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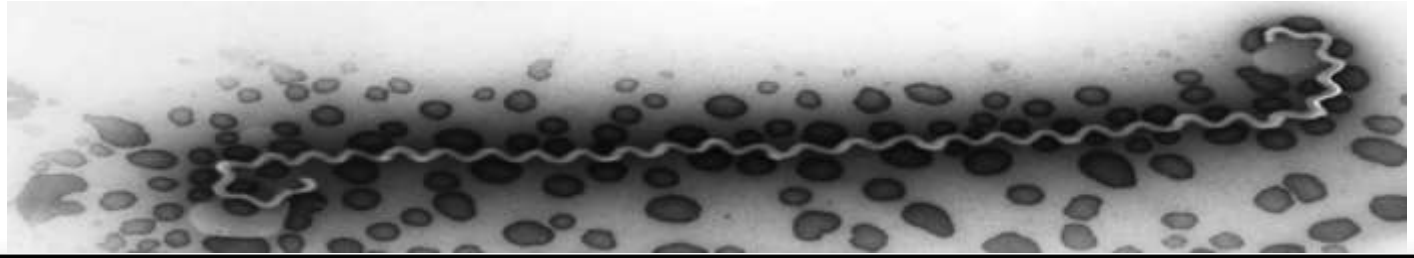
Leptospira Diagnostics in Animals

Sree Rajeev BVSc, PhD, DACVM, DACVP

1st International Workshop for Leptospirosis Translational Research Based on Country Needs
&
5th Global Leptospirosis Environmental Action Network (GLEAN) Meeting

10-12 November 2015
Rio de Janeiro, Brazil
Oswaldo Cruz Institute/FIOCRUZ




Lake Huron
Missouri
North Carolina
Norwegian Sea
United States
Atlantic Ocean
South Carolina
Bermuda
Texas
Florida
Turks & Caicos
Puerto Rico
Bahamas
Virgin Islands (US & Br.)
Anguilla
Cozumel and
Mexican Caribbean
Cuba
Dominican Rep.
St. Maarten/St. Martin
Antigua
Caribbean (Roving boats)
Cayman
Islands
Guadeloupe
Belize
Dominica
Jamaica
St. Lucia
St. Kitts
Barbados
Honduras
Aruba
Grenadines
Curaçao
Bonaire
Grenada
Venezuela
Trinidad & Tobago
Uruguay
South America
Brazil

Two island country

Located in the Leeward island chain in the Lesser Antilles and an island in West Indies

Land area -65 sq miles
Population-~45,000.

Smallest independent country in the western hemisphere

Member of Organization of Eastern Caribbean States and British Commonwealth

- Moved from sugar cane-based economy to tourism
- Ranked by World Bank as a high income country

- Volcanic island
- Two Large peaks with tropical rainforest

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RUSVM

Founded 1982

- Graduated > 3500 veterinarians
- AVMA Accredited since 2012
- Seven semester program of integrated pre-clinical and clinical studies
- Affiliated with more than 20 AVMA accredited schools of veterinary medicine to enable final year of clinical training
- Postgraduate research program instituted in 2014

Caribbean Research Network for Emerging and Neglected Diseases

- Fill gaps in knowledge regarding burden and transmission in the Caribbean Basin
- Develop capacity of a regional center for coordinating research efforts
- Support PAHO, CARPHA and other regional efforts to combat END

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RUSVM's New Research and Pathology Building expected to be completed by 2016

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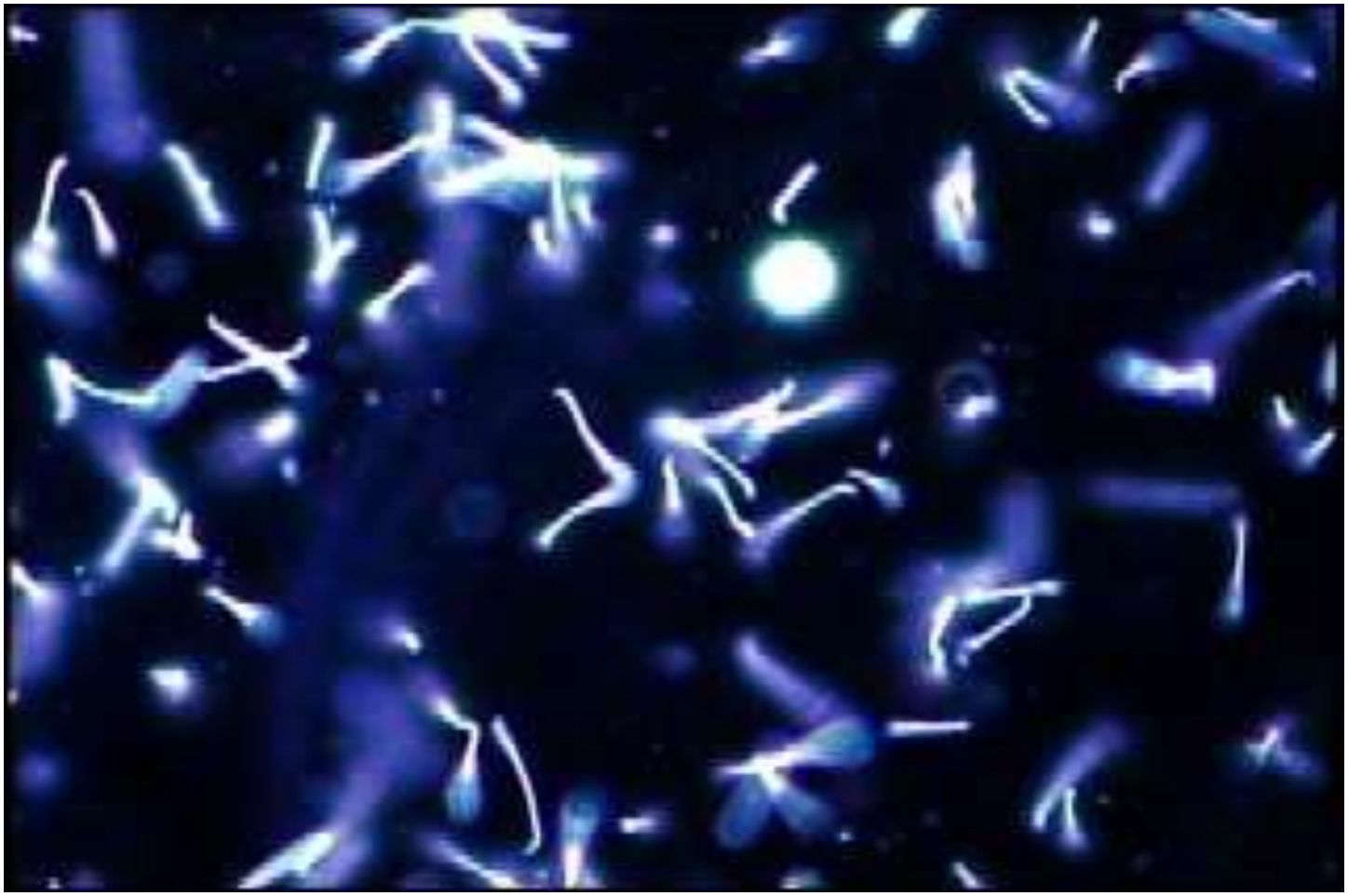
Leptospirosis in animals



