

Epidemiological investigations of leptospirosis in pigs in Vietnam

Hu Suk Lee (DVM MSc PhD)



RESEARCH PROGRAM ON
Agriculture for
Nutrition
and Health



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Climate Change,
Agriculture and
Food Security

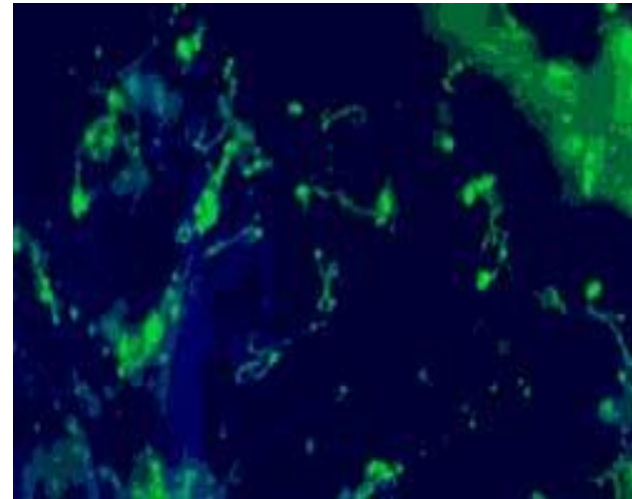
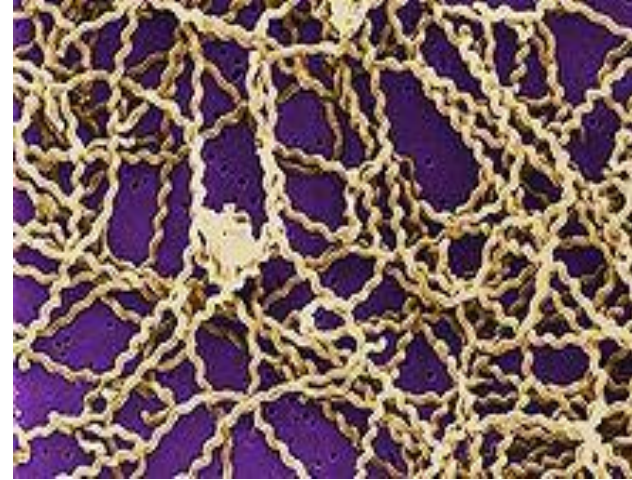


Overview

- Introduction of leptospirosis
- Research I
- Research II
- Conclusion
- Future directions

What is leptospirosis?

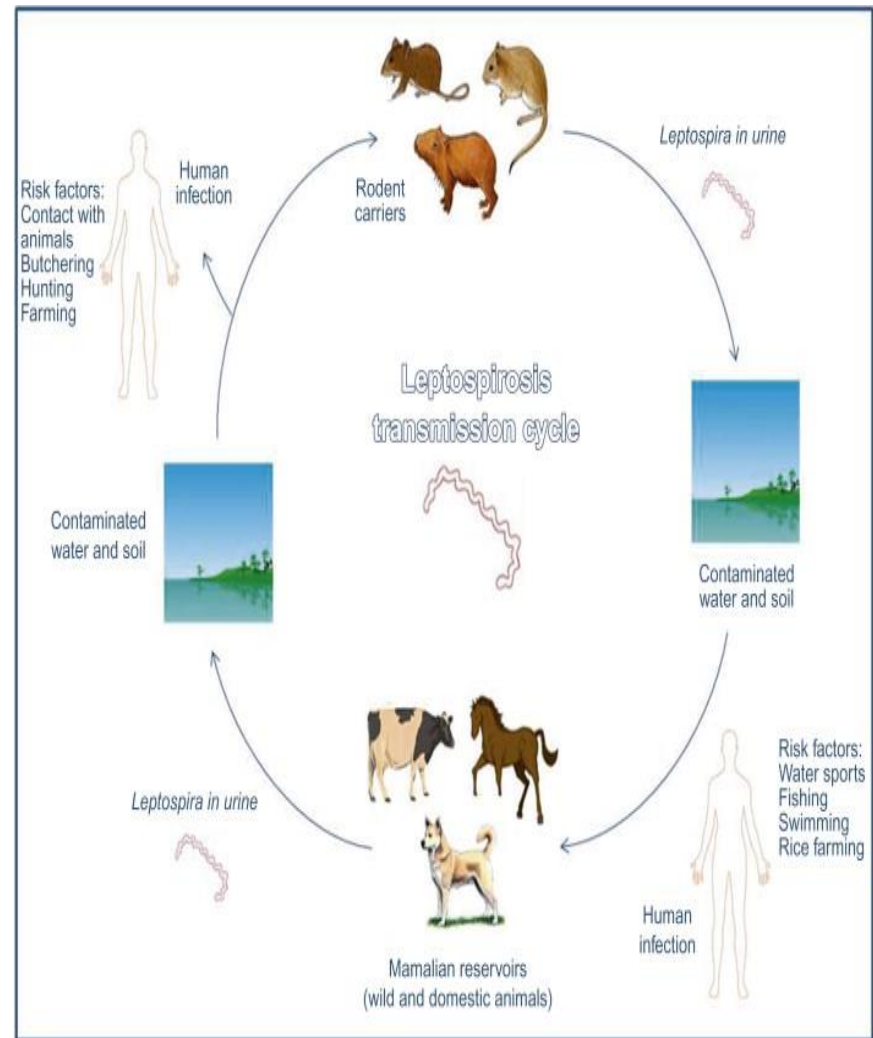
- A bacterial zoonotic disease
- The genera *Leptospira* contains three species, namely *L. interrogans*, *L. biflexa* and *L. parva*
- Pathogenic: *L. interrogans*
 - Contains 24 serogroups
 - More than 250 serovars



Source: CDC's Public Health Image Library)

Modes of transmission

- Transmitted to humans from domestic/wild animals (eg. rats, dogs, pigs and raccoon)
- Transmitted through breaks in the skin or intact mucous membranes
- Indirect contact (soil, water, feed) with infected urine
- Occupational disease of animal handling



Source: Chapter 107 - Leptospira and Leptospirosis
Molecular Medical Microbiology (Second Edition)

