those who are implementing it. If the recommendation is likely to be widely accepted or valued highly, it is likely that a strong recommendation will be made. If there is a great deal of variability or strong reasons that a recommendation is unlikely to be accepted, it is more likely that a weak recommendation will be made.
Feasibility (including resources use consideration)	Whether an intervention is achievable and sustainable in a setting where the greatest impact is expected.

Assessment for each recommendation as “strong recommendation”, “weak recommendation” or “no recommendation” was determined by the panel based on the criteria provided. A preliminary vote on every item was obtained. A consensus was arrived at when 75% or more of the votes was obtained from any recommendation.

Table 3. Assessment criteria for the strength of recommendations

Strength of recommendations	Rationale
Strong	The Panel is confident that the desirable effects of adherence to the recommendation outweigh the undesirable effects.
Weak	The Panel concludes that the desirable effects of adherence to a recommendation probably outweigh the undesirable effects. However, the recommendation is only applicable to a specific group, population or setting; OR where new evidence may result in changing the balance of risk to benefit; OR where the benefits may not warrant the cost or resource requirements in all settings.
No recommendation	Further research is required before any recommendation can be made.

Comments, feedback, and discussions that resulted from the stakeholders meeting were noted by the GWP and incorporated into the second draft. All issues that were brought up during the stakeholders meeting were resolved. The second draft was circulated to the stakeholders panel for further comments and revisions.

F. Public Forum

The revised draft was presented during the 58th Philippine Pediatric Society (PPS) Annual Convention. Minor corrections noted were incorporated into the final draft.

G. Guideline Dissemination

The final version of the guideline will be accessible through the PIDSP website.

VIII. DISCLAIMER

Recommendations are a guide and may not be appropriate for use in all situations. Healthcare providers need to use clinical judgment, knowledge, expertise, and available resources when deciding whether it is appropriate to apply the recommendations in the guideline.

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CHAPTER 2

CLINICAL AND LABORATORY FEATURES OF LEPTOSPIROSIS

Clinical Manifestations of Leptospirosis

Humans become infected through direct contact with the urine of infected animals, or indirectly with exposure to urine-contaminated environment (soil or water). The most common route is via exposure through water contaminated by urine from infected animals, usually rodents, as what happens during flooding. The bacteria enter the body through cuts or abrasions on the skin, or through the mucous membranes of the mouth, nose and eyes. Person-to-person transmission is rare (WHO, 2017). The incubation period is usually 7 to 12 days, but can range from 2 to 20 days (WHO, 2017; Nieves, 2019).

In humans, most cases are asymptomatic or mild and self-limited, but may be severe and potentially fatal (Day, 2018). The clinical course is variable and described as biphasic (Nieves, 2019; Dele Davies, 2016).

The first stage, or septicemic phase, is characterized by systemic signs, such as abrupt onset of fever, chills, headache, myalgia, conjunctival suffusion (red eyes), abdominal pain, vomiting, and/or diarrhea. The septicemic phase lasts for about 4-7 days. Clinical improvement and defervescence coincide with disappearance of leptospires from the blood, CSF, and all other tissues, except from the aqueous humor and kidneys. The second stage, or immune phase, is characterized by rapid antibody formation and lasts from 4-30 days (Nieves, 2019; Dele Davies, 2016).

Leptospirosis may present as an anicteric or icteric disease, with ninety percent or more presenting as an anicteric disease. The signs and symptoms in the septicemic phase are similar for both the anicteric and icteric disease. However, the hallmark of the immune phase of anicteric leptospirosis is meningitis, while the hallmark of the immune phase of icteric disease is characterized by impaired hepatic and renal functions (Nieves, 2019; Dele Davies, 2016). Weil syndrome, a rare (<10% of cases) severe form of leptospirosis, is characterized by impaired hepatic and renal function, vascular collapse, hemorrhage, severe alterations in consciousness, and is associated with a high mortality rate(Nieves, 2019; Dele Davies, 2016).

Laboratory Findings in Leptospirosis

Results of laboratory tests in leptospirosis are non-specific.

Although WBC counts may range from 3,000 to 26,000/microL, it is generally less than 10,000/microL and a left shift may be seen (Day, 2018). Thrombocytopenia (Chierakul, 2008) and pancytopenia (Stefos, 2005) have been noted in case series and case reports.

Proteinuria, pyuria, granular casts, and microscopic hematuria are possible findings on urinalysis (Berman, 1973). Elevated creatine kinase, indicative of renal failure characteristic of severe leptospirosis, has been observed in approximately 50% of affected patients (Johnson, 1975).

Derangements in sodium and potassium levels are seen in leptospirosis. It has been suggested that inhibition of Na^+-K^+-Cl^- co-transporter activity in the thick ascending limb of Henle by the outer membrane protein of the *Leptospira sp.* organism results in sodium wasting and hypokalemia (Wu, 2004; Krishnan, 2003).

Elevation of liver transaminases (<200 IU/L), seen in about 40% of patients, and high bilirubin concentrations (60-80 mg/dl) are the GI abnormalities particularly noted in severe disease (Day, 2018). In Chang's evaluation of 11 patients with sporadic leptospirosis in Taiwan, it was determined that progressive elevation of AST without concomitant change in ALT was indicative of an acute disease course with ensuring death. An AST/ALT Ratio (AAR) of greater than 3 means a grave prognosis (Chang, 2005).

CSF abnormalities in leptospirosis include neutrophilic pleocytosis and elevated protein concentrations. Hypoglycorrachia is rare but has been reported (Helmer, 1973).

Oliguria and WBC count above 12,900/mm^3 were among the mentioned findings associated with adverse outcomes among infected patients (Day, 2018).

Question 1: Among children with acute fever and possible exposure, what clinical manifestations should make one suspect leptospirosis?

Recommendation 1: Among children with acute fever and possible exposure, the presence of any or all of the following clinical manifestations should make one highly suspect leptospirosis:

- Renal syndrome (defined as any sign or symptom pointing to a possible kidney damage)
- Chest pain
- Cardiac syndrome (defined as any sign or symptom pointing to a possible cardiac involvement)

AND/OR

- Conjunctival suffusion/red eye

Quality of evidence: Very low
Strength of recommendation: Strong

Recommendation 2: Among children with acute fever and possible exposure, the presence of any or all of the following clinical manifestations may make one highly suspect leptospirosis:

- Arthralgia
- Myalgia
- Muscle tenderness

Quality of evidence: Very low
Strength of recommendation: Strong

Summary of Evidence

A total of seven studies evaluating signs and symptoms that may make one suspect leptospirosis in children with acute fever and possible exposure to leptospirosis were reviewed. All were cross-sectional studies (Agampodi, 2016; Ellis, 2008; Goarant, 2009; Karande, 2003; Kendall, 2010; Libraty, 2007; Morgan, 2002).

All seven studies were done in hospitals in different countries: Thailand (Libraty, 2007), India (Karande, 2003), Bangladesh (Kendall, 2010), Sri Lanka (Agampodi, 2016), Hawaii (Ellis, 2008), United States (Morgan, 2002), and New Caledonia (Goarant, 2009).

Studies were included if they had children as participants and if comparison was made between those with leptospirosis and without leptospirosis. Two studies had only children as their participants (Karande, 2003; Libraty, 2007) while five studies had both children and adults as participants (Agampodi, 2016; Ellis, 2008; Goarant, 2009; Kendall, 2010; Morgan, 2002).

Table 4. Summary of studies on clinical manifestations of leptospirosis

Study *(Study Design)*	Study Period	Patients (N)	Location	Outcome determined	Remarks
Agampodi 2016 *Cross-sectional*	Feb to May 2011	Sri Lankan children and adults 13 years old and above (mean age= 41) 48 patients were confirmed either by detection of Leptospira DNA in blood (N=26), positive MAT test (N=16), or both (N=6) and 28 who were clinically suspected case of leptospirosis (with negative or unconfirmed laboratory test) (N=76)	Teaching Hospital Anuradhapura (THA), Sri Lanka	Demographic and clinical characteristics for patients who were clinically suspected to have leptospirosis *(conjunctival suffusion (red eyes), anuria, proteinuria, oliguria and hematuria, myalgia, arthralgia, muscle tenderness, prostration, headache, positive Kernig's sign, icterus/jaundice, abdominal pain, anorexia, diarrhea, skin rash)*	There were more adults in the population studied.
Ellis 2008 *Cross-sectional*	Sep 12, 2001 to Apr 30, 2002	Hawaiian children and adults 10-67 years old 53 patients were IgM (ELISA) positive and 1106 who were negative for leptospirosis and dengue infection (N=1159)	All acute care hospitals and major clinics throughout the state of Hawaii	Demographic and clinical characteristics for patients who tested positive for leptospirosis *(Eye pain, myalgia, headache, skin rash)*	There were more adults in the population studied.
Goarant 2009 *Cross-sectional*	Jan to Jun 2008	Children and adults from New Caledonia 4-84 years old 98 cases of confirmed leptospirosis and 410 negative cases diagnosed using qPCR detection and MAT (N=508)	Health center, standard unit or ICU was obtained from the health centers and hospitals in New Caledonia	Symptoms reported from lab-confirmed leptospirosis and negative cases, risk factors *(cardiac syndrome, conjunctival suffusion/red eyes, renal syndrome, myalgia, headache, meningeal syndrome or meningismus, icterus/jaundice, hemorrhage)*	There were more adults in the population studied.

Study *(Study Design)*	Study Period	Patients (N)	Location	Outcome determined	Remarks
Karande 2003 *Cross-sectional*	Jul 24, 2000-Sep 14, 2000	Indian children 1 month-12 years old 18 children were confirmed to have leptospirosis by blood dark field microscopy and/or IgM-ELISA and 35 children with no leptospirosis children (N=53)	Outpatient or emergency care department and admitted at the Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai, India	Commonest complaints, final diagnosis of cases *(conjunctival suffusion/red eyes myalgia**,** headache, meningeal syndrome or meningismus, icterus/jaundice, abdominal pain, skin rash)*	Only hospitalized patients recruited.
Kendall 2010 *Cross-sectional*	Jan-Dec 2001	Bangladesh children, less than 5 years old and older with fever. There were 49 cases of probable or definite Leptospirosis by MAT and IgM ELISA and 500 controls with undiagnosed fever. Febrile patients were additionally evaluated for dengue, enteric fever and bloodstream infection. No overlap between the diagnoses of dengue, enteric fever and leptospirosis (N=549)	Kamalapur, a low-income neighborhood in Dhaka, Bangladesh, and referred to a field clinic	Demographic and clinical features of patients with leptospirosis and with undiagnosed fever *(chest pain, eye pain, myalgia, headache, abdominal pain, hemorrhage, skin rash)*	Only tested paired sera from febrile persons in a low-income urban community in Bangladesh. Probable cases of leptospirosis were included.
Libraty 2007 *Cross-sectional*	1994-1997	Thai children 6 months-14 years old 18 leptospirosis cases (14 definite and 4 probable) confirmed by ELISA and MAT (cases) and 214 with dengue as control (N=232)	Queen Sirikit Institute of Child Health in Bangkok, Thailand, Kamphaeng Phet Provincial Hospital, Kamphaeng Phet, Thailand	Presenting symptoms and signs between children with leptospirosis and dengue *(headache, abdominal pain, hemorrhage, skin rash)*	There were probable cases included.

Study *(Study Design)*	Study Period	Patients (N)	Location	Outcome determined	Remarks
Morgan 2002 *Cross-sectional*	mid-July 1998	245 triathlon participants and community residents 15–52 years old 52 participants had a laboratory-confirmed case of leptospirosis by 1 or more positive results using ELISA, MAT, culture or immunohistochemical staining and 193 participants with no infection who had two negative ELISA results (N=245)	Springfield, Illinois	Most common symptoms associated with fever, risk factors *(conjunctival suffusion/red eyes, eye pain, myalgia, headache)*	Cases from hospital, controls from community

The clinical manifestations that were evaluated were the following:

Cardiac Symptoms

Chest pain: One study evaluated chest pain (Kendall, 2010). Those who had leptospirosis were almost nineteen times more likely to have chest pain as compared to those without leptospirosis (OR: 18.8; 95% CI: 4.4 to 81.4). There is serious risk of bias inherent in an observational study design. There is also indirectness since there were more adults included in the study. The confidence interval is wide which is suggestive of imprecision. Even after taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 15.96), the evidence is graded as very low.

Cardiac Syndrome: One study evaluated this parameter and, per personal communication, it was defined by the author as any sign or symptom pointing to a possible cardiac involvement, e.g., arrhythmias (Goarant, 2009). Those who had leptospirosis were almost seven times more likely to have cardiac syndrome as compared to those without leptospirosis (OR: 6.7; 95% CI: 2.3 to 19.2). There is serious risk of bias inherent in an observational study design. There is also indirectness since there were more adults included in the study. The confidence interval is wide which suggests imprecision. Even after taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 6.33), the evidence is graded as very low.

Eye Symptoms

Conjunctival suffusion/red eyes: Four studies evaluated this parameter (Agampodi, 2016; Goarant, 2009; Karande, 2003; Morgan, 2002). Pooled analysis showed that those who had leptospirosis were almost six times more likely to have conjunctival suffusion or red eyes as compared to those without leptospirosis (OR: 5.64; 95% CI: 2.46 to 12.91). There is a very serious risk of bias inherent in an observational study design and inclusion of few probable cases. There is inconsistency due to heterogeneity or wide variance of point estimates across studies. There is imprecision as suggested by the wide confidence interval. There is also indirectness since there were more adults included in the studies. Even after taking into consideration the magnitude of the effect which has a strong association (Converted RR: 3.85), the evidence is graded as very low (Figure 1).

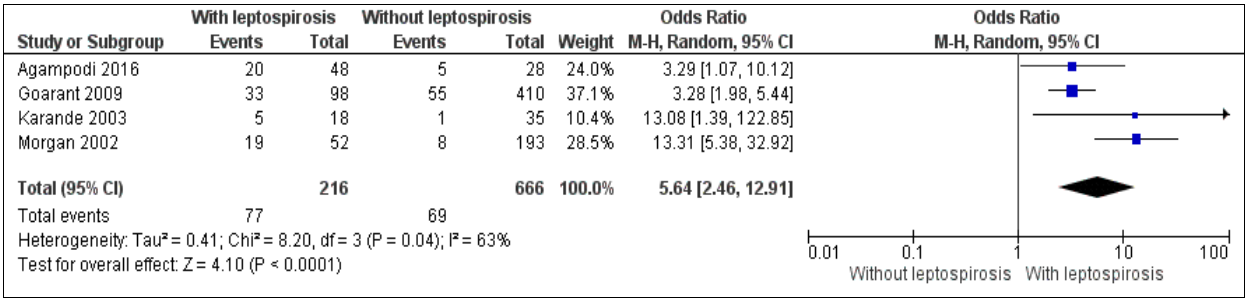

Figure 1. Forest plot of meta-analysis of data for the presence of conjunctival suffusion/red eyes comparing those with and without leptospirosis in admitted patients

Eye pain: Three studies evaluated this parameter (Ellis, 2008; Kendall, 2010; Morgan, 2002). Pooled analysis showed that those who had leptospirosis were almost three times more likely to have eye pain as compared to those without leptospirosis, but this did not reach statistical significance (OR: 2.95; 95% CI: 0.38 to 23.00). There is serious risk of bias inherent in an observational study design. There is inconsistency due to heterogeneity or wide variance of point estimates across studies. The wide confidence interval is suggestive of imprecision. There is also indirectness as there were more adults included in the studies; hence, this evidence is graded as very low (Figure 2).

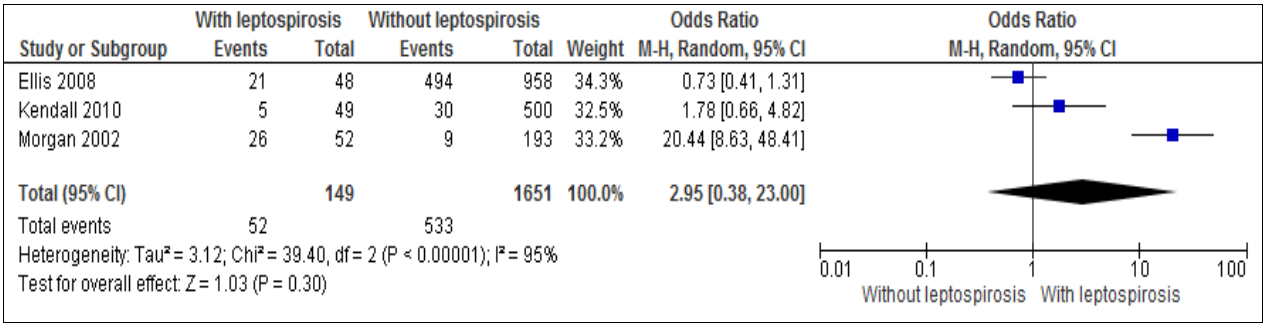

Figure 2. Forest plot of meta-analysis of data for the presence of eye pain comparing those with and without leptospirosis in admitted patients

Renal Symptoms

Renal syndrome: One study evaluated this parameter and, per personal communication, was defined by the author as any sign or symptom pointing to possible kidney damage (e.g., oliguria, anuria) (Goarant, 2009). Those who had leptospirosis were six times more likely to have renal syndrome as compared to those without leptospirosis (OR: 6.3; 95% CI: 3.3 to 12.2). There is serious risk of bias inherent in an observational study design. There is also indirectness since there were more adults included in the study. The wide confidence interval is suggestive of imprecision. After taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 5.00), the evidence is graded as very low.

Anuria: One study evaluated this parameter (Agampodi, 2016). Those who had leptospirosis were three times more likely to have anuria as compared to those without leptospirosis, but this did not reach statistical significance (OR: 3.06; 95% CI: 0.14 to 66.15). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study. There is also serious imprecision since there was an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Proteinuria: One study evaluated this parameter (Agampodi, 2016). Those who had leptospirosis were almost two times more likely to have proteinuria as compared to those without leptospirosis, but this did not reach statistical significance (OR: 1.8; 95% CI: 0.18 to 18.19). There is very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study. There is also serious imprecision since there was an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Oliguria: One study evaluated this parameter (Agampodi, 2016). Those who had leptospirosis were more likely to have oliguria as compared to those without leptospirosis, but this did not reach statistical significance (OR: 1.14; 95% CI: 0.41 to 3.16). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study. There is also serious imprecision since there was an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Hematuria: One study evaluated this parameter (Agampodi, 2016). Those who had leptospirosis were more likely to have hematuria as compared to those without leptospirosis, but this did not reach statistical significance (OR: 1.14; 95% CI: 0.41 to 3.16). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Constitutional Symptoms

Myalgia: Six studies evaluated this parameter (Agampodi, 2016; Ellis, 2008; Goarant, 2009; Karande, 2003; Kendall, 2010; Morgan, 2002). The site of myalgia was not indicated except for Karande who described myalgia as generalized (Karande, 2003). Pooled analysis showed that those who had leptospirosis were almost three times more likely to have myalgia as compared to those without leptospirosis, but this did not reach statistical significance (OR: 2.81; 95% CI: 0.92 to 8.60). There is serious risk of bias inherent in an observational study design and inclusion of few probable cases. There is inconsistency due to heterogeneity and wide variance of point estimates across studies. The wide confidence interval is suggestive of imprecision. There is also indirectness since there were more adults included in the studies; hence, this evidence is graded as very low (Figure 3).

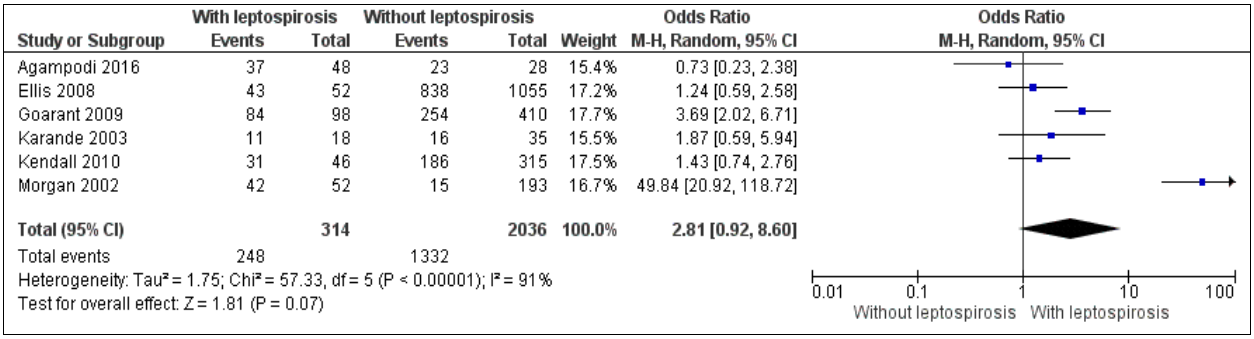

Figure 3. Forest plot of meta-analysis of data for the presence of myalgia comparing those with and without leptospirosis in admitted patients

Arthralgia: One study evaluated this parameter (Agampodi, 2016). Those who had leptospirosis were three times more likely to have arthralgia as compared to those without leptospirosis (OR: 3.4; 95% CI: 1.0 to 11.85). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study, and there is imprecision as suggested by the wide confidence interval. The evidence for arthralgia is graded as very low.

Muscle tenderness: One study evaluated this parameter (Agampodi, 2016). Those who had leptospirosis were two times more likely to have muscle tenderness as compared to those without leptospirosis, but this did not reach statistical significance (OR: 2.11; 95% CI: 0.75 to 6.00). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Prostration: One study evaluated this parameter (Agampodi, 2016). Those who had leptospirosis were two times more likely to have prostration as compared to those without leptospirosis, but this did not reach statistical significance (OR: 2.01; 95% CI: 0.68 to 5.92). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Neurological Symptoms

Headache: Seven studies evaluated this parameter (Agampodi, 2016; Ellis, 2008; Goarant, 2009; Karande, 2003; Kendall, 2010; Libraty, 2007; Morgan, 2002). Pooled analysis showed that those who had leptospirosis were almost three times more likely to have headache as compared to those without leptospirosis, but this did not reach statistical significance (OR: 2.45; 95% CI: 0.80 to 7.51). There is a very serious risk of bias inherent in an observational study design and inclusion of few probable cases. There is inconsistency due to heterogeneity or wide variance of point estimates across studies. There is indirectness as there were more adults included in the studies. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low (Figure 4).

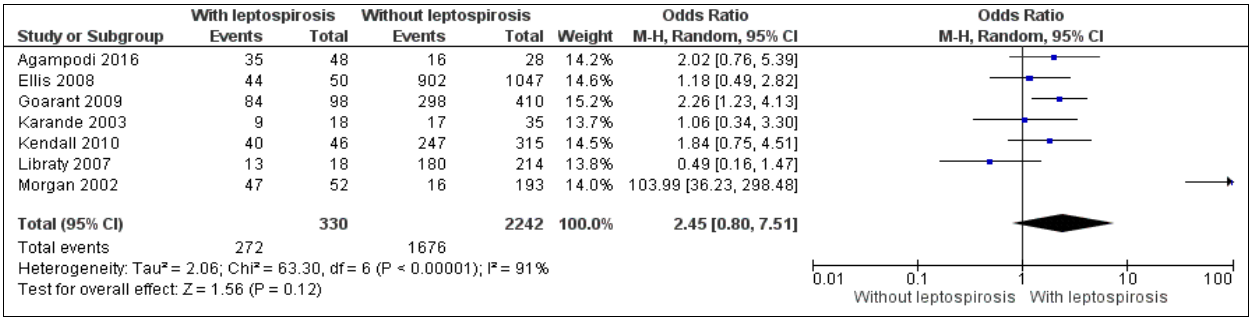

Figure 4. Forest plot of meta-analysis of data for the presence of headache comparing those with and without leptospirosis in admitted patients

Meningeal syndrome/meningismus: Two studies evaluated this parameter (Goarant, 2009; Karande, 2003). Goarant, per personal communication, defined meningeal syndrome as any sign pointing to a possible meningeal involvement such as headache, photophobia, and nuchal rigidity (Goarant, 2009). Meningismus is a constellation of signs and symptoms (e.g., headache, neck stiffness) characterized by meningeal irritation without objective findings. Pooled analysis showed that those who had leptospirosis were two times more likely to have meningeal syndrome as compared to those without leptospirosis, but this did not reach statistical significance (OR: 2.06; 95% CI: 0.40 to 10.56). There is serious risk of bias inherent in an observational study design. There is inconsistency due to heterogeneity or wide variance of point estimates across studies. There is indirectness since there were more adults included in the studies. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low (Figure 5).

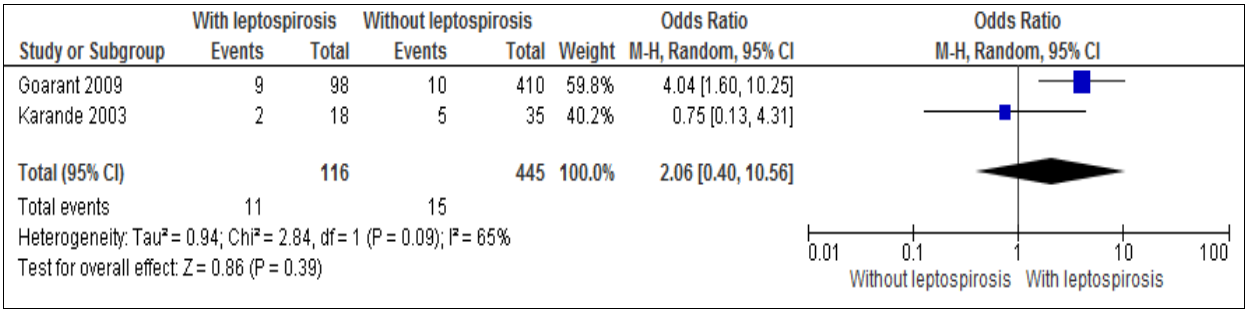

Figure 5. Forest plot of meta-analysis of data for the presence of meningeal syndrome comparing those with and without leptospirosis in admitted patients

Positive Kernig's sign: Only one study evaluated this parameter (Agampodi, 2016). Those who had leptospirosis were likely to have positive Kernig's sign as compared to those without

leptospirosis, but this did not reach statistical significance (OR: 1.37; 95% CI: 0.42 to 4.44). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is indirectness since there were more adults included in the study. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Gastrointestinal Symptoms

Icterus/jaundice: Three studies evaluated this parameter (Agampodi, 2016; Goarant, 2009; Karande, 2003). Pooled analysis showed that those who had leptospirosis were two times more likely to have icterus or jaundice as compared to those without leptospirosis, but this did not reach statistical significance (OR: 2.31; 95% CI: 0.46 to 11.50). There is a very serious risk of bias inherent in an observational study design and inclusion of few probable cases. There is inconsistency due to heterogeneity or wide variance of point estimates across studies. There is indirectness since there were more adults included in the studies. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low (Figure 6).