file1 lepto DF.mpg

Pathogenesis

Penetrates epithelial barriers (incubation 4-20 days)
Bacteremia (for up to 7 days)
Enter kidney, (liver, spleen, CNS, genital tract)
Antibodies develop (extent and duration varies)

Maintenance hosts

Privileged sites
(kidneys or reproductive tract?)

Long term shedding

Incidental hosts → **Severe Disease**

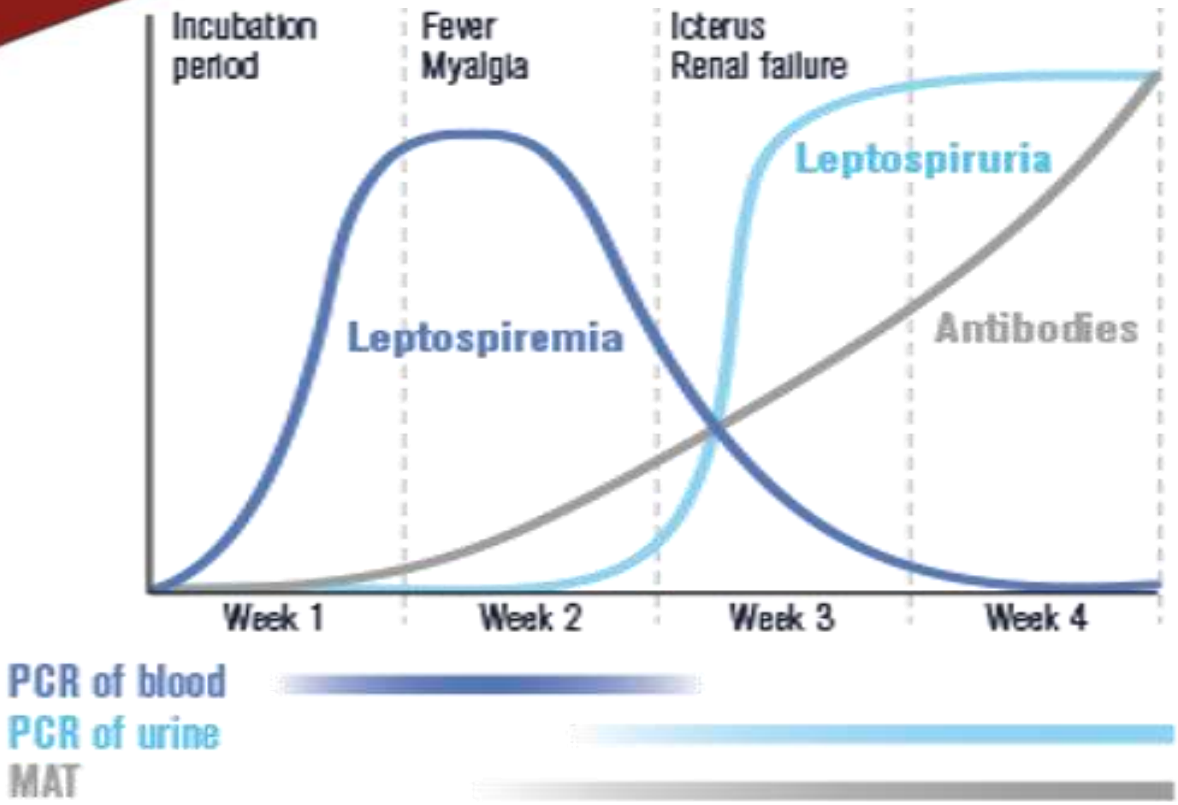
Recovery with short term shedding in urine

Leptospirosis-Dogs

Mild or no signs of disease, to severe illness or death.