Host animals

Species	Common infections	Possible others
Dogs	Canicola, Icterohemorrhagiae, Grippotyphosa, Pomona	Bratislava
Cats	rarely infected	
Cattle and deer	Hardjobovis, Pomona, Grippotyphosa, Icterohemorrhagiae	Australis, Autumnalis, Canicola, Bataviae, Hebdomadis, Krematosis, Tarassovi, Sejroe, Bratislava
Pigs	Pomona, Bratislava, Canicola, Tarassovi, Icterohemorrhagiae	Grippotyphosa, Sejroe
Sheep	Pomona, Grippotyphosa, Bratislava, Hardjo	
Horses	Pomona, Bratislava, Canicola, Icterohemorrhagiae, Sejroe	

Reference: Bharti, A.R., Nally, J.E., Ricaldi, J.N., Matthias, M.A., Diaz, M.M., Lovett, M.A., Levett, P.N., Gilman, R.H., Willig, M.R., Gotuzzo, E., 2003. Leptospirosis: a zoonotic disease of global importance. *Lancet Infect. Dis.* 3, 757-771.

Incidence rate in the Asia Pacific region

Annual incidence per 100,000	Country/region
High (>10)	Bangladesh ^a Cambodia ^a Fiji ^a French Polynesia ^a India (Andaman and Nicobar Islands) Laos ^a Nepal ^a New Caledonia Sri Lanka Thailand Vietnam ^a Wallis and Futuna ^a
Moderate (1 to 10)	American Samoa ^a China India (mainland) Indonesia Malaysia New Zealand Palau ^a Philippines Marshall Islands ^a Vanuatu ^a Mongolia ^a
Low (<1)	Australia Hong Kong SAR Japan South Korea Taiwan
Insufficient information	Bhutan Myanmar North Korea Papua New Guinea Timor-Leste Western Pacific islands not mentioned above

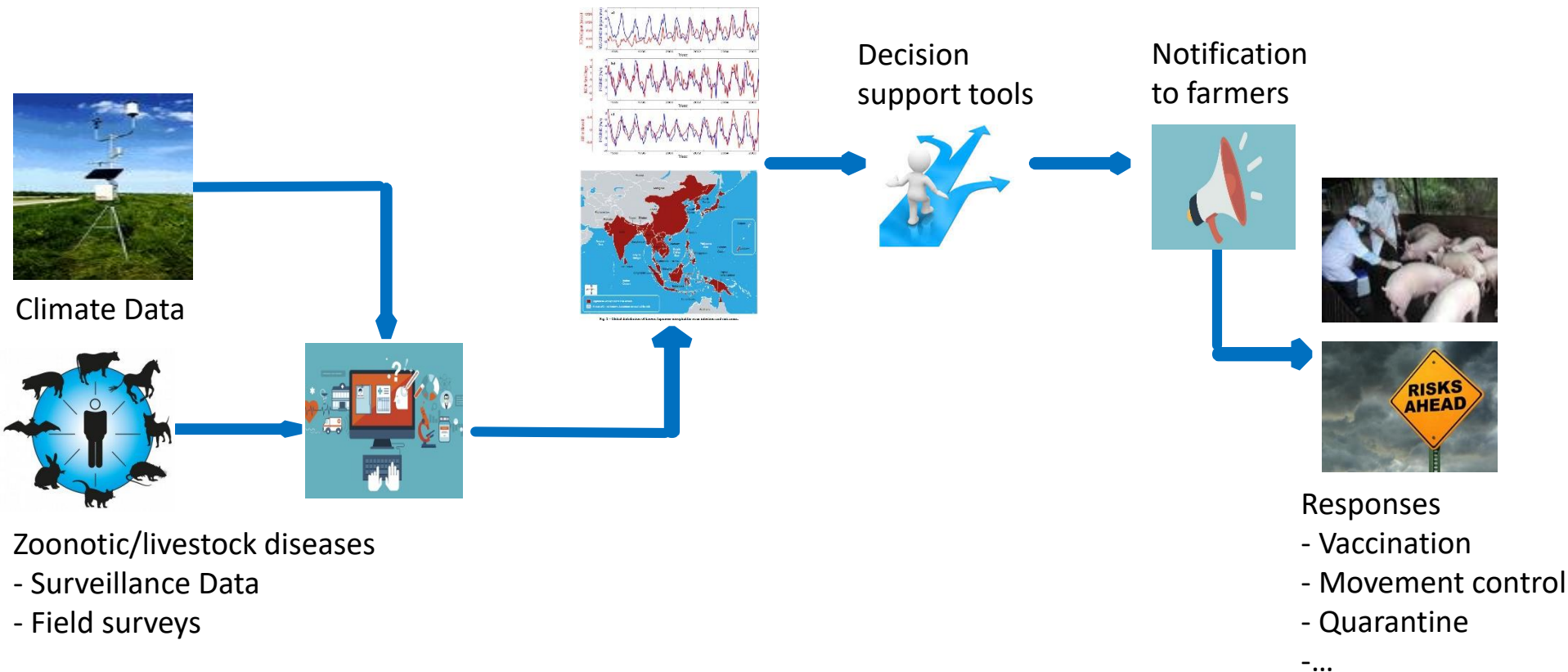
Reference: Victoriano, A. F. B., Smythe, L. D., Gloriani-Barzaga, N., Cavinta, L. L., Kasai, T., Limpakarnjanarat, K., ... & Yanagihara, Y. (2009). Leptospirosis in the Asia Pacific region. *BMC infectious diseases*, 9(1), 147.