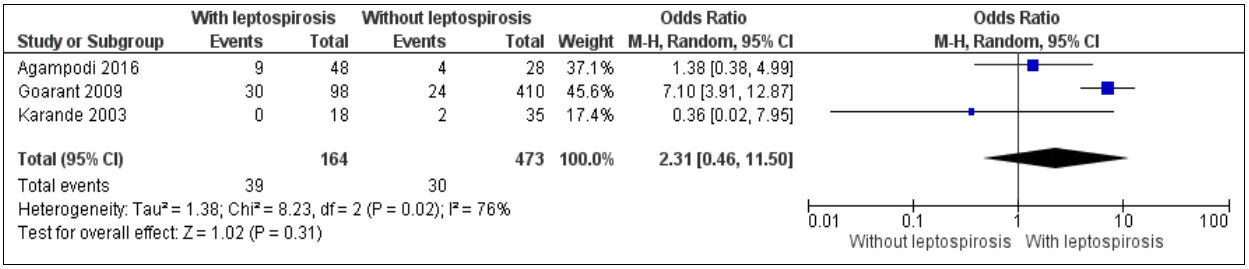

Figure 6. Forest plot of meta-analysis of data for the presence of icterus/jaundice comparing those with and without leptospirosis in admitted patients

Abdominal pain: Four studies evaluated this parameter (Agampodi, 2016; Karande, 2003; Kendall, 2010; Libraty, 2007). Pooled analysis showed that those who had leptospirosis were two times more likely to have abdominal pain as compared to those without leptospirosis, but this did not reach statistical significance (OR: 2.15; 95% CI: 0.96 to 4.85). There is a very serious risk of bias inherent in an observational study design and inclusion of few probable cases. There is inconsistency due to heterogeneity or wide variance of point estimates across studies. There is indirectness since there were more adults included in the studies. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low (Figure 7).

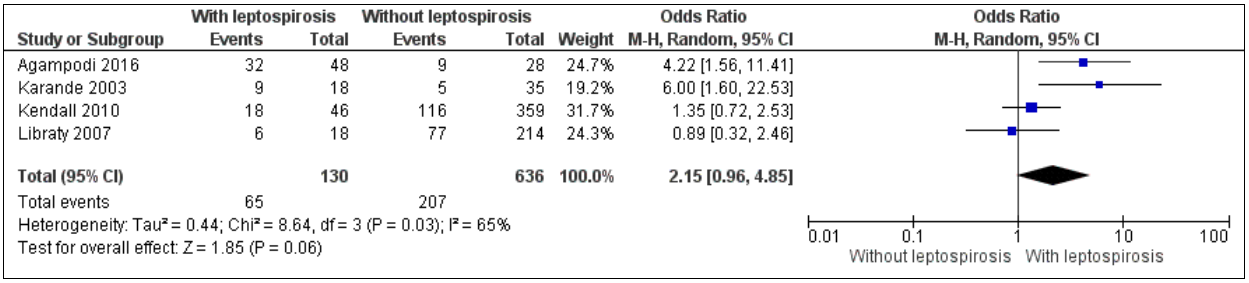

Figure 7. Forest plot of meta-analysis of data for the presence of abdominal pain comparing those with and without leptospirosis in admitted patients

Anorexia: One study evaluated this parameter (Agampodi, 2016). Those who had leptospirosis were almost two times more likely to have anorexia as compared to those without leptospirosis, but this did not reach statistical significance (OR: 1.87; 95% CI: 0.49 to 7.13). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Diarrhea: One study evaluated this parameter (Agampodi, 2016). Those who had leptospirosis were likely to have diarrhea as compared to those without leptospirosis, but this did not reach statistical significance (OR: 1.37; 95% CI: 0.42 to 4.44). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Bleeding Symptoms

Hemorrhage: Three studies evaluated this parameter (Goarant, 2009; Kendall, 2010; Libraty, 2007). However, the sites of the bleeding were not indicated. Pooled analysis showed that those who had leptospirosis were two times more likely to have hemorrhage or bleeding as compared to those without leptospirosis, but this did not reach statistical significance (OR: 2.11; 95% CI: 0.68 to 6.61). There is serious risk of bias inherent in an observational study design. There is inconsistency due to heterogeneity or wide variance of point estimates across studies. There is indirectness since there were more adults included in the studies. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low (Figure 8).

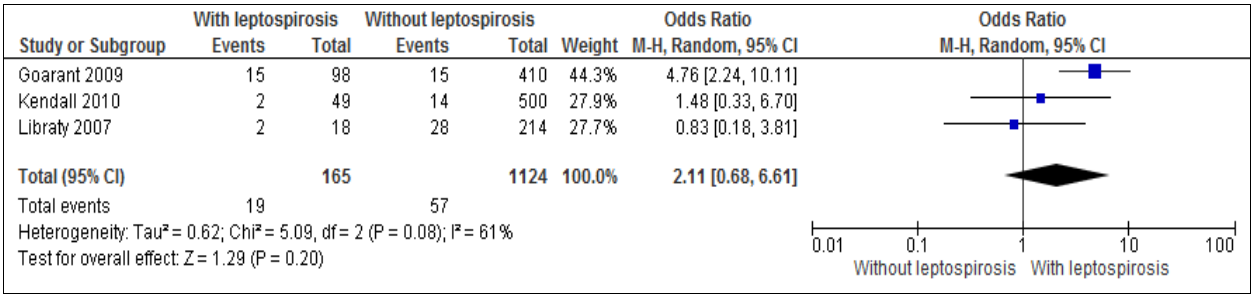

Figure 8. Forest plot of meta-analysis of data for the presence of hemorrhage/bleeding comparing those with and without leptospirosis in admitted patients

Skin rash: Five studies evaluated this parameter (Agampodi, 2016; Ellis, 2008; Karande, 2002; Kendall, 2010; Libraty, 2007). Pooled analysis showed that those who had leptospirosis were almost two times more likely to have skin rash as compared to those without leptospirosis, but this did not reach statistical significance (OR: 1.70; 95% CI: 0.59 to 4.84). There is serious risk of bias due to observational study design and inclusion of few probable cases. There is inconsistency due to heterogeneity or wide variance of point estimates across studies. There is serious imprecision as evidenced by the overlapping confidence interval with the null value. There is indirectness since there were more adults included in the studies; hence, this evidence is graded as very low (Figure 9).

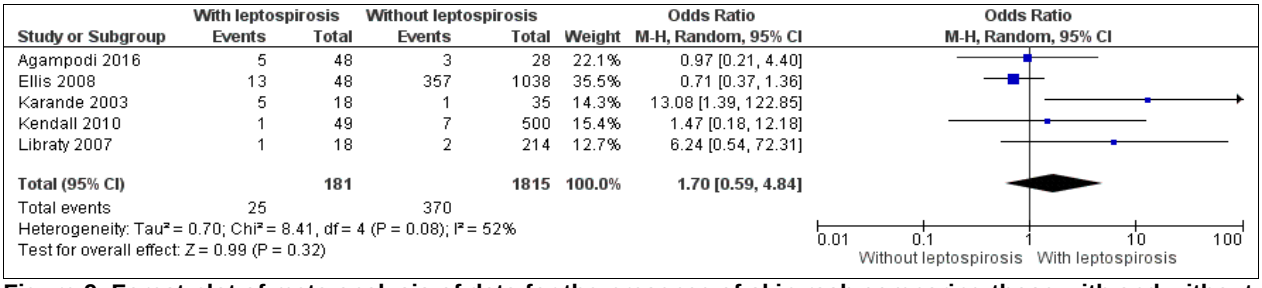

Figure 9. Forest plot of meta-analysis of data for the presence of skin rash comparing those with and without leptospirosis in admitted patients

Considerations for Recommendation Development during the Stakeholders Panel (SP) Meeting:

- The consensus panel had a long discussion on this question that even led to a postponement of the votation. The votation was subsequently done by the Delphi Method.
- The seven studies that evaluated for signs and symptoms that may make one suspect leptospirosis in children with acute fever and possible exposure suffered from risk of bias – all being observational (cross-sectional) studies, with imprecision and indirectness. Hence, the quality of evidence is graded very low.
- Despite the very low quality of evidence, a consensus was made via the Delphi Method for a strong recommendation because renal syndrome and conjunctival suffusion turned out to be statistically significant. These two manifestations, especially the renal manifestations, are what clinicians usually look for when considering the possibility of leptospirosis. According to the representative from PSN, renal syndrome is a more encompassing term, defined by the author as ANY sign or symptom of renal damage.
- Chest pain and cardiac syndrome were likewise voted for a strong recommendation, even if not commonly seen in children with leptospirosis. These were the two significant parameters from a single study that had more adult participants.
- For the second recommendation, the SP also voted on a strong recommendation for arthralgia, myalgia, and muscle tenderness despite very low quality of evidence (not statistically significant) as these are also usually seen in clinical practice among children with leptospirosis.
- A limitation of the guideline was the use of studies involving admitted patients only. There were no studies on patients seen on an outpatient basis.

Question 2: Among children with leptospirosis, what are the signs and symptoms associated with an increased risk of mortality?

Recommendation 1: In children with leptospirosis, the presence of any one of the following signs and symptoms increases the risk of mortality:

- Pallor
- Loss of consciousness
- Murmur
- Meningism
- Irregular rhythm
- Dyspnea
- Pulmonary hemorrhage
- Convulsions/seizure
- Crackles/rales on lung auscultation
- Hemoptysis
- Anuria
- Disorientation
- Jaundice
- Tachycardia

Quality of evidence: Very low
Strength of recommendation: Strong

Summary of Evidence

A total of six studies evaluated signs and symptoms that may predict disease mortality in children and adults with leptospirosis. Five studies were cross-sectional studies (Amilasan, 2012; Daher, 2010; Lopes, 2010; Mendoza, 2013; Pappachan, 2004), while the remaining study was case-control in design (Bonus, 2016). One of the studies involved adults only, but was nonetheless included because it was a local study (Mendoza, 2013).

All the studies classified their data into two categories: those who have leptospirosis and survived, and those who have leptospirosis and died.

All studies included patients who were admitted in the hospital. Three studies were done locally (Amilasan, 2012; Bonus, 2016; Mendoza, 2013), two studies were done in Brazil (Daher, 2010; Lopes, 2004), and one was done in India (Pappachan, 2004).

Table 5. Summary of studies evaluating signs and symptoms that increase the risk of mortality

Study (*Study Design)*	Study Period	Patients (N)	Location	Outcome determined	Remarks
Amilasan 2012 *Cross-sectional*	Oct 11-31, 2009	Filipino children and adult patients There were 34 who were aged <15 years old and 12 who were aged <10 years old 51 died and 420 survived (N=471)	San Lazaro Hospital (SLH)	Clinical manifestations associated with mortality	There were more adults included in the study.
Bonus 2016 *Case-control*	Jan 2008 - Dec 2012	Filipino pediatric patients ≤18 years old 14 died and 390 survived (N=404)	Philippine General Hospital (PGH), Research Institute for Tropical Medicine (RITM) and San Lazaro Hospital (SLH)	Clinical manifestations associated with mortality	There were probable cases of leptospirosis included.
Daher 2010 *Cross-sectional*	May 1985–Dec 2006	Brazilian children and adult patients 8-84 years old 31 patients died and 180 survived (N=201)	Walter Cantídio University Hospital and São José Infectious Diseases Hospital, in Fortaleza City, Northeast Brazil	Clinical manifestations associated with mortality	There were more adults included in the study.
Lopes 2010 *Cross-sectional*	1993-1997	Brazilian children and adult patients 100 pediatric and 740 adult patients 121 died and 719 survived (N=840)	Couto Maia Hospital, Salvador, BA, Brazil	Clinical manifestations associated with mortality	There were more adults included in the study.

Study (*Study Design)*	Study Period	Patients (N)	Location	Outcome determined	Remarks
Mendoza 2013 *Cross-sectional*	Sept 28 - Nov 30, 2009	Adult patients Mean age was 38.9 years old 14 died and 245 survived (N=259)	University of the Philippines-Philippine General Hospital (UP-PGH), National Kidney and Transplant Institute (NKTI), The Medical City (TMC), University of Santo Tomas Hospital (USTH), Manila Doctors Hospital (MDH), Ospital ng Maynila Medical Center (OMMC), Cardinal Santos Medical Center (CSMC), East Avenue Medical Center (EAMC), and Makati Medical Center (MMC)	Clinical manifestations associated with mortality	There were only adults included in the study.
Pappachan 2004 *Cross-sectional*	2002	Indian children and adults 12-75 years old 17 died and 265 survived (N=282)	General medicine wards of Calicut Medical College in Northern Kerala, India	Clinical manifestations associated with mortality	There were more adults included in the study.

The clinical signs and symptoms that were evaluated were the following:

Respiratory Symptoms

Pulmonary hemorrhage: Only one study evaluated this parameter (Mendoza, 2013). Those who died were almost forty-nine times more likely to have pulmonary hemorrhage as compared to those who survived (OR: 48.54; 95% CI: 13.27 to 177.51). There is serious risk of bias due to observational study design. There is also indirectness since only adults were included in the study. The wide confidence interval is suggestive of serious imprecision. Even after taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 14.58), the evidence is graded as very low.

Dyspnea: Two studies evaluated this parameter (Bonus, 2016; Pappachan, 2004). Pooled analysis showed that those who died were nine times more likely to have dyspnea as compared to those who survived (OR: 9.13; 95% CI: 4.20 to 19.88). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study. The wide confidence interval is suggestive of serious imprecision. Even after taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 5.50), the evidence is graded as very low (Figure 10).

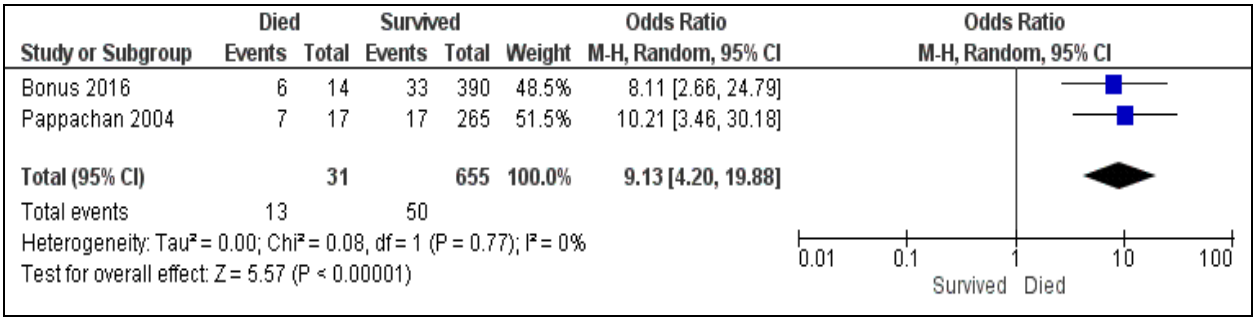

Figure 10. Forest plot of pooling of data for the presence of dyspnea comparing those with leptospirosis who died and survived

Crackles/rales on lung auscultation: Two studies evaluated this parameter (Bonus, 2016; Daher, 2010). Pooled analysis showed that those who died were seven times more likely to have crackles/rales as compared to those who survived (OR: 7.12; 95% CI: 3.28 to 15.44). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study. The wide confidence interval is suggestive of serious imprecision. Even after taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 5.20), the evidence is graded as very low (Figure 11).

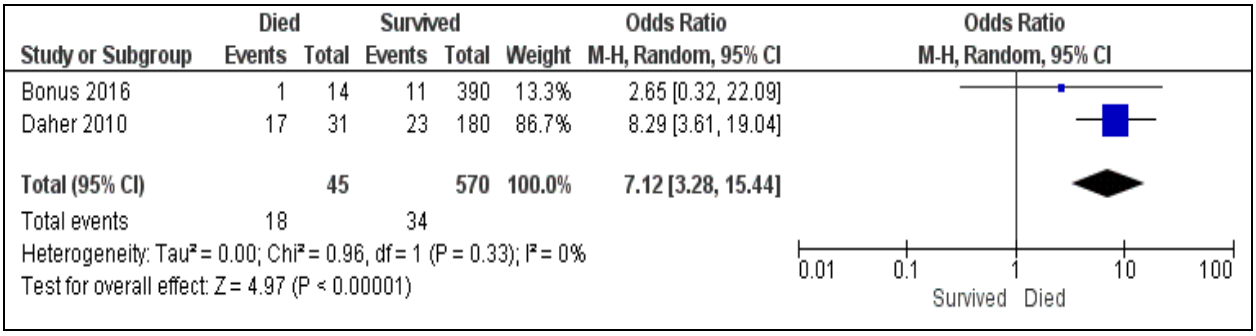

Figure 11. Forest plot of pooling of data for the presence of crackles/rales on auscultation comparing those with leptospirosis who died and survived

Hemoptysis: Three studies evaluated this parameter (Amilasan, 2012; Bonus, 2016; Pappachan, 2004). Pooled analysis showed that those who died were almost seven times more likely to have hemoptysis as compared to those who survived (OR: 6.93; 95% CI: 3.07 to 15.66). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the study. The wide confidence interval is suggestive of serious imprecision. Even after taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 6.24), the evidence is graded as very low (Figure 12).

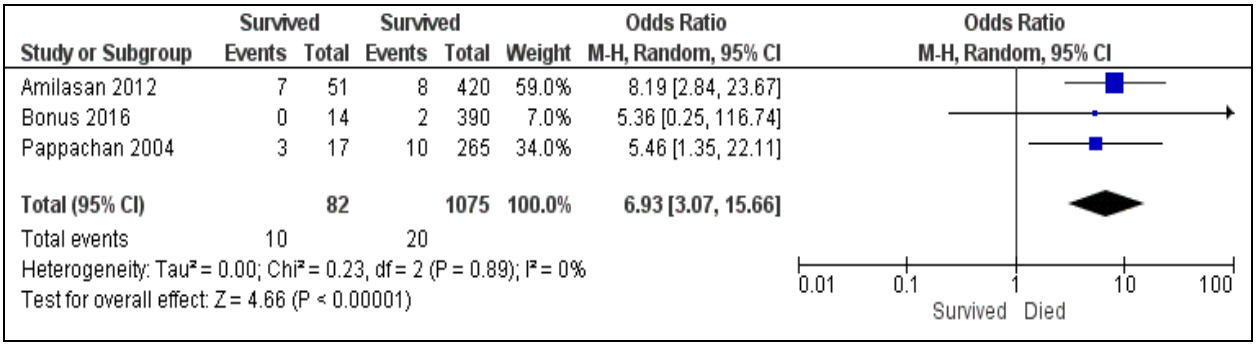

Figure 12. Forest plot of pooling of data for the presence of hemoptysis comparing those with leptospirosis who died and survived

Decreased breath sounds: Only one study evaluated this parameter (Bonus, 2016). Those who died were four times more likely to have decreased breath sounds as compared to those who survived, but this did not reach statistical significance (OR: 4.2; 95% CI: 0.5 to 36.8). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Constitutional Symptoms

Pallor: Only one study evaluated this parameter (Bonus, 2016). Those who died were almost thirty times more likely to have pallor as compared to those who survived (OR: 29.9; 95% CI: 1.8 to 505.2). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. The wide confidence interval almost inclusive of the null value is suggestive of serious imprecision. Even after taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 27.50), the evidence is graded as very low.

Malaise: Two studies evaluated this parameter (Amilasan, 2012; Bonus, 2016). Pooled analysis showed that those who died were almost two times more likely to have malaise as compared to those who survived, but this did not reach statistical significance (OR: 1.98; 95% CI: 0.46 to 8.54). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is indirectness since there were more adults included in the study. There is inconsistency due to heterogeneity or wide variance of point estimates across studies. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low (Figure 13).

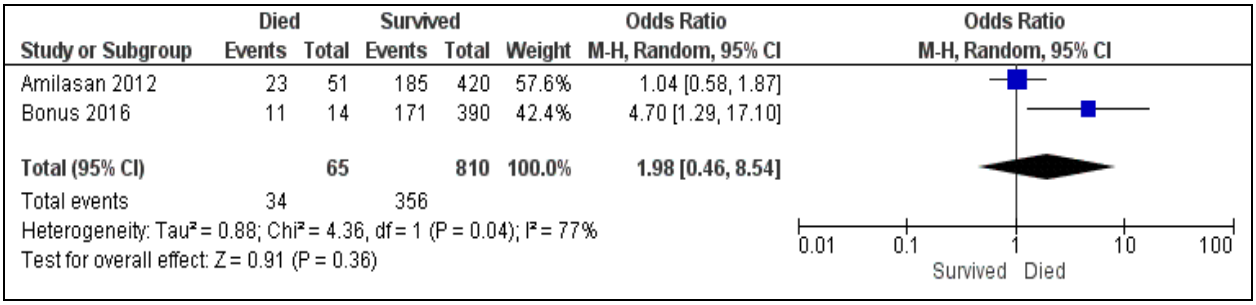

Figure 13. Forest plot of pooling of data for the presence of malaise comparing those with leptospirosis who died and survived

Chills/rigor: Two studies evaluated this parameter (Bonus, 2016; Pappachan, 2004). Pooled analysis showed that those who died were almost two times more likely to have chills or rigor as compared to those who survived, but this did not reach statistical significance (OR: 1.73; 95% CI: 0.73 to 4.13). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is indirectness since there were more adults included in the study. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low (Figure 14).

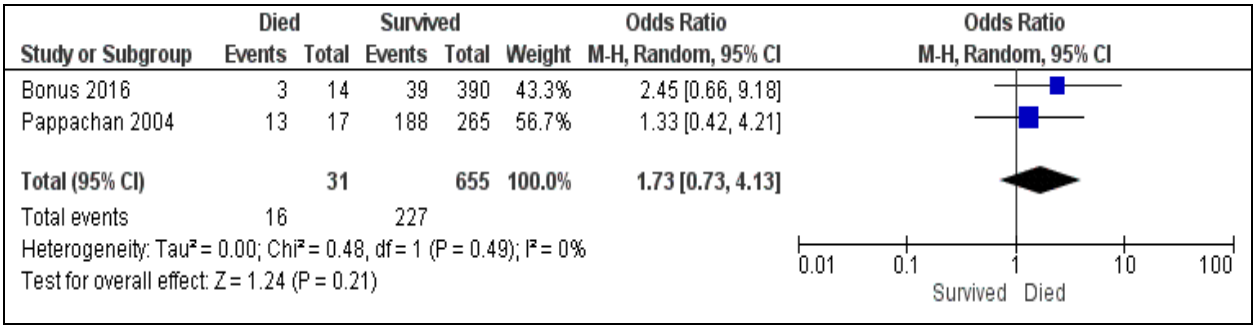

Figure 14. Forest plot of pooling of data for the presence of chills/rigor comparing those with leptospirosis who died and survived

Signs of dehydration: Only one study evaluated this parameter (Bonus, 2016). Those who died were almost three times more likely to have signs of dehydration as compared to those who survived, but this did not reach statistical significance (OR: 2.8; 95% CI: 0.7 to 10.4). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Anorexia: Only one study evaluated this parameter (Bonus, 2016). Those who died were almost two times more likely to be anorexic as compared to those who survived, but this did not reach statistical significance (OR: 1.7; 95% CI: 0.5 to 6.4). There is a very serious risk of bias inherent in observational study design and inclusion of probable cases. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Neurological Symptoms

Loss of consciousness: Only one study evaluated this parameter (Bonus, 2016). Those who died were almost thirty times more likely to have loss of consciousness as compared to those who survived (OR: 29.9; 95% CI: 1.8 to 505.2). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. The wide confidence interval is suggestive of serious imprecision. Even after taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 27.50), the evidence is graded as very low.

Meningism: Only one study evaluated this parameter (Pappachan, 2004). Those who died were almost eleven times more likely to have meningism as compared to those who survived (OR: 10.6; 95% CI: 2.3 to 48). There is serious risk of bias inherent in an observational study design. There is also indirectness since there were more adults included in the study. The wide confidence interval is suggestive of serious imprecision. Even after taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 8.23), the evidence is graded as very low.

Convulsion/seizure: Two studies evaluated this parameter (Amilasan, 2012; Bonus 2016). Pooled analysis showed that those who died were almost eight times more likely to have convulsion or seizure as compared to those who survived (OR: 7.81; 95% CI: 1.39 to 43.84). There is a very serious risk of bias inherent in an observational study and inclusion of probable cases. There is also indirectness since there were more adults included in the study. The wide confidence interval is suggestive of serious imprecision. Even after taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 7.55), the evidence is graded as very low (Figure 15).

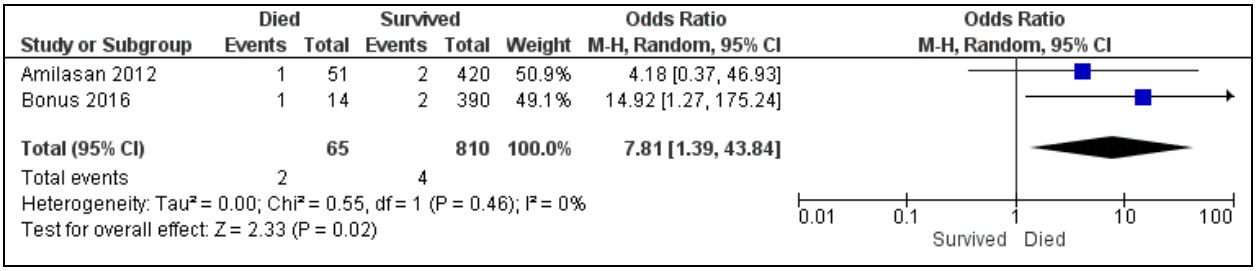

Figure 15. Forest plot of pooling of data for the presence of convulsion/seizure comparing those with leptospirosis who died and survived

Disorientation: Only one study evaluated this parameter (Pappachan, 2004). Those who died were five times more likely to have disorientation as compared to those who survived (OR: 5; 95% CI: 1.3 to 17.6). There is serious risk of bias inherent in an observational study design. There is also indirectness since there were more adults included in the study. Even after taking into consideration the magnitude of the effect which has a strong association (Converted RR: 3.75), the evidence is graded as very low.