(polyuria, polydipsia, dehydration, vomiting, diarrhea, inappetence, lethargy, or abdominal pain)

Signs of **Renal failure and** hepatic disease including icterus. Bleeding abnormalities and disseminated intravascular coagulation(DIC)

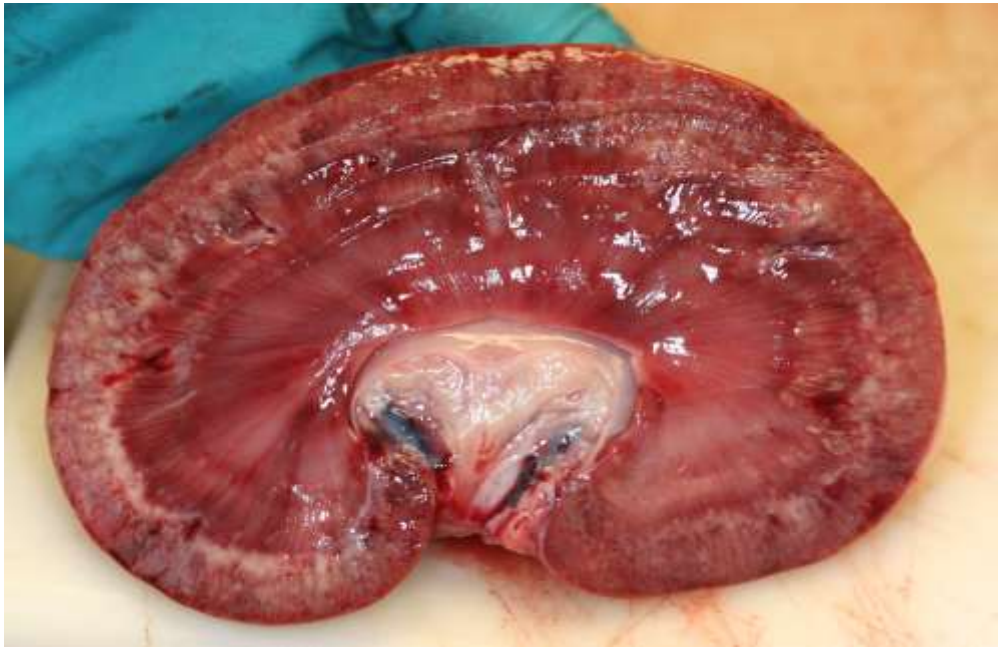


Mortality 11-27%

Chronic renal failure 33% - 40% of surviving dogs

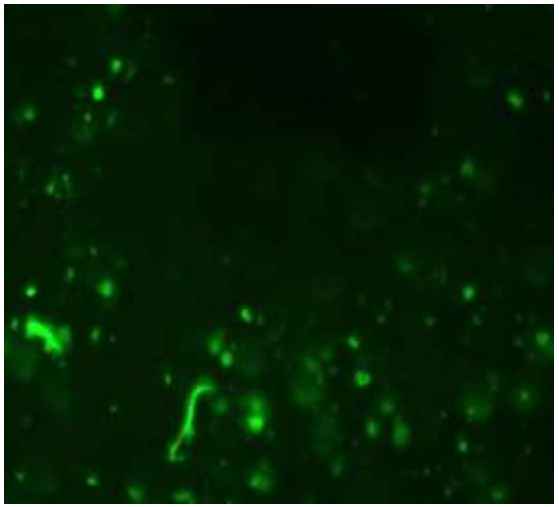
http://www.leptoinfo.com/clinics_veterinarians/lepto_articles/articleTwo2.html

Dogs surviving acute renal tubulointerstitial injury may have residual chronic kidney injury that progresses over months to years



Ante-mortem Diagnostic Tests

- FA and PCR on urine and blood
- Microscopic agglutination Test on 6 serovars



- Post-mortem Diagnosis
- Histopathology and confirmation by PCR and Immunohistochemistry