Research I

Early Warning and Forecasting System concept

Main objectives

- To develop early warning systems of zoonotic (prediction/disease models and risk maps)
- To conduct epidemiological investigations of diseases
- To support capacity building for public health




RESEARCH ARTICLE

Open Access



Sero-prevalence of specific *Leptospira* serovars in fattening pigs from 5 provinces in Vietnam

Hu Suk Lee^{1*} , Nguyen Viet Khong², Huyen Nguyen Xuan², Vuong Bui Nghia², Hung Nguyen-Viet¹ and Delia Grace³

Abstract

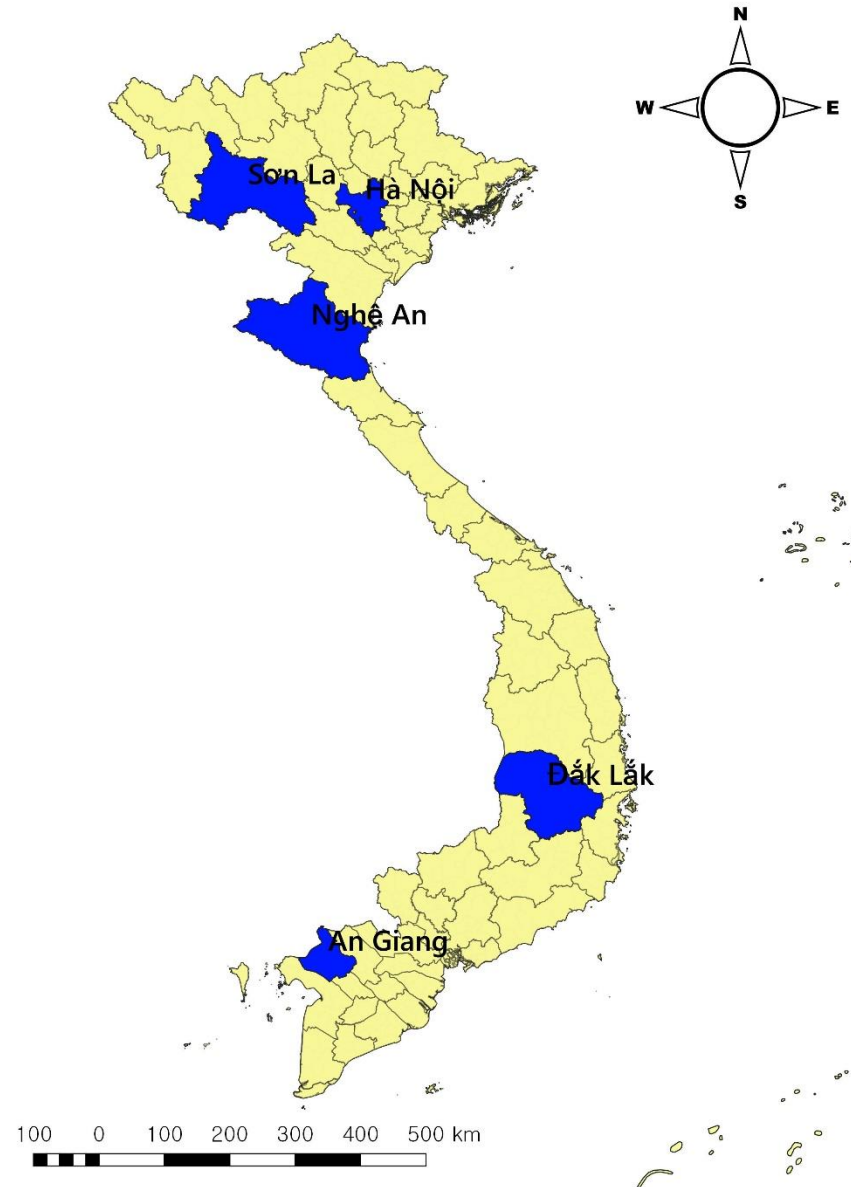
Background: Leptospirosis is a zoonotic bacterial disease with a worldwide distribution. In Vietnam, leptospirosis is considered endemic. In pigs, leptospirosis can result in reproductive problems (such as abortion and infertility) which lead to economic loss. In addition, transmission to people presents a public health risk. In Vietnam, few national studies have been conducted on sero-prevalence of leptospirosis in pigs. The main objective of this study was to evaluate the sero-prevalence and incidence of presumptive infective *leptospira* serovars in fattening pigs from 5 provinces in Vietnam.

Results: Blood samples from fattening pigs were randomly collected at slaughterhouses. We collected 1959 sera samples from 5 provinces (Son La, Hanoi, Nghe An, Dak Lak and An Giang) between January and early June 2016. The microscopic agglutination test (MAT) was used to identify the serogroups/serovars. Overall, the sero-prevalence was 8.17% (95% CI: 6.99–9.47) and serovar Tarassovi Mitis (2.19%) had the highest prevalence followed by Australis (1.94%), Javanica (1.68%) and Autumnalis (1.17%) using a cutoff ($\geq 1:100$). The sero-prevalence among female pigs (5.28%, 95% CI: 3.94–6.93) was slightly higher than among male pigs (4.88%, 95% CI: 3.51–6.58), but this difference was not statistically significant.

Introduction of study

- To evaluate the sero-prevalence of leptospirosis in pigs in Vietnam
- National Partner: National Institute of Veterinary Research (NIVR)
- Swine blood samples from 5 provinces (Jan- Jun 2016)
 - 385 samples* / province
 - Total sample: 1,925
 - Slaughterhouses (6-9 months)
 - Sampling information & Questionnaires (252 people)

*Sample size (each province): 50% prevalence, 95% CI and precision 5%



Laboratory diagnosis

- All samples were analyzed at NIVR in Hanoi
- Microscopic Agglutination Test (MAT)
 - National standard of animal leptospirosis in Vietnam
 - 15 serovars of *Leptospira* used as antigens
 - Cut-off level: $\geq 1:100$ titer (~ 800)
 - Positive titer is the greatest serum dilution (2-fold from 1:100) agglutinating $>50\%$ of live leptospire

List of *leptospira* antigens used in the MAT

No.	Genomospecies	Serogroup	Serovar
1	<i>L. interrogans</i>	Australis	Australis
2	<i>L. interrogans</i>	Autumnalis	Autumnaliss
3	<i>L. interrogans</i>	Bataviae	Bataviae
4	<i>L. interrogans</i>	Australis	Bratislava
5	<i>L. interrogans</i>	Canicola	Canicola
6	<i>L. kirschneri</i>	Grippotyphosa	Grippotyphosa
7	<i>L. interrogans</i>	Hebdomadis	Hebdomadis
8	<i>L. interrogans</i>	Icterohaemorrhagiae	Icterohaemorrhagiae
9	<i>L. borgpetersenii</i>	Javanica	Javanica
10	<i>L. noguchii</i>	Panama	Panama
11	<i>L. interrogans</i>	Pomona	Pomona
12	<i>L. interrogans</i>	Pyrogenes	Pyrogenes
13	<i>L. borgpetersenii</i>	Sejroe	Hardjo
14	<i>L. borgpetersenii</i>	Sejroe	Saxkoebing
15	<i>L. biflexa</i>	Semarang	Patoc
16	<i>L. borgpetersenii</i>	Tarassovi	Tarassovi

