



Mahidol University

Faculty of Veterinary Science

Never Overlook Leptospirosis in Dogs: Practical Diagnosis, Treatment & Prevention

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1 April 2021





Why it is important?

- Both dogs and cats can shed leptospire in their urine without showing clinical signs of the disease (Rojas et al. 2010, Fenimore et al. 2012, Llewellyn et al. 2013, Rodriguez et al. 2014)
- Public health concern!!!





Classification and Nomenclature of *Leptospira* spp.

2 Species

- *Leptospira interrogans* sensu lato (pathogenic strains)
- *Leptospira biflexa* sensu lato (saprophytic, non-pathogenic strains)

How to write according to nomenclature

- *Leptospira interrogans* serovar Australis
- *Leptospira biflexa* serovar Patoc

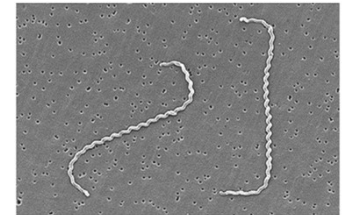
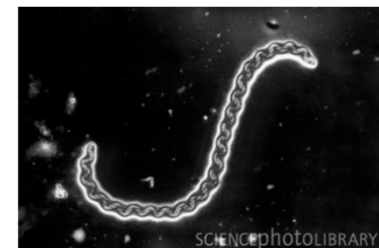
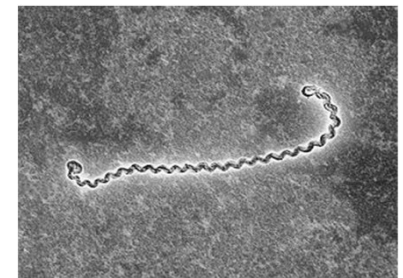


FIG 1. Scanning electron micrograph of *Leptospira interrogans* strain RGA. Image source: Public Health Image Library CDC/NCID/Rob Weyant (<http://phil.cdc.gov/phil/details.asp>)





Definitions

- **Serovar:** member of the genus *Leptospira*, which reacts with a specific monoclonal antiserum. Antisera are specific to immunogenic carbohydrate antigens of leptospiral lipopolysaccharide.
- **Serogroup:** group of antigenically closely related leptospiral serovars. Members of the same serogroup agglutinate when incubated with patient serum containing antibodies to one serovar of the same serogroup.
- **Strain:** specific isolate of a defined leptospiral serovar





	Serogroups	Serovars	Strains
<i>Leptospira interrogans</i>	Pyrogenes	Manila	LT 398
	Pyrogenes	Pyrogenes	Salinem
	Canicola	Canicola	Hond Utrecht
	Autumnalis	Autumnalis	IV
	Bataviae	Losbanos	Akiyami A
	Hebdomadis	Hebdomadis	LT 101-69
	Australis	Australis	Akiyami B
	Icterohaemorrhagiae	Copenhageni	Akiyami C
	Icterohaemorrhagiae	Icterohaemorrhagiae	M20
	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
	Pomona	Pomona	Ictero No. 1
	Sejroe	Hardjo	Pomona
	Grippotyphosa	Ratnapura	Hardjoprajitno
			UP-BL-FR13
<i>Leptospira borgpetersenii</i>	Tarassovi	Tarassovi	Perepelitsin
	Javanica	Poi	Poi
<i>Leptospira kirschneri</i>	Grippotyphosa	Grippotyphosa	Moskva V
<i>Leptospira meyeri</i>	Semaranga	Semaranga	Veldrat Semaranga 173
<i>Leptospira biflexa</i>	Semaranga	Patoc	Patoc 1

Zamora, 2015



Background – *Leptospira interrogans*

- Over 250 pathogenic serovars
- Serovar distribution in dogs
 - Historically
 - Canicola, Icterohaemorrhagiae
 - 19 serovars reported in Thailand

Anhoa, Australis, Ballum, Bataviae, Bratislava, Broomi, Canicola, Copenhageni, Coxi, Grippotyphosa, Haemolytica, Icterohaemorrhagiae, Khorat, Paidjan, Patoc, Pyrogenes, Rachmati, Saxkoebing, Sejroe

Altheimer et al. *BMC Veterinary Research* (2020) 16:89
<https://doi.org/10.1186/s12917-020-2230-0>

