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Leptospirosis as a "tool ready" disease: diagnosis and surveillance

Rudy A Hartskeerl
Head WHO/FAO/OIE and National Leptospirosis Reference Centre, Amsterdam

Presentation conducted during the *International Workshop of the Oswaldo Cruz Institute/FIOCRUZ for Leptospirosis Research Based on Country Needs & the 5th Global Leptospirosis Environmental Action Network (GLEAN) Meeting* on November 10-12, 2015, in Rio de Janeiro, Brazil .

Tool ready (or targeted) neglected tropic diseases

Infections that can be controlled or even eliminated through mass administration of safe and effective medicines (mass drug administration) or other, effective interventions.

Lymphatic filariasis
Onchocerciasis
Schistosomiasis
Soil-transmitted helminths (**ascariasis**, **hookworm**, **whipworm**)
Trachoma

http://www.cdc.gov/globalhealth/ntd/diseases/

Tool deficient NTD

- Varies in what is needed to advance it to the tool-ready category. For example:
  - No tools for early detection
  - (antibiotic) treatment absent or difficult (treatment that must be administered by highly trained professionals in specialized facilities).

Tool-deficient NTD

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World Health Organization: address tool-deficient diseases by first strengthening primary care systems in endemic areas

Leptospirosis is tool-deficient in many respects
sessions, presentations and discussions according to GLEAN pillars

Predict
- Leptospirosis as emerging and neglected disease *(session 1)*
- Prediction *(session 3)*
- Panel Discussion: The "One Health" approach

Detect
- Current tools and emerging technologies for leptospirosis diagnosis *(session 4)*
- Surveillance *(session 2)*

Prevent
- Studies and interventions in animals *(session 7 and One Health)*
- Vaccine (Key lecture Albert Ko)

Respond
- Human case management *(session 6)*
- Working Groups: Outbreak Response Guidelines
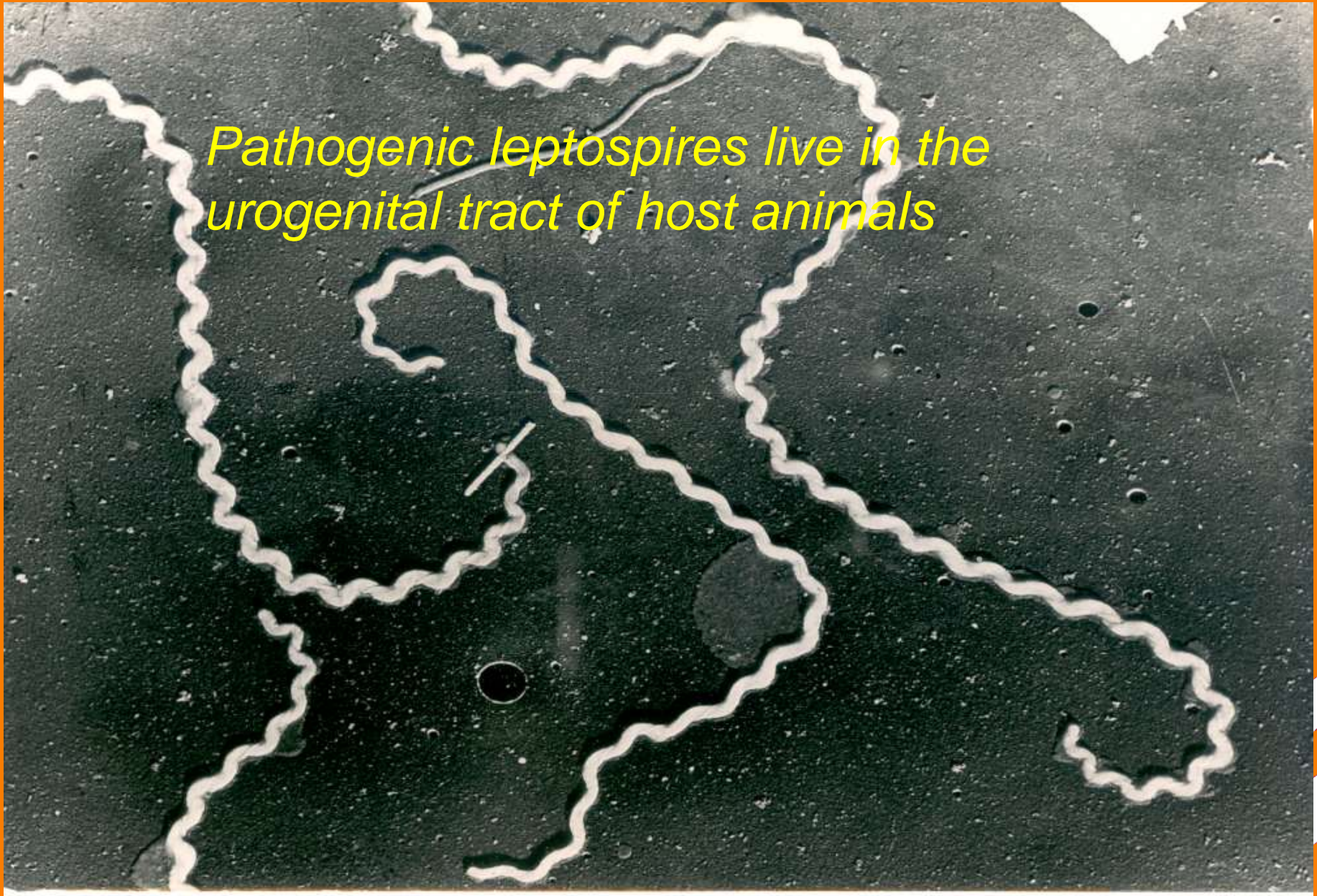
Focus of presentation

