

# Risk based surveillance of Leptospirosis in cross- species domestic animals in Bhutan

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# What is leptospirosis?

- Leptospirosis is an infectious disease caused by *Spirochaetes* bacteria belonging to the genus *Leptospira*
- 25 serogroups and 250 serovars

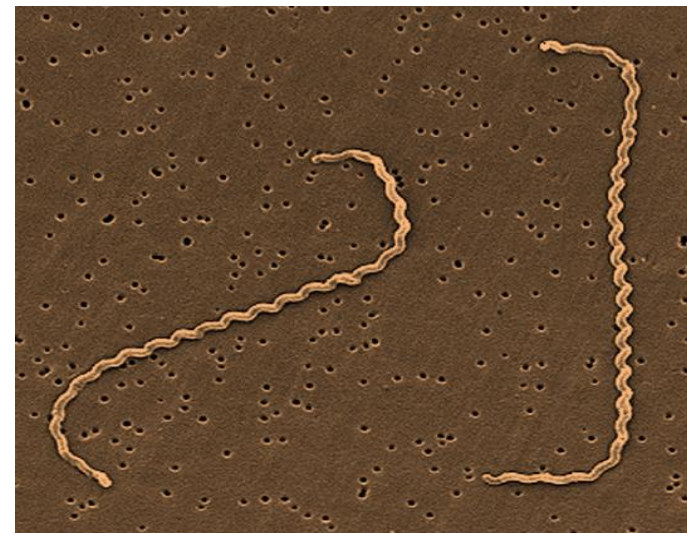


FIG. 1. Scanning electron micrograph of *L. interrogans* serovar icterohaemorrhagiae strain RGA bound to a 0.2- $\mu$ m membrane filter.

# Leptospires

*L. interrogans*

*L. biflexa*

Serovars (No. = 250)  
E.g. *L. interrogans* serovar  
Icterohaemorrhagiae strain RGA

Serovars (No. = 45)  
E.g. *L. biflexa* serovar  
Patoc Strain Patoc 1

Serogroups (No. = 25)  
E.g. *L. interrogans* serovar  
Icterohaemorrhagiae strain RGA  
in the Icterohaemorrhagiae  
serogroup

Serogroups (No. = 38)  
E.g. *L. biflexa* serovar  
Patoc strain Patoc 1 in  
the semaranga serogroup