Cardiac Symptoms

Murmur: Only one study evaluated this parameter (Bonus, 2016). Those who died were almost fifteen times more likely to have murmurs as compared to those who survived (OR: 14.9; 95% CI: 1.3 to 175.2). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. The wide confidence interval almost inclusive of the null value is suggestive of serious imprecision. After taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 13.95), the evidence is graded as very low.

Irregular rhythm: Only one study evaluated this parameter (Bonus, 2016). Those who died were almost ten times more likely to have irregular rhythm as compared to those who survived (OR: 9.9; 95% CI: 1 to 102). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Tachycardia: Only one study evaluated this parameter (Pappachan, 2004). Those who died were four times more likely to be tachycardic as compared to those who survived (OR: 4.1; 95% CI: 1.2 to 13.1). There is serious risk of bias inherent in an observational study design. There is also indirectness since there were more adults included in the study. The wide confidence interval almost inclusive of the null value is suggestive of serious imprecision. The evidence for tachycardia is graded as very low.

Hypotension: Only one study evaluated this parameter (Bonus, 2016). Those who died were two times more likely to be hypotensive as compared to those who survived, but this did not reach statistical significance (OR: 2.3; 95% CI: 0.6 to 8.7). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Renal Symptoms

Anuria: Two studies evaluated this parameter (Amilasan, 2012; Bonus, 2016). Pooled analysis showed that those who died were almost seven times more likely to be anuric as compared to those who survived (OR: 6.52; 95% CI: 2.93 to 14.51). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included. The wide confidence interval is suggestive of serious imprecision. After taking into consideration the magnitude of the effect which has a very strong association (Converted RR: 5.77), the evidence is graded as very low (Figure 16).

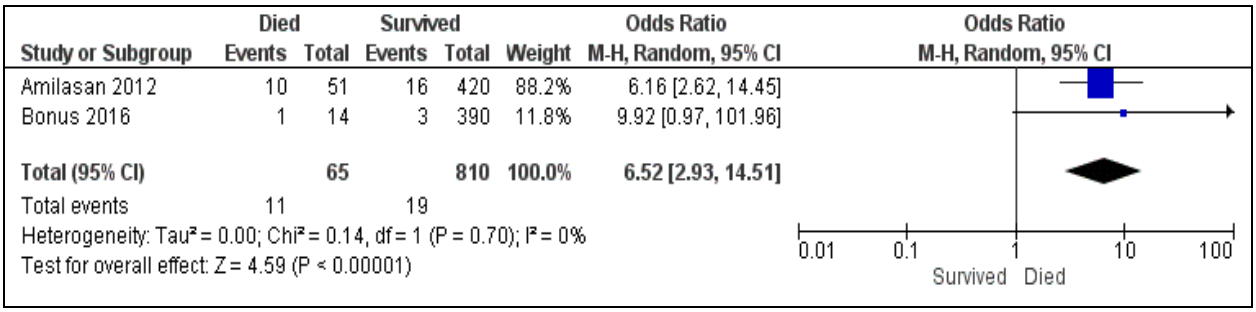

Figure 16. Forest plot of pooling of data for the presence of anuria comparing those with leptospirosis who died and survived

Oliguria: Four studies evaluated this parameter (Amilasan, 2012; Bonus, 2016; Daher, 2010; Pappachan, 2004). Pooled analysis showed that those who died were almost three times more likely to be oliguric as compared to those who survived, but this did not reach statistical significance (OR: 2.66; 95% CI: 0.68 to 10.41). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is indirectness since there were more adults included in the study. There is inconsistency due to heterogeneity or wide variance of point estimates across studies. There is also serious imprecision since there is overlapping of confidence interval with the null value; hence, this evidence is graded as very low (Figure 17).

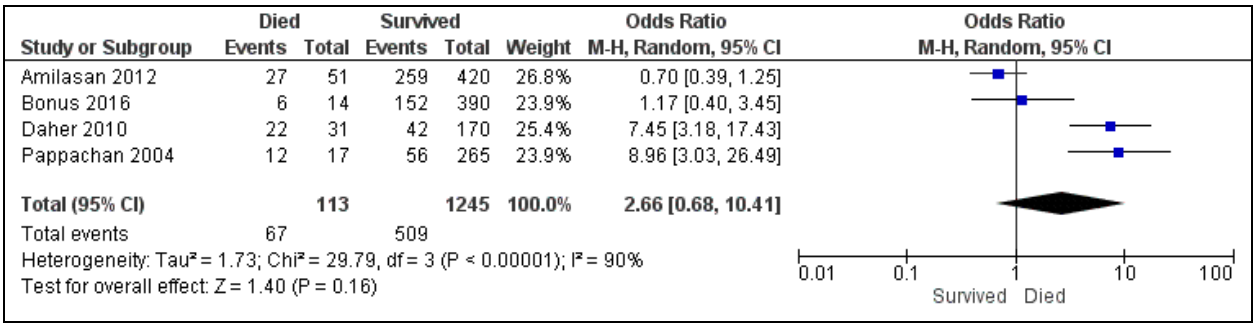

Figure 17. Forest plot of pooling of data for the presence of oliguria comparing those with leptospirosis who died and survived

Edema: Only one study evaluated this parameter (Bonus, 2016). Those who died were two times more likely to have edema as compared to those who survived, but this did not reach statistical significance (OR: 2.1; 95% CI: 0.3 to 16.9). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Dysuria: Only one study evaluated this parameter (Bonus, 2016). Those who died were almost two times more likely to be dysuric as compared to those who survived, but this did not reach statistical significance (OR: 1.6; 95% CI: 0.09 to 28.2). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Gastrointestinal Symptoms

Jaundice: Four studies evaluated this parameter (Amilasan, 2012; Bonus, 2016; Lopes, 2010; Pappachan, 2004). Pooled analysis showed that those who died were almost five times more likely to have jaundice as compared to those who survived (OR: 4.76; 95% CI: 2.99 to 7.59). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also indirectness since there were more adults included in the studies. After taking into consideration the magnitude of the effect which showed weak association (Converted RR: 1.54), this evidence is graded as very low (Figure 18).

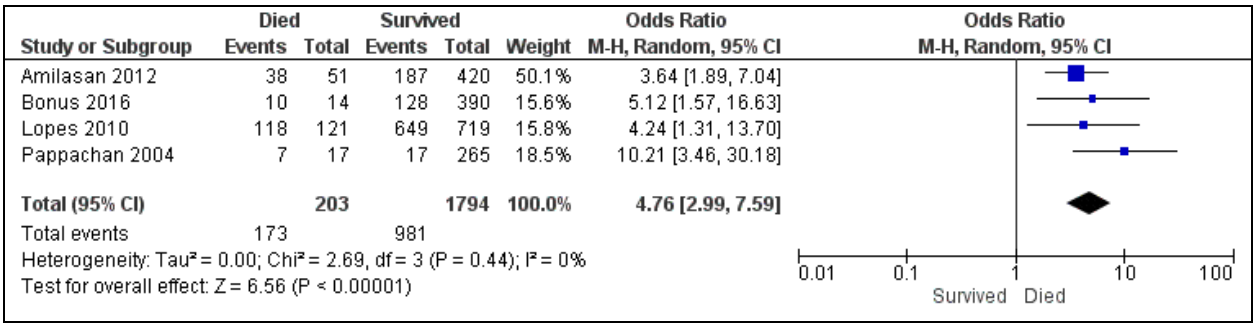

Figure 18. Forest plot of pooling of data for the presence of jaundice comparing those with leptospirosis who died and survived

Abdominal pain: Three studies evaluated this parameter (Amilasan, 2012; Bonus, 2016; Pappachan, 2004). Pooled analysis showed that those who died were likely to have abdominal pain as compared to those who survived, but this did not reach statistical significance (OR: 1.31; 95% CI: 0.53 to 3.26). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is indirectness since there were more adults included in the study. There is inconsistency due to heterogeneity or wide variance of point estimates across studies. There is also serious imprecision due to an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low (Figure 19).

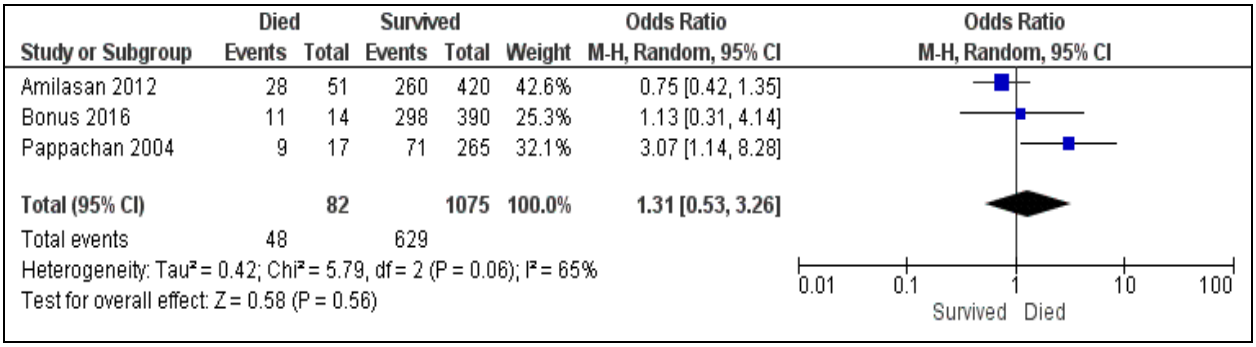

Figure 19. Forest plot of pooling of data for the presence of abdominal pain comparing those with leptospirosis who died and survived

Diarrhea: Two studies evaluated this parameter (Amilasan, 2012; Bonus, 2016). Pooled analysis showed that those who died were likely to have diarrhea as compared to those who survived, but this did not reach statistical significance (OR: 1.40; 95% CI: 0.83 to 2.34). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is indirectness since there were more adults included in the study. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low (Figure 20).

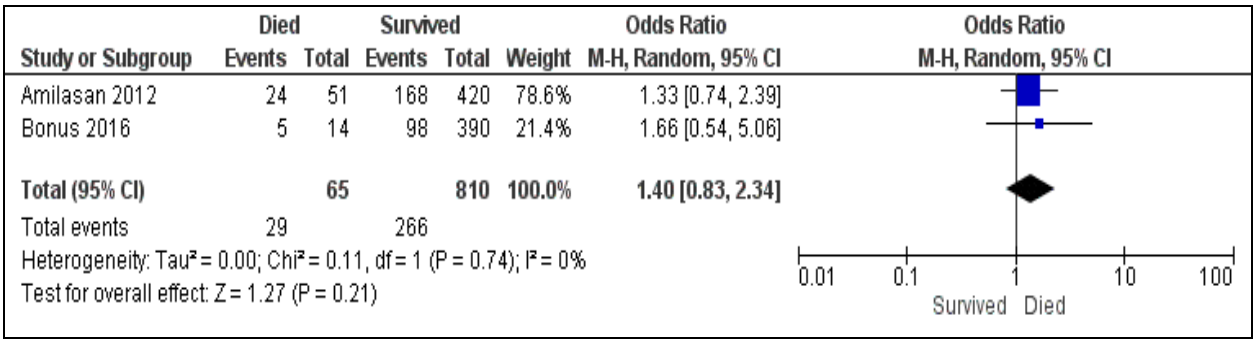

Figure 20. Forest plot of pooling of data for the presence of diarrhea comparing those with leptospirosis who died and survived

Eye Symptoms

Retro-orbital pain: One study evaluated this parameter (Bonus, 2016). Those who died were almost four times more likely to have retro-orbital pain as compared to those who survived, but this did not reach statistical significance (OR: 3.8; 95% CI: 0.2 to 77.4). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Conjunctival suffusion: Three studies evaluated this parameter (Amilasan, 2012; Bonus, 2016; Pappachan, 2004). Pooled analysis showed that those who died were likely to have conjunctival suffusion as compared to those who survived, but this did not reach statistical significance (OR: 1.40; 95% CI: 0.77 to 2.57). There is a very serious risk of bias inherent in an observational study design and inclusion of probable cases. There is indirectness since there were more adults included in the study. There is also serious imprecision since there was an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low (Figure 21).

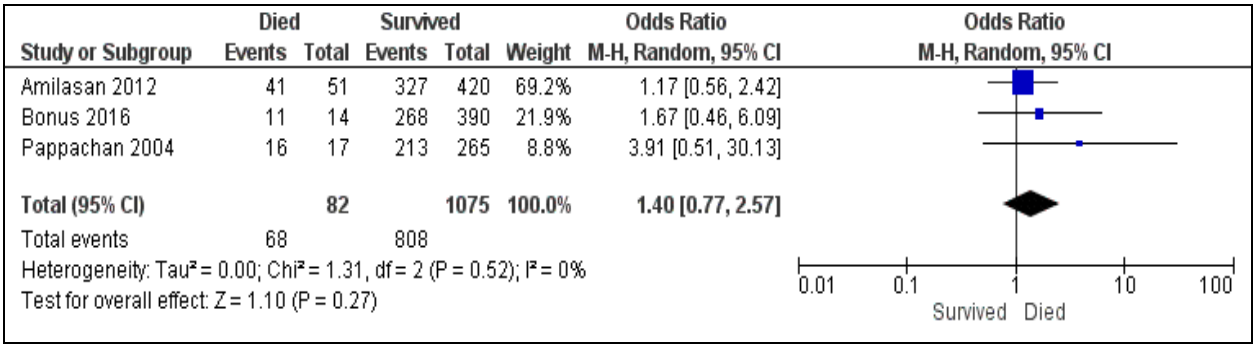

Figure 21. Forest plot of pooling of data for the presence of conjunctival suffusion comparing those with leptospirosis who died and survived

Bleeding Symptoms

Hematemesis: Only one study evaluated this parameter (Bonus, 2016). Those who died were five times more likely to have hematemesis as compared to those who survived, but this did not reach statistical significance (OR: 5.4; 95% CI: 0.2 to 116.8). There is a very serious risk of bias inherent of observational study design and inclusion of probable cases. There is also serious imprecision since there was an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Epistaxis: Only one study evaluated this parameter (Bonus, 2016). Those who died were almost three times more likely to have epistaxis as compared to those who survived, but this did not reach statistical significance (OR: 2.7; 95% CI: 0.3 to 22.1). There is a very serious risk of bias inherent of observational study design and inclusion of probable cases. There is also serious imprecision since there was an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Melena: Only one study evaluated this parameter (Bonus, 2016). Those who died were two times more likely to have melena as compared to those who survived, but this did not reach statistical significance (OR: 2.1; 95% CI: 0.3 to 16.9). There is a very serious risk of bias inherent of observational study design and inclusion of probable cases. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Gum bleeding: Only one study evaluated this parameter (Bonus, 2016). Those who died were two times more likely to have gum bleeding as compared to those who survived, but this evidence did not reach statistical significance (OR: 2; 95% CI: 0.1 to 38). There is a very serious risk of bias inherent of observational study design and inclusion of probable cases. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Skin hemorrhage: Only one study evaluated this parameter (Amilasan, 2012). Those who died were almost two times more likely to have skin hemorrhage as compared to those who survived, but this did not reach statistical significance (OR: 1.8; 95% CI: 0.1 to 38.5). There is serious risk of bias inherent of observational study design. There is indirectness as there more adults included in the study. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Other Symptoms

Presence of wound lesions: Only one study evaluated this parameter (Bonus, 2016). Those who died were almost two times more likely to have wound lesions as compared to those who survived, but this did not reach statistical significance (OR: 1.8; 95% CI: 0.1 to 38.5). There is a very serious risk of bias inherent of observational study design and inclusion of probable cases. There is also serious imprecision since there is an overlapping of the confidence interval with the null value; hence, this evidence is graded as very low.

Considerations for Recommendation Development during the Stakeholders Panel (SP) Meeting:

- All the six studies that were used to evaluate the signs and symptoms associated with an increased risk of mortality suffered from risk of bias because of their study design and serious imprecision. Also, five of the studies had indirectness. Hence, the quality of evidence is graded as very low.
- The SP however voted for a strong recommendation despite very low quality of evidence as all of these sign/symptoms turned out to be statistically significant. Any of these signs/symptoms is noted in actual practice among children with severe leptospirosis who die, reflective of leptospirosis' capability for multi-organ involvement with the potential for severity and even death.
- The representative from PAFP preferred data on clinical signs and symptoms that warrant admission. The GWP will consider including a question on admission criteria in the next edition.

Question 3: What laboratory findings are associated with severe leptospirosis?

Recommendation 1: The following laboratory parameters are ~~a~~ssociated with severe leptospirosis:

- Deranged prothrombin time (prothrombin time greater than or equal to 15 seconds; prothrombin time less than 68%)
- Elevated AST/ALT ratio (greater than or equal to 2)
- Elevated LDH (greater than or equal to 390 IU/L)
- Elevated CRP (greater than 282 mg/L)
- Elevated creatine phosphokinase (greater than 443 U/L)

Quality of evidence: Very low
Strength of recommendation: Strong

Recommendation 2: There is insufficient evidence to suggest that the following laboratory tests are associated with severe leptospirosis:

- Elevated bilirubin (greater than 49 µmol/L; total bilirubin greater than or equal to 35 µmol/L)
- Thrombocytopenia (less than 92 x 10^9/L)
- Elevated creatinine (greater than 154 µmol/L)
- Elevated BUN (greater than 9.3 mmol/L)
- Hematuria
- Decrease in hemoglobin (less than 12.2 g/dL)

Quality of evidence: Very low
Strength of recommendation: Strong

Summary of Evidence

Three studies evaluating abnormal laboratory findings in patients with severe leptospirosis were reviewed: one cross-sectional, one prospective cohort, and one retrospective case-control. One study included pediatric patients while the remaining two involved adult patients only.

Bonus conducted a case control study involving 404 patients aged 0-18 years old with probable or laboratory-confirmed leptospirosis admitted in three tertiary government hospitals in the Philippines. Patients who died were identified as the cases (non-survivor group, n=14), while those who survived (survivor group, n=390) served as the control (Bonus, 2016).

Mikulski focused on 47 adult patients with severe leptospirosis admitted at a hospital in New Caledonia, France between March 2009 and February 2011. In this study, patients were classified as having severe leptospirosis (n=22) if they developed either a fatal outcome or a need for mechanical ventilation or dialysis at any time during hospitalization. Patients without these factors were classified as the non-severe group (n=22) (Mikulski, 2015).

Hochedez included 102 adults with quantitative PCR-confirmed leptospirosis from December 2010 through February 2013 in Martinique, France. Severe leptospirosis was defined as having the presence of more than one of the following: shock treated with vasoactive drugs, acute renal failure requiring dialysis, internal bleeding requiring blood transfusion, respiratory insufficiency requiring mechanical ventilation, or death. In this study, there were no deaths. The patients being compared were those with severe disease (n=12) and those with non-severe disease (n=90) (Hochedez, 2015).

Table 6. Summary of studies evaluating laboratory findings associated with severe leptospirosis

Author *(Study Design)*	Study Period	Patients (N)	Location	Outcome determined	Remarks
Bonus 2016 *Retrospective case control study*	Jan 2008-Dec 2012	Filipino children 0-18 years old with probable or laboratory-confirmed leptospirosis (N=404)	3 tertiary hospitals (PGH, SLH, RITM) in the Philippines	Clinical profile, outcome and risk factors of leptospirosis in children	There were probable cases included in the study.
Mikulski 2015 *Prospective cohort study*	Mar 2009-Feb 2011	Adult patients with (+) PCR or serologic evidence of disease (N=47)	Nouméa Central Hospital in New Caledonia, France	Laboratory findings of severe and non-severe leptospirosis	There were only adults included in the study.
Hochedez 2015 *Cross-sectional study*	Dec 2010-Feb 2013	Adult patients 37-57 years old (N=102)	University Hospital of Martinique, France	Laboratory findings of severe and non-severe leptospirosis	There were only adults included in the study.

The following laboratory parameters are likely to be associated with severe leptospirosis:

Deranged Prothrombin Time (PT): Two studies evaluated derangement in prothrombin time values (Bonus, 2016; Hochedez, 2015). In the study of Bonus, non-survivors were twenty three times more likely to have PT greater than or equal to 15 seconds (OR: 23; 95% CI: 2.8 to189.7), while Hochedez' study showed that severe leptospirosis were almost six times more likely to have a PT value of <68% (OR 5.5; 95% CI: 1.5 to 20.1). Bonus' study is graded as very low because of serious risk of bias inherent to the study design and because of inclusion of probable cases. Both studies had wide confidence intervals suggestive of imprecision. Hochedez' study is graded as very low due to serious risk of bias inherent to the study design, and because of indirectness as only adult subjects were included.

Elevated AST/ALT Ratio: Only one study evaluated this parameter (Mikulski, 2015). Those with severe leptospirosis were seven times more likely to have an AST/ALT ratio greater than or equal to 2 (OR: 7.1; 95% CI: 1.8 to 28.1). The wide confidence interval almost inclusive of the null value is suggestive of serious imprecision. There is very low quality of evidence because of the observational study design and indirectness as only adult subjects were included.

Elevated LDH: Only one study evaluated this parameter (Mikulski, 2015). Patients with severe leptospirosis were almost six times more likely to have an LDH value greater than or equal to 390 IU/L (OR: 5.8; 95% CI: 1.3 to 25.6). There is indirectness as only adult subjects were included and serious risk of bias inherent to the study design. The wide confidence interval almost inclusive of the null value is suggestive of serious imprecision. The quality of evidence is very low.

Elevated C-Reactive Protein (CRP): Only one study evaluated this parameter (Hochedez, 2015). Those with severe leptospirosis were five times more likely to have an elevated CRP greater than 282 mg/L (OR: 5.2; 95% CI: 1.5 to 18.3). The study included adult subjects only. The wide confidence interval almost inclusive of the null value is suggestive of serious imprecision. The quality of evidence is very low due to indirectness and serious risk of bias inherent to the study design.

Elevated Creatine Phosphokinase: Only one study evaluated this parameter (Hochedez, 2015). Those with severe leptospirosis were almost five times more likely to have a creatine phosphokinase greater than 443 U/L (OR: 4.6; 95% CI: 1.1 to 19.6). The wide confidence interval almost inclusive of the null value is suggestive of serious imprecision. Quality of evidence is very low because of indirectness and risk of bias inherent to the study design.

There is insufficient evidence to say that the following laboratory parameters are associated with severe leptospirosis:

Elevated Bilirubin: Three studies evaluated this parameter (Bonus, 2016; Hochedez, 2015; Mikulski, 2015). Two studies showed that severe leptospirosis was five times more likely to have elevated bilirubin levels, with bilirubin values of greater than 49 µmol/L in Hochedez' study (OR: 5.4; 95% CI: 1.5 to 18.9), and total bilirubin greater than or equal to 35 µmol/L in Mikulski's study (OR: 5; 95% CI: 1.3 to 20.0). Both studies had very low quality of evidence due to indirectness as only adult subjects were included and due to serious risk of bias inherent to the study design. Both studies had wide confidence intervals that overlap with or almost inclusive of the null value is suggestive of serious imprecision. The study of Bonus showed that non-survivors were almost four times more likely to have total bilirubin levels of >20 umol/L (OR: 3.72; 95% CI 0.19 to 74.49); however, results did not reach statistical significance. This study has very low level of evidence due to serious risk of bias inherent to the study design, inclusion of probable cases, and imprecision.

Thrombocytopenia: Two studies evaluated this parameter (Bonus, 2016; Hochedez 2015). Hochedez' study showed that patients with severe leptospirosis were five times more likely to have a platelet count of less than 92 x 10^9/L (OR: 5.2; 95% CI: 1.5 to 18.1). There is very low quality of evidence due to serious risk of bias inherent to the study design and due to indirectness. In Bonus' study, non-survivors were twice more likely to have a platelet count of less than 150 x 10^3/mm^3, but this did not reach statistical significance (OR: 2.3; 95% CI: 0.7 to 7.6). Both studies had wide confidence interval that overlaps with or almost inclusive of the null value is suggestive of serious imprecision. The quality of evidence is graded as very low due to serious risk of bias inherent to the study design, inclusion of probable cases, and imprecision.

Elevated Creatinine: Two studies evaluated this parameter (Bonus, 2016; Hochedez, 2015). Patients with severe leptospirosis in Hochedez' study were five times more likely to have creatinine greater than 154 µmol/L (OR: 5.2; 95% CI: 1.5 to 18.1). The quality of evidence is very low due to serious risk of bias inherent to the study design and due to indirectness. The confidence interval was almost inclusive of the null value which is suggestive of imprecision. In Bonus' study, non-survivors were almost three times more likely to have an elevated creatinine for age, but this did not reach statistical significance (OR: 2.6; 95% CI: 0.3 to 21.1). The quality of evidence is very low due serious risk of bias inherent to the study design, inclusion of probable cases, and serious imprecision.

Hematuria: Only one study evaluated this parameter (Bonus, 2016). Non-survivors were five times more likely to have red blood cells greater than 5 per high power field (HPF) in the urine, but this finding did not reach statistical significance (OR: 5.4; 95% CI: 1 to 30.2). The quality of evidence is very low due to serious imprecision and serious risk of bias inherent to the study design and for inclusion of probable cases.

Decrease in Hemoglobin: Two studies evaluated this parameter (Bonus, 2016; Hochedez, 2015). Severe leptospirosis was almost four times more likely to have hemoglobin less than 12.2 g/dL in Hochedez' study, but this finding did not reach statistical significance (OR: 3.5; 95% CI: 1 to 12). The quality of evidence is very low due to serious imprecision, indirectness, and serious risk of bias inherent to the study design. In Bonus' study, hemoglobin of less than 130 mg/dl was not statistically different between non-survivors and survivors (OR: 1.2; 95% CI: 0.3 to 4.4). The quality of evidence is very low due to serious imprecision, serious risk of bias inherent to the study design, and for inclusion of probable cases.