Case detection
Surveillance

OneHealth approach needed

Pathogenic leptospires live in the urogenital tract of host animals

Leptospires are excreted with urine into environment

Penetration through broken skin and mucous membranes

Leptospira have to adapt to a wide variety of 'environments'

- Wide variety of leptospires, high genome plasticity and a wide variety of serovars
- Wide variety of clinical manifestations mimicking several other diseases
- Diagnosis on clinical grounds alone is challenging:
- Support of a lab is indispensable

- Host - serovar association
- Epidemiology complex and dynamic:
    continuous and sustainable surveillance
    is indispensable

Subjects of presentation

1. Wide variety of clinical manifestations**; Diagnosis on clinical grounds alone is challenging:**
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WHO case definition

❑**Clinical criteria:** Fever + at least two of the most common clinical signs, or
One of the following syndromes: >> aseptic meningitis
                                           >> gastro intestinal symptoms
                                           >> pulmonary complications
                                           >> renal failure, heart problems

❑**Biological criteria:**   >> identification of leptospirosis
                               >> serological tests Elisa and MAT

❑**Epidemiological criteria:**
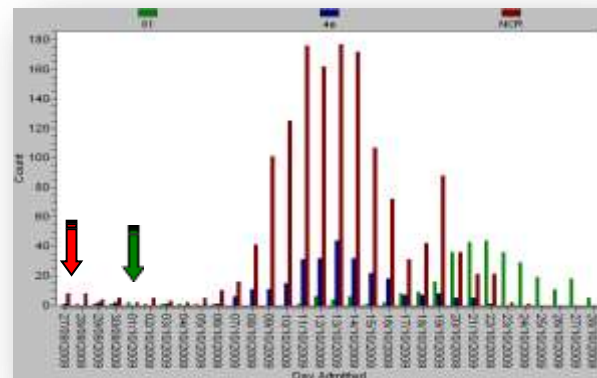Contact with water, wet soil or contaminated animals (incubation 2-20 days)
    ➢ Temperate areas: Summer-Autumn: occupational or leisure context
    ➢ Tropical areas: Floods, natural disasters, Socio economic context
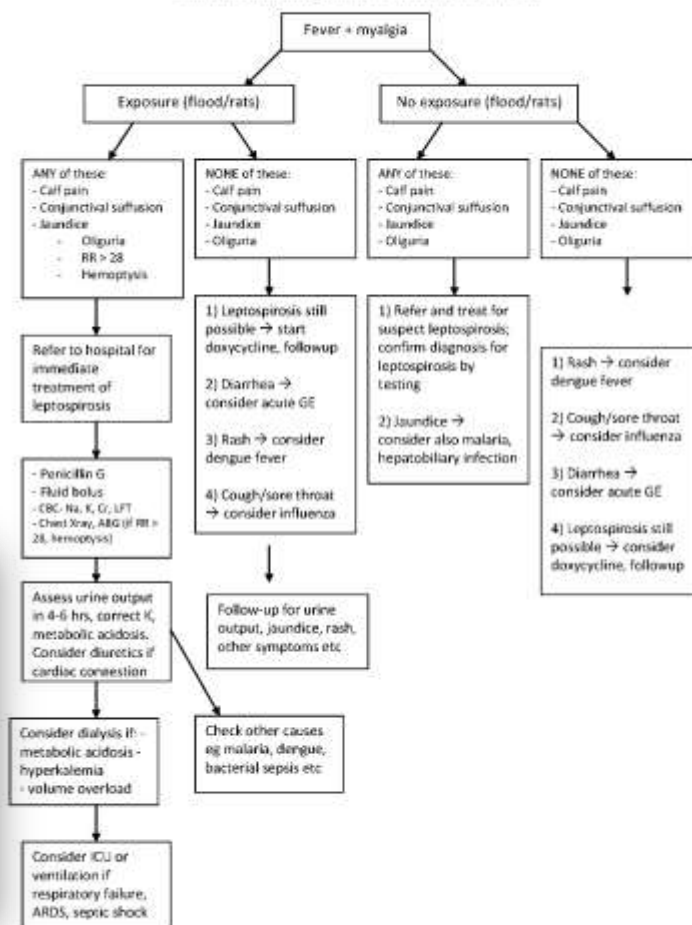
Need for locally relevant clinical algorithms