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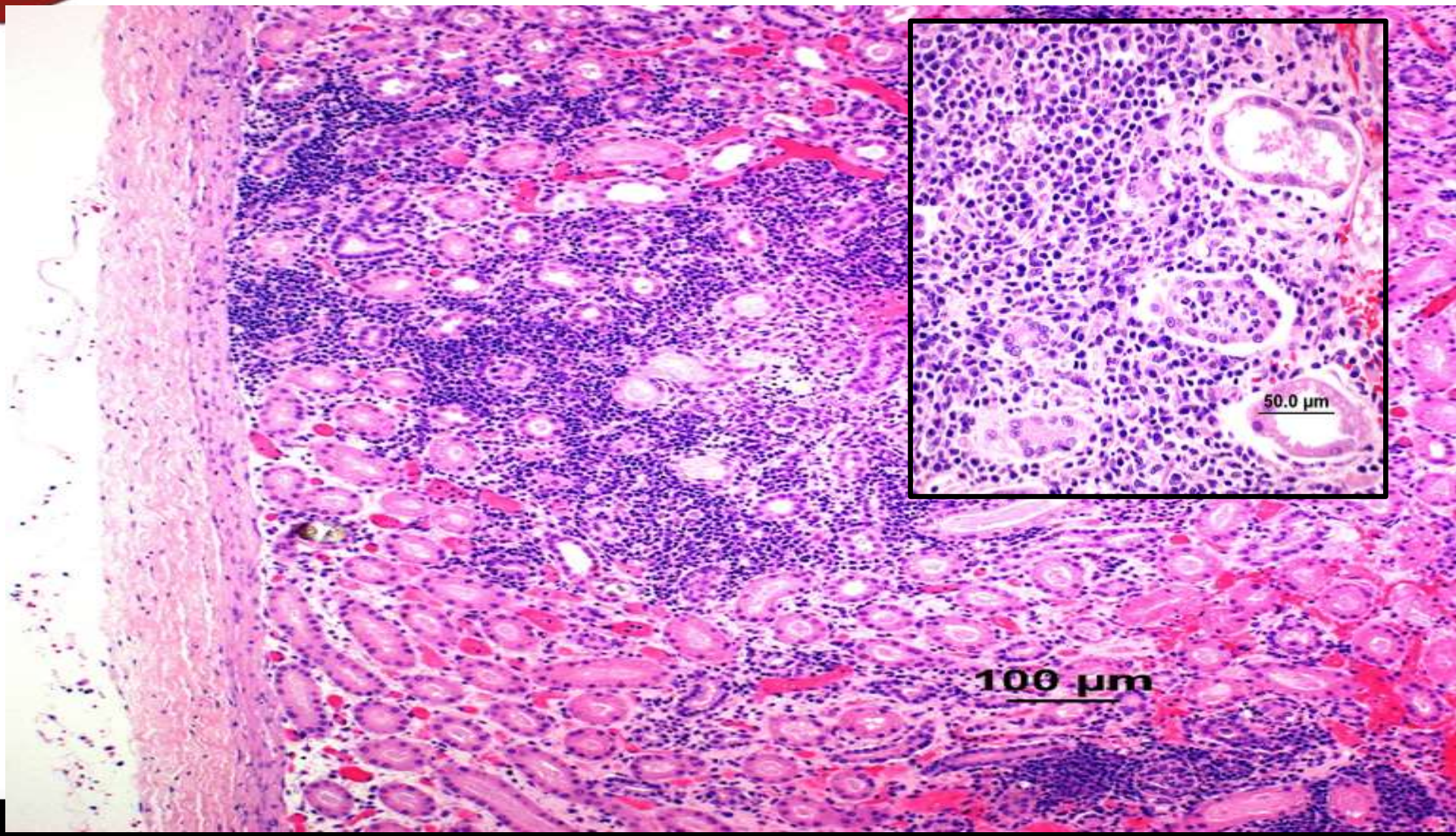
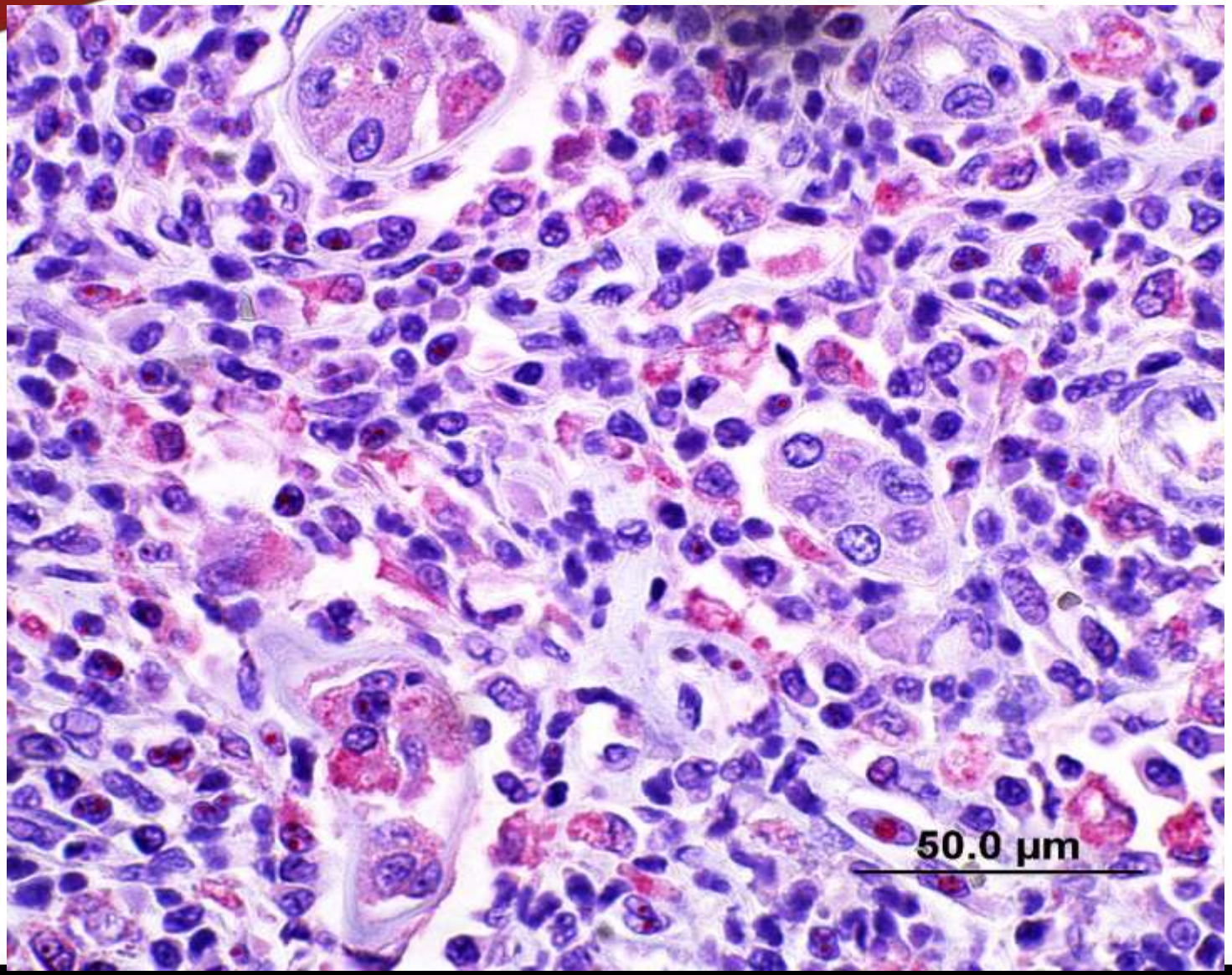
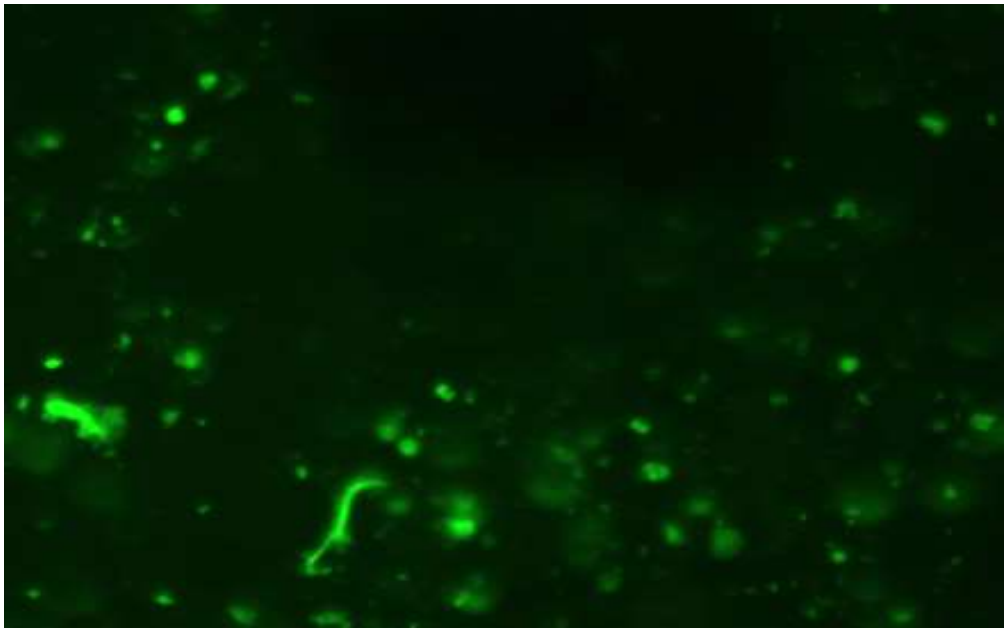
Tubulointerstitial Nephritis