Elevated Blood Urea Nitrogen (BUN): Two studies evaluated this parameter (Bonus, 2016; Hochedez 2015). Non-survivors were six times more likely to have elevated BUN for age in Bonus' study, but it did not reach statistical significance (OR: 6.2; 95% CI: 0.4 to 107.1). The quality of evidence is very low due to serious imprecision, serious risk of bias inherent to the study design, and for inclusion of probable cases. Patients with severe leptospirosis in Hochedez' study were almost four times more likely to have a BUN greater than 9.3 mmol/L, but this did not reach statistical significance (OR: 3.5; 95% CI: 0.8 to 15.4). The quality of evidence is very low due to serious risk of bias inherent to the study design, indirectness, and serious imprecision.

Considerations for Recommendation Development during the Stakeholders Panel (SP) Meeting:

- The quality of evidence for the 3 studies that looked into laboratory parameters suggestive of severe leptospirosis is very low due to serious risk of bias, serious imprecision and indirectness.
- The SP voted for a strong recommendation for deranged PT, elevated AST/ALT ratio, LDH, CRP, and CPK as laboratory findings associated with severe leptospirosis. These parameters were statistically significant and are actual laboratory findings seen in clinical practice that are reflective of multi-organ dysfunction in severe leptospirosis. Deranged PT and elevated AST/ALT are suggestive of hepatic dysfunction, elevated LDH is suggestive of tissue injury, and elevated creatinine phosphokinase is suggestive of muscle damage.
- For the second recommendation, the SP voted for a strong recommendation that elevated bilirubin, thrombocytopenia, elevated creatinine and BUN, hematuria, and decrease in hemoglobin are associated with severe leptospirosis despite the insufficient evidence. These are the other important parameters for multi-organ dysfunction.
- The representative of DOH prefers to indicate cut-off levels in the pediatric age group. Laboratory values indicated in the recommendation statement were the actual levels mentioned in the studies, majority of which included more of adult subjects. Only Bonus' study was done in the pediatric age group.
- Electrolyte determination was emphasized by the representative of PNSP as an important parameter in the evaluation of patients with leptospirosis because the disease involves the tubules which regulate electrolyte levels.

- The studies that evaluated laboratory findings in severe leptospirosis were limited to hospitalized patients; there were no studies that specifically looked at OPD patients with leptospirosis.

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CHAPTER 3

LABORATORY DIAGNOSIS OF LEPTOSPIROSIS

Leptospirosis presents similarly to other febrile infectious disease conditions. Confirmatory testing is usually carried out in those with a history of exposure coupled with symptoms suggestive of the disease. Direct detection via culture or the use of serology are the methods employed to establish evidence of infection (Lane, 2016).

Culture of appropriate clinical specimens done prior to antibiotic therapy can confirm leptospirosis (Day, 2018). This method, however, is fraught with challenges as it entails the use of special media and the organism takes 1-2 weeks (or may extend to over a month) to grow (Lane, 2016). While highly specific, culture has low sensitivity (5-50%) (Haake, 2015). During the leptospiremic phase, blood and CSF cultures are useful. However, as the immune phase begins, yield from blood culture decreases (Shreier, 2013; WHO, 2003). Urine cultures are most likely to give positive results after the second week of illness (Lane, 2016).

While isolation of leptospires is the only direct and definitive proof of infection, serological data forms an important part of diagnostic investigation, and it must be used in association with clinical presentation and epidemiologic data (WHO, 2003). Antibodies usually become detectable in the blood 5 to 10 days after symptom onset (Levett, 2001).

Microscopic Agglutination Test (MAT), considered as the cornerstone or the "gold standard" of leptospirosis serodiagnosis (WHO, 2003), is used as the reference test for the development of other assays (Day, 2018). ELISA and other rapid screening tests for leptospiral antibodies have also been developed. MAT is carried out by mixing the patient's serum with live antigen suspensions of leptospiral serovars. This mixture is then examined microscopically for agglutination and the titers are determined (Haake, 2015). MAT is usually positive 10-12 days after symptom onset, but seroconversion may sometimes occur as early as 5-7 days after onset of the disease. Antibiotic therapy may cause delay in antibody response. MAT may give an indication of the serogroup to which the infective serovar belongs to, but only rarely identifies it. Both IgM- and IgG-class antibodies are detected. MAT cannot differentiate between agglutinating antibodies due to current, recent or past infections. Paired sera are ideally used and examined for seroconversion or a four-fold or greater rise in titer (WHO, 2003). The appropriate interval between sample collections depends on the onset of symptoms and the presentation of the patient. An interval of 3-5 days may detect rising titers if the characteristic symptoms are present. Longer intervals, i.e., 10-14 days, would be needed for patients that present earlier in the course of illness or if the onset of symptoms cannot be determined (Haake, 2015). The "*WHO Recommended Standards And Strategies For Surveillance, Prevention And Control Of Communicable Diseases*" cites that confirmatory diagnosis of leptospirosis using MAT entails seroconversion or a fourfold or greater rise in titers on paired sera taken at least 2 weeks apart (WHO, 2018). The cut-off titer of a single specimen should be determined in the light of seroprevalence of persistent antibodies due to past infections in the general population, and in relation to the presence of antibodies to other diseases that may cause cross-reactions (e.g., hepatitis, autoimmune diseases, legionellosis) (WHO, 2003).

Although specific, MAT has several limitations that include the following: (1) it needs to maintain panels for live leptospires, hence it is usually carried out in reference laboratories; (2) it cannot be standardized; (3) it is time-consuming; (4) it is technically demanding; and (5) it may pose a potential hazard to the laboratory personnel (WHO, 2003; Nieves, 2019). When the causative strain is not represented in the panel used, antibodies may not be detected or only a low titer is found with a serovar antigenically resembling the absent causative strain. Results reporting "no titer" or "low titer" do not exclude the disease (WHO, 2003).

Other serodiagnostic and rapid screening antibody tests have been developed. Several assays of Enzyme-Linked Immunosorbent Assay (ELISA) are available and it can be performed with commercial kits or with an antigen produced "in house". It uses a broadly reactive genus-specific antigen to detect IgM, and sometimes also IgG, antibodies (WHO, 2003). ELISA is carried out with relative simplicity, and it can be standardized as it does not use a panel of live antigens. It gives a positive response (usually 6-8 days from the appearance of the first clinical signs) a little earlier than MAT because it is more sensitive to IgM antibodies. It can help differentiate between current and previous infection since the antibodies from the past infection may not be detectable. Some test systems, however, are less specific than MAT and weak cross-reactions due to the presence of other diseases is possible. As such, ELISA results should still be confirmed by MAT. ELISA cannot identify the infecting serovar since it is a genus-specific test (WHO, 2003). IgM ELISA is shown to be a sensitive screening test for leptospirosis in one systematic review done in Brazil (Rosa, 2017). Currently, this test is not locally available.

Most local laboratories offer IgM Immunochromatography Test (ICT). ICT has been developed as an alternative rapid screening test for leptospirosis. Some studies show IgM ICT as an acceptable early screening test, but they recommended that a follow-up confirmatory test such as MAT be done (Amran, 2018; Goris, 2013; Iwasaki, 2016;Podgorsek, 2015). One study recommended its use in resource-limited setting (Niloofa, 2015), but other studies found ICT to have limited value in the diagnosis of leptospirosis (Blacksell, 2006; Wagenaar, 2004). Performance of the test was only moderate for samples collected within the first week of illness which is the period crucial for therapeutic intervention (Rao, 2019). In a prospective cohort evaluation of rapid diagnostic tests (that included ICTs), there was low sensitivity of the test in the early acute phase of illness (until 4 days post onset of symptoms) (Goris, 2013), as antibodies are not yet at detectable levels in the early stage of the disease (Goris, 2011). Dengue, syphilis, and scrub typhus can have cross reactivity with rapid tests performed for leptospirosis (Amran, 2018).

In recent years, molecular tests such as the Polymerase Chain Reaction (PCR) are increasingly utilized in the diagnosis of infectious diseases. PCR detects the causative agent's DNA in clinical samples. Short DNA sequences specific for the organism are used as primers and, in combination with DNA polymerase, are subjected to temperature cycles that amplifies the organism's DNA (WHO, 2003). Leptospiral DNA has been detected in the blood during the first 7 days of illness (highest sensitivity between days 1 and 4), and in the urine after day 7 of illness (AAP, 2018). Aside from this, the CSF, aqueous humor, and organs post-mortem are reported sites where leptospiral DNA have been amplified (Levett, 2004). Assays designed for diagnostic purposes target either housekeeping genes such as *rrs, gyrB, or secY*, or pathogen-specific genes such as *lipL32, lig, or lfb1* (Haake, 2015). Conventional PCR for the detection of leptospiral DNA was introduced in 1989 (Ahmed, 2012), using urine samples from cattle (Van Eys, 1989). Studies on the use of conventional PCR in human leptospirosis showed that its value as a diagnostic method is not clear (Ahmed, 2012), detecting only 44% of MAT positive cases in one study (Yersin, 1999) and only in 14 cases of 200 subjects in another (Merien, 1995). A disadvantage of conventional PCR is that it is prone to contamination, and thus may

give false positive results (Ahmed, 2009; Jouglard, 2006). Real-Time (RT) PCR, on the other hand, is a PCR-based amplification of DNA that is monitored during the amplification process utilizing several types of dyes and probes. TaqMan probes, Molecular Beacons, Scorpions, Light Upon eXtension technology (LUX), and SYBR Green 1 dye are among the most available formats that detect PCR products by generation of a fluorescent signal. RT PCR has been shown to have a high degree of accuracy on blood samples during the early phase of the disease (Ahmed, 2012). In general, PCR require special equipment, a dedicated laboratory space and highly skilled personnel. In addition to its propensity for contamination giving false positive results, it may also give false negative results in the presence of inhibitors in the sample submitted (WHO, 2003).

The Research Institute for Tropical Medicine (RITM) offers the following diagnostic tests for leptospirosis:

Table 7. Diagnostic tests for Leptospirosis at the Research Institute for Tropical Medicine

Test	Specimen and Collection time	Turnaround time
Culture	Whole blood - Within 10 days after symptom onset CSF - 5-10 days after symptom onset Urine - 2nd week to 30 days after symptom onset	12 weeks
qPCR	Whole blood, CSF, Serum: within 10 days after symptom onset Urine: 2nd week up to 30 days after symptom onset	3-5 days
MAT	Serum: Acute phase: 5-10 days after onset of symptoms Convalescent phase: 5 to 20 days after acute phase of the disease	7 working days

(National Reference Laboratory for Emerging/Re-emerging Bacterial Diseases Leptospirosis Unit, RITM)
*Coordination with RITM for specimen handling (needed volume, storage and transport) is recommended.

Question 4: Can IgM Immunochromatography Test (ICT) be used as a rapid test in the diagnosis of leptospirosis in children?

Recommendation 1: IgM ICT may be used as a rapid test in the diagnosis of leptospirosis in children.
Quality of evidence: Moderate
Strength of recommendation: Strong

Summary of Evidence

Two studies included the evaluation of IgM ICT compared with MAT as a rapid test in the diagnosis of leptospirosis (Iwasaki, 2016; Niloofa, 2015). Subjects included were hospitalized patients. One was done in Manila and the other was done in Sri Lanka.

Iwasaki investigated 113 clinically-diagnosed leptospirosis patients at San Lazaro Hospital who were enrolled in the study after the August 2012 flood. Seventy seven (77) MAT-positive and 36 MAT-negative patients, age-stratified into four groups (<20, 20-40, 41-64, and >64 years old) were included. It was not clearly stated, however, how many patients were less than 20 years old and what the youngest age of the included subjects were (Iwasaki, 2016).

Niloofa included a total of 888 patients, aged 13-80 years old, with 354 MAT-positive cases and 534 controls. The patients were recruited from three hospitals in the Western Province of Sri Lanka from June 2012 to December 2013 (Niloofa, 2015).

For the evaluation of IgM ICT, forest plots were constructed to graphically assess the variability of the estimates of the tests. A random-effects meta-analysis was performed using MetaDisc software version 1.4. Inconsistency (statistical heterogeneity) among studies was assessed by the conventional Chi-squared test for heterogeneity and by calculating the I^2 statistic to highlight the effect of true variability rather than sampling error on the overall variation in diagnostic estimates.

Table 8. Summary of Sensitivity, Specificity, PPV, and NPV values of studies evaluating IgM Immunochromatographic Test (ICT)

Study	True Positive	False Positive	True Negative	False Negative	PPV	NPV	Sensitivity	Specificity	No. of participants
Iwasaki 2016	61	7	29	16	89.7	64.4	79.2 (68.5 - 87.63)	80.56 (63.98 - 91.81)	113
Niloofa 2015	248	159	436	45	60.9	90.6	84.6 (80.0 - 88.6)	73.3 (69.5 - 76.8)	888

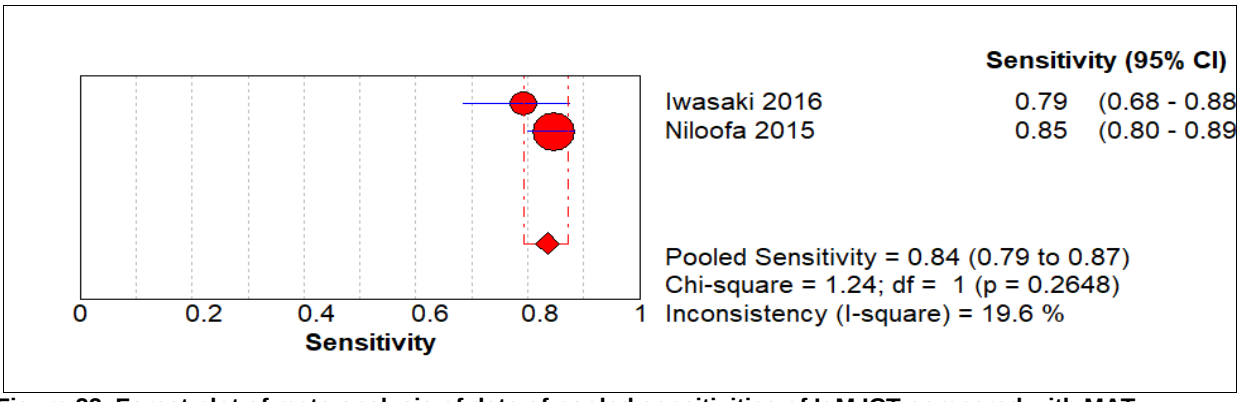

Figure 22. Forest plot of meta-analysis of data of pooled sensitivities of IgM ICT compared with MAT

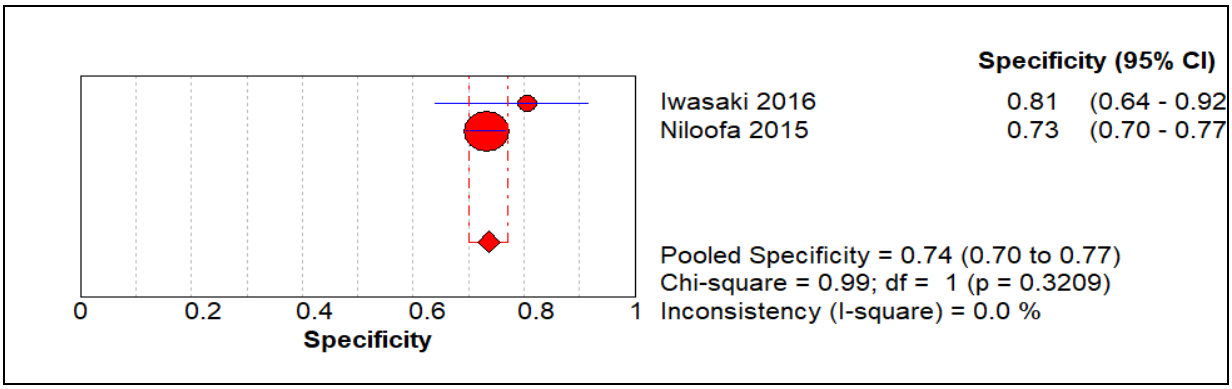

Figure 23. Forest plot of meta-analysis of data of pooled specificities of IgM ICT compared with MAT

Pooled sensitivity of IgM ICT is 84% (95% CI: 79% to 87%; I^2 = 19.6%) for all patients with leptospirosis (confirmed by MAT), while pooled specificity is 74% (95% CI: 70% to 77%; I^2 = 0%) (Figures 22-23). There is indirectness due to inclusion of more adults subjects; thus, the quality of evidence is graded as moderate.

The above data showed variable results. MAT or culture remains to be the gold standard for the diagnosis of leptospirosis.

Table 9. Summary of studies evaluating ICT as a rapid diagnostic test that can be used for the diagnosis of leptospirosis in children

Study *(Study Design)*	Patient characteristics	Location	Tests evaluated in the study	Reference standard	Remarks
Iwasaki 2016 *Cross-sectional*	Individuals, <20 to >64 years old, with clinically-diagnosed leptospirosis (N=113)	San Lazaro Hospital, Manila	*ICT, MAT, ELISA, LAMP, real time PCR	MAT[a]	Most of the subjects belong to the 20-64 year old age group
Niloofa 2015 *Cross-sectional*	Hospitalized Sri-Lankan patients, 13-80 years old, with suspected leptospirosis (based on WHO-CLERG epidemiologic criteria) (N=888)	National Hospital of Sri Lanka (NHSL), Colombo North Teaching Hospital (CNTH) and Base Hospital Homagama (BHH)	MAT, IgM-ELISA, IgM ICT** (Leptocheck-WB)	MAT[b]	More adult patients included

a: In Iwasaki's study, sensitivity and specificity of ICT and ELISA were defined with respect to MAT
b: in Niloofa's study, data analysis was performed using MAT as reference standard and using Bayesian Latent Class Model analysis
*Only results of the ICT compared to MAT were evaluated
**Only the results of IgM ICT compared to MAT were evaluated

Considerations for Recommendation Development during the Stakeholders Panel (SP) Meeting:

- There was moderate quality of evidence for the use of IgM ICT as a rapid test for leptospirosis diagnosis. Leptospirosis IgM ICT is readily available in most local hospitals. For this, the consensus panel voted for a strong recommendation.

Question 5: Can IgM Enzyme-linked Immunosorbent Assay (ELISA) be used as a rapid test in the diagnosis of leptospirosis in children?

Recommendation 1: IgM ELISA may be used as a rapid test in the diagnosis of leptospirosis in children.
Quality of evidence: Low
Strength of recommendation: Weak

Summary of Evidence

Four studies included IgM ELISA as a rapid diagnostic test in the evaluation of leptospirosis. Three were cross-sectional studies and one was a case-control study.

All studies were done in hospitals. One study was done in the Philippines (Iwasaki, 2016), one in Thailand (Desakorn, 2012), one in Sri Lanka (Niloofa, 2015), and one in mainland France and French overseas territories (Bourhy, 2013). The specific IgM ELISA evaluated in the included studies are summarized below.

Table 10. IgM ELISA used In the Included Studies(as Rapid Test in the Diagnosis of Leptospirosis in Children)

Study	IgM ELISA evaluated
Iwasaki (2016)	ELISA (Diagnostic Automation, Calabasas, CA, USA)
Niloofa (2015)	IgM-ELISA (Institut Virion\Serion GmbH, Warburg, Germany)
Desakorn (2012)	*Leptospira sp.* IgM ELISA (Panbio Pty., Ltd.,Queensland, Australia)
Bourhy (2013)	In-house IgM ELISA – developed an ELISA based on a whole-cell antigen extract obtained from *L. faineiserovar Hurstbridge*

The above studies evaluated IgM ELISA compared with MAT in the rapid diagnosis of leptospirosis.

Desakorn conducted a retrospective case-control study of 218 patients aged 15 years and older. One hundred nine (109) patients with laboratory-confirmed leptospirosis (using *Leptospira sp.* culture and/or Microscopic Agglutination Test [MAT]) were designated as cases, and 109 patients without leptospirosis served as controls. The patients were identified from a prospective cohort study of consecutive patients presenting to Udon Thani Hospital, Northeast Thailand with an acute febrile illness between 2001 and 2002. Sera on admission of two leptospirosis cases and two controls were not available to test by the IgM ELISA (Desakorn, 2012)

Bourhy tested an in-house ELISA using a total of 819 serum samples from patients originating from mainland France, Martinique, Guadeloupe and other French territories. MAT was used as the reference test. Samples were grouped into four panels consisting of confirmed cases with clinical suspicion of leptospirosis and seroconversion between paired sera, probable cases with clinical suspicion of leptospirosis and a single MAT of ≥ 400, confirmed negative cases (healthy donors and patients with infection other than leptospirosis) who were all MAT negative, and probable negative cases with clinical suspicion of leptospirosis and MAT titers of <50 on paired sera. In the analysis, samples from confirmed cases and probable cases (202 MAT-negative and 317 MAT-positive samples, N=519) were evaluated (Bourhy, 2013).

Iwasaki and Niloofa also included IgM ELISA in the evaluation of tests for the diagnosis of leptospirosis among hospitalized patients. Most of the included subjects were adults (Iwasaki, 2016; Niloofa, 2015). Description of their studies were discussed in the previous question (refer to Question No. 3).

Indirectness is rated as serious since adults were included in all the studies reviewed. Imprecision is rated as serious if there was overlapping of confidence interval with the null value.

Similar to the evaluation done for IgM ICT, forest plots were constructed to graphically assess the variability of the estimates of the tests for IgM ELISA. A random-effects meta-analysis was performed using MetaDisc software version 1.4. Inconsistency (statistical heterogeneity) among studies was assessed by the conventional Chi-squared test for heterogeneity and by calculating the I^2 statistic to highlight the effect of true variability rather than sampling error on the overall variation in diagnostic estimates.

Table 11. Summary of the results of studies that included the evaluation of IgM ELISA in the diagnosis of leptospirosis

Study	True Positive	False Positive	True Negative	False Negative	PPV	NPV	Sensitivity (CI)	Specificity (CI)	No. of participants
Desakorn 2012	56	36	71	51	60.9	58.2	52.3 (42.5-62.1)	66.4 (56.6-75.2)	214
Bourhy 2013	298	3	199	19	99	91	94.0 (90.8-96.4)	98.5 (95.7-99.7)	519
Iwasaki 2016	67	19	17	10	77.9	63.0	87.0 (77.4-93.6)	47.2 (30.4-64.5)	113
Niloofa 2015	252	92	503	41	73.3	92.5	86.0 81.5-89.8)	84.5 (81.4-87.3)	888

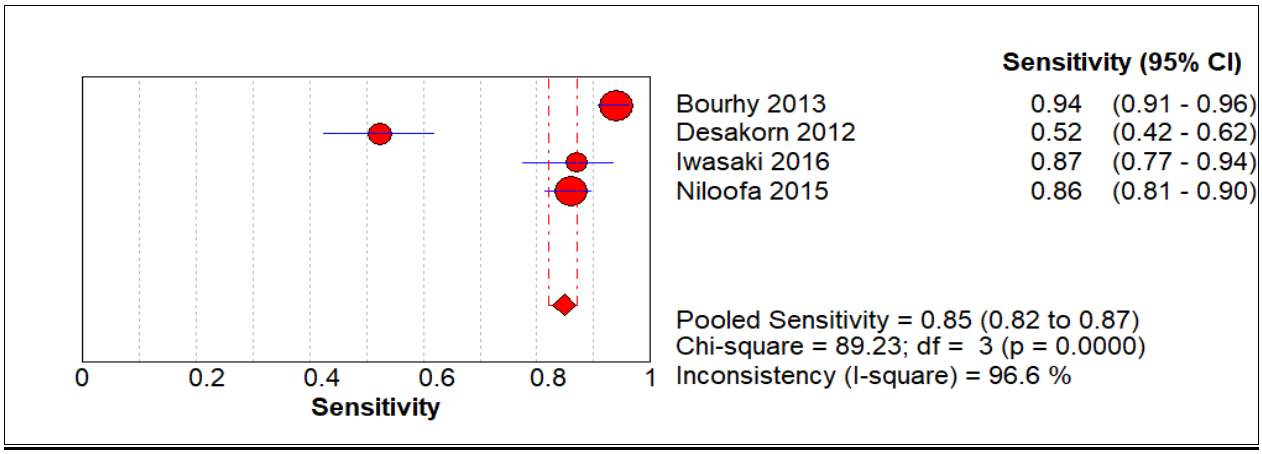

Figure 24. Forest plot of meta-analysis of data of pooled sensitivities of IgM ELISA compared with MAT

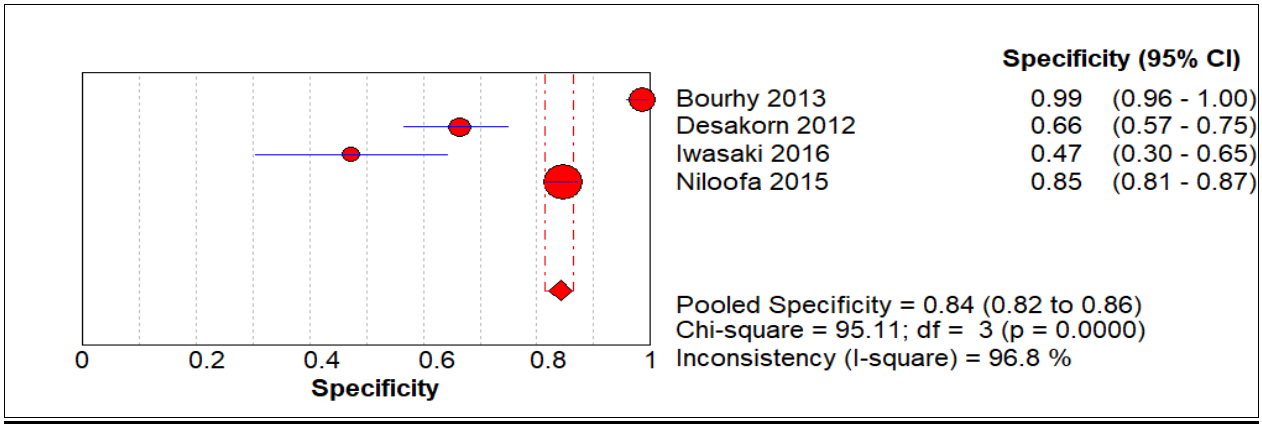

Figure 25. Forest plot of meta-analysis of data of pooled specificities of IgM ELISA compared with MAT

The pooled sensitivity of ELISA was 85% (95% CI: 82% to 87%; $I^2 = 96.6\%$) for all patients with leptospirosis (confirmed by MAT), while the pooled specificity was 84% (95% CI: 82% to 86%; $I^2 = 96.8\%$) (Figures 24-25). The evidence was graded as low due to inconsistency and indirectness.

