MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Fit for purpose: testing for leptospirosis in humans and animals

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ivabs

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Background

- New Zealand: 2.5 cases notified per 100,000 in 2012
  - occupational
  - few serovars
  - under-reported

- Clinical diagnostics: MAT >> PCR

Key Questions

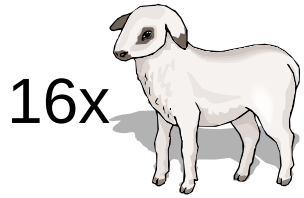
- To diagnose acute leptospirosis: how do tests perform in the early stages of disease?

1. Challenge trial: Pomona in lambs
2. Case series: humans

- How to identify carrier animals?

3. Cross-sectional study: lambs at abattoir

Study 1: Challenge Trial C



- Aim: to compare RT-PCR sensitivity on serum vs whole blood at different stages post-challenge
  - Previous work with human cases: variable results Agampodi (2012), Bourhy (2011), Stoddard (2009)…

- PCR: primers for the *gyr*B gene (Subharat 2011)

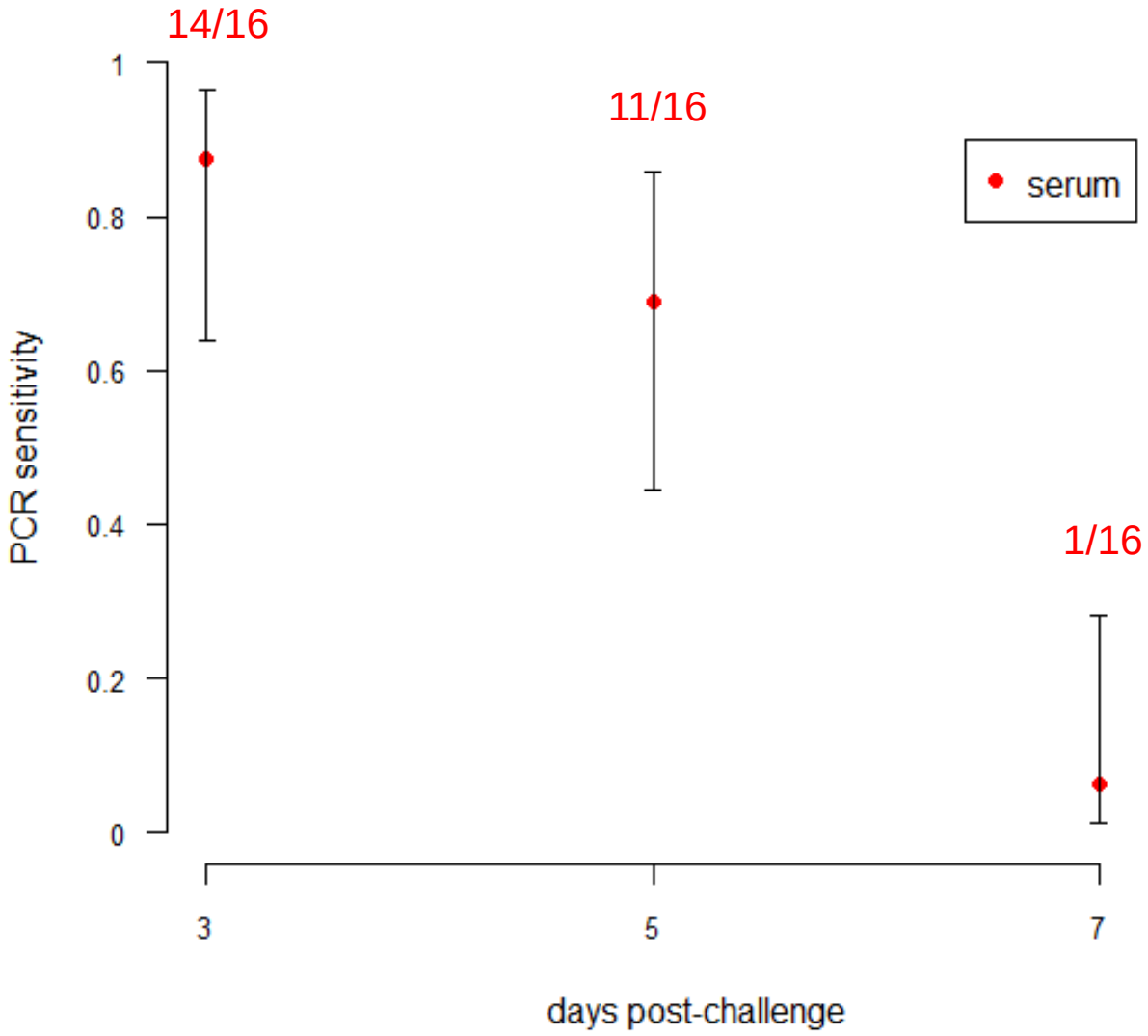
Methods: Challenge trial C

- Piggy-backed on vaccine trial
  - human Pomona isolates
  - conjunctival and nasal inoculation

- Animal model: sheep remained clinically normal for the 42 day observation period