Research Team



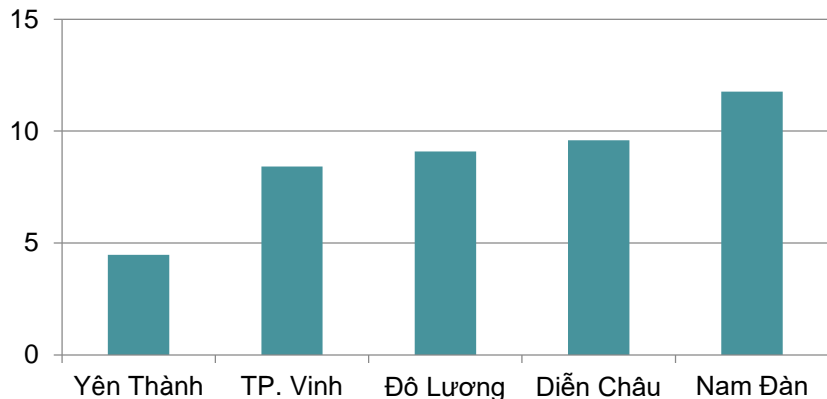
Blood Sampling



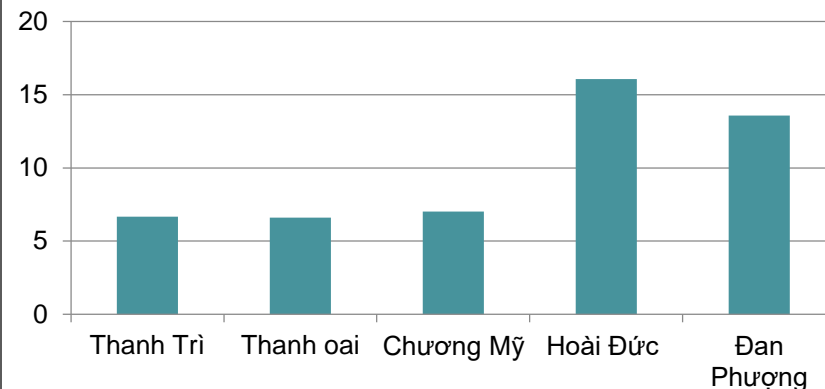
Sero-prevalence for Leptospira serovars in fattening pigs

Province	Total tested samples	Sero-positive samples (a titer \geq 1:100 for any serovars)	Sero-positive (%) with 95% CI
Hanoi	390	37	9.49 (6.77-12.84)
Son La	384	27	7.03 (4.68-10.07)
Nghe An	380	33	8.68 (6.05-11.98)
Dak Lak	385	27	7.01 (4.67-10.04)
An Giang	420	36	8.57 (6.08-11.67)
Total	1,959	160	8.17 (6.99-9.47)