The above figures show variable results. MAT or culture remains to be the gold standard for the diagnosis of leptospirosis. Furthermore, IgM ELISA is not locally available.

Table 12. Summary of studies for IgM ELISA as a rapid diagnostic test for the diagnosis of leptospirosis in children

Author *(Study Design)*	Patient Characteristics	Location	Test Evaluated*	Reference test or Gold standard used	Remarks
Iwasaki 2016 *Cross-sectional*	Individuals (<20 to > 64 yrs. old) with clinically-diagnosed leptospirosis (N=113)	San Lazaro Hospital, Manila	MAT, ELISA*, ICT, LAMP, and real time-PCR	MAT	Most of the subjects belong to the 20-64 years old age group
Niloofa 2015 *Cross-sectional*	Hospitalized Sri-Lankan patients, 13-80 years old, with suspected leptospirosis (based on WHO-CLERG epidemiologic criteria) (N=888)	National Hospital of Sri Lanka, Colombo North Teaching Hospital, and Base Hospital Homagama	MAT, IgM-ELISA* and Leptocheck-WB (ICT)	MAT	More adult patients included
Desakorn 2012 *Retrospective case-control*	Thai individuals 15 years old and above with fever of unknown cause (N=218 with 109 cases and 109 controls; sera from 2 cases and 2 controls were not available to evaluate by ELISA)	Udon Thani Hospital, Thailand	IgM ELISA* (Panbio)	*Leptospira sp.* culture and/or MAT	Included adult patients
Bourhy 2013 *Cross-sectional*	Human sera (of patients aged 9-89 yrs. old) were tested at National Reference Center for Leptospirosis were used (N=819 sera; in the analysis, 202 MAT-negative samples and 317 MAT-positive samples were evaluated)	Patients were from Mainland France, Martinique, Guadeloupe, and other French territories	in-house ELISA*	MAT	Sera from adults were included

* Only the results of ELISA were included in the evaluation

Considerations for Recommendation Development during the Stakeholders Panel (SP) Meeting:

- An issue that came up during the discussion was the availability of IgM ELISA. Currently, this test is not locally available.
- The representative from the PNSP asked why MAT was not evaluated. It was explained that MAT was used as the reference test or gold standard test in studies that evaluated IgM ELISA.
- Majority of the SP voted for a weak recommendation because IgM ELISA is not yet locally available.

Question 6: Can polymerase chain reaction (PCR) be used in the diagnosis of leptospirosis in children?

Recommendation 1: PCR may be used in the diagnosis of leptospirosis in children.
Quality of evidence: Low
Strength of recommendation: Strong

Summary of Evidence

There were two case-control studies that included the evaluation of PCR as a diagnostic test for leptospirosis (Narayanan, 2016; Thaipadunpanit, 2011). Both studies were conducted in hospitals. Although both studies involved pediatric patients, there were more adult subjects included.

The study of Narayanan identified 134 children and 443 adults with clinically suspected leptospirosis. Subjects were age-stratified into the pediatric group (ages 0-17 years old) and adult group (ages ≥18 years old). Controls consisted of age- and sex-matched healthy subjects. Sensitivity, specificity and predictive values of IgM ELISA, microscopic slide agglutination test and PCR were compared with MAT (Narayanan, 2016).

Thaipadunpanit evaluated two real-time PCR assays targeting *rrs* or *lipL32* in 266 patients (133 cases of leptospirosis and 133 controls). The diagnostic sensitivity and specificity of both assays were determined using positive culture and/or MAT as the gold standard (Thaipadunpanit, 2011).

Studies were included if they had children as participants and if the diagnostic reference standard used included MAT.

Table 13. Summary of Sensitivity, Specificity, PPV, and NPV valuesof studies that evaluated PCR

Study	TP	FP	FN	TN	PPV	NPV	Sensitivity	Specificity	No. of participants
Narayanan 2016	147	18	5	408	89	99	97 (85 - 100)	96 (91 - 99)	577
Thaipadunpanit 2011 rt PCR assay	74	14	59	119	84	67	56 (47 - 64)	90 (83 - 94)	266

TP - True Positive; FP - False Positive; FN - False Negative; TN- True Negative

For the evaluation of PCR, forest plots were constructed to graphically assess the variability of the estimates of the tests. A random-effects meta-analysis was performed using MetaDisc software version 1.4. Inconsistency (statistical heterogeneity) among studies was assessed by the conventional Chi-squared test for heterogeneity and by calculating the I^2 statistic to highlight the effect of true variability rather than sampling error on the overall variation in diagnostic estimates.

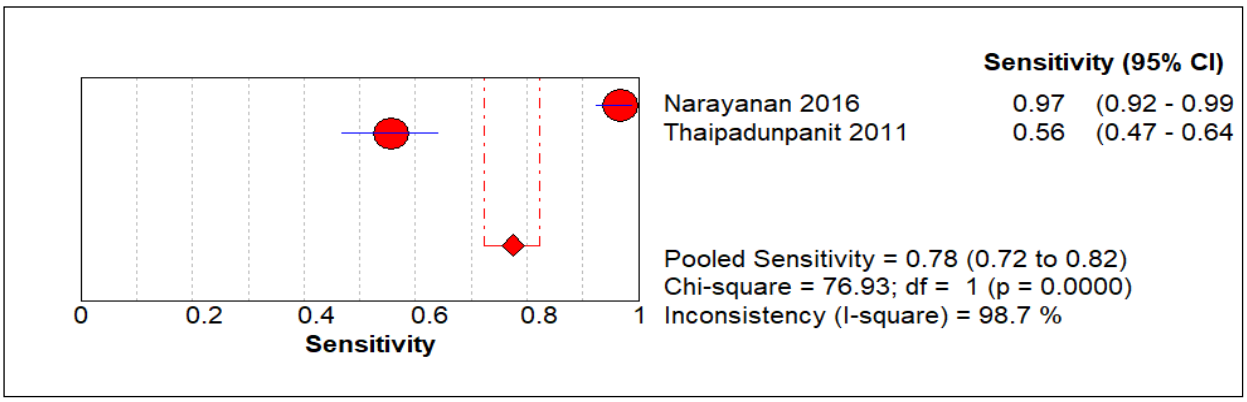

Figure 26. Forest plot of meta-analysis of data of pooled sensitivities of PCR compared with MAT

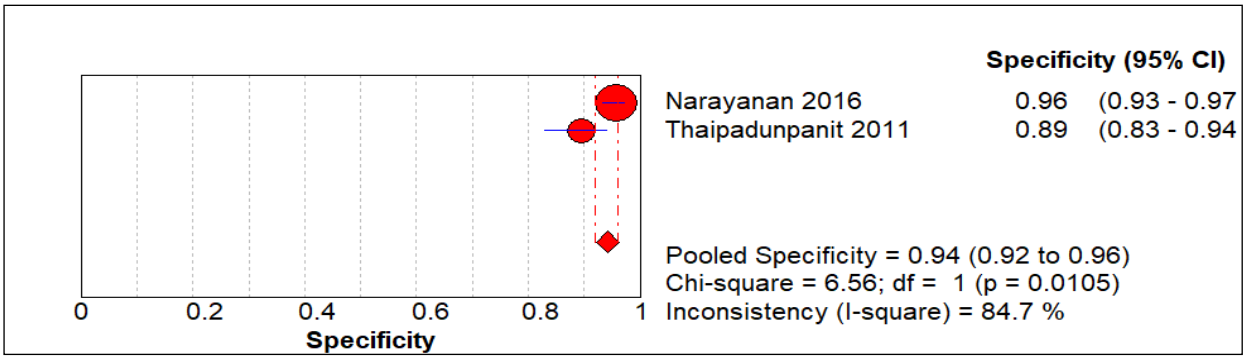

Figure 27. Forest plot of meta-analysis of data of pooled specificities of PCR compared with MAT

Pooled sensitivity of the two studies is 78% (95% CI: 72% to 82%; I^2 = 98.7%) for all patients with leptospirosis (confirmed by MAT), while pooled specificity is 94% (95% CI: 92% to 96%; I^2 = 84.7%) (Figures 26-27). There is indirectness due to inclusion of more adults and heterogeneity is significant. The quality of evidence is graded as low.

The above results show that while PCR's pooled specificity is >90%, pooled sensitivity is only 78%. In the local setting, PCR is not widely available. It is likewise technically demanding, thus limiting its accessibility only in reference laboratories.

Table 14. Summary of studies that included the evaluation of PCR as a diagnostic test for leptospirosis

Author *(Study Design)*	Patients (N)	Location	Tests evaluated	Reference standard used	Remarks
Narayanan 2016 *Case-control*	Hospitalized Indian patients 134 children aged 0-17 years old and 443 adults patients aged ≥18 years old with suspected leptospirosis (N=577)	Government Hospital, Municipality of Chennai, India	MAT, IgM-ELISA, MSAT, PCR	MAT	More adult patients included
Thaipadunpanit 2011 *Case-control*	Patients 15-79 yrs old 133 cases of leptospirosis and 133 controls (N=266)	Udon Thani Hospital, Thailand (2001-2002)	PCR assays (*rrs* and *lipL32*)	Culture and/or MAT	More adult patients included

Considerations for Recommendation Development during the Stakeholders Panel (SP) Meeting:

- The two studies that evaluated PCR as a diagnostic test for leptospirosis show low quality of evidence due to indirectness and significant inconsistency. Pooled analysis showed a higher specificity (>90%) compared to IgM ICT (74%) and IgM ELISA (84%). PCR gives positive results earlier (first 7 days from onset of illness) compared to MAT (10-12 days from onset) and ELISA/ICT (6-8 days from onset). Turnaround time is shorter for PCR (3-5 days) as compared to culture (12 weeks) and MAT (7 days). For these, the SP voted for a strong recommendation.
- A member of the GWP mentioned that PCR is available at RITM, but not readily available in other institutions. The representative from DOH mentioned that PCR for leptospirosis is also available at San Lazaro Hospital, but only for in-patients.

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CHAPTER 4

ANTIBIOTIC THERAPY FOR LEPTOSPIROSIS

The role of antibiotics in the treatment of leptospirosis based on current literature remains unclear. Available data generally reflect use of antibiotics in clinical practice.

In children and adults, severity of illness is classified as mild, moderate or severe. Based on the Department of Health National Antibiotic Guidelines of 2018, mild illness is managed with amoxicillin at 30-50 mg/kg/day divided into every 8 hours for 7 days (Max 500 mg q8) or doxycycline 2 mg/kg/day divided into 12 hours for 7 days. Azithromycin at 10 mg/kg/day PO (Max 500 mg/day) for 1 day followed by 5 mg/kg/day (Max 250 mg/day) for 2 days may be used as a second line antibiotic (DOH, 2018).

For moderate and severe disease, penicillin at 250,000-400,000 units/kg/day divided into every 4-6 hours (Max 1.5 MU q6-q8) is recommended as first line. Cefotaxime 100-150 mg/kg/day IV/IM divided every 6-8 hours (Max 1g q6), or ceftriaxone 80-100mg/kg/day IV/IM q24 (Max: 2 g/day), or azithromycin 10 mg/kg/day IV q24 (Max: 500 mg/day) followed by 5 mg/kg/day IV q24h (Max: 250 mg/day) are recommended as second line therapeutics. The antibiotic treatment in severe disease is usually 7 days (DOH, 2018).

There are two published meta-analysis by Brett-Major and Charan which provided evidence on the effectiveness of antibiotic treatment based on its ability to reduce the duration of clinical illness, reduction in complications, and prevention of mortality (Brett-Major, 2012; Charan, 2013).

The GWP decided to solely use duration of fever to evaluate the effect of antibiotics on clinical illness as it was the only measurable parameter that was consistent across all studies.

Question 7: How effective is the use of antibiotics in the treatment of children with leptospirosis?

Recommendation: The use of antibiotics may be considered in the treatment of children with leptospirosis, but there is no evidence to suggest that this may decrease mortality, duration of fever, renal complications, and the need for dialysis.
Quality of evidence: Very low
Strength of recommendation: Strong

Summary of Evidence

A systematic search of the literature did not yield studies that directly answered the clinical question - all studies on the effectiveness of antibiotics as treatment for severe leptospirosis were done on adults, with some studies including adolescents ≥16 years old. Also, the criteria used for severe leptospirosis varied among the different studies, and many studies included both severe and non-severe cases in the analysis.

Seven studies, which evaluated the use of antibiotics in different clinical outcomes, were found in the literature:

A meta-analysis by Brett-Major included randomized controlled trials on infected patients regardless of severity of illness. Seven trials (Costa, 2003; Edwards, 1988; McClain, 1984; Panaphut, 2003; Phimda, 2007; Suppitamongkol, 2004; Watt, 1988) were included in the study after a comprehensive systematic search, three of which were from the 1980s. Four studies assessed antibiotic treatment in severe leptospirosis, however, the criteria for severity were varying. There were varying antibiotics used: four trials with 403 subjects compared an antibiotic with placebo or no intervention; three trials compared at least one antibiotic regimen with another antibiotic. The trials all had a high risk of bias and the ability to group data for meta-analysis was limited. Although the authors' planned subgroup categorization for severe versus non-severe leptospirosis, these subgroups "did not overlap substantively providing data (events) to inform trial objectives". Pooling of results in the meta-analysis was possible only for death, days of clinical illness, and dialysis employed because the trial outcomes were varying and had limited reporting of data. Forest plots of these pooled data were not shown in the article, which raised concern on reporting bias. The quality of evidence for this meta-analysis was low because of inconsistency of results, indirectness, imprecision and possible reporting bias (Brett-Major, 2012).

Another meta-analysis by Charan evaluated the role of antibiotics in leptospirosis which included five studies: 4 RCTs (Costa, 2003; Edward, 1988; Fairburn, 1956; Watt, 1988) and 1 cohort study (Daher, 2000). All studies looked into the endemic population, except for Fairburn which studied British military men with leptospirosis, mostly non-severe, in the jungles of Malaya (Fairburn, 1956). All studies compared penicillin with no treatment, except for Watt who used a placebo (Watt, 1988). Outcomes were varying among studies. All studies had a high risk for bias and the ability to group the data for meta-analysis was limited. The quality of evidence for this meta-analysis was very low because of inconsistency and imprecision of results. There was indirectness as most studies were on adults (Charan, 2013).

The study by Watt was a randomized controlled trial on penicillin compared to placebo conducted in a national infectious disease hospital in the Philippines. Subjects were 16 years old and older with severe and late leptospirosis (i.e., with symptoms for >4 days) confirmed by antibody titer or isolation of the organism from blood or urine. Criteria for severity were elevated

creatinine (>177 umol/L) and/or jaundice present on admission, however, the most severe cases were excluded from the study (i.e., those with anuria, confusion, stupor, coma). Sample size was relatively small (N=42). Intravenous penicillin g at 6 million units per day for 7 days was compared with placebo. The primary outcomes were deaths, duration of fever after treatment, duration of increased serum creatinine, hematologic and biochemical variables, and duration of hospitalization. This study was included in the pooled analysis of death in the meta-analyses of both Brett-Major and Charan (Brett-Major, 2012; Charan, 2013). The quality of evidence for this study was very low. There was a serious risk of bias since randomization procedure and concealment were not described, and most severe cases were excluded from the study. There was not enough information on other forms of management concomitant with the experimental intervention that was done on the patients (Watt, 1998).

Another RCT conducted by Costa assessed the efficacy of Penicillin in 253 patients who were >15 years old with late stage leptospirosis (i.e., >4 days of symptoms) in an infectious disease hospital in Brazil. Cases that reached at least 26 points in a WHO probability score for leptospirosis and without a history of nephropathy, cardiomyopathy or diabetes mellitus were included. Almost all patients (91.6%) were in renal failure, with a creatinine of >1.5 mg/dL and had jaundice (94%) on admission, which suggested that patients in the trial had severe leptospirosis. All but one patient were confirmed leptospirosis by Microscopic Agglutination Test (MAT) and blood cultures. Intravenous penicillin (6 million units per day for 7 days) was compared with no treatment. The main outcome evaluated was mortality, however, the use of peritoneal dialysis and hospitalization were also reported. This study was included in the pooled analysis of death in the meta-analysis by Brett-Major who pooled the data to determine effectiveness of an antibiotic (penicillin or doxycycline) versus no treatment or placebo (Brett-Major, 2012). The quality of evidence of this study was very low. The risk for bias was high since randomization technique and allocation concealment were not mentioned in the study. Subjects in the two groups were not comparable at baseline, however, logistic regression was used to adjust for the differences. There is indirectness of the results because subjects were predominantly men in the 3rd to 5th decade of life and because of the use of the WHO criteria to define late stage leptospirosis (Costa, 2003).

Panaphut conducted an open-label RCT in a tertiary hospital in Thailand comparing ceftriaxone with penicillin g on 173 patients >16 years old with severe leptospirosis (presence of jaundice or serum creatinine >180umol/L, or mean arterial pressure <70 mmHg). Those who had experienced CPR before admission or were comatose or stuporous were excluded. Of the 173 patients who screened positive for leptospirosis using the IgM specific assay (LEPTO dipstick), only 72% were confirmed by MAT; no blood or urine cultures were done. Penicillin g was given at 1.5 million units every 6 hours and ceftriaxone was given at 1 gram per day. Gentamicin was also administered for patients in group P for whom septicemia to gram-negative organism could not initially be excluded, but was terminated if blood and urine cultures were negative. The primary outcome was the time to resolution of fever after treatment. Other outcomes were mortality and time to resolution of organ dysfunction. For those who did not return for follow up consult after discharge, local health care personnel were contacted to obtain the patient's physical condition. The quality of evidence of this study is low. There was no blinding of the patient, caregiver and outcome assessors. Subjects who were most severe were excluded from the study (i.e., those who were stuporous, comatose or had received CPR). Use of gentamicin for patients on penicillin increased variability. Patients were adults and not all were confirmed leptospirosis which could lead to indirectness (Panaphut, 2003).

Suputtamongkol conducted an open label RCT in 4 hospitals in Thailand comparing penicillin with doxycycline and cefotaxime on 256 adult patients with severe leptospirosis (i.e., acute fever <15 days) in the absence of an obvious focus of infection. Excluded were those with diabetes and those with treatment for >48 hours against leptospirosis. Leptospirosis was confirmed for all patients by serologic testing or culture. Some patients had coincident rickettsioses (similar in the 3 groups) or gram-negative bacteremia. Patients received either penicillin g at 1.5 million units every 6 hours, cefotaxime at 1 gram IV every 6 hours, or doxycycline at 200 mg infused for 30 minutes then 100 mg every 12 hours. Treatment was switched to oral amoxicillin or oral doxycycline if the patient was well enough. Gentamicin was administered, at the discretion of individual investigators, when gram-negative sepsis could not be excluded (Group P=8, Group D=4, Group C=3, p=0.34). Outcomes included mortality, time to defervescence, reason for subsequent antimicrobial treatment, duration of renal and/or hepatic dysfunction, and duration of hospitalization. Those who died within 48 hours after admission were excluded from all analysis of clearance of fever. Twenty patients were excluded from subsequent efficacy analysis (no explanation given). The quality of evidence of this study is very low. There was no blinding of the patient, caregiver and outcome assessors. Subjects who were most severe were excluded from the analysis (i.e., those who died within 48 hours of treatment). Use of gentamicin for patients in the three groups, presence of coincident rickettsioses, and gram-negative bacteremia, increased variability of the study. Definition of severe illness was different from other studies as it was based on the number of days of fever. It is noted that not all enrolled patients had renal dysfunction or jaundice. Patients were adults which could also lead to indirectness (Suputtamongkol, 2004).

Phimda conducted a randomized controlled trial on doxycycline versus azithromycin in 4 hospitals in Thailand. Of the 296 patients enrolled, median age was 36 years old (range: 15 to 88 years old). Only 23.3% had leptospirosis, 4.1% had leptospirosis-rickettsia co-infection. Diagnosis was confirmed by isolation from blood or by MAT, although paired sera were not obtained in some patients. Sixty nine (69) cases of non-severe leptospirosis were randomly assigned either to a 7-day course of doxycycline or a 3-day course of azithromycin. There was a high drop-out rate of 30.1% (42 in doxycycline and 47 in azithromycin). Outcomes assessed were cure rate, time to defervescence, and adverse events. The quality of evidence of this study is very low. There was no blinding of the patient, caregiver and outcome assessor, and there was a high drop-out rate. Confirmation of leptospirosis using convalescent sera was not possible in some patients. Indirectness, imprecision and reporting bias were noted (Phimda, 2007).

The effectiveness of antibiotics in children with leptospirosis on the following outcomes was studied: 1) mortality, 2) duration of fever, and 3) renal complications and/or the need for dialysis.

EFFECT ON MORTALITY

Table 15. Summary of studies on the use of antibiotics in preventing mortality in children with leptospirosis

Study *(Study Design)*	Study Period	Patients (N)	Location	Outcome determined	Remarks	Quality
Brett-Major 2012 *Meta-analysis (RCTs only)*		All infected patients, both severe and non-severe. Majority or all subjects in the studies reviewed were adults. (N=403)		Primary: Mortality, hospitalization, ventilator requirement, dialysis requirement Secondary: No. of days on mechanical ventilator, no. of days on dialysis, adverse events that resulted in dose decrease or discontinuation of treatment or registration as an AE	Four out of seven studies purported to assess treatment in severe leptospirosis. However, in most cases clear definition of severity were not given and criteria were varying. The most severe patients were excluded in some of these 4 studies.	Low
Charan 2013 *Meta-analysis (RCT & Cohort)*		All leptospirosis patients, both severe and non-severe. Majority or all subjects in the studies reviewed were adults. (N=409)		Among the predetermined outcomes availability of data, those that could be compared were mortality, fever days, oliguria, number of dialysis, number of patients needing dialysis	Five studies assessed penicillin with no treatment or placebo. All studies looked into leptospirosis in the endemic population except Fairburn 1956 who studied military men.	Very low

Study *(Study Design)*	Study Period	Patients (N)	Location	Outcome determined	Remarks	Quality
Panaphut 2003 *RCT, open*	Jul 2000 to Dec 2001	Patient ≥ 16 years old with severe leptospirosis. Of those screened positive using IgM specific LEPTO dipstick assay, 72% were confirmed by MAT. (N=173)	Tertiary hospital in Thailand	Primary: time to resolution of fever after treatment. Secondary: mortality and time to resolution of organ dysfunction	All patients were adults. Severe leptospirosis was based on presence of jaundice, raised creatinine or MAP <70 mmHg. Gentamicin was administered in Group P with gram negative sepsis.	Low
Suputtamongkol 2004 *RCT, open*	Jul 2001 to Dec 2002	Adult patients with suspected severe leptospirosis. Diagnosis was confirmed by serologic testing and blood culture of serologic test (MAT, IFAT or MCAT). (N=256)	4 hospitals in Thailand	Mortality (at > 48 hours after treatment), clinical treatment failure, duration of fever, hospitalization and organ dysfunction after treatment	Gentamicin was administered when gram negative sepsis could not be excluded. When well enough, medication was shifted to oral amoxicillin (PCN group) or oral doxycycline (Doxycycline group). Patients who died within the first 48 hours of admission were excluded from analyses of fever clearance. Some patients had coincident rickettsioses.	Very Low

Antibiotic treatment

The effectiveness of an antibiotic (doxycycline or penicillin) compared to placebo or no intervention was presented in the meta-analysis by Brett-Major (Brett-Major, 2012). Of the four included studies that had mortality as an outcome of interest, death among patients occurred in only two studies (Costa, 2003; Edwards, 1988). Treatment with an antibiotic (doxycycline or penicillin) did not prevent death (OR: 1.16; 95% CI: 0.23 to 5.95; random effects model, I^2=50%).

Penicillin

Mortality was reported in the meta-analysis of Charan which compared penicillin with no treatment or placebo (Charan, 2013). Penicillin showed no protection for death as compared with control (OR: 1.70; 95% CI: 0.75 to 3.82, fixed effect model with p=0.19) on pooled analysis of three studies (Costa, 2003; Daher, 2000; Edwards, 1988).

Ceftriaxone

Comparison of ceftriaxone as compared to penicillin on 173 patients (Panaphut, 2003) showed no advantage on mortality (RR: 1.0; 95% CI: 0.3 to 3.3).

Cefotaxime

Comparison of cefotaxime with penicillin by Supputamongkol showed that although cefotaxime appeared to protect from death, this was not statistically significant (RR: 0.3; 95% CI: 0.0 to 3.1) (Supputamongkol, 2004).

The meta-analysis by Brett-Major pooling two studies on cephalosporins (Panaphut, 2003; Supputamongkol, 2004) reported no significant difference in mortality rates with the controls (OR: 0.65; 95% CI: -23 to 1.87; fixed model) (Brett-Major, 2012).

Doxycycline

Comparison of doxycycline with penicillin in the study by Supputamongkol showed no protection against mortality (RR: 1.1; 95% CI: 0.2 to 7.4) (Supputamongkol, 2004).

Azithromycin

No study compared azithromycin with other treatment on the mortality of patients with leptospirosis.