Challenge Trial C

16x



Challenge Trial C

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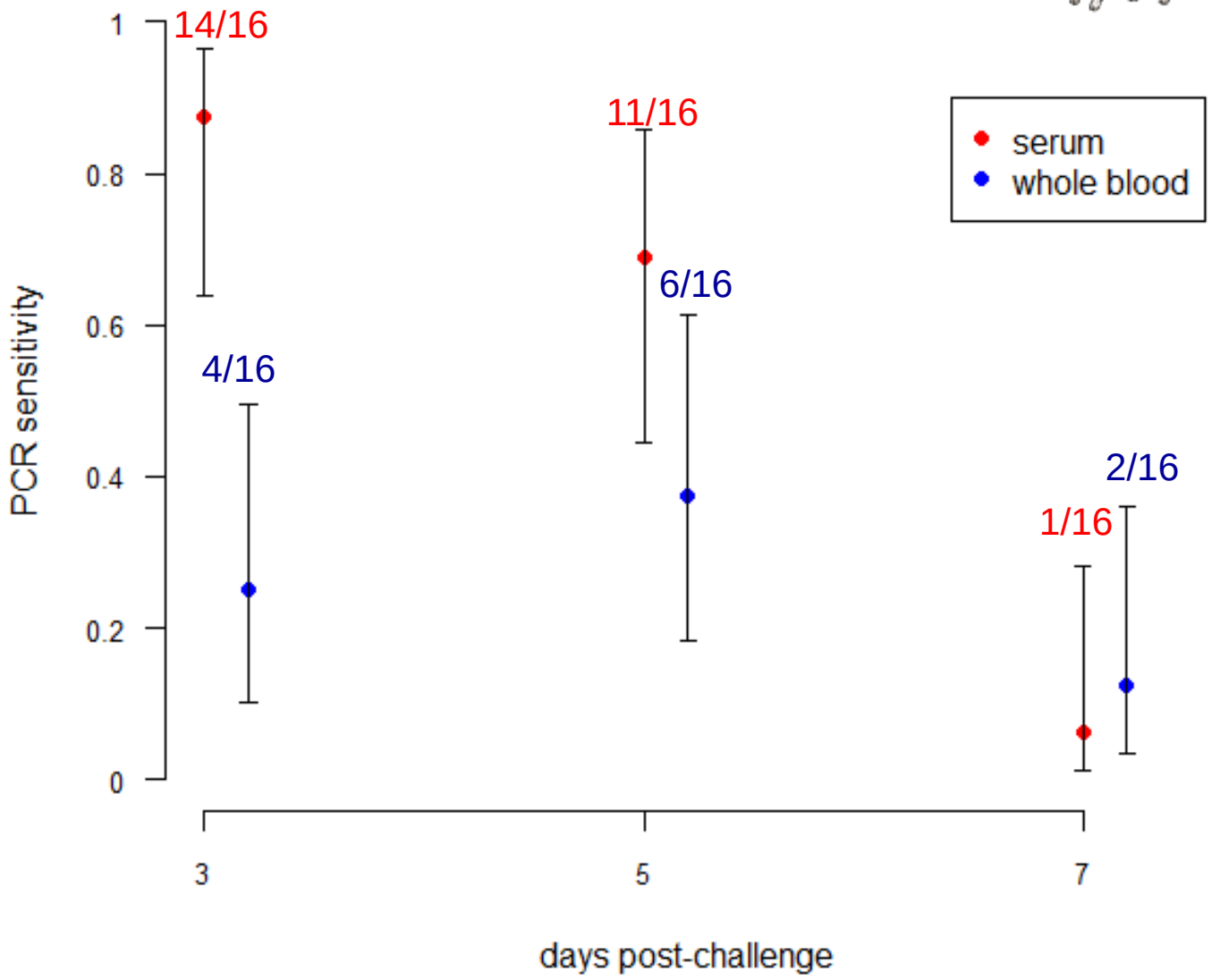

Key Questions

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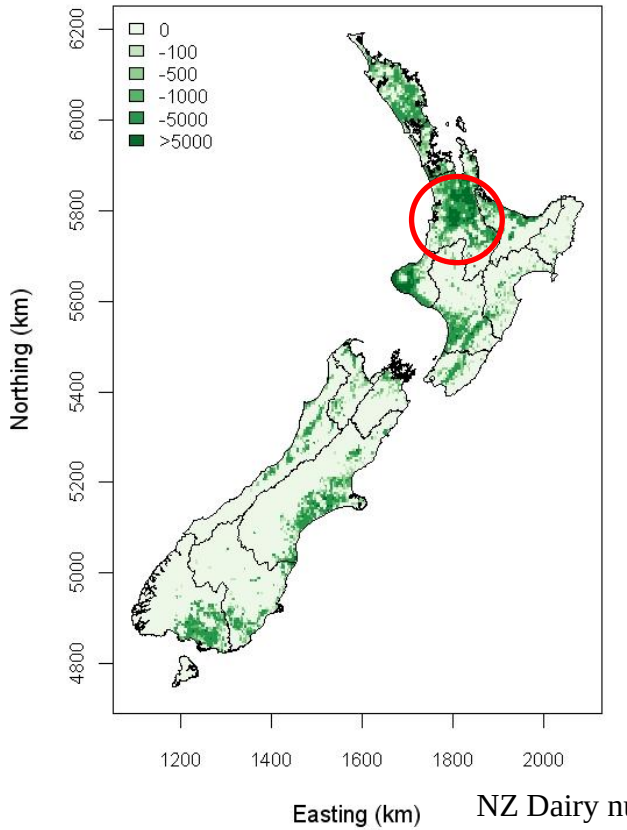
Study 2: Case series