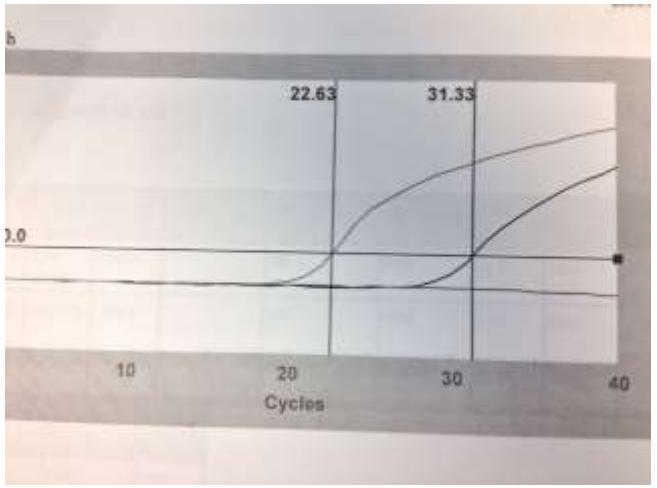



50.0 µm



Site Legend

Site ID	Sample ID	Cy3 Ct	Protocol
A4	3326-lepto/rt	31.33	LeptoRTSR
A5	N-lepto/rt	0.00	LeptoRTSR
A6	P-lepto/rt	22.63	LeptoRTSR



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Microscopic agglutination test; paired serum titers, Cross reactivity and Paradoxical reactions

	Pomona	Hardjo	Grippo typhosa	Icterohemorhagiae	Canicola	Bratislava	Autumnalis
Serum 1	400	N	3200	N	200	200	1600
Serum 2	800	N	6400	100	200	800	6400

SEROLOGY

LEPTOSPIRA

LEPTOSPIRA ANTIBODY MAT

Animal ID	POMO	HARD	GRIP	ICTE	CANI	BRAT	AUTU
GUNTHER	1:6400	NEGATI	1:6400	1:400	NEGATI	1:6400	1:6400

Ideal samples/tests for ante-mortem diagnosis to maximise the diagnostic sensitivity

Blood for PCR/FA
Urine for PCR/FA
Serum for MAT

} Based on the Stage of Presentation

Whenever possible, serum, Blood, and urine should be sent to the lab for PCR, FA and MAT

Leptospirosis in cattle and ruminants

Incidental infections with serovar Pomona results in severe acute disease

Leptospira borgpetersennii serovar Hardjo is host adapted in cattle and may result in reproductive failure due to early embryonic death and repeat breeding