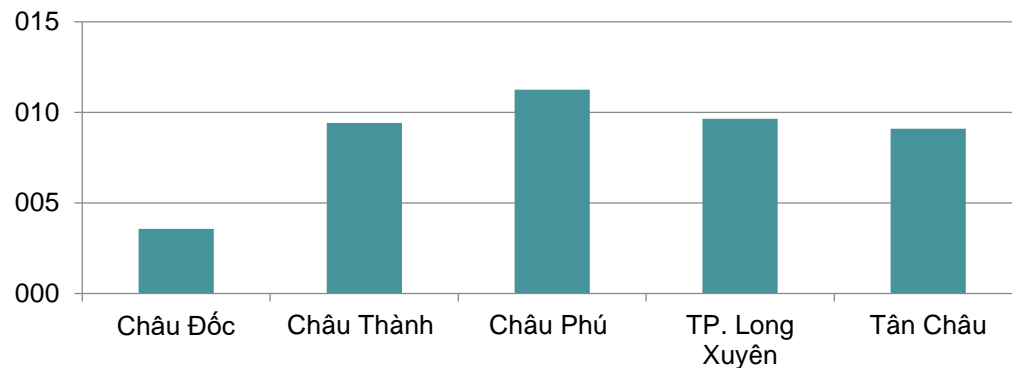
Sero-positive in Nghe An



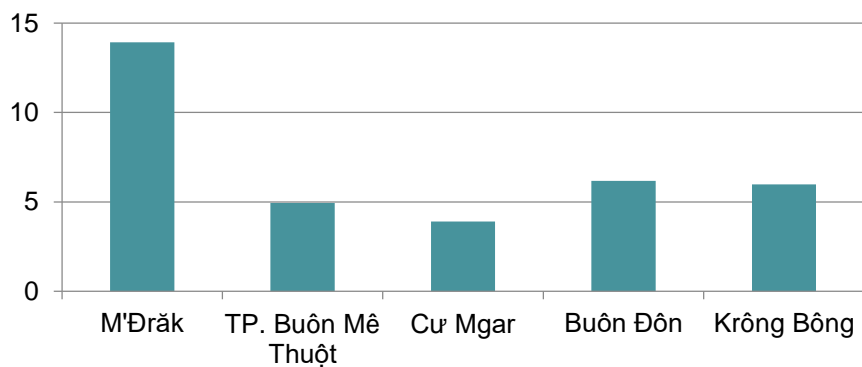
Sero-positive in Hanoi



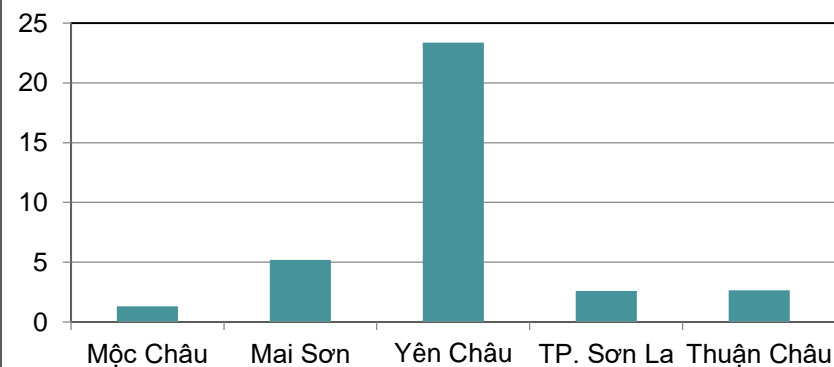
Sero-positive in An Giang



Sero-positive in Daklak



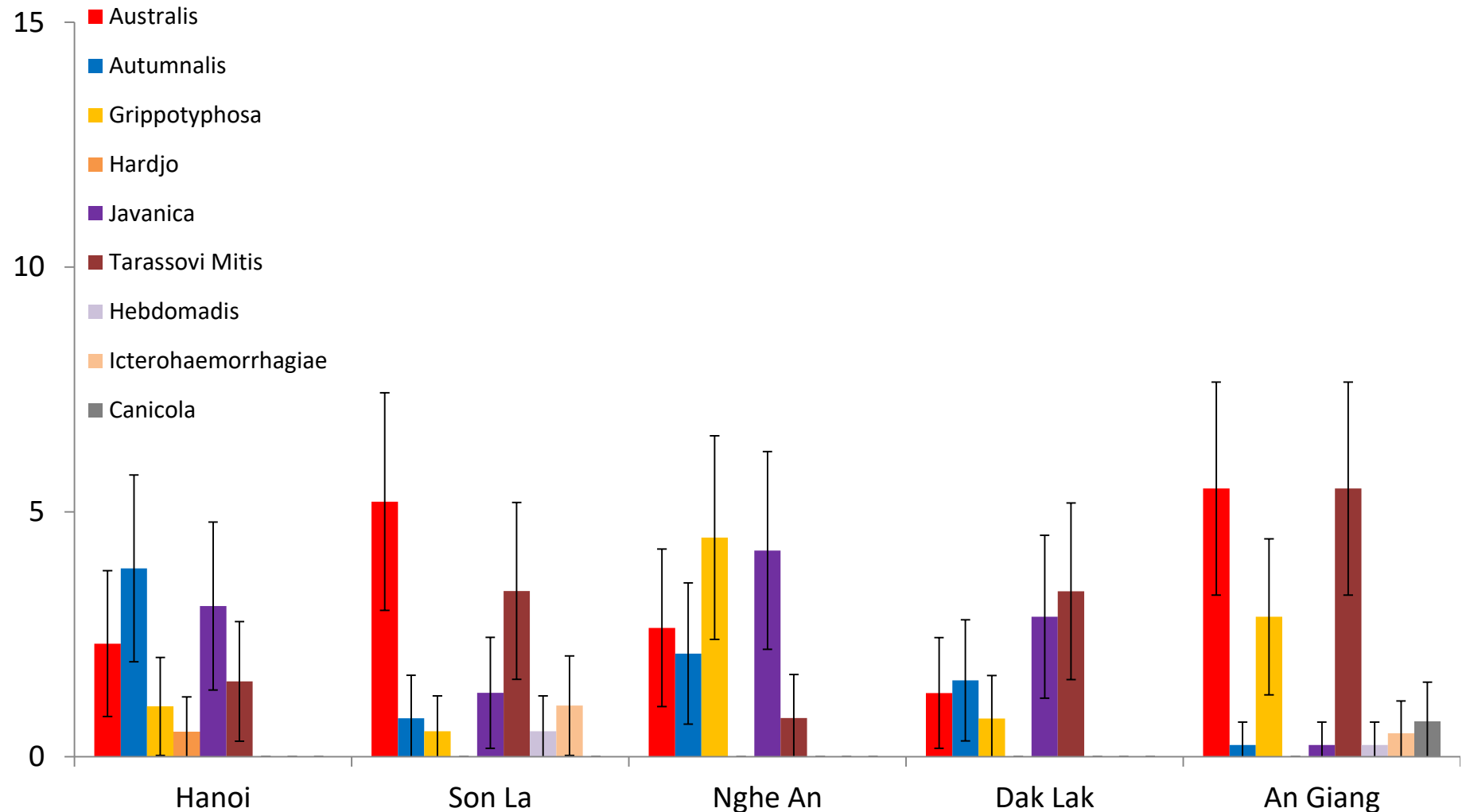
Sero-positive in Son La



MAT results for *leptospira* serovars in pigs by using 4 cutoff titers

Sero-positive results									
Seorvar	n	≥ 1:100		≥ 1:200		≥ 1:400		≥ 1:800	
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Australis	1,959	38	1.94 (1.37-2.65)	16	0.82 (0.42-1.22)	7	0.36 (0.09-0.62)	4	0.20 (0.001-0.40)
Autumnalis	1,959	23	1.17 (0.75-1.76)	7	0.36 (0.09-0.62)	2	0.10 (0-0.24)	1	0.05 (0-0.15)
Canicola	1,959	2	0.10 (0-0.24)	1	0.05 (0-0.15)	0	Null	0	Null
Grippotyphosa	1,959	21	1.07 (0.62-1.53)	9	0.46 (0.16-0.76)	4	0.20 (0.001-0.40)	4	0.20 (0.001-0.40)
Hardjo	1,959	1	0.05 (0-0.15)	1	0.05 (0-0.15)	0	Null	0	Null
Hebdomadis	1,959	2	0.10 (0-0.24)	1	0.05 (0-0.15)	0	Null	0	Null
Icterohaemorrhagiae	1,959	2	0.10 (0-0.24)	2	0.10 (0-0.24)	2	0.10 (0-0.24)	1	0.05 (0-0.15)
Javanica	1,959	33	1.68 (1.11-2.25)	8	0.41 (0.13-0.69)	3	0.15 (0-0.33)	1	0.05 (0-0.15)
Tarassovi Mitis	1,959	43	2.20 (1.55-2.84)	11	0.56 (0.23-0.89)	3	0.15 (0-0.33)	1	0.05 (0-0.15)