EFFECT ON THE DURATION OF FEVER

Table 16. Summary of studies on the effect of antibiotics in the duration of fever in children with leptospirosis

Study (Study Design)	Study Period	Patients (N)	Location	Outcome determined	Remarks	Quality
Brett-Major 2012 *Meta-analysis (RCTs only)*		All infected patients, both severe and non-severe. Majority or all subjects in the studies reviewed were adults. (N=403)		Primary: Mortality, hospitalization, ventilator requirement, dialysis requirement Secondary: No. of days on mechanical ventilator, no. of days on dialysis, adverse events that resulted in dose decrease or discontinuation of treatment or registration as an AE	Four out of seven studies purported to assess treatment in severe leptospirosis however, in most cases clear definition of severity were not given and criteria were varying. The most severe patients were excluded in some of these 4 studies.	Low
Charan 2013 *Meta-analysis (RCT & Cohort)*		All leptospirosis patients, both severe and non-severe. Majority or all subjects in the studies reviewed were adults. (N=409)		Among the predetermined outcomes availability of data, those that could be compared were mortality, fever days, oliguria, number of dialysis, number of patients needing dialysis	Five studies assessed penicillin with no treatment or placebo. All studies looked into leptospirosis in the endemic population except Fairburn 1956 who studied military men.	Very Low

Study (Study Design)	Study Period	Patients (N)	Location	Outcome determined	Remarks	Quality
Watt 1988 *RCT, placebo*	Sep-Nov,1985 and July-Oct 1986	Patients 16 years old and older with severe and late leptospirosis. Leptospirosis was confirmed by antibody titer or isolation of the organism from blood or urine. (N=42)	A national infectious disease hospital in the Philippines	Duration of fever after start of treatment, duration of increased serum creatinine, hematologic and biochemical variables, hospital duration and leptospiruria after treatment	The most severe cases: anuria, presence of confusion, stupor or coma, or a second illness were excluded.	Very Low
Panaphut 2003 *RCT, open*	Jul 2000 to Dec 2001	Patient ≥16 years old with severe leptospirosis. Of those screened positive using IgM specific LEPTO dipstick assay, 72% were confirmed by MAT. (N=173)	Tertiary hospital in Thailand	Primary: time to resolution of fever after treatment. Secondary: mortality and time to resolution of organ dysfunction	All patients were adults. Severe leptospirosis was based on presence of jaundice, raised creatinine or MAP <70 mmHg. Gentamicin was administered in Group P with gram negative sepsis.	Low

Study (Study Design)	Study Period	Patients (N)	Location	Outcome determined	Remarks	Quality
Suputtamongkol 2004 *RCT, open*	July 2001 to Dec 2002	Adult patients with suspected severe leptospirosis. Diagnosis was confirmed by serologic testing and blood culture of serologic test (MAT, IFAT or MCAT). (N=256)	4 hospitals in Thailand	Mortality (at > 48 hours after treatment), clinical treatment failure, duration of fever, hospitalization and organ dysfunction after treatment	Gentamicin was administered when gram negative sepsis could not be excluded. When well enough, medication was shifted to oral amoxicillin (PCN group) or oral doxycycline (Doxycycline group). Patients who died within the first 48 hours of admission were excluded from analyses of fever clearance. Some patients had coincident rickettsioses.	Very Low
Phimda 2007 *RCT, open*	Jul 2003 to Jan 2005	Patients suspected to have leptospirosis, non-severe between 15-88 years old. Diagnosis was confirmed by isolation from blood or MAT. (N=296)	4 hospitals in Thailand	Cure rate, time to defervescence, and adverse events	Of 296 enrolled subjects, only 23.3% had leptospirosis, and 4.1% were co-infected with rickettsia. Confirmation using convalescent sera was not possible in some patients. High drop out rate was noted.	Very Low

Antibiotic treatment

Meta-analysis of two studies by Brett-Major showed a trend for shorter duration of clinical illness by 4 days (MD: -4.04, 95% CI: -8.66 to 0.58; I^2=81%) among those given antibiotics (doxycycline or penicillin), but this was not significant (Brett-Major, 2012).

Penicillin

Watt reported an advantage with the use of penicillin showing a significantly shorter duration of fever (MD: -6.9 days; 95% CI: -2.65 to -11.15) and a greater proportion of patients who were afebrile on day 4 of Penicillin (RR: 10.4; 95% CI: 0.64 to 73.41) (Watt, 1988). However, in a meta-analysis of Charan (Charan, 2013), it was reported that fever days were similar between penicillin and controls after pooling results of three studies (MD: -0.15; 95% CI: 0.47 to 0.17; p=0.358) (Daher, 2000; Edward, 1988; Watt, 1988).

Ceftriaxone

Panaphut showed no advantage on duration of fever (MD: 0;95% CI: -0.2 to 0.2) on giving ceftriaxone as compared to penicillin (Panaphut, 2003).

Cefotaxime

Supputamongkol compared cefotaxime with penicillin and showed no advantage on time to defervescence (Median of 60 hours vs 72 hours, p=0.42) (Supputamongkol, 2004).

Meta-analysis by Brett-Major pooling studies on cephalosporins (Panaphut, 2003; Supputamongkol, 2004) reported no significant difference in fever days (MD: -0.03; 95% CI: -0.09 to 0.03, fixed model, I^2=94%) (Brett-Major, 2012).

Doxycycline

Comparison of doxycycline with penicillin in the study by Supputamongkol showed no advantage on time to defervescence (Median of 72 hours for both, p=0.42). Supputamongkol used multivariate analyses and showed that dysfunction of >2 organ systems at admission resulted in significantly longer duration of fever after treatment (p<0.001). Antimicrobial therapy (penicillin, doxycycline or cefotaxime) and onset of disease (early onset of <5 days versus late onset) were not associated with the duration of fever after treatment (p=0.56 and p=0.83, respectively) (Supputamongkol, 2004).

Azithromycin

Only one study (Phimda, 2007) compared doxycycline with azithromycin in non-severe leptospirosis. The primary outcome, cure rate, was defined as defervescence within 5 days of treatment (RR: 1.0; 95% CI: 1.0 to 1.1), and time to defervescence were comparable (Median= 45 hours vs 40 hours, p=0.45) in both groups.

EFFECT ON RENAL OUTCOMES

Table 17. Summary of studies on the use of antibiotics in reducing renal complications or the need for dialysis in children with leptospirosis

Study (Study Design)	Study Period	Patients (N)	Location	Outcome Determined	Remarks	Quality
Brett-Major 2012 *Meta-analysis (RCTs only*)		All infected patients, both severe and non-severe. Majority or all subjects in the studies reviewed were adults. (N=403)		Primary: Mortality, hospitalization, ventilator requirement, dialysis requirement Secondary: No. of days on mechanical ventilator, no. of days on dialysis, adverse events that resulted in dose decrease or discontinuation of treatment or registration as an AE	Four out of seven studies purported to assess treatment in severe leptospirosis however, in most cases clear definition of severity were not given and criteria were varying. The most severe patients were excluded in some of these 4 studies.	Low
Charan 2013 *Meta-analysis (RCT & Cohort)*		All leptospirosis patients, both severe and non-severe. Majority or all subjects in the studies reviewed were adults. (N=409)		Among the predetermined outcomes availability of data, those that could be compared were mortality, fever days, oliguria, number of dialysis, number of patients needing dialysis	Five studies assessed penicillin with no treatment or placebo. All studies looked into leptospirosis in the endemic population except Fairburn 1956 who studied military men.	Very Low

Study (Study Design)	Study Period	Patients (N)	Location	Outcome Determined	Remarks	Quality
Watt 1988 *RCT, placebo*	Sept-Nov, 1985 and July-Oct. 1986	Patients 16 years old and older with severe and late leptospirosis. Leptospirosis was confirmed by antibody titer or isolation of the organism from blood or urine. (N=42)	A national infectious disease hospital in the Philippines	Duration of fever after start of treatment, duration of increased serum creatinine, hematologic and biochemical variables, hospital duration and leptospiruria after treatment	The most severe cases: anuria, presence of confusion, stupor or coma, or a second illness were excluded.	Very Low
Daher 2000 *Cohort, prospective*	May 1996 to June 1998	Patients admitted with confirmed leptospirosis by antibody titers. All patients were on ARF (pl creatinine >1.5 mg/dl) and jaundice on admission. (N=35)	Nephrology service of a university hospital in Brazil	Mortality, oliguria, dialysis, days of hospitalization, days of fever, days required for serum creatinine, bilirubin, platelet count to reach normal	Most cases were males and ≥18 years old. Four patients who died within the first 48 hours of admission were excluded from the study.	Very Low
Panaphut 2003 *RCT, open*	Jul 2000 to Dec 2001	Patient ≥16 years old with severe leptospirosis. Of those screened positive using IgM specific LEPTO dipstick assay, 72% were confirmed by MAT. (N=173)	Tertiary hospital in Thailand	Primary: time to resolution of fever after treatment. Secondary: mortality and time to resolution of organ dysfunction	All patients were adults. Severe leptospirosis was based on presence of jaundice, raised creatinine or MAP <70 mmHg. Gentamicin was administered in Group P with gram negative sepsis.	Low

Antibiotic treatment

Pooling of two studies by Brett-Major showed that the rate of dialysis was comparable with no treatment or placebo, with a trend towards increased dialysis requirement noted when given antibiotics (OR: 1.54; 95% CI: 0.91 to 2.60; Fixed Effect) (Brett-Major, 2012).

Penicillin

The study of Watt showed that penicillin significantly shortened the duration of rise in creatinine by 5.6 days (MD: 5.6; 95% CI: 1.9 to 9.2) (Watt, 1988). However, those given penicillin had comparable risk for dialysis (OR: 1.59; 95% CI: 0.92 to 2.73) and oliguria (OR: 1.79; 95% CI: 0.32 to 9.93) as the no treatment or placebo group in the meta-analysis of Charan (Charan, 2013). Daher demonstrated that the days to normalization of creatinine was likewise comparable between penicillin and no antibiotic (MD: -1.0; 95% CI: -3.1 to 5.1) (Daher, 2000).

Ceftriaxone

Comparison of ceftriaxone with penicillin showed no advantage on renal failure rate (RR: 1.0; 95% CI: 0.7 to 1.4) (Panaphut, 2003).

Cefotaxime, doxycycline, and azithromycin

No studies reported the effectiveness of cefotaxime, doxycycline and azithromycin on renal outcomes of the patients with leptospirosis.

Considerations for Recommendation Development during the Stakeholders Panel (SP) Meeting:

- The strong recommendation from the stakeholders panel for the use of antibiotics despite the very low quality of evidence was based on the possibility of leptospirosis having serious complications being of a bacterial etiology.
- The availability of inexpensive antibiotics, absence of evidence to suggest harm, and bacterial etiology lend strength to the recommendation.

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CHAPTER 5

PREVENTION OF LEPTOSPIROSIS

Prevention of leptospirosis remains the priority since eradication is not a realistic goal (Illangasekera, 2008). Control strategies can target any of the nodal points in the transmission cycle: the animal carriers, the environment or the host (Sehgal, 2000). For resource-limited developing counties where the disease exists, the use of protective clothing, safe animal husbandry and immunization are financially not sustainable. Controlling rat populations is practically impossible (Illangasekera, 2008).

Vaccination against leptospirosis in humans does not seem possible due to the existence of more than 200 serovars of leptospires and due to the difference in geographical locations with different circulating serovars (Sehgal, 2000).

Currently, chemoprophylaxis is the only practical preventive measure against leptospirosis. However, the efficacy of chemoprophylaxis has not been sufficiently established because of few clinical trials. Limited studies have shown that chemoprophylaxis with doxycycline at 200 mg weekly, to start 1-2 days before and continuing through the period of exposure, might be effective in preventing clinical disease in adults and could be considered for those at high risk and with short-term exposures. In this chapter, we attempted to determine the usefulness of doxycycline as pre-exposure prophylaxis (Sehgal, 2000; Takafugi, 1984) and as post-exposure prophylaxis (Chusri, 2014; Gonsalez, 1998) for conferring protection against laboratory-identified leptospiral infection and symptomatic leptospirosis. Unfortunately, there are no published studies on the use of doxycycline as prophylaxis for leptospirosis in pediatric patients.

Definition of Terms:

Asymptomatic (laboratory-identified) leptospiral infection: presence of at least a four-fold seroconversion to a leptospiral serovar on the Microscopic Agglutination Test, or a positive culture, or both (Gonsalez, 1998; Takafuji, 1984).

Symptomatic leptospirosis: if the criteria for asymptomatic (laboratory-identified) leptospirosis infection is met and had symptoms of fever, chills, myalgia, headache conjunctival suffusion, meningitis, jaundice, or renal insufficiency (Gonsalez, 1998; Takafuji, 1984).

Question 8: How effective is doxycycline as pre-exposure prophylaxis in the prevention of leptospirosis in children?

Recommendation 1: Doxycycline as pre-exposure prophylaxis may be used to prevent both asymptomatic laboratory-identified leptospiral infection and symptomatic leptospirosis in those who live in, and intend to visit, highly endemic areas.
Quality of evidence: Very low
Strength of recommendation: Strong

Summary of Evidence

There were only two studies (Sehgal, 2000; Takafuji, 1984) that assessed the efficacy of pre-exposure prophylaxis with doxycycline. One study was among an indigenous population during an outbreak period (Sehgal, 2000), and the other study was among deployed soldiers for military training in the jungles (Takafuji, 1984).

Table 18. Summary of studies evaluating doxycycline as pre-exposure prophylaxis in the prevention of leptospirosis in children

Study *(Study Design)*	Study Period	Patients (N)	Location	Intervention	Outcome determined	Remarks
Sehgal 2000 *Single site prospective randomized placebo-controlled trial*	Sept-Dec 1998	Mix of residents including agricultural workers and adolescent school children from ages10 years old and above (N=782)	Diglipur town and adjoining villages in North Andaman, India	386 received doxycycline at 200 mg/week 396 received placebo (Vitamin B complex) Duration: started 2 weeks before the outbreak and continued for 12 weeks	Asymptomatic laboratory-identified leptospiral Infection Symptomatic leptospirosis Mortality Adverse Event	Diglipur is highly endemic for leptospirosis which might be the reason for the lack of impact of the drug regimen on the infection rates.
Takafuji 1984 *Single site prospective double-blind placebo-controlled randomized trial*	Fall of 1982	Active duty army soldiers deployed, younger and healthier population (N=940)	Fort Sherman training area in Panama	469 received doxycycline at 200 mg/week 471 received placebo Duration: 2-3 weeks from start of training to completion of military exercises	Asymptomatic laboratory-identified leptospiral infection Symptomatic leptospirosis Adverse Event	Only adults were included in this study.

Sehgal randomized all healthy persons aged 10 years old and above into two groups from North Andaman, India where leptospirosis was highly endemic. Group A was given doxycycline 200 mg/week (N=386) and Group B was given Vitamin B complex as placebo (N=396). The difference in the laboratory-identified leptospiral infection rates detected by Microscopic Agglutination Test between the two groups was not statistically significant (RR: 1.14; 95% CI: 0.90 to 1.43). However, the proportion of symptomatic leptospirosis was statistically significant between the two groups. There was a lower incidence of symptomatic leptospirosis among those given doxycycline (12, 3.11%) compared to placebo (27, 6.82%) ($p<0.05$). The ones given doxycycline had 54% reduction in the risk of developing symptomatic leptospirosis compared to those given placebo (RR: 0.46; 95% CI: 0.23 to 0.89). In addition, none from the doxycycline group developed complications, as compared with three patients from the placebo group who developed severe pulmonary complications and died (RR: 0.15; 95% CI: 0.0076 to 2.83). The results of the study showed that use of doxycycline as a pre-exposure prophylaxis did not reduce the incidence of asymptomatic laboratory-identified leptospiral infection in an endemic area, but had beneficial effect in reducing symptomatic leptospirosis and mortality (Sehgal, 2000).

Takafuji studied military personnel who were training in the jungles of the Republic of Panama for three weeks and were randomly assigned into two groups: doxycycline group and placebo group. Among the 469 participants from the doxycycline group, only one developed symptomatic leptospirosis. Among the 471 participants from the placebo group, there were 20 people with leptospiral infections who developed symptomatic leptospirosis. There was 95% protective efficacy ($p<0.001$) with doxycycline for both asymptomatic laboratory-identified leptospiral infection and symptomatic leptospirosis (RR: 0.05; 95% CI: 0.01 to 0.37) (Takafuji, 1984).

Pooled results from the two trials (Sehgal, 2000; Takafuji, 1984) show that as pre-exposure prophylaxis, doxycycline reduced the risk of developing asymptomatic laboratory-identified infection by 72% compared to placebo, but did not reach statistical significance (RR: 0.28; 95% CI: 0.01 to 7.16) (Figure 28).

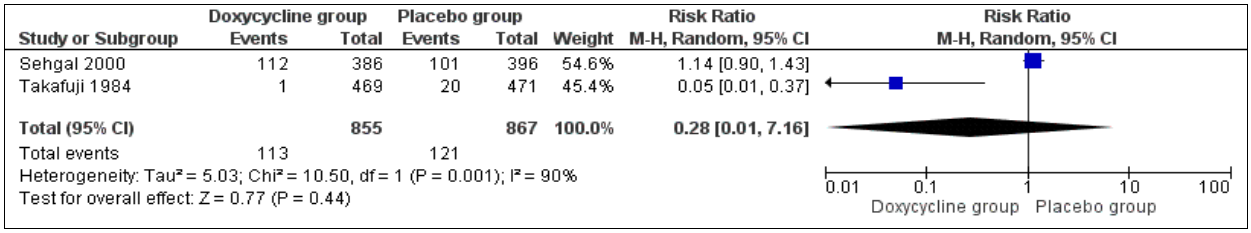

Figure 28. Forest plot of meta-analysis of data for the presence of asymptomatic laboratory-identified infection comparing those who were given pre-exposure doxycycline and those who were given placebo

Pooled data from the two trials (Sehgal 2000; Takafuji 1984) show protective efficacy of 82% in the prevention of symptomatic leptospirosis, but this did not reach statistical significance (RR: 0.18; 95% CI: 0.02 to 1.80). However, this result is non-inferior with a trend of benefit for doxycycline as pre-exposure prophylaxis to prevent symptomatic leptospirosis (Figure 29).

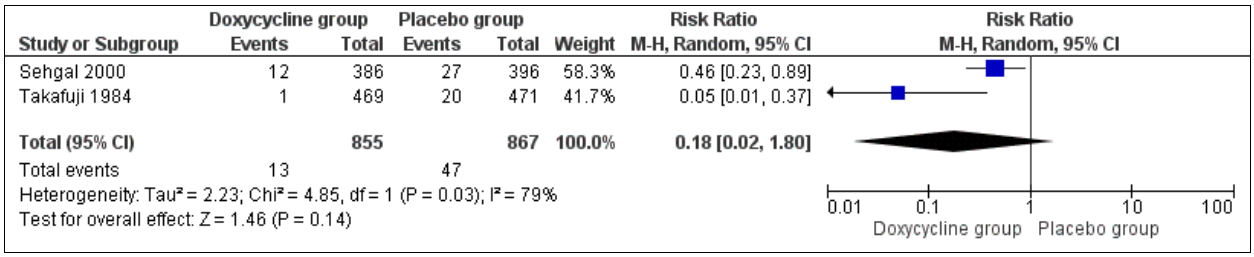

Figure 29. Forest plot of meta-analysis of data for the presence of symptomatic leptospirosis comparing those who were given pre-exposure doxycycline and those who were given placebo

In Takafuji's study, those in the doxycycline group were thirteen times more likely to experience nausea and vomiting, while only 1 had vomiting in the placebo group ($p<0.01$) (Takafuji, 1984). In Sehgal's study, adverse events could not be evaluated because there was no specific number of participants mentioned who experienced adverse events in both groups (Sehgal, 2000). Therefore, pooled data analysis for adverse events is not feasible.

It is important to note that the studies evaluated by the technical working group for leptospirosis pre-exposure prophylaxis involved children ≥10 years of age and adults. There were no published studies that looked into the benefits of pre-exposure prophylaxis with doxycycline for leptospirosis in pediatric patients, even in the local setting.

The quality of evidence for these two trials is very low because of inconsistency of results, indirectness as studies were on adults, and imprecision.

Considerations for Recommendation Development during the Stakeholders Panel (SP) Meeting:

- There is insufficient evidence to recommend the use of doxycycline as pre-exposure prophylaxis in children. However, the SP voted for a strong recommendation as the two studies done mostly in adults showed a trend of benefit towards the use of doxycycline as pre-exposure prophylaxis, even if the results were not statistically significant.
- Nausea and vomiting are strongly associated with the use of doxycycline, but are considered as non-serious side effects.

Question 9: How effective is doxycycline as post-exposure prophylaxis in preventing leptospirosis in children?

Recommendation 1: The use of doxycycline may be considered as post-exposure prophylaxis but there is no evidence in children to suggest that it can prevent symptomatic leptospirosis.
Quality of evidence: Very low
Strength of recommendation: Strong

Summary of Evidence

There were only two studies that evaluated patients aged 18 years old and above that were given doxycycline and placebo as post-exposure prophylaxis for leptospirosis (Chusri, 2014; Gonsalez, 1998). One is a randomized, double-blinded, placebo-controlled trial (Gonsalez, 1998) and the other is a non-randomized controlled trial (Chusri, 2014).

Table 19. Summary of studies on doxycycline as post-exposure prophylaxis in the prevention of leptospirosis in children

Study (Study Design)	Study Period	Patients (N)	Location	Intervention	Outcome determined	Remarks
Gonzalez 1998 *Double-blinded place randomized placebo-controlled trial*	After the Mar 29, 1992 flood	Among residents aged 18-74 years old after exposure to flooding, (N=82)	Cabucu District, Sao Paolo, Brazil	40 received doxycycline 200 mg as a single dose 42 received placebo as a single dose Given until 48 hours of exposure	Asymptomatic laboratory-identified leptospiral infection Symptomatic leptospirosis	Cabucu District is endemic for leptospirosis which might be the reason for the lack of impact of the drug regimen on the infection rates. Only adults were included in this study.
Chusri 2014 *Non-randomized controlled trial*	Oct 8 - 10, 2010	All residents 18 years old and above exposed to flood water since Oct 3,2010 (N=641)	Hat Yai City, Southern Thailand	600 received doxycycline 200 mg as a single dose 41 did not receive doxycycline Given 5-7 days from exposure	Asymptomatic laboratory-identified leptospiral infection Symptomatic leptospirosis Adverse Event	Hat Yai City is endemic for leptospirosis which might be the reason for the lack of impact of the drug regimen on the infection rates. Only adults were included in this study.

Chusri investigated the efficacy of a single dosage of 200 mg doxycycline against leptospirosis in residents aged 18 years old and above who were exposed to flooding in Southern Thailand. As post-exposure prophylaxis, doxycycline reduced the risk of developing asymptomatic laboratory-identified leptospiral infection by 77% compared to placebo (RR: 0.23; 95% CI: 0.08 to 0.66), while the risk for developing symptomatic leptospirosis was reduced by 86% (RR: 0.14; 95% CI: 0.2 to 1.1) (Chusri, 2014).

In addition, the study by Chusri found that having a lacerated wound was associated significantly with asymptomatic laboratory-identified leptospiral infection (OR: 37.20; P<0.001) and symptomatic leptospirosis (OR: 18.24; P=0.003). Those who had ≤3 hours exposure to flood per day was also associated with asymptomatic laboratory-identified leptospiral infection (OR: 3.70; P=0.038). The use of doxycycline as prophylaxis, even among those with lacerated wound, showed a protective efficacy of 92% (95% CI: 81.2% to 96.6.%) for asymptomatic laboratory-identified leptospiral infection, and 95.6% (95% CI: 78.2% to 99.3%) for symptomatic leptospirosis. The use of doxycycline among those with exposure to flood waters of ≤3 hours, showed a protective efficacy of 89.2% (95% CI: 63.6% to 96.67%) against asymptomatic laboratory-identified leptospiral infection but, there was no mention of protection against symptomatic leptospirosis. Twelve participants in the doxycycline group developed gastrointestinal symptoms, ten of whom developed nausea without vomiting. However, none of these twelve patients developed symptomatic leptospirosis or asymptomatic laboratory-identified leptospiral infection. One participant had skin rash involving the anterior chest wall and neck, which resolved spontaneously. The proportion of gastrointestinal and skin problems was not significantly different between the two groups (P=0.54 and P=0.33, respectively) (Chusri, 2014).

Gonsalez, on the other hand, conducted a trial to determine the effectiveness of single dose of doxycycline among participants 18-74 years old in preventing leptospirosis after high-exposure to flooding of potentially contaminated water in Sao Paulo, Brazil. Among those who were given doxycycline (40 subjects), eleven (11) had asymptomatic laboratory-identified leptospiral infection, while two had symptomatic leptospirosis. In the placebo group (42 subjects), six (6) had asymptomatic laboratory-identified leptospiral infection while five had symptomatic leptospirosis. The risk of having asymptomatic laboratory-identified leptospiral infection among those who were given doxycycline was almost twice as compared to placebo (RR: 1.92; 95% CI: 0.79 to 4.71). The risk of developing symptomatic leptospirosis after being given doxycycline was reduced by 58% as compared to those given placebo (RR: 0.42; 95% CI: 0.86 to 2.04). However, the association was not statistically significant, and the study did not have statistical power to determine more accurate estimates of the magnitude of the potential protection (Gonsalez, 1998).

Pooled analysis of the two trials (Chusri, 2014; Gonsalez, 1998) showed that as post-exposure prophylaxis, doxycycline had no effect in reducing the risk of developing asymptomatic laboratory-identified leptospiral infection compared to placebo (RR: 0.67; 95% CI: 0.08 to 5.59) (Figure 30).

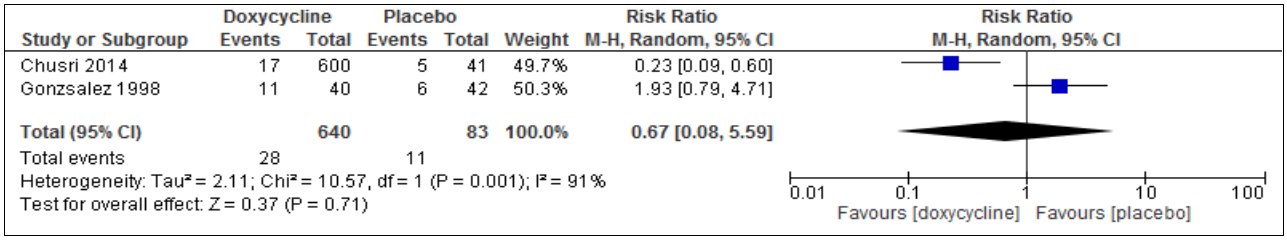

Figure 30. Forest plot of meta-analysis of data for the presence of asymptomatic laboratory-identified leptospiral infection comparing those who were given post-exposure doxycycline and those who were given placebo

The protective efficacy of doxycycline against symptomatic leptospirosis on pooled data was 75%, and statistically significant (RR: 0.25; 95% CI: 0.08 to 0.78) (Figure 31).