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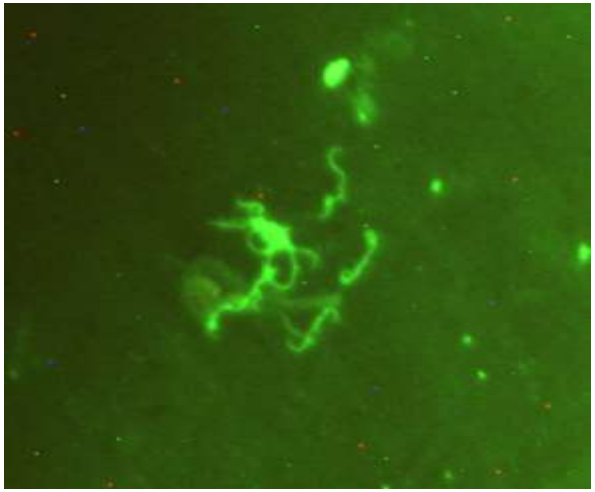
Diagnosis in cattle



- Urine FA and PCR
- Collect mid stream urine preferably after administration of a diuretic such as Lasix
- Overnight shipping of urine under refrigeration conditions is recommended
- Serum for MAT testing
- Usually chronically infected cows have low antibody titers

Horses

- Serovars pomona and grippotyphosa Bratislava(?) are the most common causes of equine leptospirosis.
- Clinical leptospirosis in horses is most commonly associated with abortions, systemic illness in foals
- severe forms including hemolysis and vasculitis with petechial hemorrhages hemoglobinuria, anemia, icterus.
- Renal failure and hepatopathy may also occur.
- The role in Equine Recurrent Uveitis??

- The first USDA-approved equine-specific vaccine to prevent leptospirosis in horses:Zoetis -Lepto EQ Innovator

Diagnosis: by MAT/FA/PCR

Other species

Pigs(Serovars Pomona and Bratislava)

Reproductive failure as evidenced by infertility and sporadic abortion

Serovar Pomona is endemic in California sea lions

Cats are considered resistant to clinical Leptospirosis

Many wild animal species are infected, however, burden of disease is not documented

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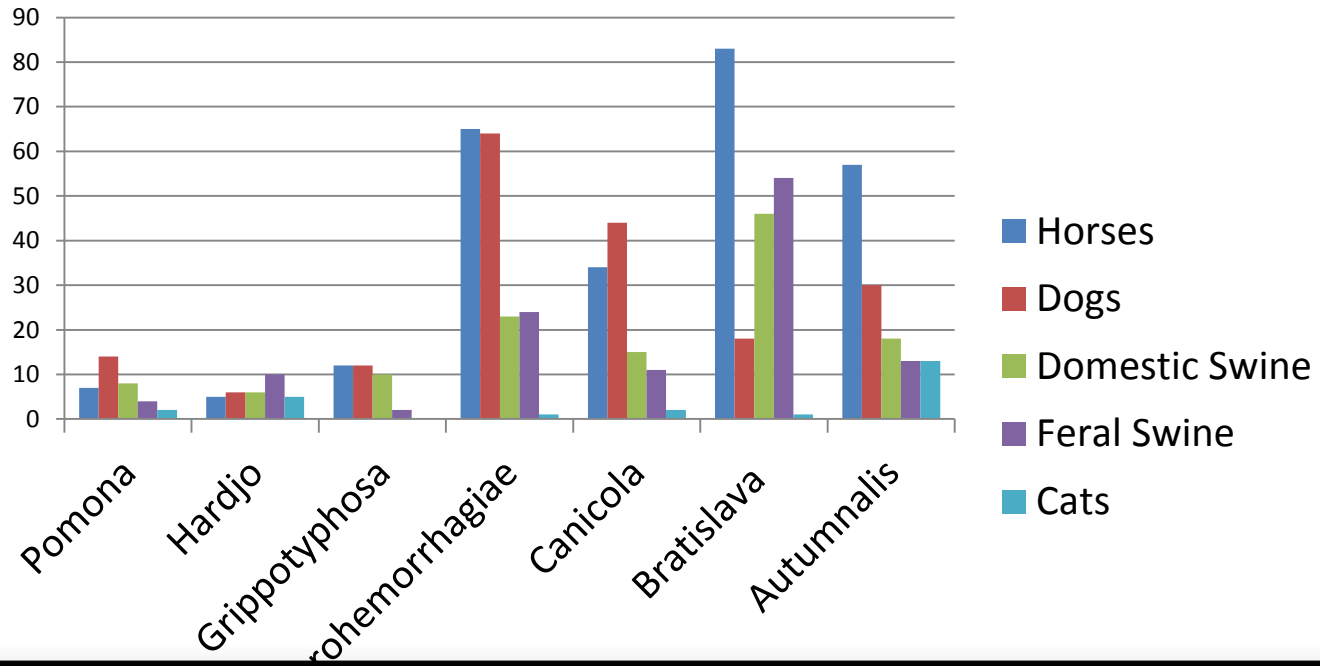
Overall Seroprevalence To Serovars

	Total tested	Positive	%positive
Horses	179	120	67%
Canine	287	93	32.40%
Domestic swine	160	59	36.80%
Feral Swine	169	79	46.70%
Cats	116	18	16%



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Dairy cattle surveillance Summary