**Table 1. Serogroups, serovars and genomospecies**

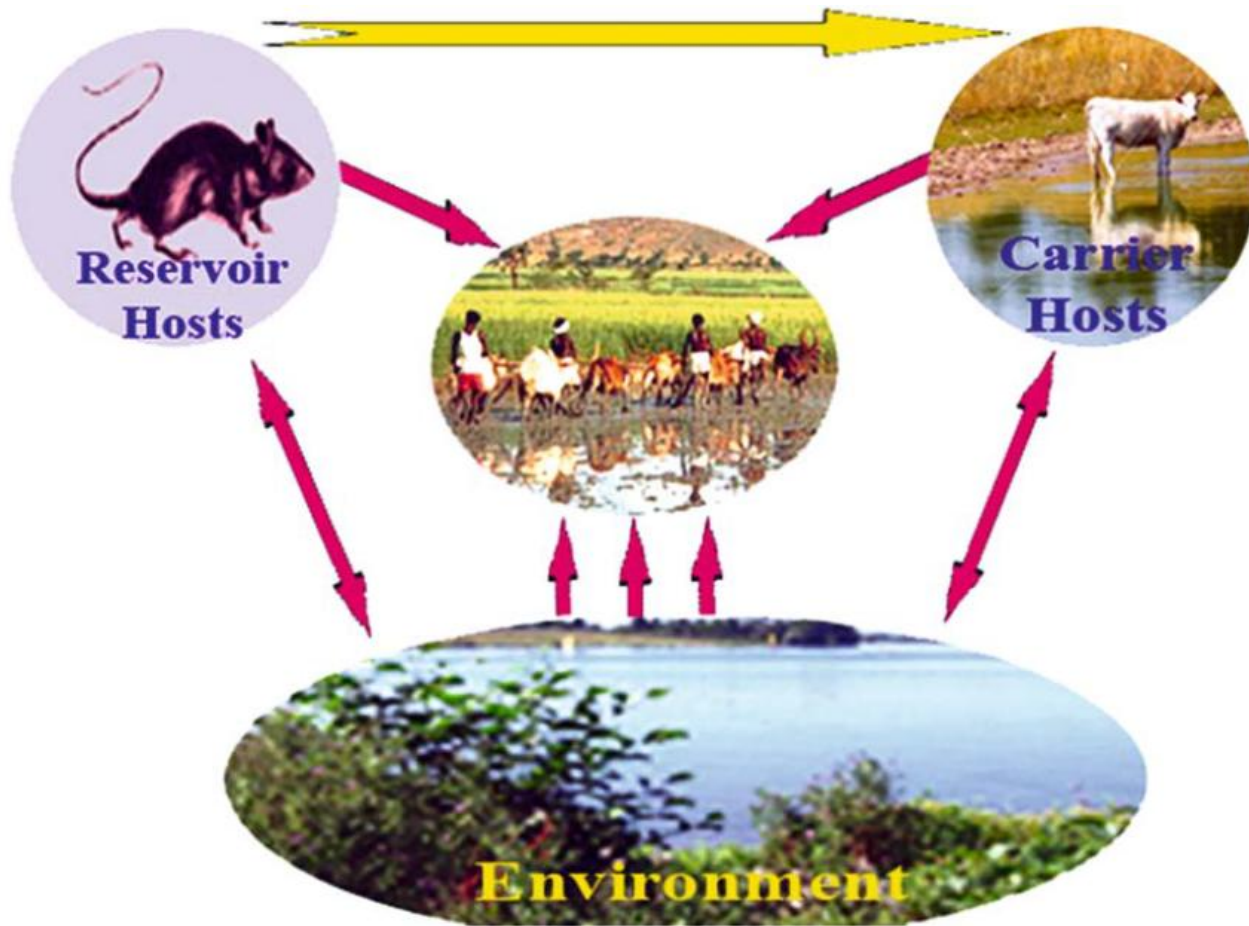
Serogroup	Representative serovar	Reference strain	Species
Australis	australis	Ballico	<i>L. interrogans</i>
Autumnalis	Rachmati	Rachmat	<i>L. interrogans</i>
Ballum	Ballum	Mus 127	<i>L. borgpetersenii</i>
Bataviae	Bataviae	Swart	<i>L. santarosai</i>
Canicola	Canicola	H.Uterecht IV	<i>L. interrogans</i>
Celledoni	Cellodoni	Cellodoni	<i>L. celledoni</i>
Cynopteri	Cynopteri	3522 C	<i>L. kirschneri</i>
Djasiman	Djasiman	Djasinman	<i>L. interrogans</i>
Grippotyphosa	grippotyphosa	Moskva V	<i>L. interrogans</i>
Hebdomadis	hebdomadis	Hebdomadis	<i>L. interrogans</i>
Icterohaem.	icterohaem.	RGA	<i>L. interrogans</i>
Javanica	poi	Poi	<i>L. borgpetersenii</i>
Louisiana	louisiana	LSU 1945	<i>L. naguchii</i>
Manhao	manhao	L 60	<i>Leptospira</i>
Mini	mini	Sari	<i>L. borgpetersenii</i>
Panama	panama	CZ 214 K	<i>L.noguchii</i>
Pomona	pomona	Pomona	<i>L. interrogans</i>
Pyrogenes	pyrogenes	Salinem	<i>L. interrogans</i>
Sarmin	rio	Rr 5	<i>L.weilii</i>
Sejroe	hardjo	Hardjopraj.	<i>L. borgpetersenii</i>
Shermani	shermani	1342 K	<i>L. santarosai</i>
Tarassovi	bakeri	LT 79	<i>L. krishneri</i>
Ranarum	ranarum	ICF	<i>L. interrogans</i>
Sichuan	sichuan	Sichuan	<i>Leptospira</i>
Sehgali	portblairi	DS 2	<i>L. interrogans</i>