Clinical Assessment Algorithm for Leptospirosis Outbreaks

Philippines outbreaks 2009

Pepeng, 3 Oct

Ondoy, 26 Sept

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Current diagnostic tests

Conventional tests

Rapid diagnostic tests (RDT)

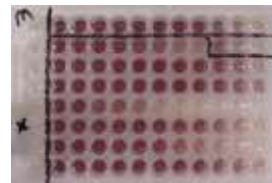**Molecular (amplification-based) tests**

Conventional tests

- **Microscopic Agglutination Test (MAT)**
  - ❖ Gold or reference standard
  - ❖ Requires panels of 'locally' representative serovars
  - ❖ May provide indication for infecting serogroup

- **ELISA**
  - ❖ IgM antibodies against leptospires
  - ❖ Genus specific

- **Isolation** by culturing confirms leptospirosis but is too slow for individual diagnosis and has mainly epidemiological value

- **Darkfield Micrsocopy** is unreliable and always should be confirmed

Rapid Diagnostic Tests

•Confirmation by conventional tests is required
- • Relatively low diagnotic accuracy
- • Varying diagnostic accuracy => repeated local evaluations
- • Need for improved QA at production and/or **test performer** (<u>easy</u> does not exclude <u>experience</u>)

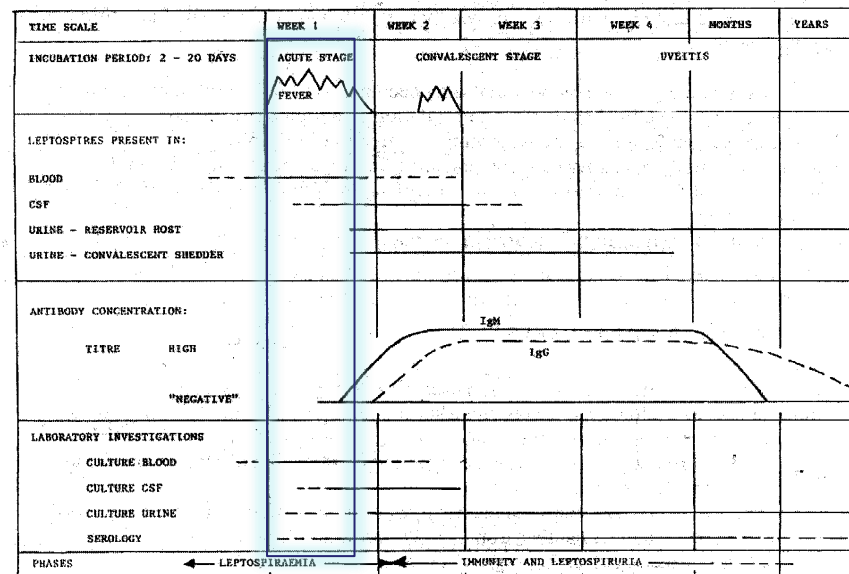•<u>Rapid</u> diagnosis is different from **<u>early</u> diagnosis**!