7/10 farms(70%) had at least one FA positive cow

All herds had at least one cow with MAT titer >100 for one or more serovars

There is a significant association with infertility and Leptospira positivity

No clear significant association with vaccination and positivity

Testing methods (MAT) should be revisited and improved

Leptospira prevalence in Beef Herds

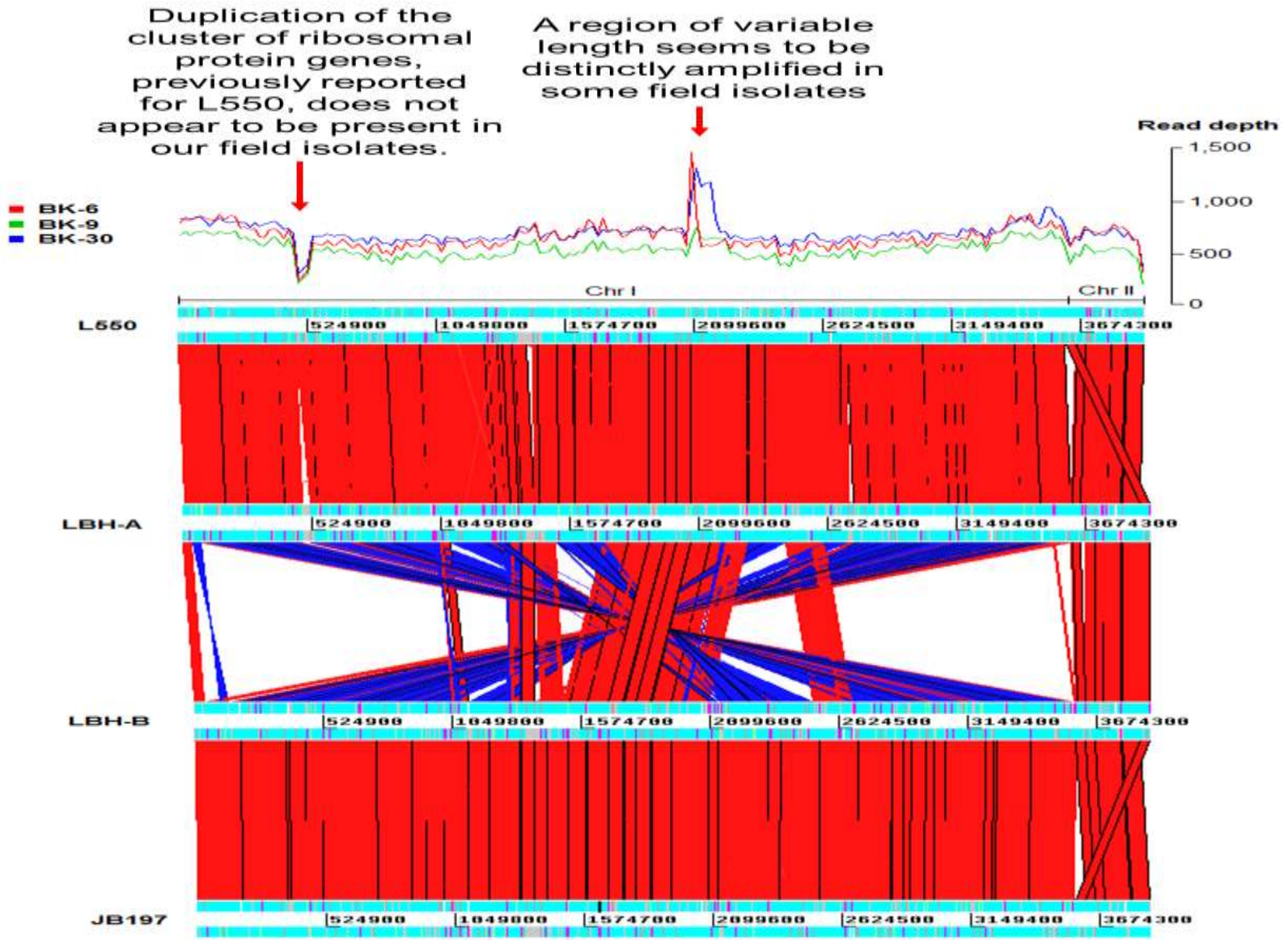
Table 1. Summary of Leptospira testing results for 37 bovine kidney samples

Test	Positive	Negative	Not Evaluated	Total
DFM	23 (62.2%)	11 (29.7%)	3 (8.1%)	37 (100%)
DFA	30 (81.1%)	5 (13.5%)	2 (5.4%)	37 (100%)
PCR	11 (29.7%)	26 (70.3%)	0 (0.0%)	37 (100%)
Culture	3 (8.1%)	34 (91.9%)	0 (0.0%)	37 (100%)

Read mapping and variant calling

Label	Total reads	Reference	Reads mapped	Reads unmapped	Total variants	SNP	INS	DEL
BK-6	9,467,714	L550	99.8%	0.2%	52	51	0	1
BK-9	7,716,262	L550	99.8%	0.2%	45	42	0	3
BK-30	9,903,708	L550	99.8%	0.2%	42	39	0	3
LBH-A	8,224,194	L550	99.9%	0.1%	43	40	0	3
LBH-B	7,467,366	JB197	99.6%	0.4%	3,848	3,777	58	13
LIH	7,929,346	Lai	97.4%	2.6%	28,828	28,561	192	75
		Cop	97.5%	2.5%	28,402	28,223	114	65

L. borgpetersenii serovar Hardjo (Hardjobovis)



Although L550 and LB197 genomes where used as template for contiguation of the LBH-A and LBH-B genomes, fragments that could be assembled *de novo* were very similar in structure to those of the corresponding reference genomes.