# Where does leptospirosis occur?

- Leptospirosis occurs worldwide but is most common in tropical and subtropical areas with high rainfall
- Highly endemic in Asia Pacific Region
- The disease is found mainly wherever humans come into contact with the urine of infected animals or a urine-polluted environment



# Transmission dynamic of leptospira



# Leptospirosis in animals

- Leptospirosis in animal could be totally unapparent or may result in acute febrile illness or severe complications
- Chronically infected animals may remain carriers for years or life and serve as reservoirs of the infection for other animals and humans.

# Leptospirosis in animals

## Acute leptospirosis:

- sudden onset of agalactia (in adult milking cattle);
- icterus and haemoglobinuria, especially in young animals;
- meningitis;
- acute renal failure or jaundice in dogs.
- vomiting, dehydration, bloodstained faeces, mucosal sloughing and death in dogs

## Chronic leptospirosis:

- abortion, stillbirth, birth of weak offspring (may be premature);
- infertility;
- chronic renal failure or chronic active hepatitis in dogs;
- cases of periodic ophthalmia in horses



# Leptospira situation in animals in Bhutan

- In 2009, sero-screening of cattle at National Jersey Breeding Centre, Samtse indicated a *L. hardo bovis* infection (5%).
- Differential diagnosis of Porcine Reproductive and Respiratory Syndrome using sera samples revealed presence of Leptospira antibodies in pig
- No systematic studies have been conducted in animals in Bhutan