Aim: comparison of diagnostic tests on patients suspected to have acute leptospirosis

- MAT (serum)
- culture (blood)
- PCR x3 (blood and serum)



NZ Dairy numbers 2010

Lab confirmed case definition

- Any one of
  - isolation of leptospires from a clinical specimen
  - detection of leptospiral nucleic acid from a clinical specimen
  - a four-fold or greater rise in MAT between acute and convalescent sera
  - single high MAT antibody titre of ≥400

NZ MoH Communicable Disease Control Manual May 2012

Case series results

- 6/17 suspects (35%) lab confirmed by 1 or more tests

Patient ID	ESR RT-PCR		HLRL RT-PCR		CHL PCR	Culture		MAT (serovar/titre)		Interval (days)
	serum	blood	serum	blood	blood	ESR	HLRL	Acute serum	Convalescent serum	
6	–	–	–	–	–	–	–	+ (Pom/800)	+ (Pom/800)	53
10	+	+	–	+	+	+	–	–	+ (Ball/200)	4
12	–	–	–	–	–	–	–	–	+ (Pom/800)	4
13	–	–	–	+	+	–	–	–	+ (Tar /800)	2
14	–	–	–	–	–	–	–	–	+ (Har + Pom/400)	5
15	–	+	–	–	+	+ (Pom)	–	–	Not Done	1

Case series results

- 6/17 suspects (35%) lab confirmed by 1 or more tests

Patient ID	ESR RT-PCR		HLRL RT-PCR		CHL PCR	Culture		MAT (serovar/titre)		Interval (days)
	serum	blood	serum	blood	blood	ESR	HLRL	Acute serum	Convalescent serum	
6	–	–	–	–	–	–	–	+ (Pom/800)	+ (Pom/800)	53
10	+	+	–	+	+	+	–	–	+ (Ball/200)	4
12	–	–	–	–	–	–	–	–	+ (Pom/800)	4
13	–	–	–	+	+	–	–	–	+ (Tar /800)	2
14	–	–	–	–	–	–	–	–	+ (Har + Pom/400)	5
15	–	+	–	–	+	+ (Pom)	–	–	Not Done	1

- 3/6 of lab positives were detected early by PCR
  - Results available ~ 3 days after samples were taken

Clinical signs study population

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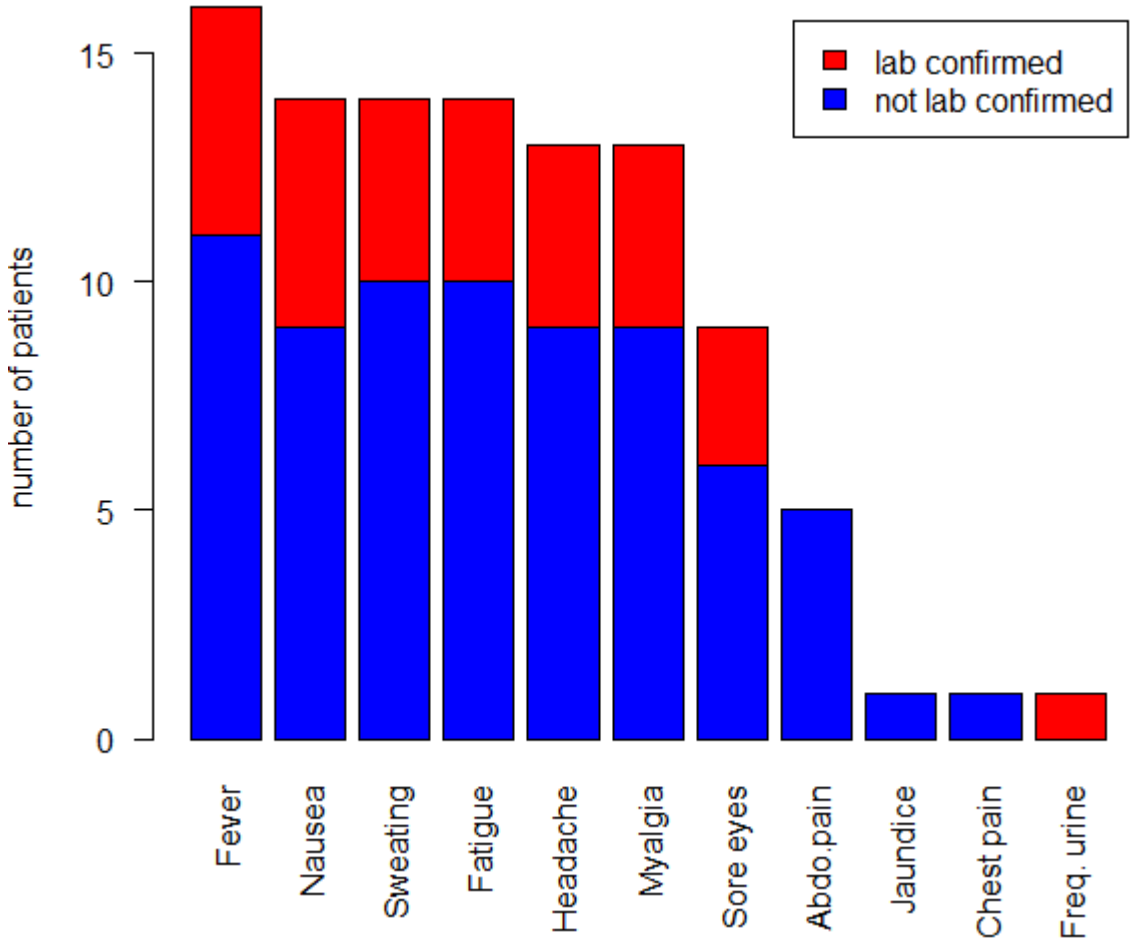
Data available
for 16/17 patients