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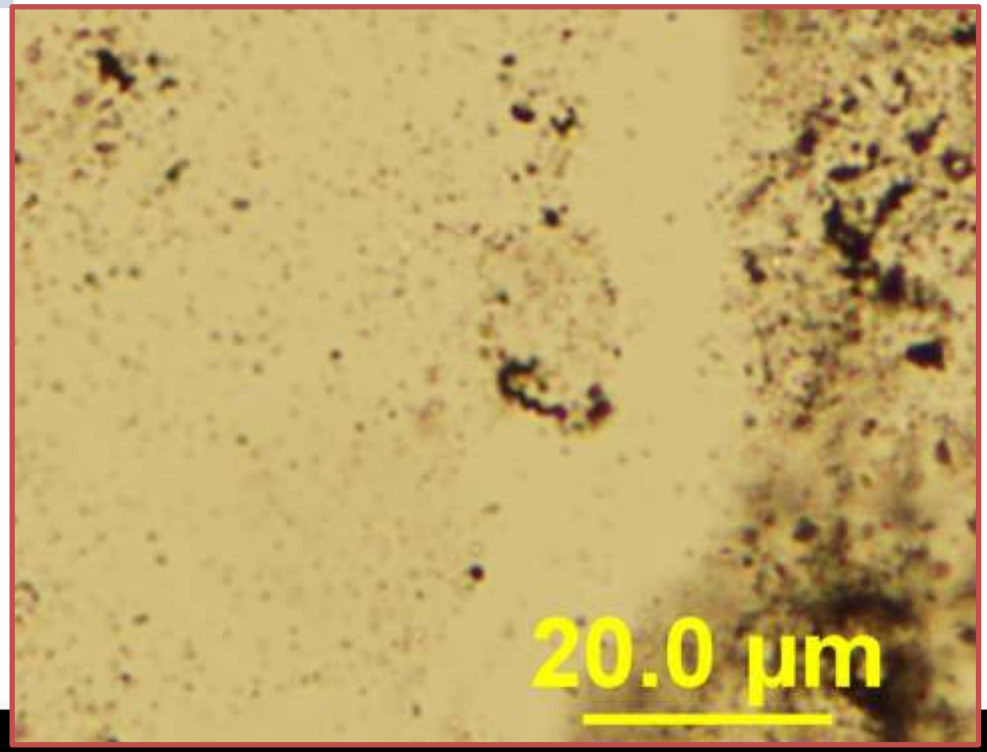
Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing. N Engl J Med 2014; 370:2408-2417June 19, 2014 DOI: 10.1056/NEJMoa1401268

Wild animals

Animal type	Tested	PCR Positive
Bobcats	5	3
Coyotes	2	1
Opossums	5	2



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Monkeys in St Kitts

Green vervet monkeys (*Cercopithecus aethiops sabaeus*)

Serovar	No. of Positive Samples	Sero-Prevalence (%)	Approx. 95% Confidence Interval (CI) (%)	No. of Positive Samples Wild	Sero-Prevalence (%)	CI (%)	No. Of Positive Samples Captive	Sero-Prevalence	CI (%)	P Value (Fisher)
Bratislava	**26**	**16.0**	**10.4-21.7**	**22**	**27.1**	**17.5-36.8**	**4**	**4.9**	**0.2-9.7**	0.0002
Autumnalis	6	3.7	0.8-6.6	3	3.7	0-7.8	3	3.7	0-7.8	1
Ballum	30	18.5	12.5-24.5	13	16	8.1-24	17	21	12.1-29.9	0.5
Bataviae	**38**	**23.5**	**16.9-30**	1	**1.2**	**0-3.6**	**37**	**45.7**	**34.8-56.5**	<0.00001
Canicola	7	4.3	1.2-7.8	4	4.9	0.2-9.7	3	3.7	0-7.8	1
Cynopteri	2	1.2	0-2.9	2	2.5	0-5.8	0			0.5
Djasiman	2	1.2	0-2.9	2	2.5	0-5.8	0			0.5
Borincana	1	0.6	0-1.8	1	1.2	0-3.6	0			1
Ictero	6	3.7	0.8-6.6	2	2.5	0-5.8	4	4.9	0.2-9.7	0.7
Mankarso	12	7.4	3.4-11.4	6	7.4	1.7-13.1	6	7.4	1.7-13.1	1
Pomona	1	0.6	0-1.8	0			1	1.2	0-3.6	1
Alexi	3	1.9	0-3.9	0			3	3.7	0-7.8	0.2
Tarassovi	5	3.1	0.4-6.8	0			5	6.1	0.9-11.4	0.06

Titers	100	200	400	800	1600	3 200
Bratislava(Australis)	23(88%)	2(7.6%)	1(3.84%)			
Autumnalis(autumnalis)	6					
Ballum (Ballum)	17 (57%)	7 (23%)	1(3.3%)	3(10%)	2(6.6%)	
Bataviae (Bataviae)	17 (45%)	11 (29%)	7 (38%)	1(2.6%)	1(2.6%)	1(2.6%)
Canicola (canicola)	7					
Cynopteri (cynopteri)	2					
Djasiman (Djasimen)	2					
Borincana (Hebdomadis)	1					
Icterohemorrhagiae (Icterohemorrhagiae	3 (50%)	2(33%)		1(16%)		
Icterohemorrhagiae Mankarso	3 (25%)	4 (33%)	1(8.3%)	3 (25%)	1(8.3%)	
Pomona (Pomona)	1					
Alexi (pyrogenes)	3					
Tarassovi (Tarassovi)	4 (80%)		1(20%)			

Seroprevalence in Monkeys

Overall seroprevalence of 49.4%
Captive monkeys – 60.5%
Wild monkeys – 38.3%

Serovar	Seroprvalence Wild Monkey	Seroprvalence Captive Monkey
Ballum	17%	21%
Bataviae	1.2%	45.7%
Bratislava	27.2%	4.9%


Dr. Cruz


Dr. Cruz

Seroprevalence in Mongoose

Overall seroprevalence 2.4%

Serovar	Seroprevalence
Bratislava	1.2% (1/83)
Icterohemorrhagiae/Mankarso	1.2% (1/83)


THANK YOU
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