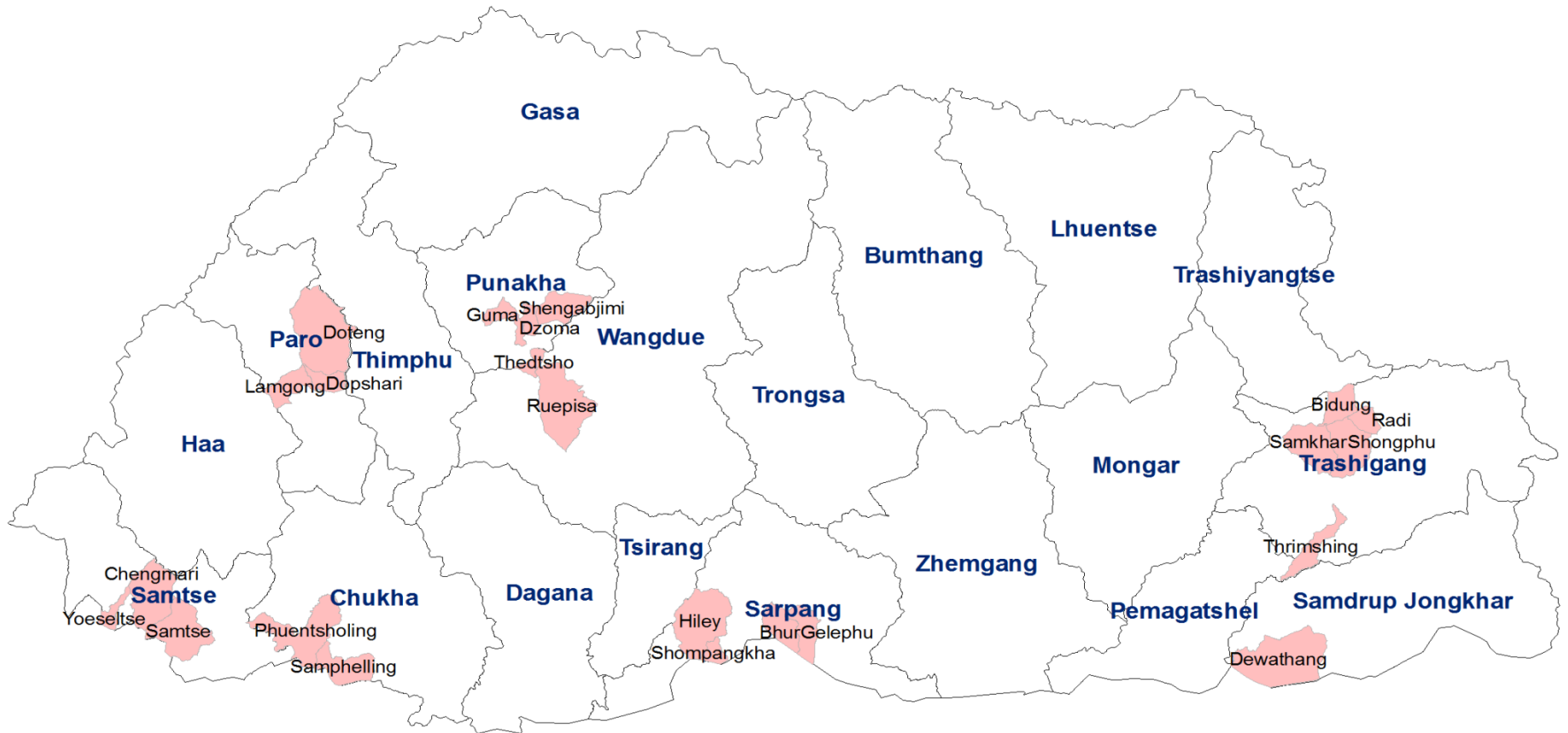
# Objectives

- To estimate the seroprevalence of Leptospirosis in cross-species domestic animals in Bhutan
- To compare the seroprevalence in domestic animal population between tropical areas (south Bhutan), paddy cultivating interior areas of Bhutan and dairy cattle in east Bhutan
- To determine seroprevalence in rodent species
- To estimate the seroprevalence of Leptospirosis in febrile cases of humans presented to the medical hospital for treatment

# Study area (domestic animal)

- Tropical areas of south Bhutan (Samtse, Chukha, Sarpang and Samdrup Jongkhar) Dzongkhags
- Paddy cultivating areas of interior Bhutan (Paro-Punakha-Wangdue valley)
- Dairy farming intensive areas of east Bhutan (dairy groups in east Bhutan)

# Surveillance sites



- Dzongkhag = 7
- Geog = 23
- Villages = 51

# Sample Referral

REGIONAL MEDICAL RESEARCH  
CENTRE (Indian council of  
Medical Research)  
Department of Health Research  
Ministry of Health & Family  
Welfare, Govt. of India

WHO Collaborating Centre for  
Diagnosis, Reference, Research  
and Training in Leptospirosis

PORT BLAIR, ANDAMAN &  
NICOBAR ISLANDS, INDIA



# Sample Referral

- Referred 592 samples (only cattle sample)
- Only 520 samples could be tested
- 72 samples have dried up



# Panel of Serovars used for MAT

<b>S.No</b>	<b>Serogroup</b>	<b>Serovar</b>	<b>Strain</b>	<b>Genomospecies</b>
1	Australis	Australis	Ballico	<i>Leptospira interrogans</i>
2	Autumnalis	Bangkinang	Bangkinang I	<i>Leptospira interrogans</i>
3	Canicola	Canicola	Hond Uterecht IV	<i>Leptospira interrogans</i>
4	Grippotyphosa	Grippotyphosa	Moskva V	<i>Leptospira interrogans</i>
5	Grippotyphosa	Grippotyphosa	CH 31	<i>Leptospira interrogans</i>
6	Hebdomadis	Hebdomadis	Hebdomadis	<i>Leptospira interrogans</i>
7	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA	<i>Leptospira interrogans</i>
8	Icterohaemorrhagiae	Lai Like	AF 61	<i>Leptospira interrogans</i>
9	Pomona	Pomona	Pomona	<i>Leptospira interrogans</i>
10	Pyrogenes	Pyrogenes	Salinem	<i>Leptospira interrogans</i>
11	Sejroe	Hardjo	Hardjoprajitno	<i>Leptospira interrogans</i>