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Discussion: Case series (comparison of diagnostic tests)

Recruiting through GPs is very challenging

PCR is a useful addition to diagnostic suite

Convalescent MATs missing (5/17)

Latent class analysis for tests in the absence of a gold standard

Key Questions

- To diagnose acute leptospirosis: how do tests perform in the early stages of disease?

1. Challenge trial: Pomona in lambs
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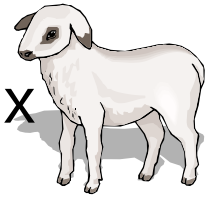
3. Cross-sectional study: lambs at abattoir

Study 3: cross-sectional (abattoir)

396 x 

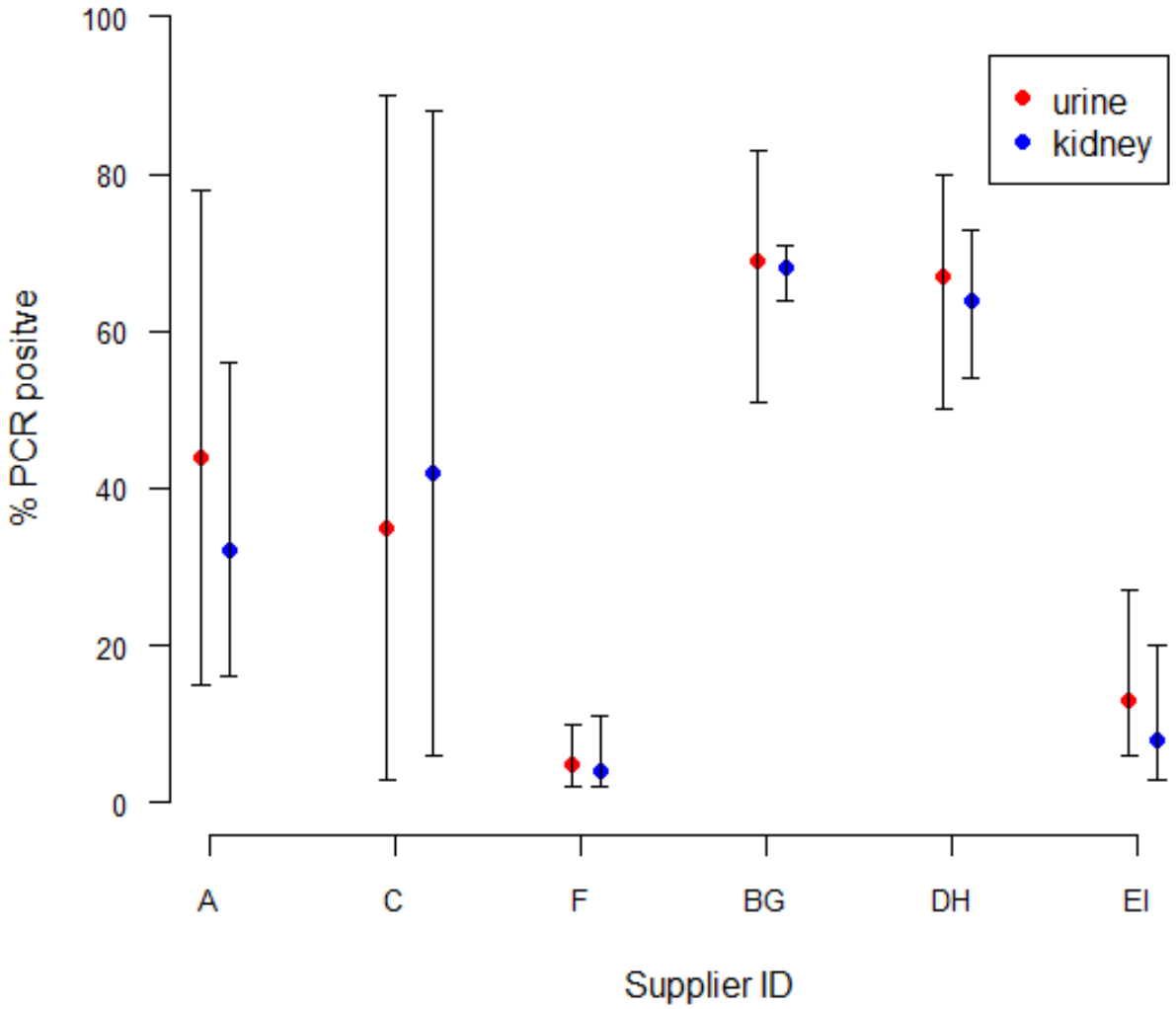
- Aim: to determine the utility of the MAT to inform animal carrier status.