BMC Veterinary Research

RESEARCH ARTICLE

Open Access

Leptospira infection and shedding in dogs in Thailand



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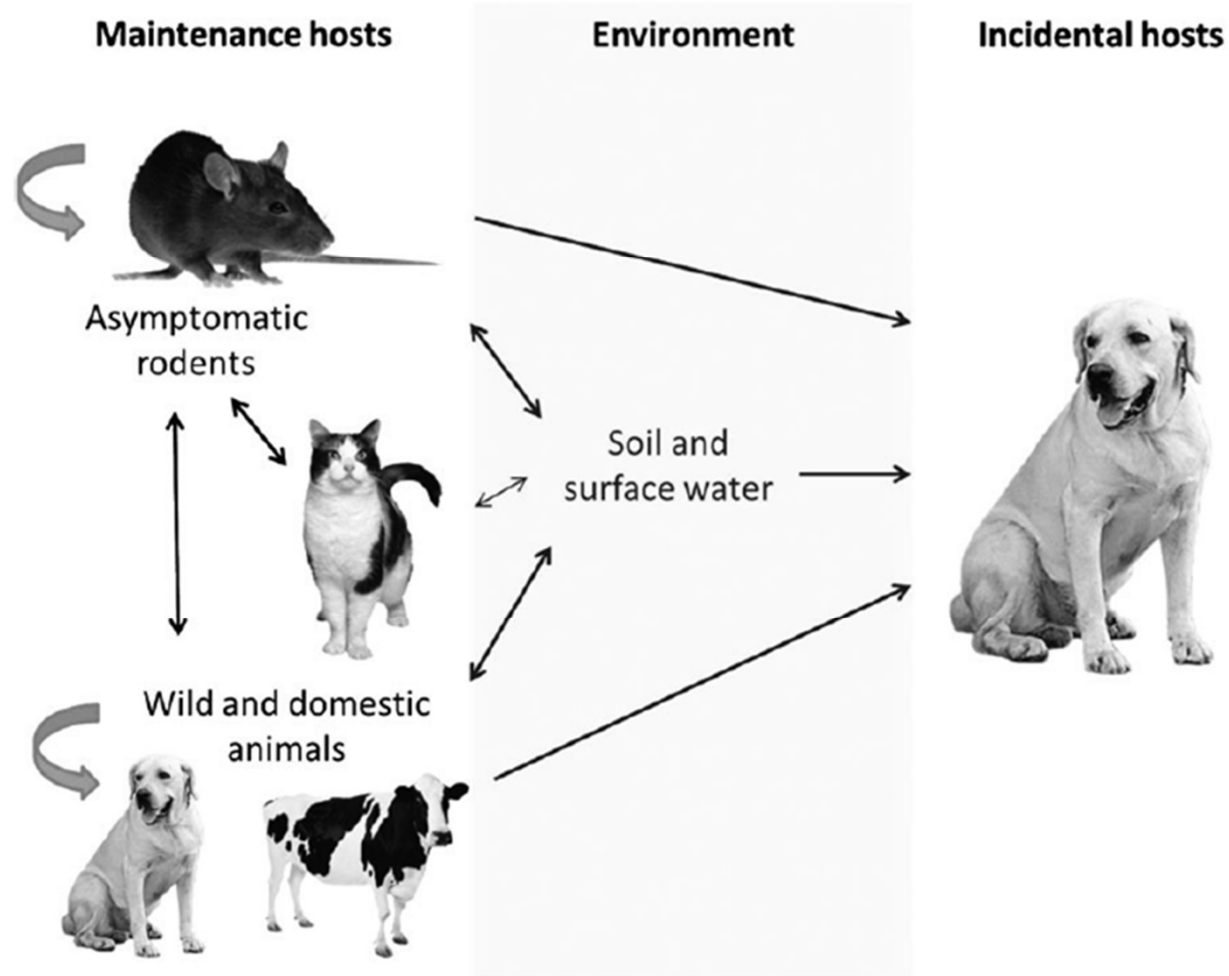


FIG 2. Transmission cycle of pathogenic *Leptospira* spp. Pathogenic leptospires are maintained in the environment by wild or domestic reservoir hosts. Incidental hosts become infected via either direct contact with reservoir hosts or contaminated soil and surface water. Cats are probably more likely to become infected via contact with prey due to their natural aversion to water. The role of dogs and cats as reservoir hosts requires further study

European consensus statement
on leptospirosis in dogs and cats, 2015



Epidemiology



- Definitive or reservoir host
- Chronic colonization of renal tubules
- Infected at young age
- No clinical signs or chronic clinical disease



Epidemiology



Table 3: Typical reservoir hosts of common leptospiral serovars (adapted from Bharti *et al.*, 2003).

Reservoir host	Host-adapted serovars
Pig	Pomona, Tarassovi
Cattle	Hardjo, Pomona
Horse	Bratislava
Dog	Canicola
Sheep	Hardjo
Rat	Icterohaemorrhagiae, Copenhageni
Mouse	Ballum, Arborea, Bim
Bat	Cynopteri, Wolffi

European consensus statement
on leptospirosis in dogs and cats, 2015



Epidemiology



- Incidental host exposure
 - Contaminated water or moist soil
 - Direct contact with contaminated urine
 - The bacteria survives but does not replicate outside the host