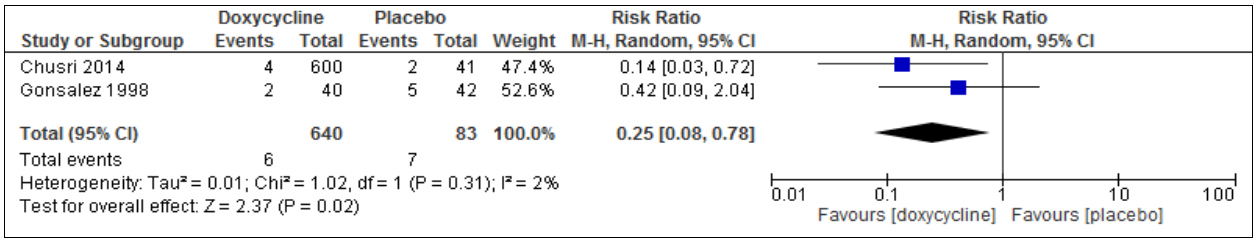

Figure 31. Forest plot of meta-analysis of data for the presence of symptomatic leptospirosis comparing those who were given post-exposure doxycycline and those who were given placebo

In Chusri's study, the use of doxycycline was associated with an increased risk of gastrointestinal adverse events. Minor adverse events occurred twice as more in those given doxycycline (12 had nausea and/or vomiting) (RR: 1.75; 95% CI: 0.11 to 29). There was no increased risk of rash among those given doxycycline (RR: 0.21; 95% CI: 0.01 to 5.07) (Chusri, 2014).

The quality of evidence for these two trials is very low because of risk of bias, inconsistency of results, indirectness as studies were on adults, and imprecision.

Considerations for Recommendation Development during the Stakeholders Panel (SP) Meeting:

- There is insufficient evidence to recommend the use of doxycycline as post-exposure prophylaxis in children as the studies evaluated included mostly adults. However, the SP voted for a strong recommendation since the studies showed protective efficacy of doxycycline against symptomatic leptospirosis and the results were statistically significant.
- Despite adverse events associated with doxycycline and its contraindication for use in children <8 years of age, it may still be used as prophylaxis considering that the dose (4 mg/kg) and duration (single dose) for this indication is unlikely to cause dental staining.

Question 10: Is there evidence to recommend the use of antibiotics other than doxycycline as post-exposure prophylaxis for leptospirosis in children?

Recommendation 1: Oral penicillin may be used for post-exposure prophylaxis to prevent symptomatic leptospirosis in high transmission areas but there are no studies in children.
Quality of evidence: Very low
Strength of recommendation: Strong

Summary of Evidence

There was only one study that used another antibiotic other than doxycycline as post-exposure prophylaxis (Illangasekera, 2008). This study evaluated whether oral penicillin can be used as chemoprophylaxis against leptospirosis in high transmission areas in central Sri Lanka in October 2005. The study recruited full-time farmers, ages 20 to 80 years old, who engaged in active farming on most days during the study period. Subjects were randomly assigned to take either oral penicillin 500 mg twice daily or placebo over a month during the active farming season. There were 152 farmers given penicillin and 167 farmers given placebo. In the treatment group, none developed symptomatic leptospirosis. In the placebo group, three had symptomatic leptospirosis. Since there was a small number of patients included, statistical analysis was not achievable (Illangasekera, 2008).

Penicillin, as post-exposure prophylaxis, reduced the risk of developing symptomatic leptospirosis by 85%, but this did not reach statistical significance (RR: 0.15; 95% CI: 0.01 to 2.92). There was no mention of asymptomatic laboratory-identified leptospiral infection in both study groups.

The quality of evidence for this study is very low due to indirectness as the study involved adults, and due to imprecision.

There were no clinical studies on the use of azithromycin, amoxicillin, ampicillin, ciprofloxacin, erythromycin, clarithromycin, streptomycin, ceftriaxone, cefotaxime, cefepime, imipenem-cilastatin, moxifloxacin, and levofloxacin as post-exposure prophylaxis.

Table 20. Summary of the study on penicillin as post-exposure prophylaxis for leptospirosis

Study *(Study Design)*	Study Period	Patients (N)	Location	Intervention	Outcome determined	Remarks
Illangasekera 2008 *Randomized double blinded placebo-controlled trial*	Oct 2005	Full-time farmers who engaged in active farming on most days, ages 20-80 years old (N=602)	High transmission area in the Medical Officer of Health (MOH) division of Yatinuwara and Udunuwara in the Central Province, Sri Lanka	Oral penicillin 500 mg twice daily or placebo beginning the day before farming 292 on oral penicillin, 143 with poor compliance 310 on placebo,143 with poor compliance Duration: beginning the day before farming and continued over a month during active farming season	Symptomatic leptospirosis	There were only adults in this study.

Considerations for Recommendation Development during the Stakeholders Panel (SP) Meeting:

- There is insufficient evidence to recommend the use of penicillin as post-exposure prophylaxis in children as the only study available was on adults. However, the SP voted for a strong recommendation despite insufficient evidence since the study showed a trend of benefit towards the use of penicillin as post-exposure prophylaxis against symptomatic leptospirosis, even if the results were not statistically significant.

References for Chapter 5

Background on Prevention of Leptospirosis:

Chusri S, McNeil EB, Hortiwakul T, Charernmak B, Sritrairatchai S, Santimaleeworagun W, et al. Single dosage of doxycycline for prophylaxis against leptospiral infection and leptospirosis during urban flooding in southern Thailand: A non-randomized controlled trial. *Journal of Infection and Chemotherapy.* 2014;20(11):709-15.

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Illangasekera VL, Kularatne SA, Kumarasiri PV, Pussepitiya DM, Premaratne MD. Is oral penicillin an effective chemoprophylaxis against leptospirosis? A placebo controlled field study in the Kandy District, Sri Lanka. *Southeast Asian Journal of Tropical Medicine and Public Health.* 2008;39(5):882.

Sehgal SC, Sugunan AP, Murhekar MV, Sharma S, Vijayachari P. Randomized controlled trial of doxycycline prophylaxis against leptospirosis in an endemic area. *International journal of antimicrobial agents*. 2000;13(4):249-55.

Takafuji ET, Kirkpatrick JW, Miller RN, Karwacki JJ, Kelley PW, Gray MR, et al. An efficacy trial of doxycycline chemoprophylaxis against leptospirosis. *New England Journal of Medicine.* 1984;310(8):497-500.

Question No. 8:

Sehgal SC, Sugunan AP, Murhekar MV, Sharma S, Vijayachari P. Randomized controlled trial of doxycycline prophylaxis against leptospirosis in an endemic area. *International journal of antimicrobial agents*. 2000;13(4):249-55.

Takafuji ET, Kirkpatrick JW, Miller RN, Karwacki JJ, Kelley PW, Gray MR, et al. An efficacy trial of doxycycline chemoprophylaxis against leptospirosis. *New England Journal of Medicine.* 1984;310(8):497-500.

Question No. 9:

Chusri S, McNeil EB, Hortiwakul T, Charernmak B, Sritrairatchai S, Santimaleeworagun W, et al. Single dosage of doxycycline for prophylaxis against leptospiral infection and leptospirosis during urban flooding in southern Thailand: A non-randomized controlled trial. *Journal of Infection and Chemotherapy.* 2014;20(11):709-15.

Gonsalez CR, et al. Use of doxycycline for leptospirosis after high-risk exposure in Sao Paulo, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo.* 1998;40(1): 59-61.

Question No. 10:

Illangasekera VL, Kularatne SA, Kumarasiri PV, Pussepitiya DM, Premaratne MD. Is oral penicillin an effective chemoprophylaxis against leptospirosis? A placebo controlled field study in the Kandy District, Sri Lanka. *Southeast Asian Journal of Tropical Medicine and Public Health.* 2008;39(5):882.

APPENDIX A

SUMMARY OF EVIDENCE TABLES WITH GRADE ASSESSMENT FOR OVER-ALL QUALITY

Question 1: Among children with acute fever and possible exposure, what clinical manifestations should make one suspect leptospirosis?

Bibliography:

1. Agampodi SB, Dahanayaka NJ, Nöckler K, Anne MS, Vinetz JM. Redefining gold standard testing for diagnosing leptospirosis: further evidence from a well-characterized, flood-related outbreak in Sri Lanka. *Am J Trop Med Hygiene*. 2016;95(3):531-6.
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Summary of Findings Table

Clinical Manifestations	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations *	Over-all Quality	OR/RR/HR or MD	Importance
Chest pain	Cross-sectional (Kendall, 2010)	549 hospitalized children & adults	Serious [1]	None	Serious[3]	Serious[4]	Very strong association[6] (Converted RR: 15.96)	VERY LOW	OR 18.8 (4.4 - 81.4)	Important

Clinical Manifestations	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations *	Over-all Quality	OR/RR/HR or MD	Importance
Cardiac syndrome	Cross-sectional (Goarant, 2009)	508 hospitalized children & adults	Serious [1]	None	Serious[3]	Serious[4]	Very strong association[6] (Converted RR: 6.33)	VERY LOW	OR 6.7 (2.3 - 19.2)	Important
Renal syndrome	Cross-sectional (Goarant, 2009)	508 hospitalized children & adults	Serious [1]	None	Serious[3]	Serious[4]	Very strong association[6] (Converted RR: 5.00)	VERY LOW	OR 6.3 (3.3 - 12.2)	Important
Conjunctival suffusion/red eyes	Cross-sectional (Agampodi, 2016; Goarant, 2009; Karande, 2003; Morgan, 2002)	882 hospitalized children & adults	Very Serious [1,5]	Serious[2]	Serious[3]	Serious[4]	Strong association[6] (Converted RR: 3.85)	VERY LOW	OR 5.64 (2.46 - 12.91)	Important
Arthralgia	Cross-sectional (Agampodi, 2016)	76 hospitalized children & adults	Very Serious [1,5]	None	Serious[3]	Serious[4]	None	VERY LOW	OR 3.4 (1.0 - 11.85)	Important
Anuria	Cross-sectional (Agampodi, 2016)	76 hospitalized children & adults	Very Serious [1,5]	None	Serious[3]	Serious[4]	None	VERY LOW	OR 3.06 (0.14 - 66.15)	Important
Eye pain	Cross-sectional (Ellis, 2008; Kendall, 2010; Morgan, 2002)	1,800 hospitalized children & adults	Serious [1]	Serious[2]	Serious[3]	Serious[4]	None	VERY LOW	OR 2.95 (0.38 - 23.00)	Important

Clinical Manifestations	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations *	Over-all Quality	OR/RR/HR or MD	Importance
Myalgia	Cross-sectional (Agampodi, 2016; Ellis, 2008; Goarant, 2009; Karande, 2003; Kendall, 2010; Morgan, 2002)	2,350 hospitalized children & adults	Very Serious [1, 5]	Serious[2]	Serious[3]	Serious[4]	None	VERY LOW	OR 2.81 (0.92 - 8.60)	Important
Headache	Cross-sectional (Agampodi, 2016; Ellis, 2008; Goarant, 2009; Karande, 2003; Kendall, 2010; Libraty, 2007; Morgan, 2002)	2,572 hospitalized children & adults	Very Serious [1, 5]	Serious[2]	Serious[3]	Serious[4]	None	VERY LOW	OR 2.45 (0.80 - 7.51)	Important
Icterus/jaundice	Cross-sectional (Agampodi, 2016; Goarant, 2009; Karande, 2003)	637 hospitalized children & adults	Very Serious [1, 5]	Serious[2]	Serious[3]	Serious[4]	None	VERY LOW	OR 2.31 (0.46 - 11.50)	Important

Clinical Manifestations	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations *	Over-all Quality	OR/RR/HR or MD	Importance
Abdominal pain	Cross-sectional (Agampodi, 2016; Karande, 2003; Kendall, 2010; Libraty, 2007)	766 hospitalized children & adults	Very Serious [1, 5]	Serious[2]	Serious[3]	Serious[4]	None	VERY LOW	OR 2.15 (0.96 - 4.85)	Important
Hemorrhage	Cross-sectional (Goarant, 2009; Kendall, 2010; Libraty, 2007)	1289 hospitalized children & adults	Serious [1]	Serious[2]	Serious[3]	Serious[4]	None	VERY LOW	OR 2.11 (0.68 - 6.61)	Important
Muscle tenderness	Cross-sectional (Agampodi, 2016)	76 hospitalized children & adults	Very Serious [1, 5]	None	Serious[3]	Serious	None	VERY LOW	OR 2.11 (0.75 - 6.00)	Important
Meningeal syndrome/meningismus	Cross-sectional (Goarant, 2009; Karande, 2003)	561 hospitalized children & adults	Serious [1]	Serious[2]	Serious[3]	Serious[4]	None	VERY LOW	OR 2.06 (0.40 - 10.56)	Important
Prostration	Cross-sectional (Agampodi, 2016)	76 hospitalized children & adults	Very Serious [1,5]	None	Serious[3]	Serious[4]	None	VERY LOW	OR 2.01 (0.68 - 5.92)	Important

Clinical Manifestations	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations *	Over-all Quality	OR/RR/HR or MD	Importance
Anorexia	Cross-sectional (Agampodi, 2016)	76 hospitalized children & adults	Very Serious [1,5]	None	Serious[3]	Serious[4]	None	VERY LOW	OR 1.87 (0.49 - 7.13)	Important
Proteinuria	Cross-sectional (Agampodi, 2016)	76 hospitalized children & adults	Very Serious [1, 5]	None	Serious[3]	Serious[4]	None	VERY LOW	OR 1.8 (0.18 - 18.19)	Important
Skin rash	Cross-sectional (Agampodi, 2016; Ellis, 2008; Karande, 2002; Kendall, 2010; Libraty, 2007)	1,976 hospitalized children & adults	Very VSeriou s[1,5]	Serious[2]	Serious[3]	Serious[4]	None	VERY LOW	OR: 1.70 (0.59 - 4.84)	Important
Diarrhea	Cross-sectional (Agampodi, 2016)	76 hospitalized children & adults	Very Serious [1,5]	None	Serious[3]	Serious[4]	None	VERY LOW	OR 1.37 (0.42 - 4.44)	Important
Positive Kernig's sign	Cross-sectional (Agampodi, 2016)	76 hospitalized children & adults	Very Serious [1,5]	None	Serious[3]	Serious[4]	None	VERY LOW	OR 1.37 (0.42 - 4.44)	Important
Oliguria	Cross-sectional (Agampodi, 2016)	76 hospitalized children & adults	Very Serious [1,5]	None	Serious[3]	Serious[4]	None	VERY LOW	OR 1.14 (0.41 - 3.16)	Important

Clinical Manifestations	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations *	Over-all Quality	OR/RR/HR or MD	Importance
Hematuria	Cross-sectional (Agampodi, 2016)	76 hospitalized children & adults	Very Serious [1,5]	None	Serious[3]	Serious[4]	None	VERY LOW	OR 1.14 (0.41 - 3.16)	Important

Rating of Quality of Evidence: Not Serious, Serious, and Very Serious. Inconsistency is rated as Serious if the heterogeneity is I^2 = <50%
OR: Odds ratio; RR: Relative Risk; HR: Hazard Ratio; MD: Mean Difference
*Factors that can decrease or increase the quality of the evidence include reporting bias, large magnitude of an effect, dose-response gradient, and effect of plausible residual confounding

ENDNOTES:

1. Risk of bias inherent due to study design
2. Heterogeneity is significant
3. Indirectness due to inclusion of more adults in the population studied.
4. Imprecision due to overlapping of the confidence interval with the null value and due to wide confidence interval.
5. Risk of bias due to inclusion of probable cases
6. Magnitude of effect is large (RR >2 or <0.5) or very large effect (RR >5 or <0.2)

Question 2: Among children with leptospirosis, what are the signs and symptoms associated with an increased risk of mortality?

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Summary of Findings Table

Clinical Signs or Symptoms	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations	Over-all Quality	OR/RR/ HR or MD	Importance
Pulmonary hemorrhage	Cross-sectional (Mendoza, 2013)	259 adults	Serious [1]	None	Serious[4]	Serious[5]	Very strong association[6] (Converted RR: 14.58)	VERY LOW	OR 48.54 (13.27 - 177.51)	Critical
Pallor	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	Very strong association[6] (Converted RR: 27.50)	VERY LOW	OR 29.9 (1.8 - 505.2)	Important

Clinical Signs or Symptoms	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations	Over-all Quality	OR/RR/ HR or MD	Importance
Loss of consciousness	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	Very strong association[6] (Converted RR: 27.50)	VERY LOW	OR 29.9 (1.8 - 505.2)	Critical
Murmur	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	Very strong association[6] (Converted RR: 13.95)	VERY LOW	OR 14.9 (1.3 - 175.2)	Important
Meningism	Cross-sectional (Pappachan, 2004)	282 children & adults	Serious[1]	None	Serious[4]	Serious[5]	Very strong association[6] (Converted RR: 8.23)	VERY LOW	OR 10.6 (2.3 - 48)	Important
Irregular Rhythm	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 9.9 (1 - 102)	Important
Dyspnea	Case-control Cross-sectional (Bonus, 2016; Pappachan, 2004)	686 children &adults	Very serious[1,2]	Not Serious	Serious[4]	Serious[5]	Very strong association[6] (Converted RR: 5.50)	VERY LOW	OR 9.13 (4.20 - 19.88)	Critical
Convulsion/ Seizure	Cross-sectional Case-control (Amilasan, 2012; Bonus, 2016)	875 children & adults	Very serious[1,2]	Not Serious	Serious[4]	Serious[5]	Very strong association[6] (Converted RR: 7.55)	VERY LOW	OR 7.81 (1.39 - 43.84)	Critical

Clinical Signs or Symptoms	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations	Over-all Quality	OR/RR/ HR or MD	Importance
Crackles/rales on lung auscultation	Cross-sectional Case-control (Bonus, 2016; Daher, 2010)	605 children & adults	Very serious[1,2]	Not Serious	Serious[4]	Serious[5]	Very strong association[6] (Converted RR: 5.20)	VERY LOW	OR 7.12 (3.28 - 15.44)	Important
Hemoptysis	Cross-sectional Case-control (Amilasan, 2012; Bonus, 2016; Pappachan, 2004)	1,157 children & adults	Very serious[1,2]	Not Serious	Serious[4]	Serious[5]	Very strong association[6] (Converted RR: 6.24)	VERY LOW	OR 6.93 (3.07 - 15.66)	Important
Anuria	Cross-sectional Case-control (Amilasan, 2012; Bonus, 2016)	875 children &adults	Very serious[1,2]	Not Serious	Serious[4]	Serious[5]	Very strong association[6] (Converted RR: 5.77)	VERY LOW	OR 6.52 (2.93 - 14.51	Critical
Hematemesis	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 5.4 (0.2 - 116.8)	Important
Disorientation	Cross-sectional (Pappachan, 2004)	282 children & adults	Serious[1]	None	Serious[4]	Not Serious	Strong association[6] (Converted RR: 3.75)	VERY LOW	OR 5 (1.3 - 17.6)	Critical

Clinical Signs or Symptoms	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations	Over-all Quality	OR/RR/ HR or MD	Importance
Jaundice	Cross-sectional Case-control (Amilasan, 2012; Bonus, 2016; Lopes, 2010; Pappachan, 2004)	1,997 children & adults	Very serious[1,2]	Not Serious	Serious[4]	Not Serious	None (Converted RR: 1.54)	VERY LOW	OR 4.76 (2.99 - 7.59)	Important
Tachycardia	Cross-sectional (Pappachan, 2004)	282 children & adults	Serious[1]	None	Serious[4]	Not Serious	None	VERY LOW	OR 4.1 (1.2 - 13.1)	Important
Decreased Breath Sounds	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 4.2 (0.5 - 36.8)	Important
Retroorbital pain	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 3.8 (0.2 - 77.4)	Important
Signs of Dehydration	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 2.8 (0.7 - 10.4)	Important
Epistaxis	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 2.7 (0.3 - 22.1)	Important

Clinical Signs or Symptoms	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations	Over-all Quality	OR/RR/ HR or MD	Importance
Oliguria	Cross-sectional Case-control (Amilasan, 2012; Bonus, 2016; Daher, 2010; Pappachan, 2004)	1,358 children & adults	Very serious[1,2]	Serious[3]	Serious[4]	Serious[5]	None	VERY LOW	OR 2.66 (0.68 - 10.41)	Critical
Hypotension	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 2.3 (0.6 - 8.7)	Critical
Edema	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 2.1 (0.3 - 16.9)	Important
Melena	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 2.1 (0.3 - 16.9)	Important
Gum Bleeding	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 2 (0.1 - 38)	Important
Malaise	Cross-sectional Case-control (Amilasan, 2012; Bonus, 2016)	875 children &adults	Very serious[1,2]	Serious[3]	Serious[4]	Serious[5]	None	VERY LOW	OR 1.98 (0.46 - 8.54)	Important
Skin Hemorrhage	Cross-sectional (Amilasan, 2012)	471 children &adults	Serious[1]	None	Serious[4]	Serious[5]	None	VERY LOW	OR 1.8 (0.1 - 38.5)	Important

Clinical Signs or Symptoms	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations	Over-all Quality	OR/RR/ HR or MD	Importance
Presence of Wound Lesions	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 1.8 (0.1 - 38.5)	Important
Chills/rigor	Case-control Cross-sectional (Bonus, 2016; Pappachan, 2004)	686 children & adults	Very serious[1,2]	Not Serious	Serious[4]	Serious[5]	None	VERY LOW	OR 1.73 (0.73 - 4.13)	Important
Anorexia	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR 1.7 (0.5 - 6.4)	Not Important
Dysuria	Case-control (Bonus, 2016)	404 children	Very serious[1,2]	None	Not Serious	Serious[5]	None	VERY LOW	OR: 1.6 (0.09 - 28.2).	Important
Conjunctival suffusion	Cross-sectional Case-control (Amilasan, 2012; Bonus, 2016; Pappachan, 2004)	1,157 children & adults	Very serious[1,2]	Not Serious	Serious[4]	Serious[5]	None	VERY LOW	OR 1.40 (0.77 - 2.57)	Important
Diarrhea	Cross-sectional Case-control (Amilasan, 2012; Bonus, 2016)	875 children & adults	Very serious[1,2]	Not Serious	Serious[4]	Serious[5]	None	VERY LOW	OR 1.40 (0.83 - 2.34)	Not Important

Clinical Signs or Symptoms	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations	Over-all Quality	OR/RR/ HR or MD	Importance
Abdominal pain	Cross-sectional Case-control (Amilasan, 2012; Bonus, 2016; Pappachan, 2004)	1,157 children & adults	Very serious[1,2]	Serious[3]	Serious[4]	Serious[5]	None	VERY LOW	OR 1.31 (0.53 - 3.26)	Important

Rating of Quality of Evidence: Not Serious, Serious, and Very Serious.
OR: Odds ratio; RR: Relative Risk; HR: Hazard Ratio; MD: Mean Difference
*Factors that can decrease or increase the quality of the evidence include reporting bias, large magnitude of an effect, dose-response gradient, and effect of plausible residual confounding

ENDNOTES:

1. Risk of bias inherent due to study design
2. Risk of bias due to inclusion of probable cases
3. Heterogeneity is significant
4. Indirectness due to inclusion of more adults in the population studied
5. Imprecision due to overlapping of the confidence interval with the null value or due to wide confidence interval
6. Magnitude of effect is large (RR >2 or <0.5) or very large effect (RR >5 or <0.2)

Question 3: What laboratory findings are associated with severe leptospirosis?

Bibliography:

1. Bonus RB, Maramba-Lazarte C, Gomez-Go GD, De Jesus J, Asinas-Tan M. Predictors of mortality among pediatric patients with leptospirosis: a multicenter retrospective study. *Ped Infect Dse Soc Phil Journal.* 2016; 17(1): 14-28.
2. Hochedez P, et al. Factors associated with severe leptospirosis, Martinique, 2010–2013. *Emerging infectious diseases.* 2015;21(12):2221-4.
3. Mikulski M, et al. Severity markers in severe leptospirosis: a cohort study. *European Journal of Clinical Microbiology & Infectious Diseases.* 2015;34(4):687-695.