- The MAT remains the veterinarians most frequently used test.

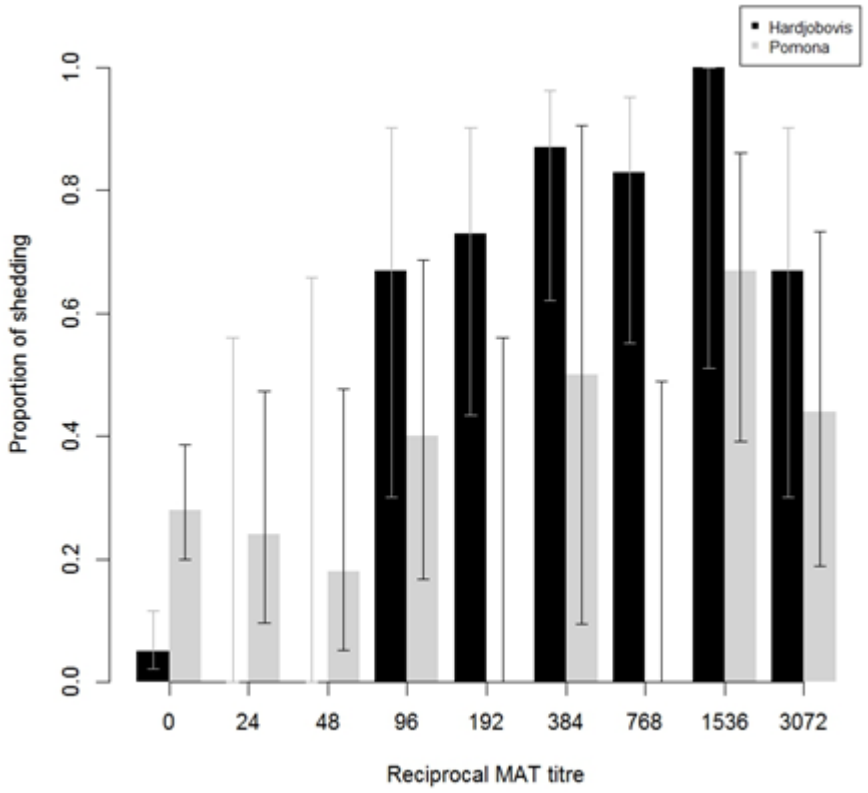
- Prime lambs from 6 suppliers
  - blood, urine and kidney

Waikato Region

Urinary shedding and renal colonisation



Association between MAT and shedding



Sheep with Hardjobovis MAT titres ≥ 1:96 were 15 times more likely to be shedding (compared with those with titres < 1:96)

Discussion: MAT and shedding

- The strong association is seen for Hardjobovis in sheep, to a lesser extent in cattle
  - not with Pomona
  - cut-off important

- Sheep as a reservoir species for Hardjobovis in New Zealand

Fit for purpose: testing for leptospirosis in humans and animals

- Suite of tests
- Consideration to
  - cut-off
  - resources available
  - stage of disease

Acknowledgements

Health Research Council

AgResearch Limited

Tertiary Education Commission

Rural Women NZ

Gribbles Veterinary

Canterbury Health Laboratory

ESR: Leptospiral Reference Laboratory

Waikato District Health Board

IVABs Postgraduate research funding

Massey University Doctoral scholarship

Staff and students Massey University : Neville Haack

Participating GPs and patients

mEpiLab

OIE Collaborating Centre for Veterinary Epidemiology and Public Health

Strains	Sheep ID	Blood					Serum					MAT Pomona			
		Day					Day					Day			
		0	3	5	7	14[b]	0	3	5	7	14	0	5	7	14
LPC 04/08	C1	–	–	–	–	–	–	P	P	–	–	–	1:96	1:3072	1:800
	C2	–	–	P	P	–	–	P	P	–	–	–	1:96	1:192	1:50
	C3	–	–	–	–	–	–	–	–	–	–	–	1:24	1:768	1:100
	C4	–	C	–	–	–	–	P	P	–	–	–	1:48	1:3072	1:800
	C5	–	C	P	–	–	–	P	P	–	–	–	1:24	1:1536	1:800
	C6	–	C	C	–	–	–	P	P	–	–	–	–	1:1536	1:800
	C7	–	P,C	P	–	–	–	P	–	–	–	–	1:48	1:3072	1:800
	C8	–	C	P	–	–	–	P	P	–	–	–	1:48	1:3072	1:200
LPC 04/04	C9	–	P,C	–	–	–	–	P	P	P	–	–	1:24	1:384	1:400
	C10	–	C	C	P	–	–	P	P	–	–	–	–	1:384	1:400
	C11	–	C	P	–	–	–	P	P	–	–	–	1:48	1:1536	1:400
	C12	–	P,C	–	–	–	–	P	–	–	–	–	1:24	1:768	1:800
	C13	–	P,C	P	–	–	–	P	P	–	–	–	1:48	1:1536	1:400
	C14	–	–	–	–	–	–	P	–	–	–	–	1:48	1:768	1:1600
	C15	–	C	–	–	–	–	P	P	–	–	–	–	1:384	1:800
	C16	–	–	–	–	–	–	–	–	–	–	–	1:48	1:768	1:400

Detection of leptospires in blood and serum by culture (C) and PCR (P) from each sampling day after Pomona challenge for sheep trial.