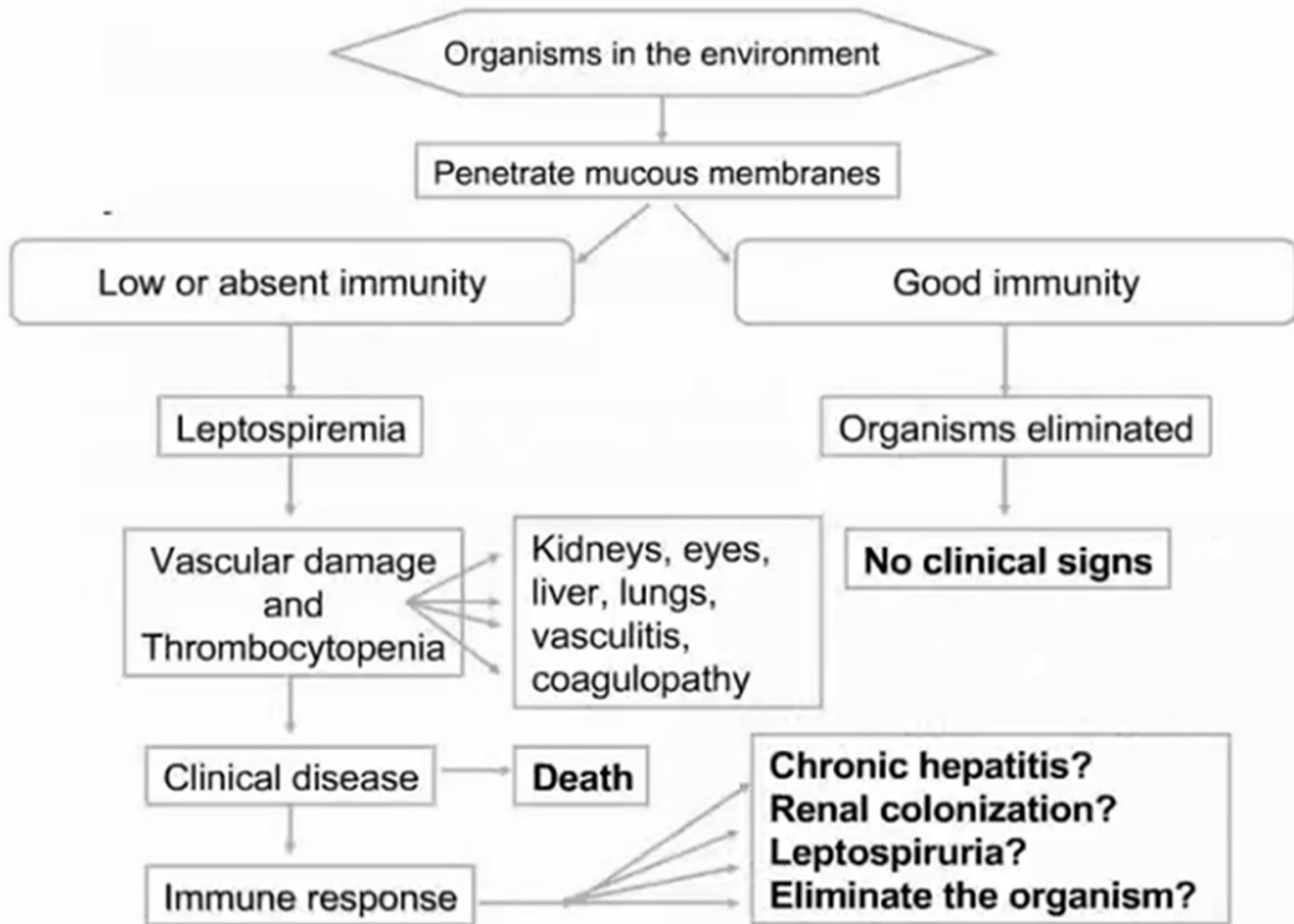




Pathogenesis

- Enter to the body through small cuts or abrasions, via mucous membranes
- Spread via the blood
- Multiply and reach target tissues such as the liver and kidneys
- Damage to the endothelium of small blood vessels leading to localized ischemia in organs





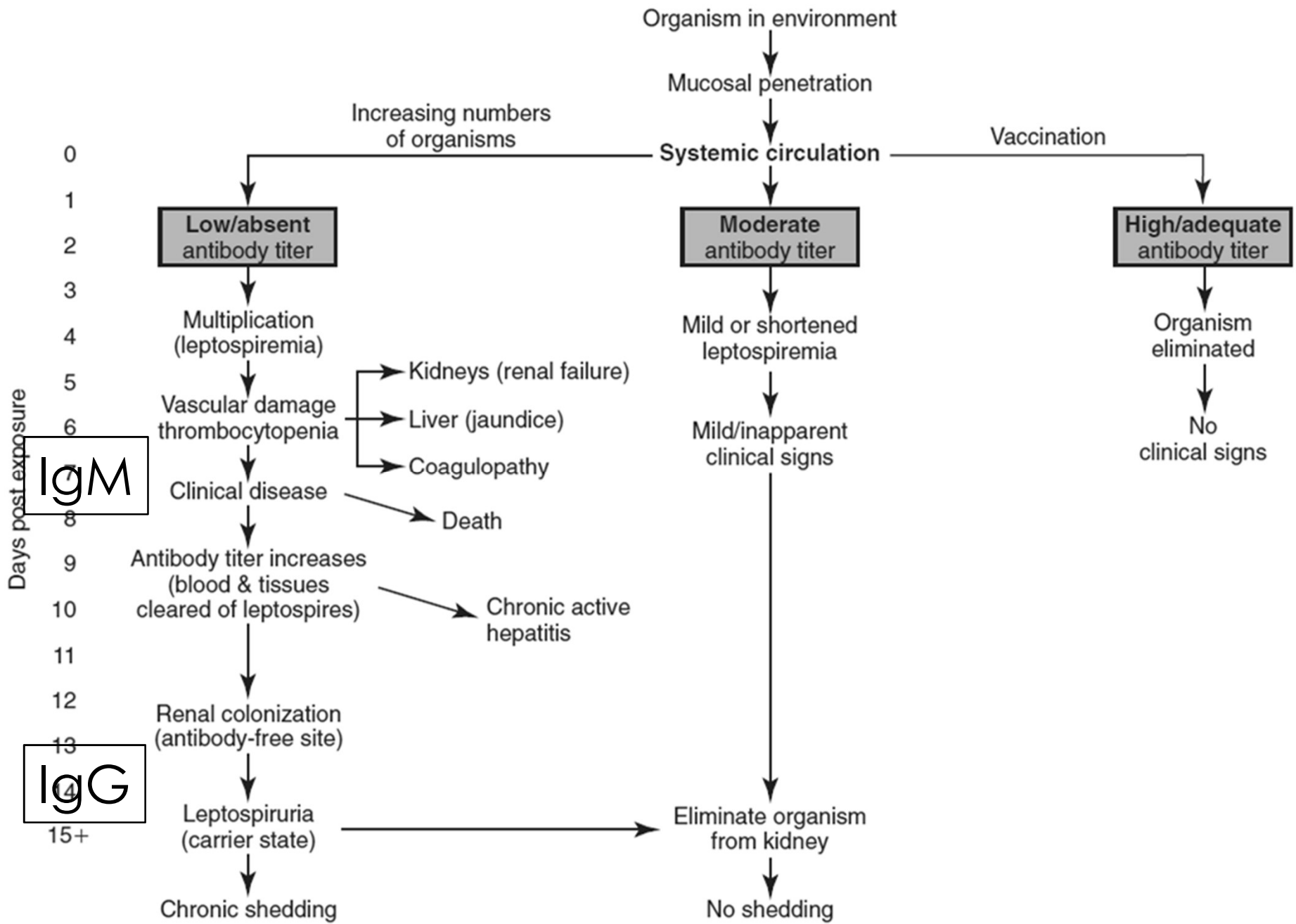


FIG. 42-2 Pathogenesis of leptospiremosis.