Early diagnosis: Molecular tests
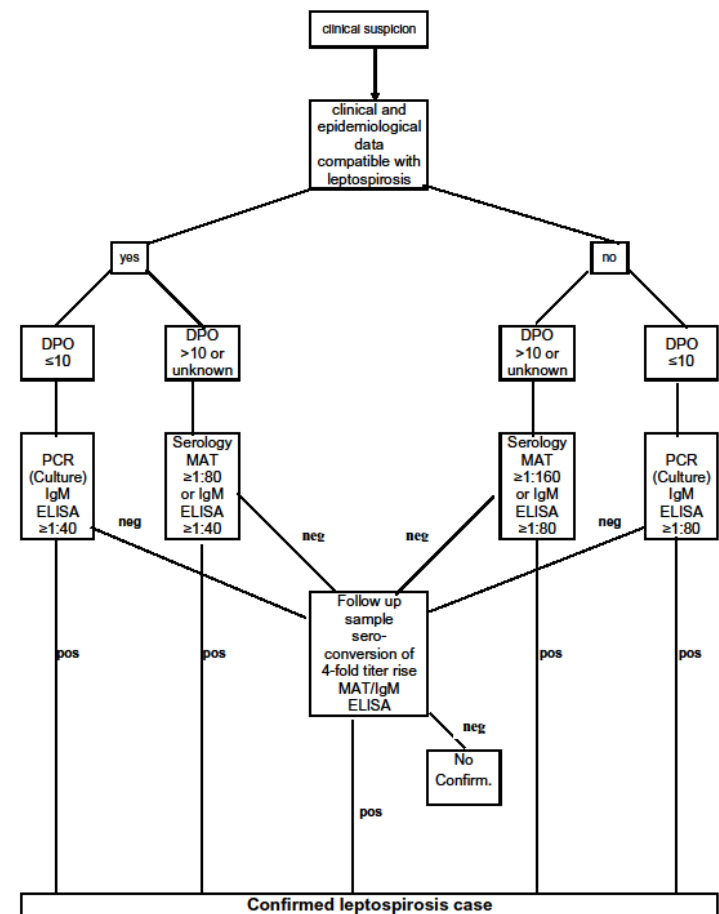

Hand-held
Battery driven
affordable

T-COR 4 qPCR

•Leptospires circulate in the blood notably first 4-7 days after onset of disease: Several NA amplification techniques on blood samples like **Polymerase Chain Reaction (PCR)** can fill in this gap.

•Use validated ones (in-house real-time PCRs or commercial ones)

Case definition: laboratory algorithms

Lacking in most situations

- Local laboratory case definition: algorithm
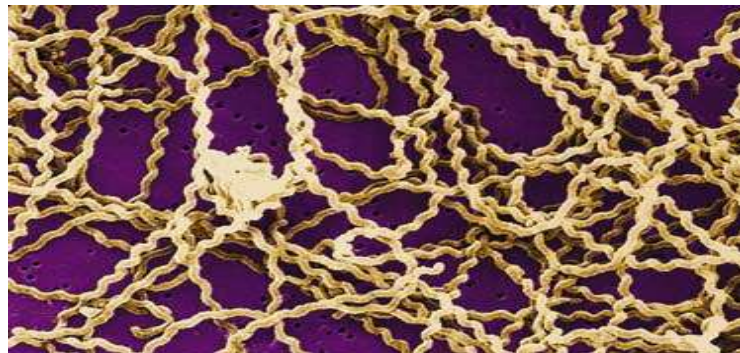- Local evaluation of (ANY) diagnostic test

Establishment of Valid Laboratory Case Definition for Human Leptospirosis

Marga G.A. Goris[1*], Mariska M.G. Leeflang[2], Kimberly R. Boer[1], Marco Goeijenbier[4], Eric C.M. van Gorp[4], Jiri F.P. Wagenaar[1,3] and Rudy A. Hartskeerl[1]

[1]Royal Tropical Institute (KIT), KIT Biomedical Research, Amsterdam, the Netherlands
[2]Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, Amsterdam, the Netherlands
[3]Slotervaart Hospital, Department of Internal medicine, Amsterdam, the Netherlands
[4]Department of Virology, Erasmus Medical Center, Rotterdam, the Netherlands

Abstract

Laboratory case definition of leptospirosis is scarcely defined by a solid evaluation that determines cut-off values in the tests that are applied. This study describes the process of determining optimal cut-off titers of laboratory tests for leptospirosis for a valid case definition of leptospirosis. In this case the tests are the microscopic agglutination test (MAT) and an in-house IgM enzyme-linked immunosorbent assay (ELISA) both on single serum and paired samples using a positive culture as the reference test in the Dutch population. The specificity was assessed using panels of sera from healthy donors, cases with known other diseases and non-leptospirosis cases with symptoms compatible with leptospirosis. Cases were divided into three periods corroborating the acute phase (1-10 days post onset of illness (DPO)), the early convalescent (11-20 DPO) and the late convalescent phase (>20 DPO). Cut-off titers for MAT and IgM ELISA were determined as 1:160 and 1:60 respectively for all three periods. These cut-off titers combined 100% specificity with a sensitivity that changed according to the stage of disease for both tests. The low sensitivities in the early acute phase are consistent with the dynamics of the humoral immune response. IgM ELISA yielded higher sensitivities compared to MAT in the acute and early convalescent stages. Moreover, the optimal sensitivity of MAT, the gold standard was <82%, implying that a significant part of global cases is missed by this recommended test. MAT and IgM ELISA manifested partly complementary, resulting in a higher sensitivity when combining the results of these two tests. The availability of paired samples and of adequate clinical and epidemiological data are other parameters that will significantly increase the sensitivity of laboratory confirmation. This study enables fine-tuning of the current laboratory

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Classification of Leptospira

- 2 classification systems
  - Based on serology (conventional)
 - Based on DNA (sequence) composition

- The two classification systems do not correlate

Characterization approaches: some considerations

- Serological (serovar) characterization is tedious, laborious and expensive (€ 500- 5000 per sample) and involves shipment of pathogens

- Molecular characterization (PFGE, MLVA/VNTR, typing array) is affordable (€ 25 - € 200 per sample)

- Whole genome sequencing becomes increasingly affordable (now < € 400 per genome): whole genome sequence typing becomes attractive.
  - Advantages: e.g. enables assessment of horizontal variome

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Characterization approaches: some considerations

- Some current molecular techniques (e.g. PFGE, MVLA) largely coincide with serovar status but cannot define these!