Sero-positive samples by serovar using cutoff titer $\geq 1:100$

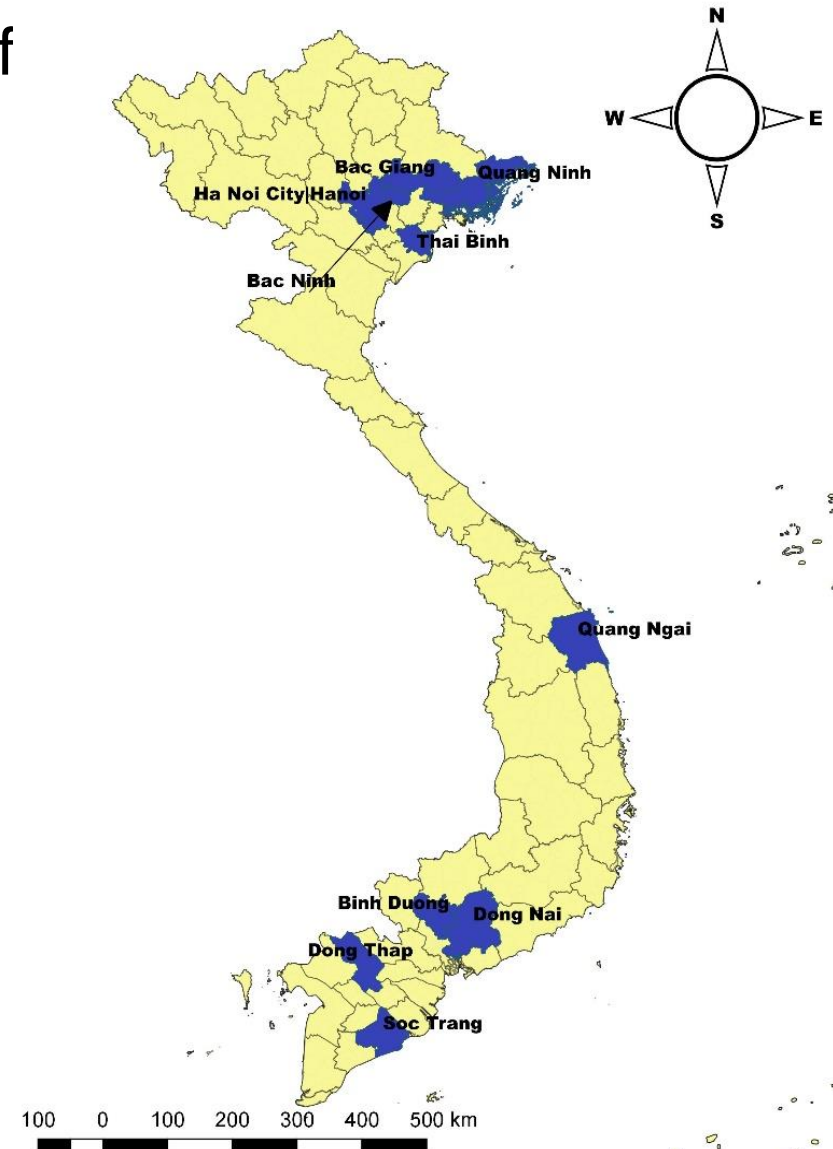


Percentage with 95% confidence interval of sero-positive samples by serovar in each province using cut-off titer ≥ 100

Research II

Introduction of study

- To evaluate the sero-prevalence of leptospirosis in pigs in Vietnam
- National Partner: National Center for Veterinary Diagnostics (NCVD)
- Swine blood samples from 10 provinces (Mar-Apr 2017)
 - 200 samples / province
 - Total sample: 2,000
 - Small/medium size farms
- Microscopic Agglutination Test was used for diagnosis



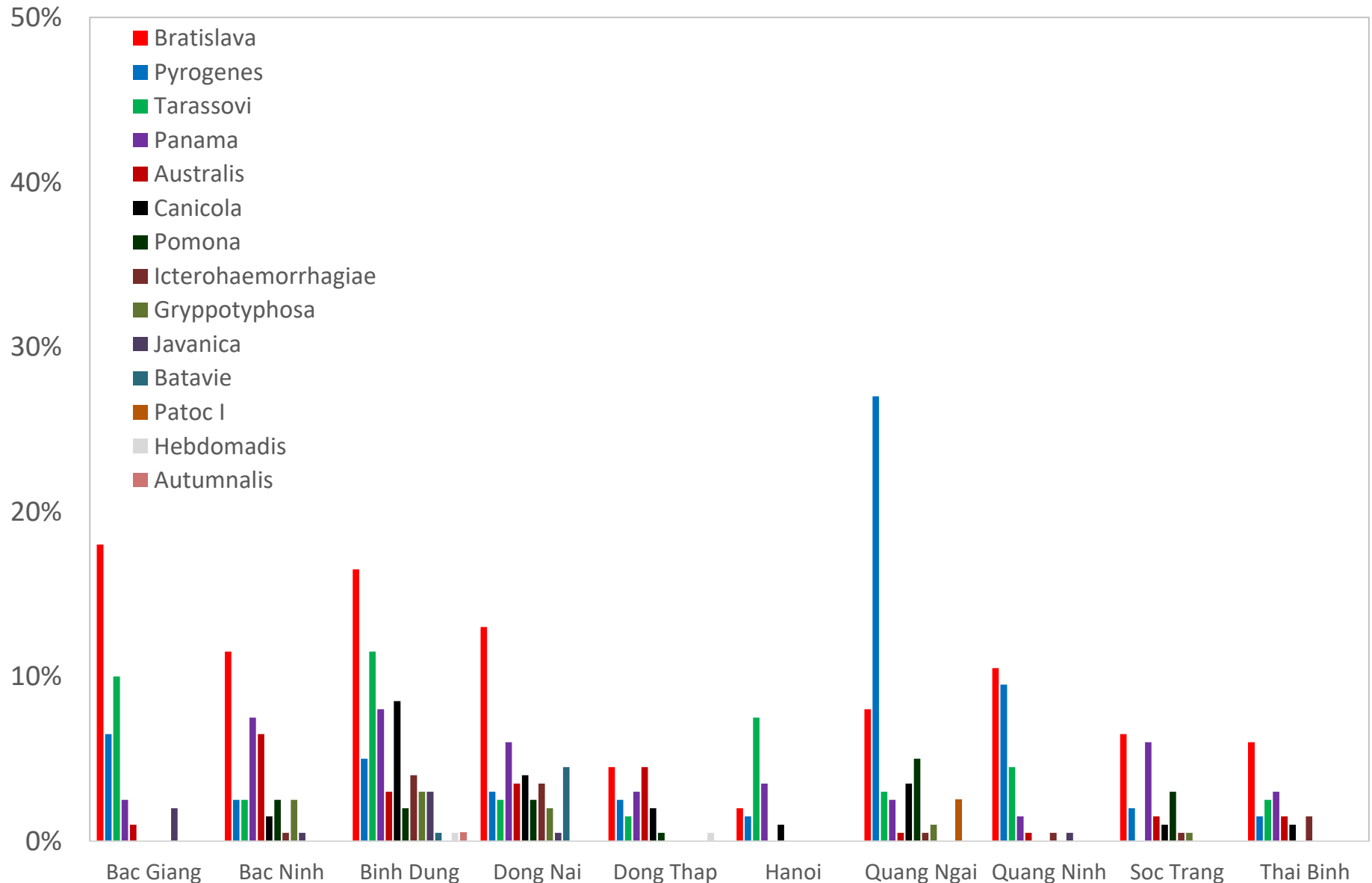
Sero-positive (%) for Leptospira serovars by province