Why is leptospirosis problematic and overlooked?

- Risk factors: inconclusive
 - Ingestion of raw meat
 - Expose or drink from rivers
 - Roaming
 - Access to sewage
 - Contact with rodent

- Nonspecific clinical signs
 - No signs to life threatening





Table 2. Clinical signs and thoracic radiographs in surviving (group 1) and non-surviving (group 2) dogs with leptospirosis on admission day

Parameter	Survivors group	Non-survivors group
	1 (n=67) n (%)	2 (n=32) n (%)
Lethargy	64 (96)	31 (97)
Anorexia	59 (88)	28 (88)
Vomiting	56 (84)	28 (88)
Abdominal pain	26 (39)	13 (41)
Diarrhoea	22 (33)	16 (50)
Oliguria	11 (16)	16 (50)
Dyspnoea grade 1*	7 (10)	6 (19)
Dyspnoea grade 2†	12 (18)	25 (78)
Delayed capillary refill time	7 (10)	11 (34)
Pallor	12 (18)	5 (16)
Fever	10 (15)	5 (16)
Hypothermia	11 (16)	4 (13)
Icterus	2 (3)	8 (25)
Lymphadenopathy	6 (9)	4 (13)
Stiff gait	6 (9)	2 (7)
Severe thoracic radiographic abnormalities‡	8 (12)	16 (50)

*Respiratory rate of >35/minute

†Respiratory rate of > 40/minute, open mouth breathing, cyanotic mucous membranes, with or without haemoptysis

‡Generalized severe reticulonodular interstitial pattern with patchy alveolar consolidations

**Knöpfler et al
2017**





Common clinical manifestations

- Lethargy
- Polydipsia and polyuria
- Oliguria or anuria
- Altered hydration status (overhydration with oliguria/anuria or dehydration with polyuria)
- Gastrointestinal abnormalities (decreased appetite, vomiting, diarrhea)
- Icterus
- Bleeding tendency (petechia, melena, hematochezia, epistaxis)
- Tachypnea
- Conjunctivitis





Probably thinking of *E.canis*

- Lethargy
- Fever
- Lameness
- Ocular signs (uveitis)
- Bleeding disorder
- Neurologic signs





Table 3. Laboratory parameters in surviving (group 1) and non-surviving (group 2) dogs with leptospirosis (at the time of maximal deviation from the reference range)

Parameters	Survivors group 1 (n=67) median (range)	Non-survivors group 2 (n=32) median (range)
Platelets (G/L) (n=99)	98 (7 to 478)	50 (5 to 639)
Haematocrit (L/L) (n=99)	0.31 (0.16 to 0.65)	0.33 (0.2 to 0.5)
Leucocytes (G/L) (n=99)	24.7 (9.1 to 87.9)	23.1 (6.6 to 104)
aPTT (s) (n=75)	18.1 (9.2 to 31.4)	21 (12.1 to 43)
PT (s) (n=76)	18.7 (14.3 to 50)	18.6 (12 to 31.2)
Creatinine ($\mu\text{mol/L}$) (n=99)	300 (70 to 1608)	635 (137 to 1273)
Urea (mmol/L) (n=98)	35.8 (4.7 to 111)	61.6 (7.6 to 300)
ALT (U/L) (n=97)	142 (36 to 16,176)	173 (20 to 757)
AP (U/L) (n=97)	238 (23 to 4590)	343 (33 to 3298)
AST (U/L) (n=93)	72 (20 to 16,733)	180 (18 to 1499)
Bilirubin ($\mu\text{mol/L}$) (n=97)	11.4 (1.4 to 604)	28 (3 to 765)
Albumin (g/L) (n=94)	27 (19 to 35)	25 (21 to 47)
Total protein (g/L) (n=97)	74 (40 to 98)	68 (41 to 90)
Sodium (mmol/L) (n=99)	136 (114 to 162)	134 (118 to 149)
Chloride (mmol/L) (n=44)	99 (81 to 124)	97 (87 to 108)
Phosphate (mmol/L) (n=97)	2.6 (0.73 to 9.3)	5.0 (1.3 to 10.1)
Potassium (mmol/L) (n=99)	2.9 (1.7 to 6.0)	3.8 (2.3 to 6.9)
Calcium (mmol/L) (n=96)	2.5 (1.0 to 3.9)	2.6 (1.6 to 3.8)
Urine protein/creatinine ratio (n=64)	2.1 (0.24 to 18.4)	2.5 (0.45 to 11.3)

**Knöpfler et al
2017**





Laboratory findings (n=99)

Tests	%
Thrombocytopenia	63
Anemia	63
Leukocytosis	57
Prolonged aPTT	53
Prolong PT	22
Increased BUN	84
Increased creatinine	81
Hyperbilirubinemia	69
Increased ALP	68
Increased ALT	54
Hypoalbuminemia	55
Hyperphosphatemia	67



Probably thinking of *E.canis*

- Thrombocytopenia
- Nonregenerative anemia
- Leukopenia
- Lymphocytosis
- Pancytopenia
- Hyperglobulinemia
- Hypoalbuminemia
- Increased ALT
- Increased ALP





