# Serological survey on Leptospirosis of cattle in Cu Chi, Ho Chi Minh City

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### ABSTRACT

Research Paper	Leptospirosis is a zoonotic disease of global importance, especially in tropical countries. The purpose of this study to evaluate the
Received: March 30, 2020 Revised: May 15, 2020 Accepted: June 19, 2020	seroprevalence of <i>Leptospira</i> in cattle in Ho Chi Minh city. Serum samples from 446 animals were tested by microscopic agglutina- tion test (MAT) using a panel of 24 <i>Leptospira</i> serovars kits pro- vided by Ho Chi Minh City Pasteur Institute. Results showed that seroprevalence of <i>Leptospira</i> at herd-level was $61.54\%$ ; and
	at individual-level was $31.17\%$ . In which, the percentage of beef cattle (29.77%) infected with <i>Lentosnira</i> was lower than that of
Keywords	dairy cattle (31.75%). Cattle from 2 to 3 years of age had the
C + 11	highest rate of <i>Leptospira</i> infection in was 37. 72%. According to
Cattle	herd size, the results found that medium-scale farming (from 25
Leptospira	to 50 animals) had the lowest seroprevalence rate with <i>Leptospira</i>
MAT	spp. (28.65%). No significant differences were found among aged
Prevalence	groups of cattle. There were a totally 7 serovars that were detected
Serovar	in cattle, in which, the two most popular serovars were L. Hardjo
	bovis $(31.37\%)$ and L. Hebdomadis $(30.26\%)$ . There were twelve
	animals infected with four <i>Leptospira</i> serovars (8.63%); meanwhile,
*	the majority of animals infected with one serovar were 42.45%. The
Corresponding author	ratio of positive animals at antibody titer of 1:200 was 53.70%.
	These results indicated a very high exposure of Cu Chi cattle to
Duong Chi Mai	Leptospira spp. which consequently posed a definite risk for people
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#### 1. Introduction

Leptospirosis is a neglected zoonotic disease and can be devastating to both human and animal health globally. It is caused by pathogenic bacteria of the *Leptospira* genus from the family Leptospiraceae (Levett, 2001). This disease is mostly endemic in humid tropical or subtropical countries. Animals or humans can be infected with this pathogen from exposure to contaminated reservoirs such as carrier mammals, contamination of soil and water. Domestic animals act as a reservoir for pathogenic *Leptospira* represents a significant health risk to a wide range of workers, veterinarians and slaughterhouse workers (Adler & Adela, 2010). It was estimated that seven to ten million people were infected by leptospirosis per year and almost 58,900 deaths occurred per year (Costa et al., 2015). *Leptospira* was first identified in 1930 and is known to be endemic for leptospirosis with a peak during the rainy season (Laras et al., 2002). In dairy cattle, though Leptospirosis is sub-clinical or mild in most cases, severe illness can sometimes end fatally, a series of clinical signs such as abortion, mastitis, loss of milk are found. According to WHO (2011), "there is little research on the economic impact of leptospirosis, and information is lacking about the societal costs of the disease, including the costs of health care, lost productivity

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Regions	Number of households	Number of householdsNumber of householdscontained positive cattle	
An Nhon Tay	7	2	28.57
An Phu	10	7	70.00
Binh My	3	2	66.67
Hoa Phu	5	0	0.00
Nhuan Duc	3	3	100.00
Pham Van Coi	1	1	100.00
Phu Hoa Đong	6	2	33.33
Phu My Hung	5	2	40.00
Phuoc Hiep	2	1	50.00
Phuoc Thanh	5	5	100.00
Phuoc Vinh An	4	0	0.00
Tan Thanh Dong	23	17	73.91
Tan Thanh Tay	4	2	50.00
Thai My	2	2	100.00
Trung An	5	5	100.00
Trung Lap Ha	2	2	100.00
Trung Lap Thuong	4	3	75.00
Total	91	56	61.54

Table 1. The seroprevalence of *Leptospira* at herd-level by region

caused by sequelae, and death of livestock". However, it showed a significant public health problem and the economic losses result from infertility, abortion, poor milk yield (Lloyd-Smith et al., 2007). The aim of this study to assess the seroprevalence of *Leptospira* in cattle in Ho Chi Minh City. This information will be reference documents to determine the strategy of leptospirosis prevention and control in humans and animals in Ho Chi Minh City.