Summary of Findings Table

Clinical Manifestations	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Reporting Bias	Over-all Quality	OR	Importance
Deranged Prothrombin time	Case control (Bonus, 2016)	404 children	Serious[1]	Not Serious	Not Serious	Serious[3]	Undetected	VERY LOW	OR 23 (2.8 - 189.7)	Important
	Cross-sectional (Hochedez, 2015)	102 adults	Serious[1]	Not Serious	Very Serious[2]	Serious[3]	Undetected	VERY LOW	OR 5.5 (1.5 - 20.1)	Important
Elevated AST/ALT ratio	Cohort (Mikulski, 2015)	47 adults	Serious[1]	Undetected	Very Serious[2]	Serious[3]	Undetected	VERY LOW	OR 7.1 (1.8 - 28.1)	Important
Elevated LDH	Cohort (Mikulski, 2015)	47 adults	Serious[1]	Undetected	Very Serious[2]	Serious[3]	Undetected	VERY LOW	OR 5.8 (1.3 - 25.6)	Important
Elevated C-reactive protein	Cross-sectional (Hochedez, 2015)	102 adults	Serious[1]	Undetected	Very Serious[2]	Serious[3]	Undetected	VERY LOW	OR 5.2 (1.5 - 18.3)	Important
Elevated creatine phosphokinase	Cross-sectional (Hochedez, 2015)	102 adults	Serious[1]	Undetected	Very Serious[2]	Serious[3]	Undetected	VERY LOW	OR 4.6 (1.1 - 19.6)	Important

Clinical Manifestations	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Reporting Bias	Over-all Quality	OR	Importance
Elevated bilirubin	Case-control (Bonus, 2016)	404 children	Serious[1]	Not Serious	Not Serious	Serious[3]	Undetected	VERY LOW	OR 3.72 (0.19 - 74.49)	Important
	Cross-sectional (Hochedez, 2015)	102 adults	Serious[1]	Not Serious	Very Serious[2]	Serious[3]	Undetected	VERY LOW	OR 5.4 (1.5 - 18.9)	Important
	Cross-sectional (Mikulski, 2015)	47 adults	Serious[1]	Undetected	Very Serious[2]	Serious[3]	Undetected	VERY LOW	OR 5 (1.3 - 20.0)	Important
Thrombocytopenia	Case-control (Bonus, 2016)	404 children	Serious[1]	Not Serious	Not Serious	Serious[3]	Undetected	VERY LOW	OR 2.3 (0.7 - 7.6)	Important
	Cross-sectional (Hochedez, 2015)	102 adults	Serious[1]	Undetected	Very Serious[2]	Serious[3]	Undetected	VERY LOW	OR 5.2 (1.5 - 18.1)	Important
Elevated creatinine	Case-control (Bonus, 2016)	404 children	Serious[1]	Not Serious	Not Serious	Serious[3]	Undetected	VERY LOW	OR 2.6 (0.3 - 21.1)	Important
	Cross-sectional (Hochedez, 2015)	102 adults	Serious[1]	Undetected	Very Serious[2]	Serious[3]	Undetected	VERY LOW	OR 5.2 (1.5 - 18.1)	Important
Elevated BUN	Case-control (Bonus, 2016)	404 children	Serious[1]	Not Serious	Not Serious	Serious[3]	Undetected	VERY LOW	OR 6.2 (0.4 - 107.1)	Important
	Cross-sectional (Hochedez, 2015)	102 adults	Serious[1]	Undetected	Very Serious[2]	Serious[3]	Undetected	VERY LOW	OR 3.5 (0.8 - 15.4)	Important

Clinical Manifestations	Study Design	Quality Assessment						Summary of Findings		
		Participants	Risk of Bias	Inconsistency	Indirectness	Imprecision	Reporting Bias	Over-all Quality	OR	Importance
Hematuria	Case-control (Bonus, 2016)	404 children	Serious[1]	Not Serious	Not Serious	Serious[3]	Undetected	VERY LOW	OR 5.4 (1 - 30.2)	Important
Decrease in hemoglobin	Case-control (Bonus, 2016)	404 children	Serious[1]	Not Serious	Not Serious	Serious[3]	Undetected	VERY LOW	OR 1.2 (0.3 - 4.4).	Important
	Cross-sectional (Hochedez, 2015)	102 adults	Serious[1]	Undetected	Very Serious[2]	Serious[3]	Undetected	VERY LOW	OR 3.5 (1 - 12)	Important

ENDNOTES:

1. Risk of bias due to inclusion of probable cases
2. Indirectness due to inclusion of only adults in the population studied.
3. Imprecision due to overlapping of the confidence interval with the null value or due to wide confidence interval

Question 4: Can IgM Immunochromatography Test (ICT) be used as a rapid test in the diagnosis of leptospirosis in children?

Bibliography:

1. Iwasaki H, Chagan-Yasutan H, Leano PS, Koizumi N, Nakajima C, Taurustiati D, et al. Combined antibody and DNA detection for early diagnosis of leptospirosis after a disaster. *Diagnostic microbiology and infectious disease.* 2016;84(4):287-91.
2. Niloofa R, Fernando N, de Silva NL, Karunanayake L, Wickramasinghe H, Dikmadugoda N, et al. Diagnosis of leptospirosis: comparison between microscopic agglutination test, IgM-ELISA and IgM rapid immunochromatography test. *PloS One*. 2015;10(6):e0129236.

Summary of Findings Table

Diagnostic Test	Study Design	Participants	Quality Assessment					Summary of Findings			
			Risk of Bias	Inconsistency	Indirectness	Imprecision	Reporting Bias	Over-all Quality	Sensitivity	Specificity	Importance
IgM ICT	Cross-sectional (Iwasaki, 2016; Niloofa, 2015)	1001 children & adults	Not Serious	Not Serious	Serious[1]	Not Serious	Undetected	Moderate	84.0 (79.0 – 87.0)	74.0 (70.0 – 77.0)	Important

ENDNOTES:

1. Indirectness due to inclusion of more adult subjects in the population studied.

Question 5: Can IgM Enzyme-linked Immunosorbent Assay (ELISA) be used as a rapid test in the diagnosis of leptospirosis in children?

Bibliography:

1. Bourhy P, Vray M, Picardeau M. Evaluation of an in-house ELISA using the intermediate species *Leptospira fainei*for diagnosis of leptospirosis. *Journal of medical microbiology*. 2013;62(6):822-7.
2. Desakorn V, Wuthiekanun V, Thanachartwet V, Sahassananda D, Chierakul W, Apiwattanaporn A, et al. Accuracy of a commercial IgM ELISA for the diagnosis of human leptospirosis in Thailand. *American journal of tropical medicine and hygiene.* 2012;86(3):524-7.
3. Iwasaki H, Chagan-Yasutan H, Leano PS, Koizumi N, Nakajima C, Taurustiati D, et al. Combined antibody and DNA detection for early diagnosis of leptospirosis after a disaster. *Diagnostic microbiology and infectious disease*. 2016;84(4):287-91.
4. Niloofa R, Fernando N, de Silva NL, Karunanayake L, Wickramasinghe H, Dikmadugoda N, et al. Diagnosis of leptospirosis: comparison between microscopic agglutination test, IgM-ELISA and IgM rapid immunochromatography test. *PloS One.* 2015;10(6):e0129236.

Summary of Findings Table

Diagnostic Test	Study Design	Participants	Quality Assessment					Summary of Findings			
			Risk of Bias	Inconsistency	Indirectness	Imprecision	Reporting Bias	Over-all Quality	Sensitivity	Specificity	Importance
IgM ELISA	Cross-sectional (Bourhy, 2013; Iwasaki, 2016; Niloofa, 2015) Case-control (Desakorn, 2012)	1,925 children& adults	Not Serious	Serious[1]	Serious[2]	Not Serious	Undetected	LOW	85.0 (82.0-87.0)	84.0 (82.0 - 86.0)	Important

ENDNOTES:

1. Heterogeneity is significant
2. Indirectness due to inclusion of more adults in the population studied.

Question 6: Can Polymerase Chain Reaction (PCR) be used in the diagnosis of leptospirosis in children?

Bibliography:

1. Narayanan R, Sumathi G, Prabhakaran SG, Shanmughapriya S, Natarajaseenivasan K. Paediatric leptospirosis: A population based case-control study from Chennai, India. *Indian journal of medical microbiology.* 2016;34(2):228.
2. Thaipadunpanit J, Chierakul W, Wuthiekanun V, Limmathurotsakul D, Amornchai P, Boonslip S, et al. Diagnostic accuracy of real-time PCR assays targeting 16S rRNA and lipL32 genes for human leptospirosis in Thailand: a case-control study. *PLoS One*. 2011;6(1):e16236.

Summary of Findings Table

Diagnostic Test	Study Design	Quality Assessment						Summary of Findings			
		Participants	Risk of bias	Inconsistency	Indirectness	Imprecision	Reporting bias	Over-all Quality	Sensitivity	Specificity	Importance
Polymerase chain reaction (PCR)	Case control (Narayanan, 2016; Thaipadunpanit , 2011)	843 children & adults	Not Serious	Serious[2]	Serious[3]	Not Serious	Undetected	LOW	77.5 (72.2 – 82.3)	94.3 (92.0 - 96.1)	Important

ENDNOTES:

1.Risk of bias inherent due to study design
2.Heterogeneity is significant
3.Indirectness due to inclusion of more adults in the population studied.
4.Imprecision due to overlapping of the confidence interval with the null value or due to wide confidence interval.
5.Risk of bias due to inclusion of probable cases

Question 7: How effective is the use of antibiotics in the treatment of children with leptospirosis?

Bibliography:

1. Brett-Major DM, Coldren R. Antibiotics for leptospirosis. *Cochrane Database Syst Rev* [Internet]. 2012;(2):CD008264. DOI: 10.1002/14651858.CD008264.pub2. PMID: 22336839.
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3. Costa E, Lopes AA, Sacramento E, Costa YA, Matos ED, Lopes MB, et al. Penicillin at the late stage of leptospirosis: a randomized controlled trial. *Rev. Inst. Med trop. S. Paulo*. 2003;45(3):141-145.
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5. Panaphut T, Domrongkitchaiporn S, Vibhagool A, Thinkamrop B, Susaengrat W. Ceftriaxone compared with sodium penicillin g for treatment of severe leptospirosis. *CID.* 2003;36:1507-13.
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8. Watt G, Tuazo ML, Santiago E, Padre LP, Calubaquib C, Ranoa CP, et al. Placebo-controlled trial of intravenous penicillin for severe and late leptospirosis. *Lancet.* 1988;1(8583):433-5.

Summary of Findings Table - Use of antibiotics in preventing mortality in children with leptospirosis

QUALITY ASSESSMENT							NO. OF PATIENTS or MEAN		EFFECT		QUALITY	IMPORTANCE
No. of studies	**Study Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Reporting Bias**			**Relative (95%CI)**	**Absolute (95%CI)**		
1 ANTIBIOTIC (PENICILLIN OR DOXYCYCLINE)							Antibiotic*	Placebo or no treatment				
Death 4	RCT (Brett-Major, 2012)	Not serious	Serious [1]	Very Serious [2.3]	Serious [4]	Serious[9]			OR: 1.16 (0.23 - 5.95)		Low	Critical

QUALITY ASSESSMENT							NO. OF PATIENTS or MEAN		EFFECT		QUALITY	IMPORTANCE
No. of studies	**Study Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Reporting Bias**			**Relative (95%CI)**	**Absolute (95%CI)**		
PENICILLIN							Penicillin	Placebo or no treatment				
Death 3	RCT, Cohort (Charan, 2013)	Serious [5]	Serious [1]	Very Serious[2,3]	Serious[4]	Serious[9]	17/179	11/188	OR: 1.70 (0.75 - 3.82)	ARR=37 more per 1000 (from 14 fewer to 133 more)	Very Low	Critical
CEFTRIAXONE							Ceftriaxone	Penicillin				
Death 1	RCT (Panaphut, 2003)	Serious[5, 8]	Not detected	Serious [2, 7]	Serious	Not detected	5/87	5/86	RR: 1.0 (0.3 - 3.3)	ARR=1 fewer per 1000 (from 70 fewer to 69 more)	Low	Critical
CEFOTAXIME							Cefotaxime	Penicillin				
Death 1	RCT (Suputtamongkol, 2004)	Very Serious[4, 8]	Not detected	Very Serious[2,3,6]	Serious	Not detected	0/88	2/87	RR: 0.3 (0.0 - 3.1)	ARR=23 lower per 1000 (from 66 lower to 21 more)	Very Low	Critical

QUALITY ASSESSMENT							NO. OF PATIENTS or MEAN		EFFECT		QUALITY	IMPORTANCE
No. of studies	**Study Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Reporting Bias**			**Relative (95%CI)**	**Absolute (95%CI)**		
DOXYCYCLINE							Doxycycline	Penicillin				
Death 1	RCT (Suputtamongkol, 2004)	Very Serious[4,8]	Not detected	Very Serious[2,3,6]	Serious	Not detected	2/81	2/87	RR: 1.1 (0.2 - 7.4)	ARR= 2 more per 1000 (from 44 less to 48 more)	Very Low	Critical
AZITHROMYCIN							Azithromycin	Doxycycline				
NO MORTALITY OUTCOME REPORTED IN STUDIES REVIEWED												

*Penicillin or Doxycycline

ENDNOTES:

1. High I^2, effects are opposite
2. Majority or all adult subjects
3. Severe and non-severe cases were included
4. Confidence interval spans no effect
5. Randomization not described fully and/or allocation not mentioned and/or unblended
6. Definition of severity variable
7. Not all subjects were verified leptospirosis cases
8. The most severe (i.e., anuric, comatose) were excluded
9. Forest plots and actual values not shown

Summary of Findings Table - Effect of antibiotics on the duration of fever in children with leptospirosis

QUALITY ASSESSMENT							NO. OF PATIENTS or MEAN		EFFECT		QUALITY	IMPORTANCE
No. of studies	**Study Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Reporting Bias**			**Relative (95%CI)**	**Absolute (95%CI)**		
ANTIBIOTIC (PENICILLIN OR DOXYCYCLINE)							Antibiotic*	Placebo or no treatment				
Days of Clinical Illness (specifically duration of fever) 2	RCT (Brett-Major, 2012)	Not serious	Very Serious [1]	Very Serious [2,3]	Serious [4]	Serious[9]				MD: 4.04 fewer (8.66 fewer to 0.58 more)	Very Low	Important
PENICILLIN							Penicillin	Placebo or no treatment				
Days of fever 3	RCT, Cohort (Charan, 2013) [includes Watt, 1988; Edward, 1988; Daher, 2000]	Serious [5]	Serious [1]	Very Serious[2,3]	Serious[4]	Serious[9]				MD: 0.15 day fewer (from 0.47 less to 0.17 more)	Very Low	Important
Duration of fever	RCT (Watt, 1988)	Very serious[5,8]	Not detected	Very serious[2,3,6]	Not serious	Not detected	4.7 days (4.19)	11.6 days (8.34)		MD: 6.90 days fewer (2.65 less to 11.15 less)	Very low	Important

QUALITY ASSESSMENT							NO. OF PATIENTS or MEAN		EFFECT		QUALITY	IMPORTANCE
No. of studies	**Study Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Reporting Bias**			**Relative (95%CI)**	**Absolute (95%CI)**		
Afebrile by day 4	RCT (Watt, 1988)	Very serious[5,8]	Not detected	Very serious[2,3,6]	Not serious	Not detected	12/20	1/19	RR: 10.4 (0.64 - 73.41)	ARR = 547 fewer per 1000 (from 259 fewer to 734 fewer)	Very low	Important
CEFTRIAXONE							Ceftriaxone	Penicillin				
Rate of Fever Abatement 1	RCT (Panaphut, 2003)	Serious[5, 8]	Not detected	Serious [2, 7]	Serious	Not detected			HR: 0.9 (0.7 - 1.3)		Low	Important
Duration of Fever 1	RCT (Panaphut, 2003)	Serious[5, 8]	Not detected	Serious [2, 7]	Serious	Not detected	3	3		MD= 0 days less (from 0.2 less to 0.2 more)	Low	Important
CEFOTAXIME							Cefotaxime	Penicillin				
Time to Deferves cence 1	RCT (Suputtamongkol, 2004)	Very Serious[4,8]	Not detected	Very Serious[2,3,6]	Serious	Not detected	Median=60	Median=72	P= 0.42		Very Low	Important
DOXYCYCLINE							Doxycycline	Penicillin				
Time to Deferves cence 1	RCT (Suputtamongkol, 2004)	Very Serious[4,8]	Not detected	Very Serious[2,3,6]	Serious	Serious[9]	Median = 72 hours	Median = 72 hours	P= 0.42		Very Low	Important

QUALITY ASSESSMENT							NO. OF PATIENTS or MEAN		EFFECT		QUALITY	IMPORTANCE
No. of studies	**Study Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Reporting Bias**			**Relative (95%CI)**	**Absolute (95%CI)**		
AZITHROMYCIN							Azithromycin	Doxycycline				
Afebrile for 48 hrs 1	RCT (Phimda, 2007)	Very Serious[5 7]	Not detected	Serious[2]	Serious	Not detected	34/34	34/35	RR: 1.0 (1.0 - 1.1)	ARR=29 more per 1000 (27 less to 84 more)	Very Low	Important
Time to Deferves cence 1	RCT (Phimda, 2007)	Very Serious[5 7]	Not detected	Serious[2]	Serious	Not detected	Median = 45 hours	Median = 40 hours	P=0.45		Very Low	Important

*Penicillin or Doxycycline

ENDNOTES:

1. High I^2, effects are opposite
2. Majority or all adult subjects
3. Severe and non-severe cases were included
4. Confidence interval spans no effect
5. Randomization not described fully and/or allocation not mentioned and/or unblended
6. Definition of severity variable
7. Not all subjects were verified leptospirosis cases
8. The most severe (i.e., anuric, comatose) were excluded
9. Forest plots and actual values not shown

Summary of Findings Table - Use of antibiotics in reducing renal complications or the need for dialysis in children with leptospirosis

QUALITY ASSESSMENT							NO. OF PATIENTS or MEAN		EFFECT		QUALITY	IMPORTANCE
No. of studies	**Study Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Reporting Bias**			**Relative (95%CI)**	**Absolute (95%CI)**		
ANTIBIOTIC (PENICILLIN OR DOXYCYCLINE)							Antibioitic *	Placebo or no treatment				
Dialysis Employed 2	RCT (Brett-Major, 2012)	Not serious	Serious [1]	Very Serious [2,3]	Serious [4]	Serious[9]			OR: 1.54 (0.91-2.60)		Low	Important
PENICILLIN							Penicillin	Placebo or no treatment				
Oliguria 2	RCT, Cohort (Charan, 2013)	Serious [5]	Serious [1]	Very Serious[2,3]	Serious[4]	Serious[9]	4/37	3/50	OR: 1.79 (0.32 - 9.93)	ARR=43 more per 1000 (from 40 fewer to 328 more)	Low	Critical
Need for Dialysis 2	RCT, Cohort (Charan, 2013)	Serious [5]	Serious [1]	Very Serious [2]	Serious[4]	Serious[9]	43/141	33/147	OR: 1.59 (0.92 - 2.73	ARR=90 more per 1000 (from 14 fewer to 217 more)	Very Low	Important
Days to Normalization of Creatinine 1	Cohort (Daher, 2000)	Very serious [5]	Not detected	Serious [2]	Serious	Not detected	10 (SD=6)	9 (SD=6)		MD= 1.0 more days (from 3.1 less to 5.1 more)	Very Low	Important

QUALITY ASSESSMENT							NO. OF PATIENTS or MEAN		EFFECT		QUALITY	IMPORTANCE
No. of studies	**Study Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Reporting Bias**			**Relative (95%CI)**	**Absolute (95%CI)**		
Days with rise in Creatinine 1	RCT (Watt, 1988)	Very serious [5,8]	Not detected	Very serious[2,3,6]	Not Serious	Not detected	2.7 (SD=1.9)	8.3 (SD=8.46)		MD= 5.6 fewer days (from 1.9 fewer to 9.2 fewer)	Very Low	Important
CEFTRIAXONE							Ceftriaxone	Penicillin				
Renal Failure 1	RCT (Panaphut, 2003)	Serious [5, 8]	Not detected	Serious [2, 7]	Serious	Not detected			RR: 1.0 (0.7 - 1.4)			

*Penicillin or Doxycycline

ENDNOTES:

1. High I^2, effects are opposite
2. Majority or all adult subjects
3. Severe and non-severe cases were included
4. Confidence interval spans no effect
5. Randomization not described fully and/or allocation not mentioned and/or unblended
6. Definition of severity variable
7. Not all subjects were verified leptospirosis cases
8. The most severe (i.e., anuric, comatose) were excluded
9. Forest plots and actual values not shown

Question 8: How effective is doxycycline as pre-exposure prophylaxis in the prevention of leptospirosis in children?

Bibliography:

1. Sehgal SC, Sugunan AP, Murhekar MV, Sharma S, Vijayachari P. Randomized controlled trial of doxycycline prophylaxis against leptospirosis in an endemic area. *International journal of antimicrobial agents*. 2000;13(4):249-55.
2. Takafuji ET, Kirkpatrick JW, Miller RN, Karwacki JJ, Kelley PW, Gray MR, et al. An efficacy trial of doxycycline chemoprophylaxis against leptospirosis. *New England Journal of Medicine.* 1984;310(8):497-500.

Summary of Findings Table

No. of Studies	Study Design	Outcome	Quality Assessment					No. of Patients		Summary of Findings		
			Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations	Doxycycline	Placebo	Over-all Quality	OR/RR/HR or MD	Importance
2	Randomized trials	Asymptomatic laboratory-identified leptospiral infection	Not Serious	Serious[2]	Serious[4]	Serious[5]	None	113/855 (13.2%)	121/867 (14.0%)	VERY LOW	RR: 0.28 (0.01 - 7.16)	Important
2	Randomized trials	Symptomatic leptospirosis	Not Serious	Serious[2]	Serious[4]	Serious[5]	None	13/855 (1.5%)	47/867 (5.4%)	VERY LOW	RR: 0.18 (0.02 - 1.8)	Important
1	Randomized trial (Sehgal, 2000)	Adverse events, minor (nausea and vomiting)	Not Serious	Undetected	Serious[4]	Serious[5]	None	Cannot be determined; no exact number stated	Cannot be determined; no exact number stated	VERY LOW	Cannot be calculated	Important
1	Randomized trial (Takafuji, 1984)	Adverse events, minor (nausea and vomiting)	Not Serious	Undetected	Serious[4]	Serious[5]	Very strong association[6]	13/469 (2.77%)	1/471 (0.21%)	VERY LOW	RR: 13.06 (1.71 - 99.40)	Important

Rating of Quality of Evidence: Not Serious, Serious, and Very Serious.
OR: Odds ratio; RR: Relative Risk; HR: Hazard Ratio; MD: Mean Difference
*Factors that can decrease or increase the quality of the evidence include reporting bias, large magnitude of an effect, dose-response gradient, and effect of plausible residual confounding

ENDNOTES:

1. Risk of bias inherent due to study design
2. Risk of bias due to inclusion of probable cases
3. Heterogeneity is significant
4. Indirectness due to inclusion of more adults in the population studied.
5. Imprecision due to overlapping of the confidence interval with the null value or due to wide confidence interval.
6. Magnitude of effect is large (RR >2 or <0.5) or very large effect (RR >5 or <0.2)

Question 9: How effective is doxycyline as post-exposure prophylaxis in the prevention of leptospirosis in children?

Bibliography:

1. Chusri S, McNeil EB, Hortiwakul T, Charernmak B, Sritrairatchai S, Santimaleeworagun W, et al. Single dosage of doxycycline for prophylaxis against leptospiral infection and leptospirosis during urban flooding in southern Thailand: A non-randomized controlled trial. *Journal of Infection and Chemotherapy.* 2014;20(11):709-15.
2. Gonsalez CR, et al. Use of doxycycline for leptospirosis after high-risk exposure in Sao Paulo, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo.* 1998;40(1): 59-61.

Summary of Findings Table

No. of Studies	Study Design	Outcome	Quality Assessment					No. of Patients		Summary of Findings		
			Risk of Bias	Inconsistency	Indirectness	Imprecision	Reporting Bias	Doxycycline	Placebo	Over-all Quality	OR/RR/ HR or MD	Importance
2	Non-randomized trial and randomized trial	Asymptomatic laboratory-identified leptospiral infection	Serious[1]	Serious[2]	Very Serious[5]	Serious[4]	Undetected	28/640 (2.8%)	11/83 (12.2%)	VERY LOW	RR: 0.67 (0.08 - 5.59)	Important
2	Non-randomized trial and randomized trial	Symptomatic leptospirosis	Serious[1]	Serious[2]	Very Serious[5]	Serious[4]	Undetected	6/640 (1%)	7/83 (5%)	VERY LOW	RR: 0.25 (0.08 - 0.78)	Important
1	Non-randomized trial (Chusri, 2014)	Adverse events, minor (gastrointestinal symptoms)	Serious[1]	Undetected	Very Serious[5]	Serious[4]	Undetected	12/600 (2%)	0/41 (0%)	VERY LOW	RR: 1.75 (0.11 - 29)	Important
1	Non-randomized trial (Chusri, 2014)	Adverse Events, minor (skin rash)	Serious[1]	Undetected	Very Serious[5]	Serious[4]	Undetected	1/600 (0.17%)	0/41 (0%)	VERY LOW	RR 0.21 (0 - 5.07)	Important

*Exported from gradepro.org

ENDNOTES:

1. Risk of bias inherent due to lack of randomization or blinding in one of the studies
2. Heterogeneity is significant
3. Indirectness due to inclusion of more adults in the population studied.
4. Imprecision due to overlapping of the confidence interval with the null value or due to wide confidence interval.
5. Indirectness due to inclusion of only adults in the population studied.

Question 10: Is there evidence to recommend the use of antibiotics other than doxycycline as post-exposure prophylaxis for leptospirosis in children?

Bibliography:

1. Illangasekera VL, Kularatne SA, Kumarasiri PV, Pussepitiya DM, Premaratne MD. Is oral penicillin an effective chemoprophylaxis against leptospirosis? A placebo controlled field study in the Kandy District, Sri Lanka. *Southeast Asian Journal of Tropical Medicine and Public Health.* 2008;39(5):882.

Summary of Findings Table - Quality assessment of the study on penicillin versus placebo as post-exposure prophylaxis for the prevention of leptospirosis.

Intervention: Penicillin 500 mg (twice a day) for a month versus placebo as post-exposure prophylaxis

No. of Studies	Study Design	Outcome	Quality Assessment					No. of Patients		Summary of Findings		
			Risk of Bias	Inconsistency	Indirectness	Imprecision	Reporting Bias	Penicillin	Placebo	Over-all Quality	OR/RR /HR or MD	Importance
1	randomized double blind placebo controlled trial	Symptomatic leptospirosis	Not Serious	Undetected	Very Serious[5]	Serious[4]	Undetected	0/292 (0.0%)0	3/310 (0.97 %)	VERY LOW	RR 0.15 (0.01 to 2.92)	Important

*Exported from gradepro.org

ENDNOTES:

1. Risk of bias inherent due to lack of randomization or blinding in one of the studies
2. Heterogeneity is significant
3. Indirectness due to inclusion of more adults in the population studied.
4. Imprecision due to overlapping of the confidence interval with the null value or due to wide confidence interval.
5. Indirectness due to inclusion of only adults in the population studied

APPENDIX B

DECLARATION OF CONFLICT OF INTEREST

MA. ANNA P. BAÑEZ, M.D.	Has been a facilitator in industry-sponsored continuing medical education activities involving antibiotics
MELBA V. MASIGAN, M.D.	Has been a resource speaker in industry-sponsored continuing medical education activities on antibiotics
MA. LIZA ANTOINETTE M. GONZALES, M.D.	Has been a resource speaker in industry-sponsored continuing medical education activities on antibiotics
FATIMA I. GIMENEZ, M.D.	Has been a facilitator and resource speaker industry-sponsored continuing medical education activities involving antibiotics
GRACE DEVOTA G. GO, M.D.	No potential conflict of interest
MARY ANTONETTE C. MADRID, M.D.	Has been a resource speaker in industry-sponsored continuing medical education activities on antibiotics
JOHN ANDREW T. CAMPOSANO, M.D.	No potential conflict of interest
MA. LUCILA M. PEREZ, M.D.	No potential conflict of interest
JENNIFER M. NAILES, M.D., M.S.P.H.	No potential conflict of interest