Clues for diagnosis

- Non specific clinical manifestations, hematology and serum biochemistry!!!
- Suspect if
 - Signs of vasculitis
 - Acute kidney injury +/- hepatic injury
 - Fever
 - Pulmonary hemorrhage
 - Uveitis
 - Myositis
 - Reproductive failure

Quick treatment initiated,
increased survival rates

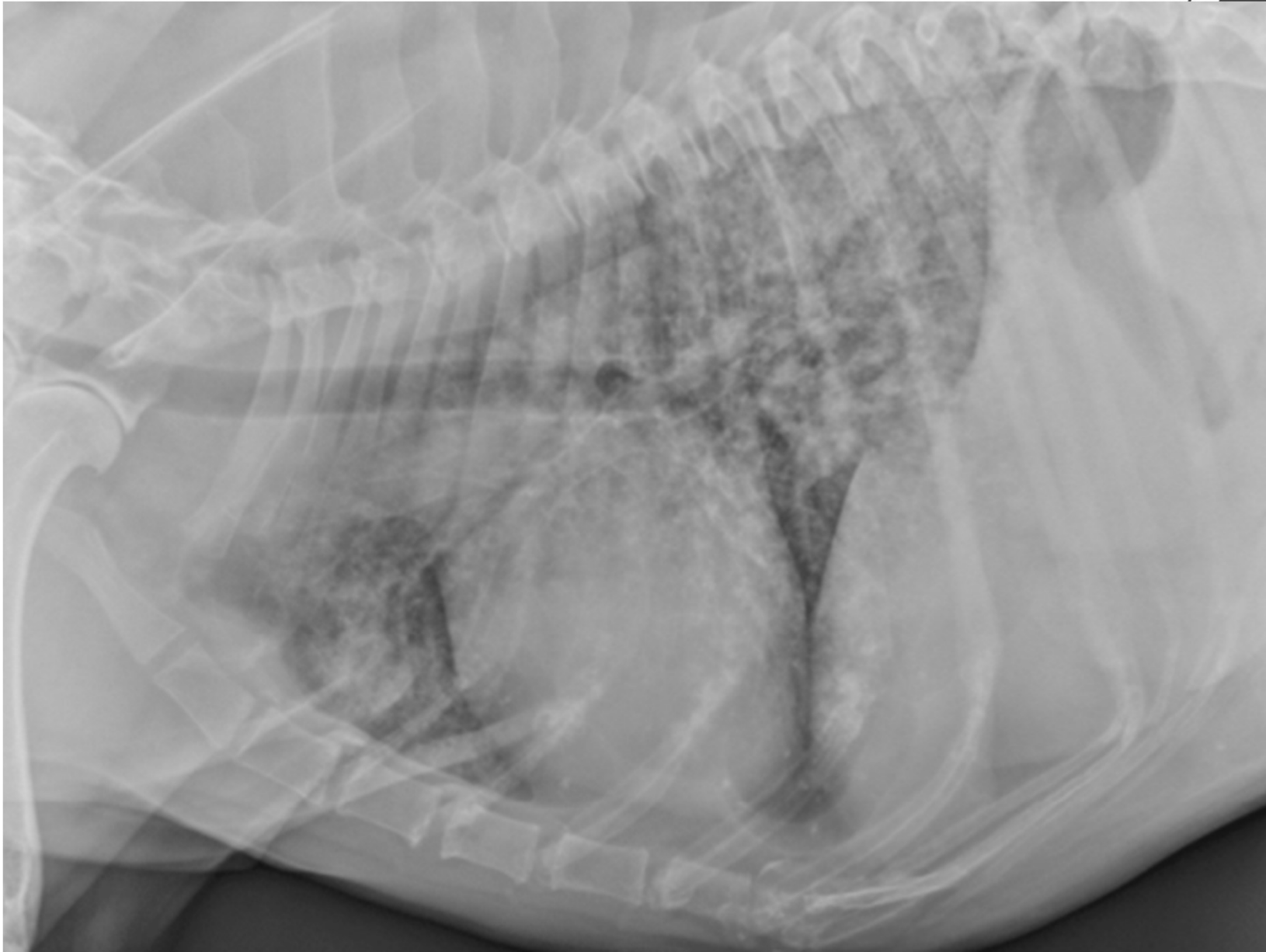


Do you think diagnostic
imaging necessary?





<https://todaysveterinarypractice.com/diagnosis-and-treatment-of-leptospirosis-in-dogs/>



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Thoracic radiography

- Abnormality reported 70% of cases (Knöpfler et al 2017)
- Abnormalities found in with or without clinical signs
- Most common: mild to moderate interstitial pattern
- Pulmonary hemorrhage syndrome: severe nodular interstitial to alveolar pattern





Abdominal ultrasonography

- 85-100% found nonspecific changes associated with kidney injury
 - Increased cortical echogenicity
 - Renomegaly
 - Mild pyelectasia
 - Medullary rim sign
 - Perirenal effusion