Province (no.)	Sero-positive samples (a titer \geq 1:100 for any serovars)	Sero-positive (%) with 95% CI
Bac Giang (200)	61	30.5 (24.20-37.39)
Bac Ninh (200)	45	22.5 (16.91-28.92)
Binh Duong (200)	65	32.5 (26.06-39.47)
Dong Nai (200)	38	19.0 (13.81-25.13)
Dong Thap (200)	23	11.5 (7.43-16.75)
Hanoi (200)	23	11.5 (7.43-16.75)
Quang Ngai (200)	75	37.5 (30.77-44.61)
Quang Ninh (200)	43	21.5 (16.02-27.85)
Soc Trang (200)	20	10.0 (6.22-15.02)
Thai Binh (200)	28	14.0 (9.51-19.59)
Total (2,000)	421	21.05 (19.28-22.90)

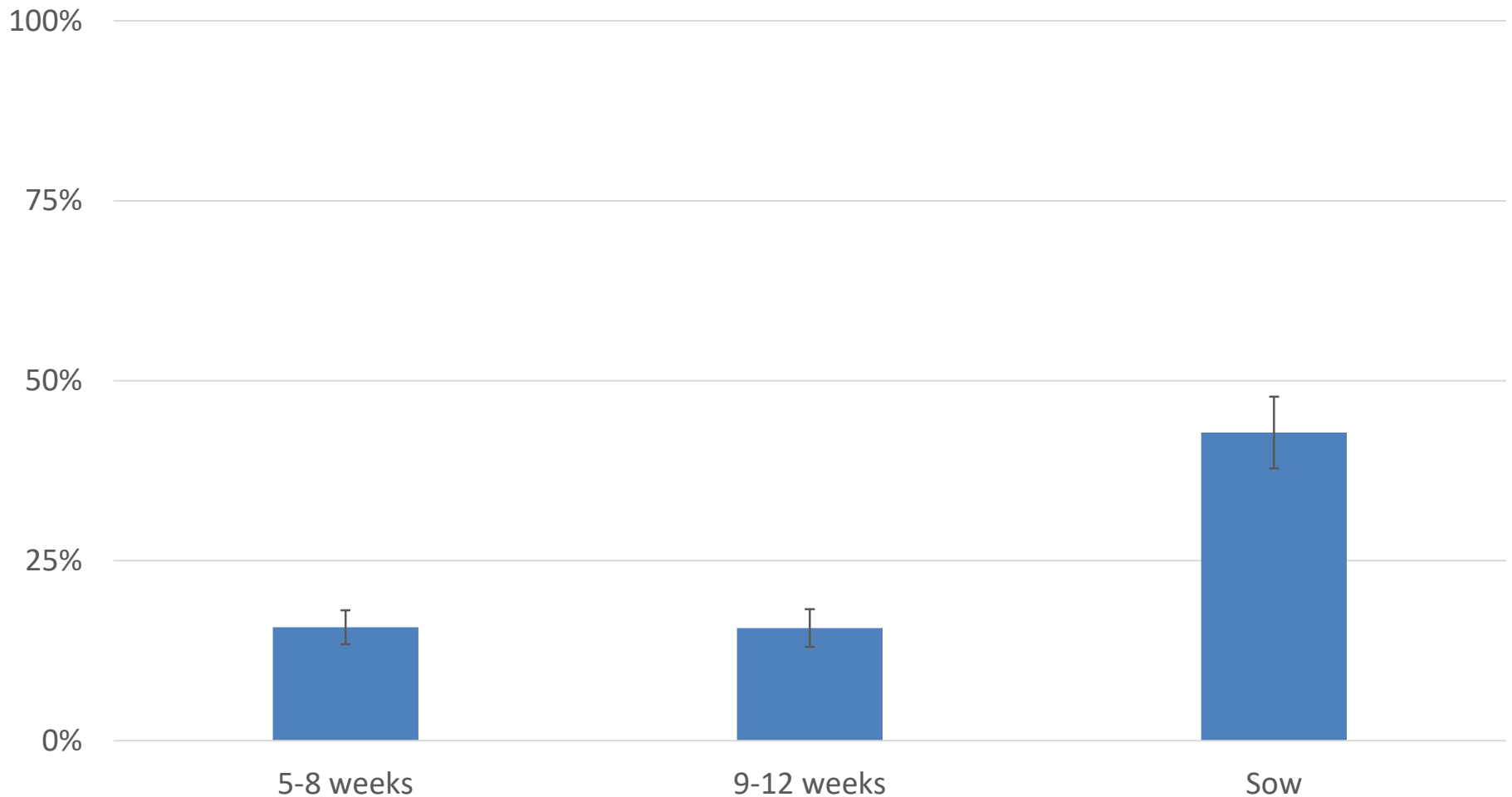
MAT results by using 3 cutoff titers

Serovar	Total samples	≥ 1:100	≥ 1:200	≥ 400
		N (% , 95% CI)	N (% , 95% CI)	N (% , 95% CI)
Australis	2,000	45 (2.25, 1.65-3.00)	4 (0.2, 0.005-0.05)	0
Autumnalis	2,000	1 (0.05, 0.0001-0.28)	0	0
Bataviae	2,000	10 (0.5, 0.24-0.92)	1 (0.05, 0.0001-0.28)	0
Bratislava	2,000	193 (9.65, 8.39-11.03)	39 (1.95, 1.39-2.66)	8 (0.4, 0.17-0.79)
Canicola	2,000	45 (2.25, 1.65-3.00)	2 (0.1, 0.01-0.36)	0
Grippotyphosa	2,000	18 (0.9, 0.53-1.42)	2 (0.1, 0.01-0.36)	0
Icterohaemorrhagiae	2,000	22 (1.1, 0.69-1.66)	3 (0.15, 0.03-0.44)	0
Javanica	2,000	13 (0.65, 0.35-1.11)	4 (0.2, 0.005-0.05)	0
Panama	2,000	87 (4.35, 3.50-5.34)	15 (0.75, 0.42-1.23)	2 (0.1, 0.01-0.36)
Pomona	2,000	31 (1.55, 1.06-2.19)	3	0
Pyrogenes	2,000	122 (6.1, 5.09-7.24)	8 (0.4, 0.17-0.79)	0
Hardjo	2,000	0	0	0
Sakoebing	2,000	0	0	0
Tarassovi	2,000	91 (4.55, 3.68-5.56)	8 (0.4, 0.17-0.79)	1 (0.05, 0.0001-0.28)
Patoc	2,000	5 (0.25, 0.08-0.58)	1 (0.05, 0.0001-0.28)	0

Sero-positive samples by serovar using cutoff titer $\geq 1:100$



Sero-positive by age group in pigs using cut off titer $\geq 1:100$



Conclusion

- Leptospirosis in pigs may be a useful indicator of the human/animal burden in Vietnam
- Wildlife may play an important role in the transmission of leptospirosis to domesticated pigs in Vietnam

Future directions

- Currently, which types of serovars are commonly detected in humans and wildlife?
 - Possible transmission of leptospirosis between wildlife and domestic animals/humans
- To evaluate the association between lepto and potential environmental risk factors (e.g. climate factors, rural vs urban)
- To evaluate the seasonal and temporal patterns of leptospirosis in humans and animals

Example: seasonality of lepto

J Vet Intern Med 2014;28:779-788