Strains	Sheep ID	Blood					Serum				
		Day					Day				
		0	3	5	7	14[b]	0	3	5	7	14
LPC 04/08	C1	–	–	–	–	–	–	P	P	–	–
	C2	–	–	P	P	–	–	P	P	–	–
	C3	–	–	–	–	–	–	–	–	–	–
	C4	–	C	–	–	–	–	P	P	–	–
	C5	–	C	P	–	–	–	P	P	–	–
	C6	–	C	C	–	–	–	P	P	–	–
	C7	–	P,C	P	–	–	–	P	–	–	–
	C8	–	C	P	–	–	–	P	P	–	–
LPC 04/04	C9	–	P,C	–	–	–	–	P	P	P	–
	C10	–	C	C	P	–	–	P	P	–	–
	C11	–	C	P	–	–	–	P	P	–	–
	C12	–	P,C	–	–	–	–	P	–	–	–
	C13	–	P,C	P	–	–	–	P	P	–	–
	C14	–	–	–	–	–	–	P	–	–	–
	C15	–	C	–	–	–	–	P	P	–	–
	C16	–	–	–	–	–	–	–	–	–	–

RT-PCR: serum had higher sensitivity (88%, 14/16) at day 3 compared to whole blood (25%, 4/16).

RT-PCR: serum had higher sensitivity (88%, 14/16) at ≤5 days compared to whole blood (50%, 8/16).

HLRL RT-PCR methods

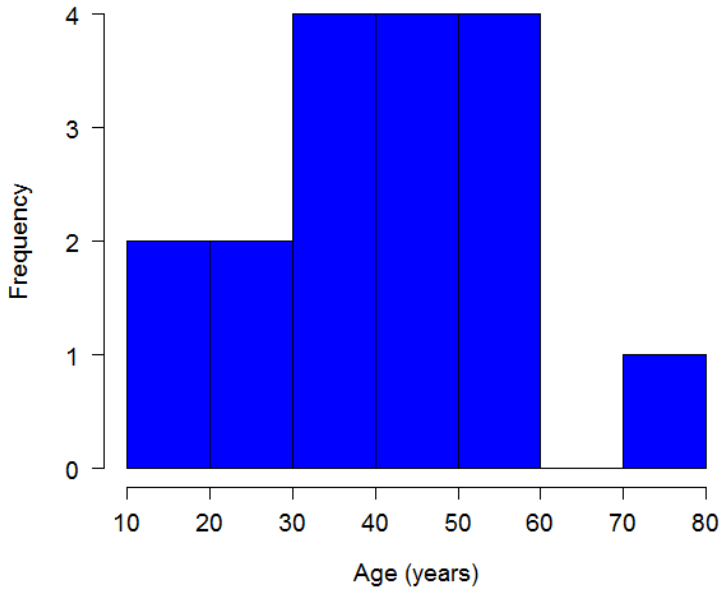
- QIAamp DNA mini kit for extraction
- Primer: DNA gyrase subunit B (gyrB) gene (Subharat et al., 2011).
- Thermal cycling and samples were considered as positive if a similar melting temperature to the positive controls was recorded.
- Massey's RT-PCR protocol is based on Slack 2006, using primers from gyrB gene and intercalating Dyes SYTO 9 green fluorescent nucleic acid stain (5mM solution in DMSO) as chemistries
-

ESR's RT-PCR 'modified based on the assay described by Slack et al., 2007 using the Roche LightCycler version 1'.

The CHL used the same set of primer (Lept-A and B) as Merien et al. 1992, targeted at rrs gene for the first round of PCR, while primers Lept-F4 (designed at Canterbury Health Laboratories) and Lept-B were used for the second round of PCR (nested).

Case series: patient demographics

- 17 recruited
- 6/17 lab confirmed
- 16 Males
- Middle-age



- 10/17 occupation recorded
- All had animal contact

Clinical signs reported from patients suspected with leptospirosis

Patient ID	Fever	Myalgia	Headaches	Sweating	Tiredness	Sore eyes	Jaundice	Conjunctival suffusion	Stomach pains	Nausea	Other
1	+	+	+	–	+	+	–	–	–	+	–
2	+	–	+	+	+	+	–	–	–	+	–
3	+	+	+	+	+	–	–	–	–	+	chest pains
4	+	+	–	+	+	–	–	–	+	+	–
5	–	–	–	+	–	–	–	–	–	–	–
6	/	/	/	/	/	/	/	/	/	/	/
7	+	+	+	+	+	+	–	–	+	+	–
8	+	+	+	+	+	+	–	–	+	+	–
9	+	+	+	+	+	+	–	–	–	–	–
10	+	–	–	–	–	–	–	–	–	+	–
11	+	+	+	+	+	–	–	–	+	+	–
12	+	+	+	+	+	+	–	–	–	+	–
13	+	+	+	+	+	+	–	–	–	+	–
14	+	+	+	+	+	+	–	–	–	+	–
15	+	+	+	+	+	–	–	–	–	+	frequent urination
16	+	+	+	+	+	+	+	–	–	+	–
17	+	+	+	+	+	–	–	–	+	+	–