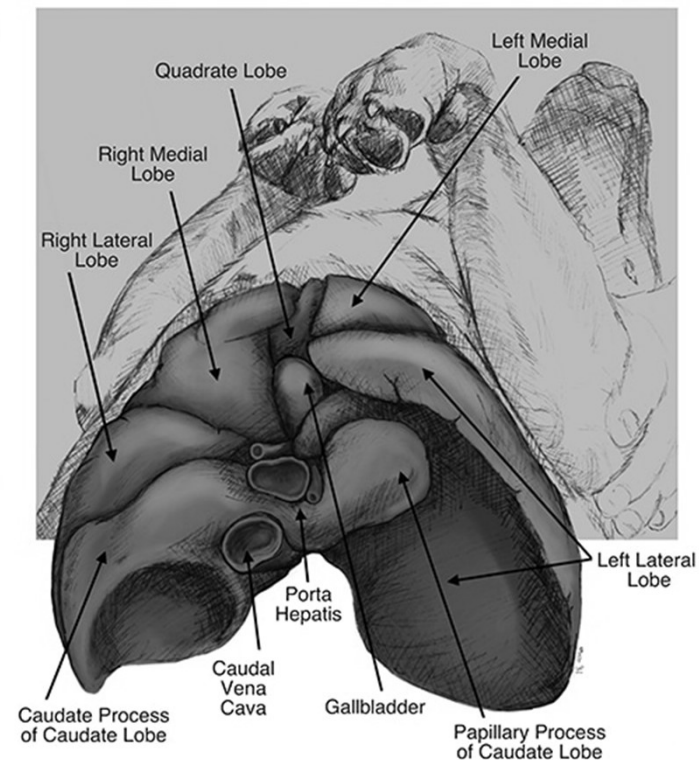
FIG. 3. Kidney from figure 2 in a water bath demonstrating the medullary rim sign (open arrow) and the chronic depressed infarct (white arrow).

Biller et al, 1992

Abdominal ultrasonography



- Hepatic changes
 - Hypoechoic parenchyma
 - Hepatomegaly
 - Evidence of biliary sludge



Pamela Boutilier, in Small Animal Internal Medicine



Diagnostic tests

- Detect bacteria directly
 - Culture
 - Dark field microscopy
 - PCR for bacterial DNA

- Detect Ab against *Leptospira*
 - Microscopic agglutination test (MAT)
 - Point-of-care assays

Timing of specimen collection & optimum specimens to be submitted



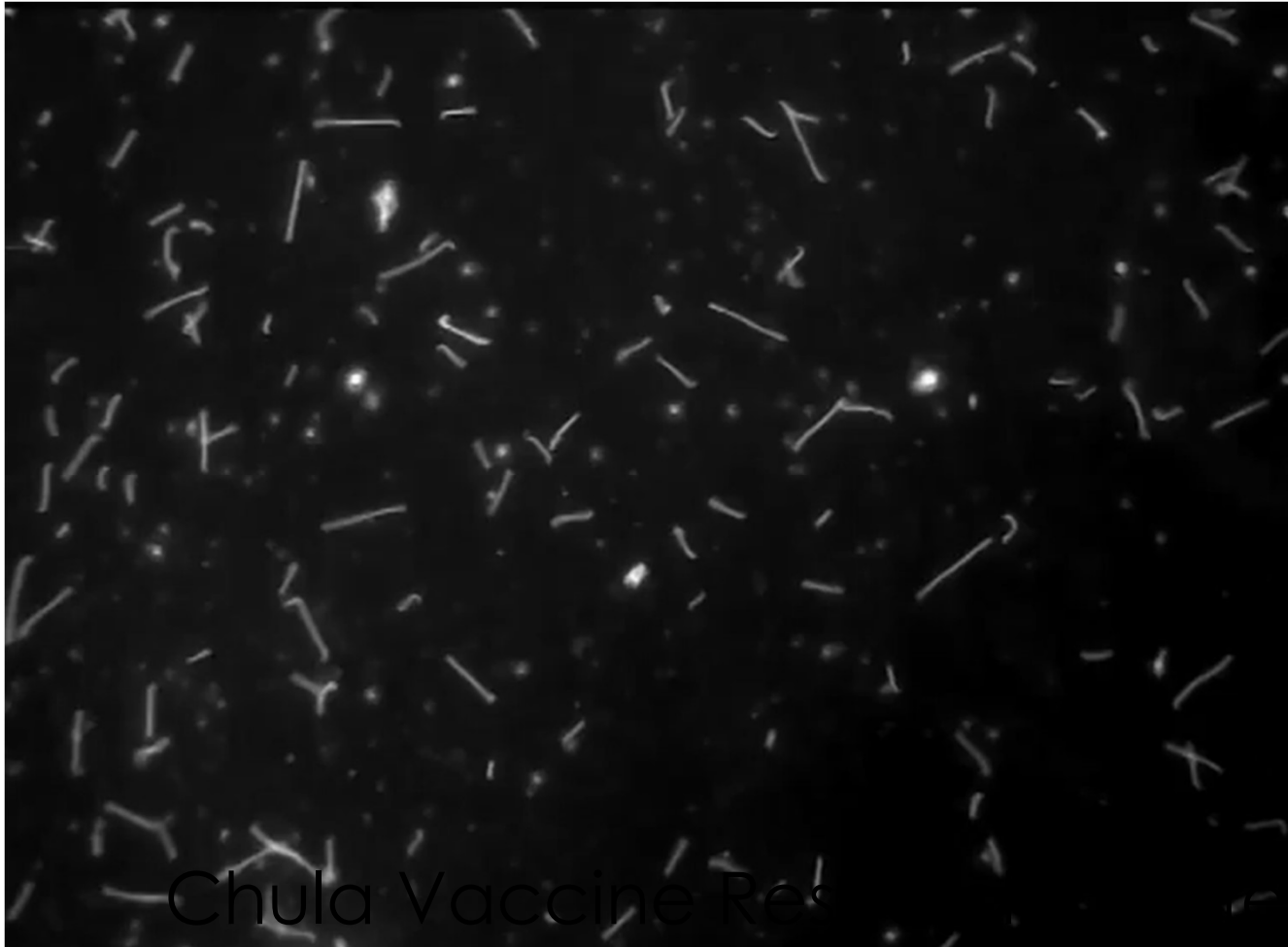


Bacterial culture

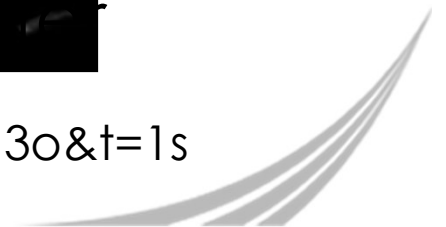
- Technically difficult
 - Need special media
 - Culture incubation times: months
 - Recommendation
 - First 10 days: blood
 - Later: urine