- Serovar status based on agglutination features mainly based on antibody-LPS interactions
- Eligible molecular typing of serovars be based on LPS coding sequences.
- Proof of principle presented: availability of sequences is limiting factor (Da Silva et al., Use of selective PCR amplification of LPS biosythesis genes for molecular typing of *Leptospira* at the serovar level. Current Microbiol 2011)
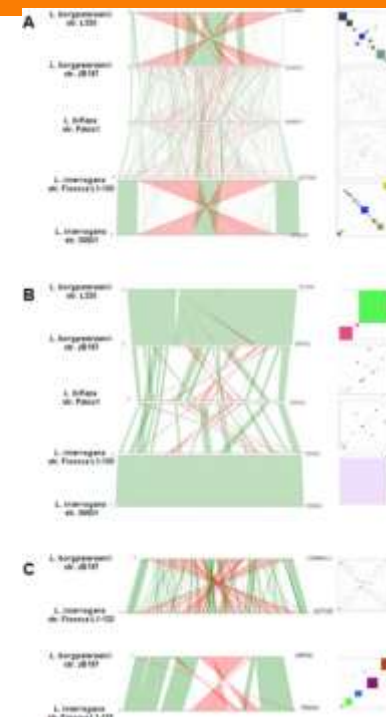
Whole genome sequencing will provide tools for concomitant determination/deduction of genotypic and serological features

Structure of Leptospira rfb locus gene clusters (LPS coding) of 20 serovars.
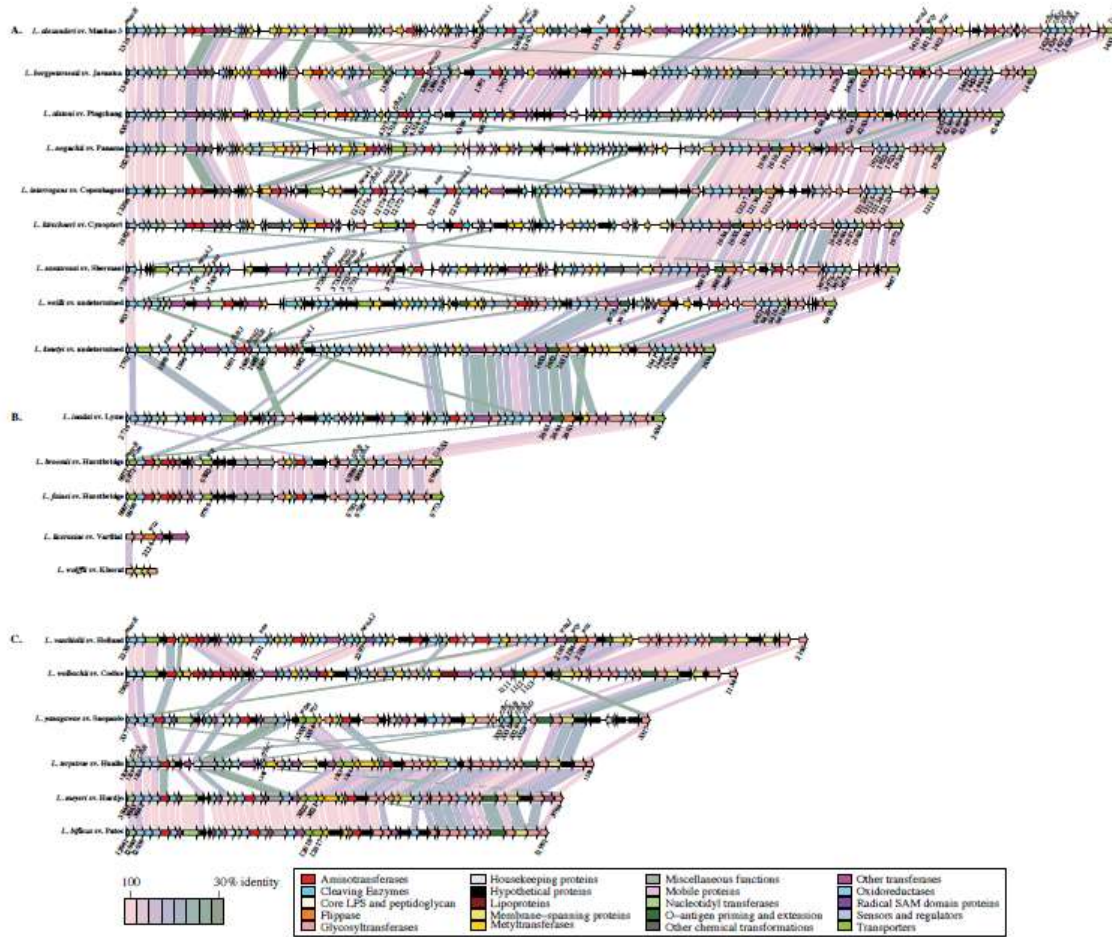
Fouts et al., What Makes a Bacterial Species Pathogenic?: Comparative Genomic Analysis of the Genus Leptospira" (#PNTD-D-15-01081