Regional and Temporal Variations of *Leptospira* Seropositivity in Dogs in the United States, 2000-2010

H.S. Lee, M. Levine, C. Guptill-Yoran, A.J. Johnson, P. von Kamecke, and G.E. Moore

Background: Previous studies have reported a seasonal increased risk for leptospirosis, but there is no consistent seasonality reported across regions in the United States.

Objectives: To evaluate and compare seasonal patterns in seropositivity for leptospirosis in dogs for 4 US regions (northeast [NE], midwest [MW], south-central [SC], and California-southern west coast [CS]).

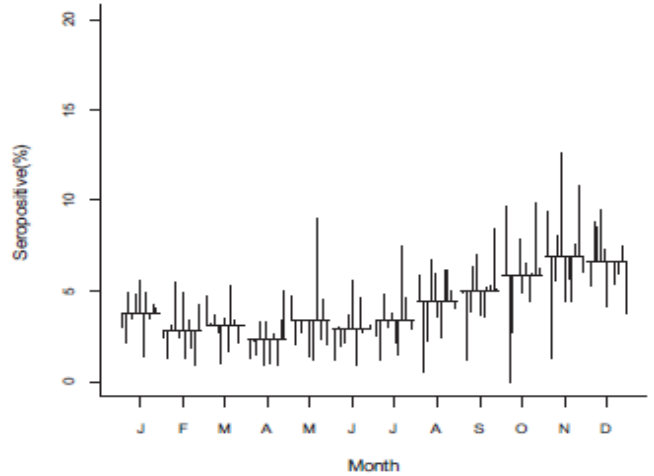
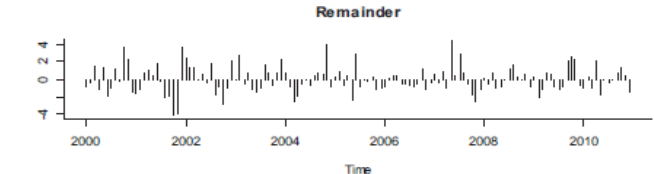
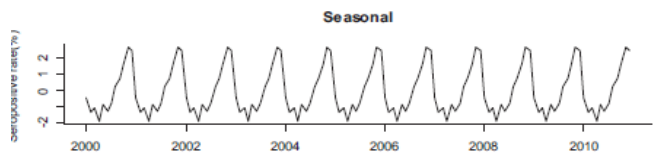
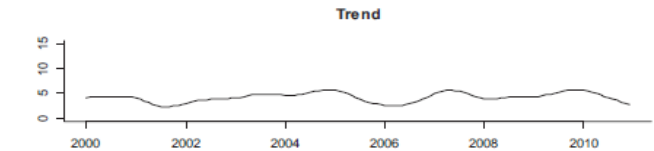
Animals: Forty four thousand nine hundred and sixteen canine serum samples submitted to a commercial laboratory for microscopic agglutination tests (MAT) from 2000 through 2010.

Methods: In this retrospective study, positive cases were defined as MAT titers $\geq 1 : 3,200$ for at least one of 7 tested serovars. Four geographic regions were defined, and MAT results were included in regional analyses based on hospital zip-code. A seasonal-trend decomposition method for times series was utilized for the analysis. Monthly variation in the seropositive rate was evaluated using a seasonal cycle subseries plot and logistic regression.

Results: Two thousand and twelve of 44,916 (4.48%) samples were seropositive. Compared to seropositive rates for February, significantly higher monthly rates occurred during the 2nd half of the year in the MW (OR 3.92-6.35) and NE (OR 2.03-4.80) regions, and only in January (OR 2.34) and December (OR 1.74) in the SC region. Monthly seropositive rates indicative of seasonality were observed earlier in the calendar year for both CS and SC regions.

Conclusions and Clinical Importance: Seasonal patterns for seropositivity to leptospires differed by geographic region. Although risk of infection in dogs can occur year round, knowledge of seasonal trends can assist veterinarians in formulating differential diagnoses and evaluation of exposure risk.

Key words: Dogs; *Leptospira*; Microscopic agglutination tests; Seasonal cycle subseries plot; Seasonal-trend decomposition procedure based on loess; Seropositive.



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Any Questions?

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