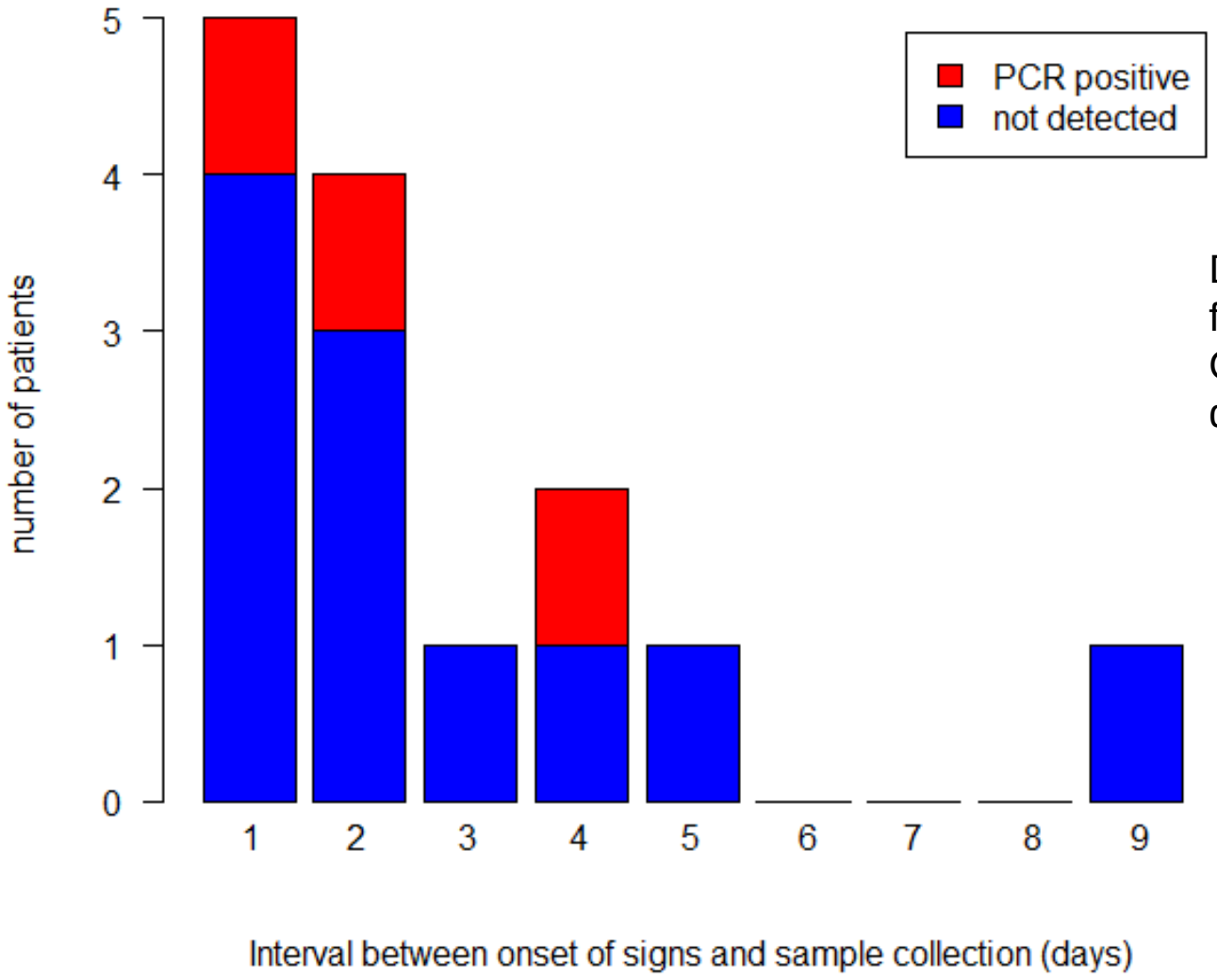
'/' = not recorded

Patient ID	ESR RT-PCR		HLRL RT-PCR		CHL PCR	Culture		MAT (serovar/titre)		Onset date	Recruitment date	Interval
	serum	blood	serum	blood	blood	ESR	HLRL	Acute serum	Convalescent serum			
1	–	–	–	–	–	–	–	–	–	16/08/2011	18/08/2011	2
2	–	–	–	–	–	ND	ND	–	–	2/09/2011	5/09/2011	3
3	–	–	–	–	–	–	–	–	–	23/10/2011	25/10/2011	2
4	–	–	–	–	–	–	–	–	– [a]	3/02/2012	4/02/2012	1
5	–	–	–	–	–	–	–	E (Pomona/50)	E (Pomona/50)	/	27/04/2012	/
6	–	–	–	–	–	–	–	+ (Pomona/800)	+ (Pomona/800)	15/03/2012	7/05/2012	53
7	–	–	–	–	–	–	–	–	ND	22/05/2012	23/05/2012	1
8	–	–	–	–	–	–	–	–	ND	/	25/05/2012	/
9	–	–	–	–	–	–	–	–	ND	13/06/2012	15/06/2012	2
10	+	+	–	+	+	+	–	–	+ (Ballum/200) [a]	14/06/2012	18/06/2012	4
11	–	–	–	–	–	–	–	–	ND	11/07/2012	20/07/2012	9
12	–	–	–	–	–	–	–	–	+ (Pomona/800)	6/08/2012	10/08/2012	4
13	–	–	–	+	+	–	–	–	+ (Tarrassovi /800)	28/10/2012	30/10/2012	2
14	–	–	–	–	–	–	–	–	+ (Hardjo and Pomona/400)	4/11/2012	9/11/2012	5
15	–	+	–	–	+	+ (Pomona)	–	–	ND	22/04/2013	23/04/2013	1
16	–	–	–	–	–	–	–	–	–	23/06/2013	24/06/2013	1
17	–	–	–	–	–	–	–	–	–	2/07/2013	3/07/2013	1