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Surveillance

ILS worldwide survey (1987-1997)   150.000 cases per year

ILS worldwide survey (1998-2001) ⎤   350.000-500.000 cases
                                 ⎥   per year, CFR ~10%
LeptoNet; online global surveillance ⎦

WHO-LERG Systematic review   1 million cases per year,
                                      CFR 6%
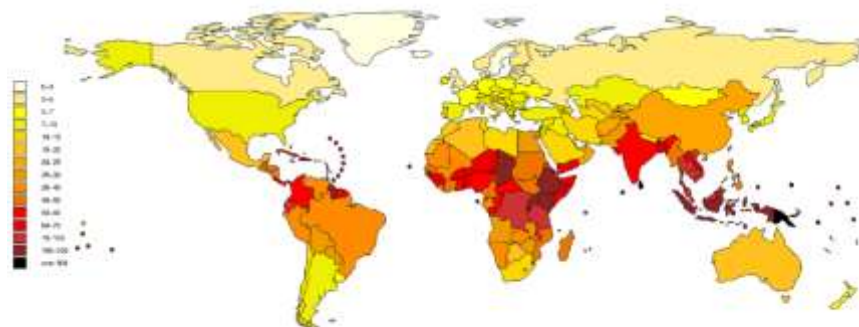
Fig 4. Burden of leptospirosis in terms of DALYs/100,000 per year.

doi:10.1371/journal.pntd.0094122.g004

Global burden of disease study 2010 of listed NTDs

Disease	Number of cases (expected in 2010)	Deaths	DALYs (millions) Disability adjusted life years
Intestinal nematode infections	1,723 million	2,700	5.19
Leishmaniasis	10 million	51,600	3.32
Schistosomiasis	252 million	11,700	3.31
Leptospirosis*	1 million	**58,900**	2.90** ⬅
Lymphatic filariasis	36 million	-	2.78
Food-borne trematodiasis	16 million	-	1.88
Rabies	1,100	26,400	1.46
Dengue	197,000# (~200 million)	14,700	0.83

*Is not a listed NTD (severe cases only); **Torgerson et al., PLoS NTD 2015
Incident (acute) symptomatic cases only
Hotez et al., PLoS NTD 2014, e2865 (top 7 listed NTDs only)

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Tool-ready

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Still underestimation

Need for national (laboratory) and surveillance networks focusing on OneHealth requirements