# Prevalent serovar in cattle sample in Bhutan

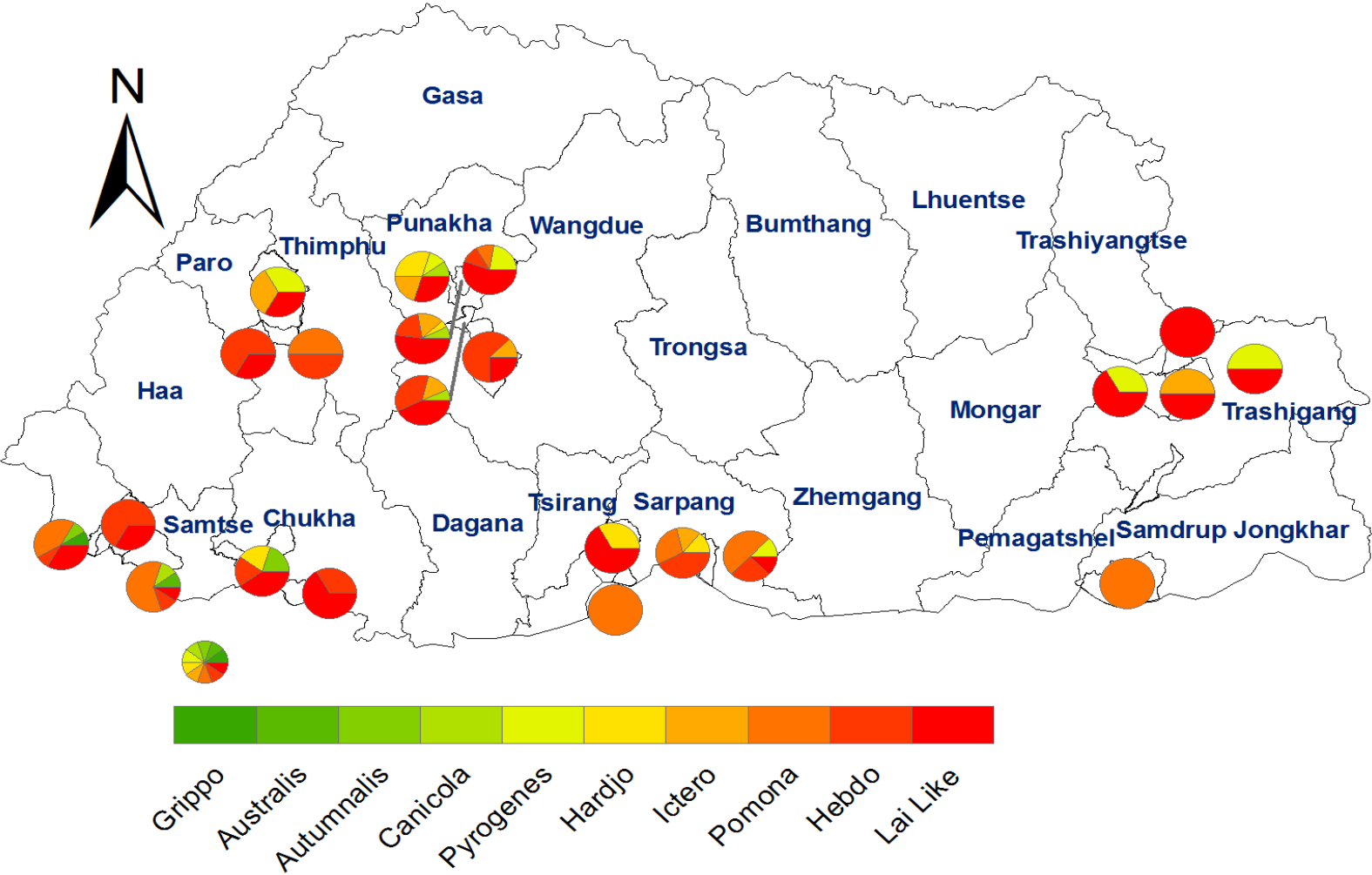
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Serovar	No positive	Percent
Australis	2	1.45
Autumnalis	1	0.72
Canicola	5	3.62
Grippotyphosa	1	0.72
<b>Hebdomadis</b>	<b>30</b>	<b>21.74</b>
Icterohaemorrhagiae	12	8.70
<b>Lai Like</b>	<b>50</b>	<b>36.23</b>
<b>Pomona</b>	<b>22</b>	<b>15.94</b>
Pyrogenes	8	5.80
Hardjo	7	5.07
<b>Total</b>	<b>138</b>	<b>100</b>