ND: Tests were not performed

[a] MAT was performed by Waikato District Health Board

Acute serum= samples within 5days of symptom onset; convalescent serum= samples taken 3weeks later. ESR = Environmental Science and Research, Wellington (reference lab); HLRL = Hopkirk Leptospirosis Research Laboratory, Massey University; CHL = Canterbury Health Laboratory, Christchurch



Data was available for 15/17 patients. One interval of 52 days since excluded

Case series results

- Is it useful to use MAT as gold standard?
  - Five convalescent MATs missing

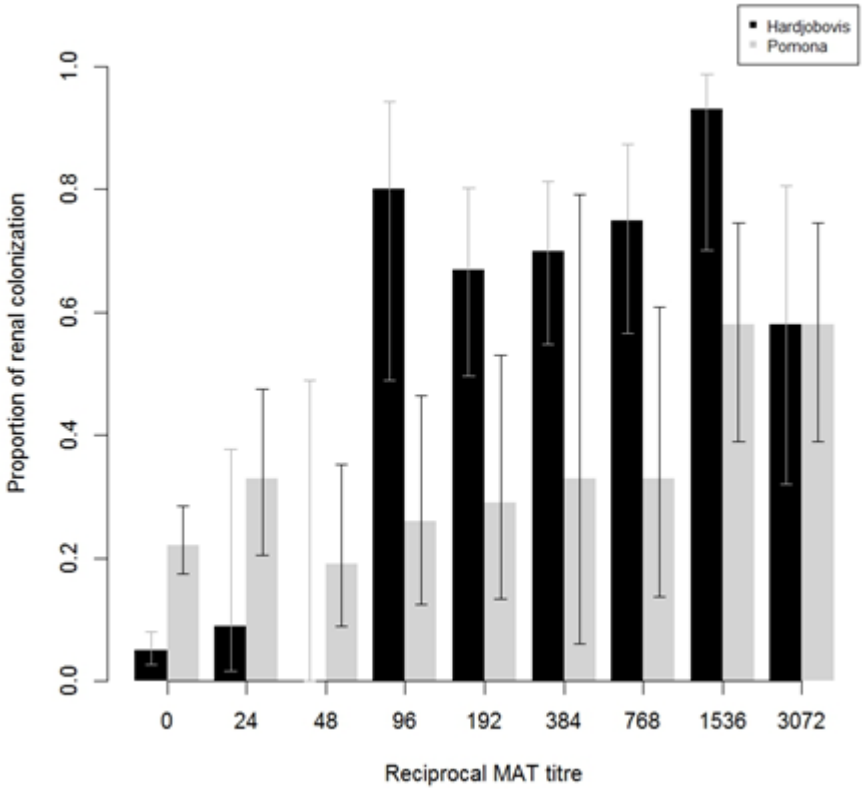
	MAT pos	MAT neg	
PCR pos	2	0	2
PCR neg	3	7	10
	5	7	

- PCR highly specific but low sensitivity (40%: 95% CI 12% – 77%)

Number of sheep sampled, number tested by urine, kidney RT-PCR and MAT (≥1:48 positive), number of samples tested positive, and percentage positive by supplier.

Supplier		Sample size	Urine RT-PCR			Kidney RT-PCR			MAT (Hardjo or Pomona)		
			N	N pos	% (95% CI)	N	N pos	% (95% CI)	N	N pos	% (95% CI)
Sheep only	A	77	43	19	44 (15-78)	77	25	32 (16-56)	77	56	73 (70-76)
	C	60	17	6	35 (3-90)	60	25	42 (6-88)	60	32	53 (8-94)
	F	90	41	2	5 (2-10)	90	4	4 (2-11)	90	35	39 (13-73)
Mixed sheep and cattle	BG	40	13	9	69 (51-83)	40	27	68 (64-71)	39	37	95 (88-98)
	DH	42	12	8	67 (50-80)	42	27	64 (54-73)	40	28	70 (63-76)
	EI	90	30	4	13 (6-27)	90	7	8 (3-20)	90	36	40 (19-65)
	Total	399	156	48	33 (19-52)	399	115	31(18-48)	396	224	57 (40-73)

Association between colonisation and MAT titres



Sheep with Hardjobovis MAT titres ≥ 1:96 were 14 times more likely to be renally colonised
(compared with those with titres < 1:96)