Not routinely use in clinical practice

Dark field microscopy



https://www.youtube.com/watch?v=3UD9B3_Vq3o&t=1s





Dark field microscopy

- Need special equipment & training
- Rapid diagnosis
- Low sensitivity
 - Short duration of leptospiremia
 - Previous antimicrobial treatment
- False positive from debris

Not routinely use in clinical practice



PCR for bacterial DNA

- Specimen: blood, urine

Transient

- Leptospiremia: first 10 days post infection

- Leptospiuria: after leptospiremic phase **Intermittent**

- Negative result: cannot rule out leptospirosis

- Low bacteria

- Immune response

- Administration of antibiotics





PCR for bacterial DNA

- Positive result with a compatible clinical manifestation suggests leptospirosis
- Positive in healthy dog = chronic carrier state
 - Rate 0-25% based on region
- Positive result should interpret in the light of clinical signs
- Recent vaccination does not interfere with PCR results





Microscopic Agglutination Test (MAT)

- Provides a quantitative antibody titer
- Panel (typically 6–8) of serovars
 - False negative if not include the infecting serogroup
- Paired serum samples: 7-14 days apart
 - 4X increase in titer = seroconversion, confirmed leptospirosis
 - Acute- or convalescent- phase

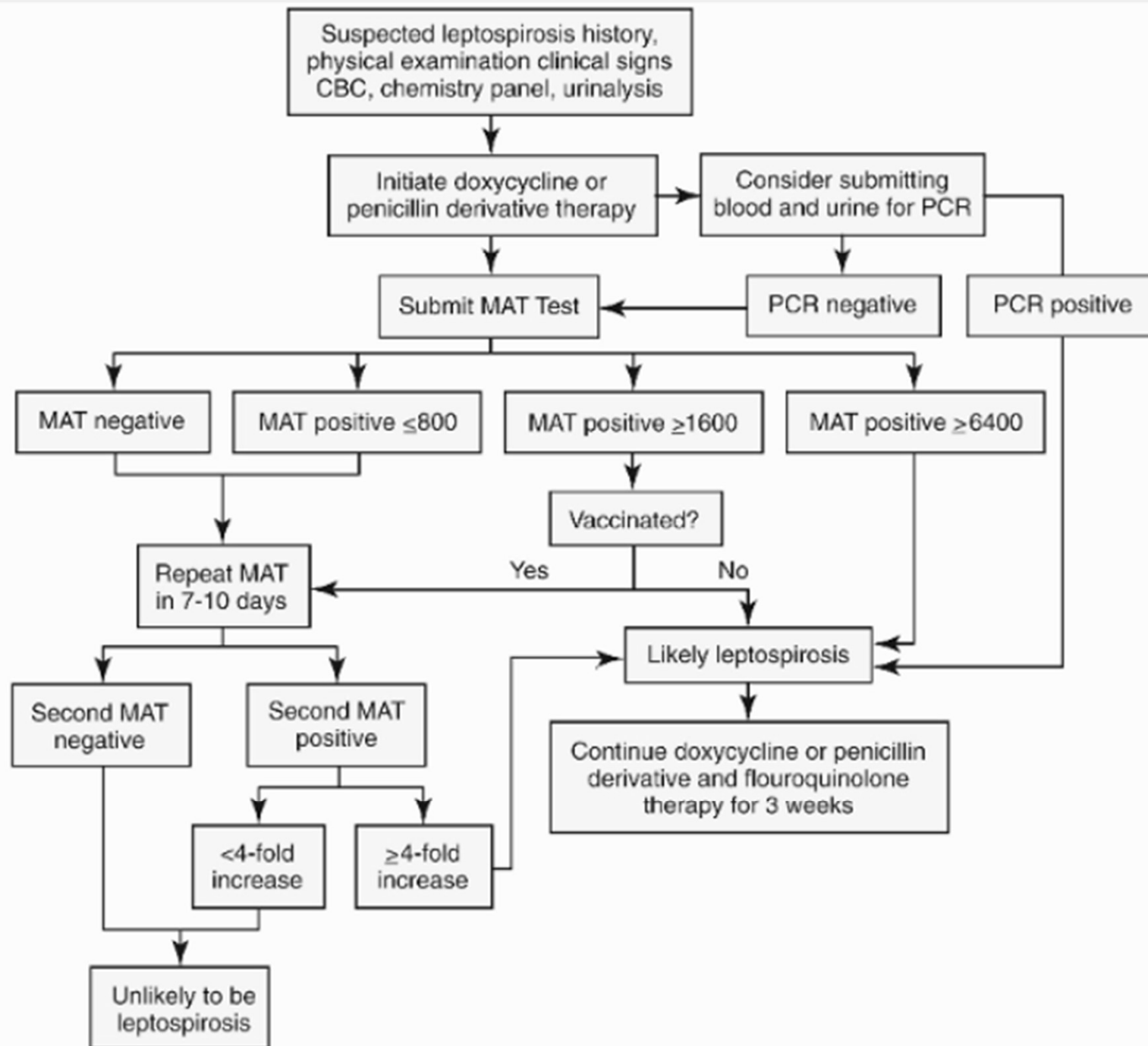




Microscopic Agglutination Test (MAT)

- Careful interpretation: previous exposure vs. vaccination status
- Single high titer + clinical signs suggest leptospirosis
- Vaccination can induce a positive MATs to both vaccinal and nonvaccinal serogroups
- Serologic cross-reaction among serovars





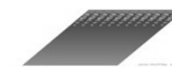


Point-of-care assays

- Provide rapid diagnostic results
- Modified ELISA & qualitative detect lepto Ab
- Limitation as MAT
- Should be confirmed Paired MAT or PCR
- Need more studies



GenBody
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Animal Diagnostics



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Leptospira Ab
Leptospira Antibody Test

Rapid immunochromatographic test for the detection of the antibodies to Leptospira in serum, plasma or whole blood from cats and dogs.