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Positive samples were found with titers between **1:40** and **1:2560**, with the highest titers being in the **serogroup Lai Like**

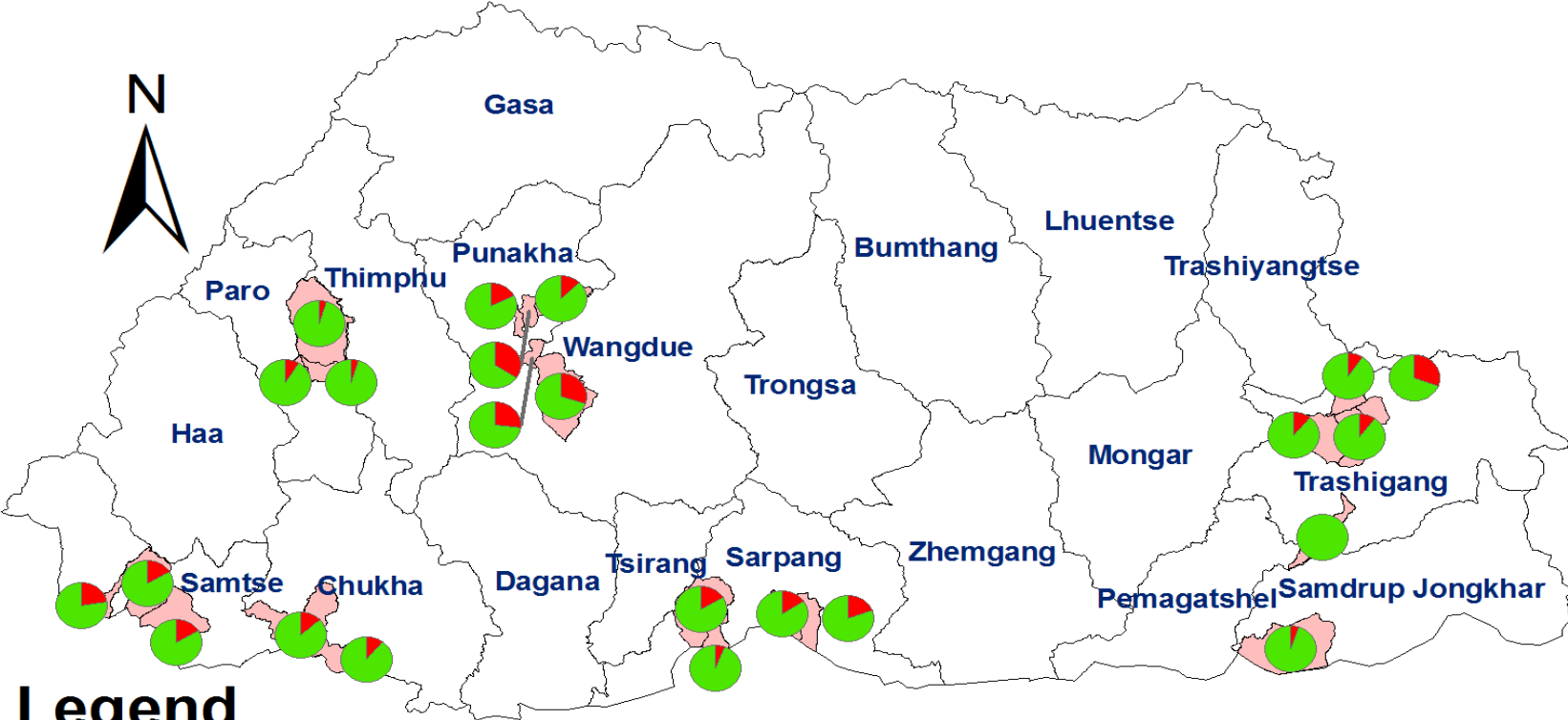
# Distribution of serovar in Bhutan






# Different Leptospira serovar antibody titres according to dilution of serum

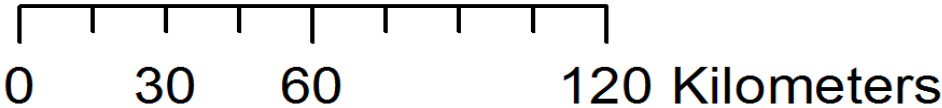
	<b>Australis</b>	<b>Autumnalis</b>	<b>Canicola</b>	<b>Grippityphosa</b>	<b>Hebdomadis</b>	<b>Ictero</b>	<b>Lai Like</b>	<b>Pomona</b>	<b>Pyrogenes</b>	<b>Hardjo</b>	<b>Total</b>
<b>Titre</b>											
1:40	1	2	3	1	18	7	20	9	7	3	<b>71</b>
1:80			1		4	3	13	5	1	1	<b>28</b>
1:160			1		5	2	10	5		3	<b>26</b>
1:320					1		3	2			<b>6</b>
1:640					1		1	1			<b>3</b>
1:1280					1		2				<b>3</b>
1:2560							1				<b>1</b>
<b>Total</b>	<b>1</b>	<b>2</b>	<b>5</b>	<b>1</b>	<b>30</b>	<b>12</b>	<b>50</b>	<b>22</b>	<b>8</b>	<b>7</b>	<b>138</b>

# Distribution of sample and seropositive



## Legend

-   Seropositive
-  Total sample tested



# Results

Dzongkhag	Geog	Total tested	Seropositive	Prevalence (%)
Sarpang	Gelephu	25	6	24.00
Sarpang	Shompangkha	16	1	6.25
Sarpang	Gakidling	15	3	20.00
Sarpang	Samtenling	22	4	18.18
Samtse	Samtse	45	9	20.00
Samtse	Yoeseltse	31	9	29.03
Samtse	Trashicholing	20	4	20.00
Chukha	Phuentsholing	19	3	15.79
Chukha	Sampheling	25	3	12.00
S/Jongkhar	Deothang	39	2	5.13
Paro	Dopshari	22	1	4.55