Need for international (regional) exchange

Tailor-made approaches but common organizational structures

Levels of tests and surveillance tasks

Screening and early warning:
Peripheral labs (hospitals, health centres, GP): Clinical suspicion, RDT, inoculation of culture media (shipment to expert centre) and collection of clinical and demographic data
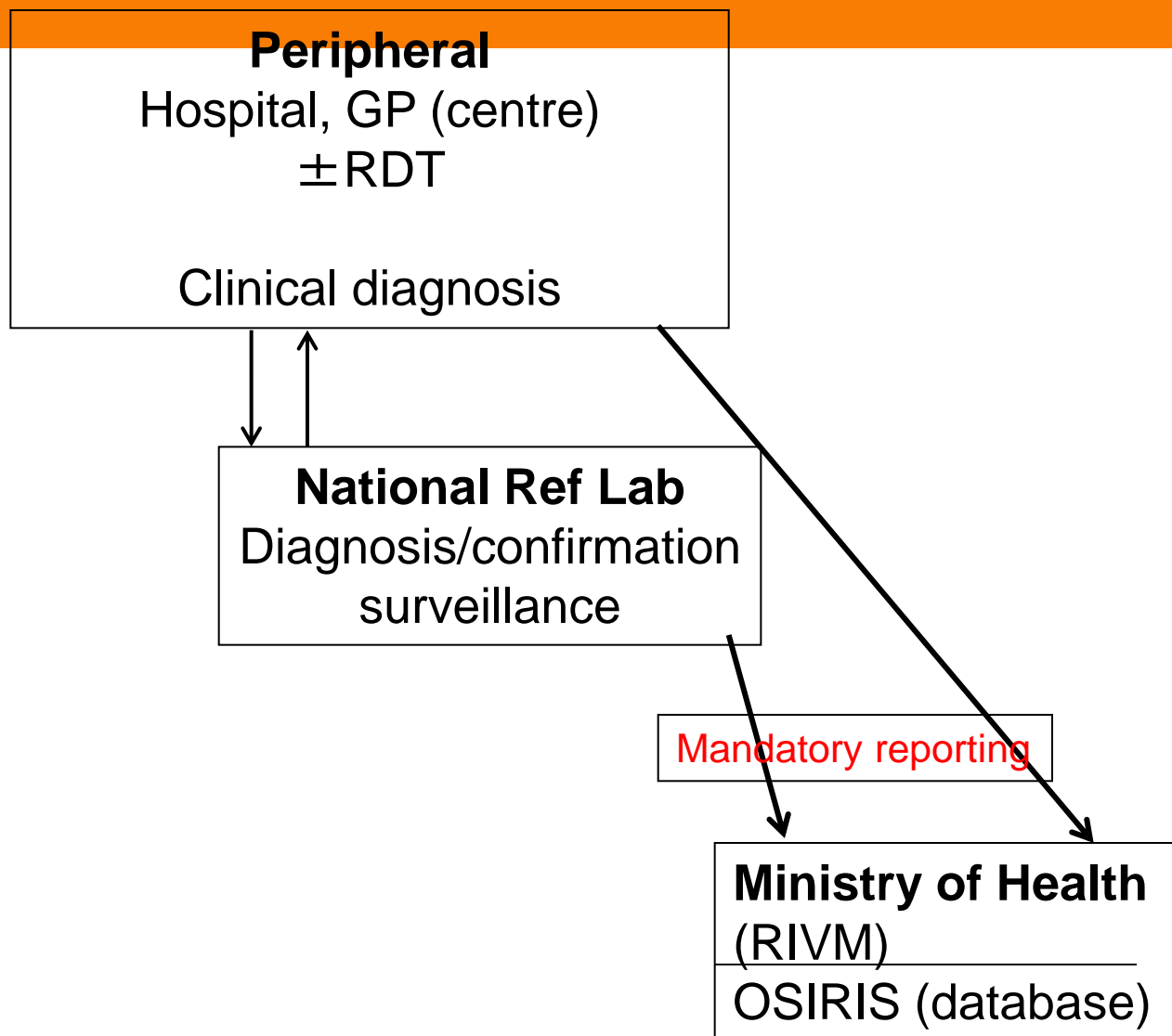
Confirmation, surveillance, research:
National Reference Centre: MAT (ELISA), PCR, culturing, bacterial typing, preparation of culture medium. Collation of data.
The centre forms a link between MoH (and MoA) and periphery and is primary partner to execute source investigations

Epidemiology:
MoH in concerted action with MoA and close communication with the Reference Centre

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Peripheral
Hospital, GP (centre)
±RDT

Clinical diagnosis

National Ref Lab
Diagnosis/confirmation
surveillance

Mandatory reporting

Ministry of Health
(RIVM)
OSIRIS (database)

Peripheral
Hospital, GP (centre)
±RDT

Clinical diagnosis

National Ref Lab
Diagnosis/confirmation
surveillance

(semi)domestic animals suspected
Source investigation

Mandatory reporting

Ministry of Agriculture

Ministry of Health
(RIVM)
OSIRIS (database)

ECDC

Surveillance

- Only in few countries, notification and mostly not mandatory

- Lack of base-line data required for timely recognizing outbreaks

- Unawareness on disease occurrence, distribution, risk and transmission factors and burden

- Unawareness locally circulating serovars and hosts
  $\Longrightarrow$ control (vaccine; Keynote Albert Ko)

Besides: need for guidelines

Epidemic situation: save lives !

Outbreak confirmation; Risk communication to public and health services; adequate diagnosis; empirical treatment (primary care); individual protection; community protection (targeted prophylaxis); [rodent control & vaccination (too slow, too late, serovar specific)]

Recommendations for high risk countries with an unknown epidemiological situation

1. Background investigation (Epidemiological survey; Passive surveillance; Laboratory training)
2. Recognising problems (Information/communication; Syndromic surveillance and empirical treatment; Risk mapping with planning for crisis situations)

National control programs: endemic and epidemic countries

Legislation (notification); Incidence mapping; Laboratory network and guidelines; Syndromic surveillance; Case management and decision algorithm; sanitation; Rodent control; Animal vaccination (livestock, pets); targeted human vaccination; Information/communication; Epidemic management

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In summary

Lack of well validated diagnostic tools and clinical and laboratory algorithms

Lack of adequate operating surveillance systems

Lack of baseline data and awareness

Major infection sources and local serovars are unknown

Short of memory after outbreaks (governments); Much attention and activities during and shortly after outbreaks but no sustained commitments and follow-up of recommendations

It is not a matter of IF a new outbreak occurs but a matter of WHEN

Future and dreams

Next generation sequencing and metagenomics

Future (tests)

•Early and rapid – antigen detection based?