Only for veterinary use

Store at 2-30°C



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Diagnostic strategy

- No perfect test
- Need combination of tests or series
- 2 Consensuses recommendation (all 3 of these)
 - Appropriate clinical signs
 - PCR (blood & urine) before antibiotic
 - Paired MAT titers
- Point of care: valuable while waiting for PCR or MAT





Treatment of Leptospirosis

- Human = controversy
- Dogs = sparse studies
- Recommendation
 - Doxycycline 5 mg/kg q12h or 10 mg/kg q24h PO for 14 days
 - Dogs with gastrointestinal signs initially should be treated with an
 - Ampicillin 20–30 mg/kg IV q6–8h
 - Penicillin G 25,000–40,000 U/kg IV q6–8h
 - Amoxicillin 20–30 mg/kg IV q6–8h





Supportive care

- Depends on severity and affected organs
- Maintain hydration with IV fluid therapy
- Correcting electrolyte & acid-base imbalance
- Anti-emetics
- Anti-hypertension
- Pain control
- Nutritional support





Treatment of AKI

- Can be
 - Polyuria **10x maintenance, > 20ml/kg/h**
 - Anuria/oliguria **1/2x maintenance, 1ml/kg/h**
- Monitor UOP, body weight, RR, SBP q4-6h
- GI signs: anti-emetics & gastroprotectants
- Pain control: fentanyl, buprenorphine
- Dialysis when oliguria or anuria with subsequent life-threatening hyperkalemia or severe volume overload and advanced uremia refractory to medical management





Treatment of Hepatopathy

- Signs severe liver failure with hepatoencephalopathy, hypoglycaemic seizures or ascites
- Mostly supportive to improve hepatic function





Treatment of leptospiral pulmonary hemorrhage syndrome (LPHS)

- Main cause of death
- Plasma or whole blood transfusion
- Oxygen therapy & (mechanical ventilation, if available)



Treatment of hemostatic disorders



- Hemostatic disorders in leptospirosis are multi-factorial origin

Plasma transfusion

- Hypocoagulable conditions from DIC
- Failure of coagulation factor synthesis
- Thrombocytopenia

No specific therapy





Treatment and prophylaxis for dogs living in the same household as infected dogs

- Doxycycline 5 mg/kg q12h or 10 mg/kg q24h for 2 weeks





Hospitalized Patients

- Daily monitor: clinical signs and
 - Renal function
 - Electrolytes
 - Acid-base status
 - PCV
 - Plasma protein
- CBC q 48 h to assess thrombocytopenia





Precautions to Take Around Hospitalized Leptospirosis Patients

- Place warning signs on patient's cage
- Limit movement of patient through the hospital (although isolation is not needed)
- Wear personal protective equipment (gloves, disposable gown, eyewear/facemask) when handling the patient
- Do not pressure wash animal cages (to avoid aerosolization of leptospire)
- Minimize urinary contamination (walk the patient frequently)
- Clean with disinfectant solutions that will inactivate leptospire (e.g., bleach, iodine-based products, accelerated hydrogen peroxide, quaternary ammonium)
- Launder bedding normally with hot water and detergent





Monitoring patients

- Discharge dogs, if PU resolved with adequate hydration without additional fluid support
- Re-examination
 - 1 week after discharge
 - Then 1-3 weeks until clinical stabilization
- Long term monitoring of renal function: q 1, 3, 6 months





Environmental control

- Minimize exposure to carriers
 - Urine PCR
- Minimize exposure to standing water
- Isolate possible-shedding dogs
 - Treat carrier states with doxycycline
 - Bacteria sensitive to detergent & drying
 - Iodine-based disinfectants are best
- Preventing of zoonotic transfer
 - Treat carrier states with doxycycline





Vaccination

- Prior to 2000
 - Bivalent bacterins: canicola, icterohaemorrhagiae
- Since 2000 (in USA) 4 serovar vaccines
 - Canicola, Icterohaemorrhagiae
 - Pomona, Grippotyphosa
- 2000 – purified subunit vaccine
- 2005 – microfiltered vaccine
- 2010 – two new culture-based vaccine





Vaccination questions

- Duration of immunity – Do the vaccines last for a year?
 - Yes! 15 months (Grosenbaugh & Pardo, 2018)
- Side-effect (Type I hypersensitivity)
 - Breed-associated?
- Do vaccinated dogs remain nonclinical carriers?
 - No
- Can MAT predict immunity?
 - No



Do vaccines interfere with RT-PCR?



Midence et al 2012

- 2 groups of 10 dogs – each vaccinated with one or two different 4-serovar vaccines
- Blood collected pre-vaccinated, 3, 7, weekly to 8 weeks
- RT-PCR on blood of all dogs was negative for *L. interrogans* at all time points
- MAT ... typical vaccinated dogs
- Conclusion: recent vaccination does not interfere with the use of RT-PCR for the diagnosis of leptospirosis



Take home message

- Nonspecific clinical signs and laboratory parameters leading to overlook
- No perfect tests but need the combination to confirm
- Treatment of other diseases may also treat lepto!
- Monitoring carefully and continuously
- Duration of immunity last for 15 months