Paro	Lamgomg	30	3	10.00
Paro	Doteng	22	1	4.55
Punakha	Guma	24	5	20.83
Punakha	Dzomi	33	17	51.52
Punakha	Shengana	35	5	14.29
Wangdue	Thedtsho	24	9	37.50
Wangdue	Rubesa	16	7	43.75
Trashigang	Radhi	9	4	44.44
Trashigang	Shongphu	9	1	11.11
Trashigang	Samkhar	24	3	12.50
Trashigang	Thrimshing	5	0	0.00
Trashigang	Bidung	10	1	10.00
<b>Total</b>		<b>520</b>	<b>101</b>	<b>19.42</b>



# Conclusions

- Leptospirosis is present in cattle in different agro ecological zone and farming system in Bhutan
- Constitute baseline data for further study of leptospira infections in animals in Bhutan
- The survey findings will form the basis for review and development of Leptospirosis control program and assist decide to institute awareness on zoonotic implications of Leptospirosis in human population

# Recommendations

- Develop diagnostic facilities in Bhutan (PHL/NCAH) and test cross-species samples to determine the seroprevalence
- Conduct study in rodent species to determine the prevalence of *Leptospira* since rodents are the main reservoir of leptospira infection



# Acknowledgements

- World Health Organization, SEARO, New Delhi for providing fund support to conduct the survey in Bhutan both animal and human
- WHO Collaborating Centre for Diagnosis, Reference, Research and Training in Leptospirosis, PORT BLAIR, ANDAMAN & NICOBAR ISLANDS, INDIA
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