•Discriminative (multiplex testing/differential diagnosis)

•Traceable (online data transfer to e.g. MoH) - early outbreak warning (RDT with QR/barcodes or chip; data and location recording by smart phone?)

- Development of clinical/laboratory algorithm: Validation of diagnostics locally and/or on globally representative sample banks
- Assessment of epidemic thresholds: Collection of base-line data (surveillance)

Sequencing: reversed trends in capacities and costs
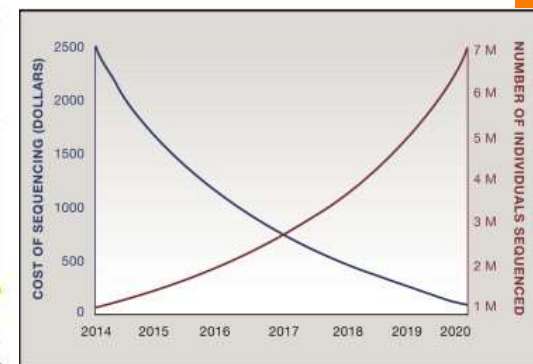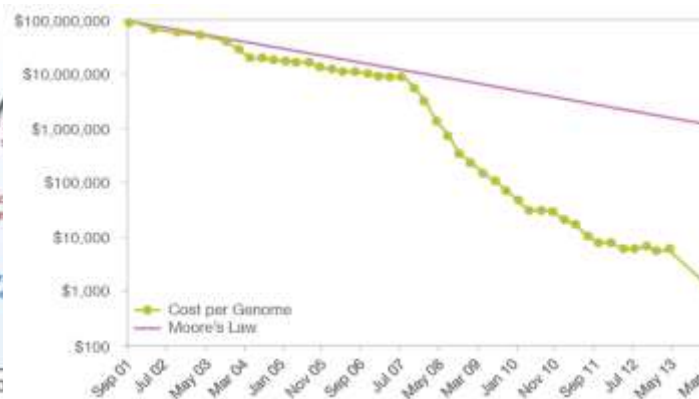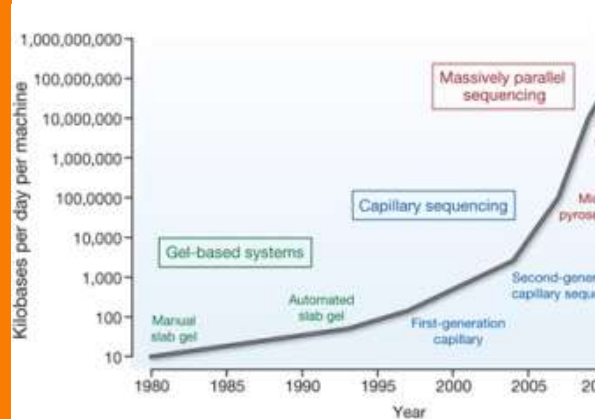
Leptospira genome is ~4 Mb (0.004 Gb

Platform Features

Increased availability of small, portable NGS

Feature	HiSeq2500 - Highoutput	HiSeq2500 – Rapid mode	MiSeq	PacBio RSII
Number of reads	150-180M/lane	100-150M/lane	12-15M (v2) 20-25M (v3)	50-80K/SMRT cell
Read length	2 x 100 bp	2 x 150 bp	2 x 300 bp (v3)	~ 10-20 kb
Yield per lane (PF data)	up to 35 Gb	up to 45Gb	up to 15 Gb	up to 0.4 Gb
Instrument Time	~12-14 days	~2 days	~2 days	~2 hours
Pricing per Gb	$59 (PE100)	$53 (PE150)	$108 (PE300)	$697

Next generation sequencing (NGS)

- Identity of (micro)organisms will be based on whole genome sequence
- Reversed genomics: e.g. serovar can be deduced from genome sequence
- It will be possible to sequence genome DNAs from various microorganisms in a sample in a single run and thus establish their presence (multiplex sequencing: meta genomics)
- It will also be possible to identify yet unknown micro-organisms (de novo sequencing)

Comparison with sequences of all (known) organisms in databases for identification

Meta epidemiological data
Big data
GIS
Remote sensing
Weather, climate
Conventional data

Diagnosis within one day

sequences

- Online surveillance
- Early outbreak warning
- Identification outbreak factors (prediction and prevention)
- Identification of vaccine components

Clinical sample

Future diagnosis and surveillance

From single disease diagnosis and surveillance to multi-disease diagnosis and surveillance
Within the coming 5 – 10 years

Thanks