# 2. Materials and Methods

Serum samples from 446 cattle were collected from 90 households in Cu Chi district, Ho Chi Minh city from July to October, 2020. Information about the cattle including breed, age, gender, day of vaccination and day of sample collection were recorded. There are about 30 - 40% cattle of total number of cattle per household chosen randomly to collect blood sample. Then, blood samples were left to clot naturally at room temperature. Sera from those tubes after blood clotting were centrifuged to be completely clear and free of hemolyzed blood cells. All sera were labeled with the animal identification number before being stored at -70°C until testing using MAT. Total of twenty four serovars were chosen based on the test kit provided by the Pasteur Institute, Ho Chi Minh City. Each tube of *Leptospira* contained

approximately 2 x  $10^8$  leptospires/mL. The principle of the MAT reaction is the agglutination reaction occurred between the surface of live *Leptospira* and the specific antibody in the test serum against *Leptospira*. The degree of agglutination of the antigen-antibody complex was assessed under a dark-field microscope. This test detects antibodies specific for the *Leptospira* bacteria and is the World Health Organization gold standard test to diagnose leptospirosis.

For data analysis, the overall seroprevalence, prevalence of each serovar, and 95% confidence intervals were calculated by utilizing Excel 2016. Chi-square tests were used to compare the differences in the proportion of seroprevalence of each serovar according to herd size, age group and other variables according to the Minitab 16.0 software.

## 3. Results and Discussion

According to Table 1 the result showed that the highest percentage of households with cattle infected with *Leptospira* spp. was 100% in Nhuan Duc, Pham Van Coi, Phuoc Thanh, Thai My, Trung An and Trung Lap Ha. The statistical difference was found in the proportion of households with infected animals among regions (P < 0.05). Previously using an agglutination test with the

53

			Seropositive	
		Number of tested	samples (a titer $\geq$	Ratio (%)
		samples	1/100 IOF any	
	An Nhon Tay	32	4	12 50
	An Phu	70	25	35.71
	Binh My	11	3	27.27
	Hoa Phu	23	0	0.00
	Nhuan Duc	14	7	50.00
	Pham Van Coi	3	2	66.67
	Phu Hoa Đong	28	3	10.71
	Phu My Hung	18	4	22.22
Regions	Phuoc Hiep	10	2	20.00
	Phuoc Thanh	26	12	46.15
	Phuoc Vinh An	14	0	0.00
	Tan Thanh Dong	117	48	41.03
	Tan Thanh Tay	19	6	31.58
	Thai My	8	5	62.50
	Trung An	25	5	20.00
	Trung Lap Ha	9	4	44.44
	Trung Lap Thuong	19	9	47.37
Hord size	< 25	218	68	31.19
(heads)	$\geq 25$ - $< 50$	171	49	28.65
(neads)	$\geq 50$	57	22	38.60
Types of	Beef cattle	131	39	29.77
$\operatorname{cattle}$	Dairy cattle	315	100	31.75
	< 1	34	11	32.35
Ago	$\geq 1$ - $\leq 2$	137	33	24.09
(vears)	$> 2$ - $\leq 3$	114	43	37.72
(years)	$> 3$ - $\leq 4$	85	29	34.18
	> 4	76	23	30.26

Table 2. The seroprevalence of Leptospira spp. in cattle

same panel of *Leptospira* spp. strains provided by the Pasteur Institute, Ho Chi Minh City, this result was consistent with the results of Pham (2016), the rate of household with cattle infected with *Leptospira* was 61.22% in 6 districts (Cu Chi, Binh Chanh, Hoc Mon, Thu Duc, 9 and 12) of Ho Chi Minh City.

In Table 2, of 446 serum samples were tested by MAT method, there were 139 seropositive samples with a titer  $\geq 1/100$  for any serovars were found, accounting for 31.17%. No significant differences were found among groups with herd size and type of cattle (P > 0.05). Risk factors, including the use of well or stream water, minding livestock, walking barefoot, and the presence of rats and cats at home, were associated with being exposed to *Leptospira* (Ganoza et al., 2006). It is likely related to the increased risk of exposure, transmission and persistence of infections in larger intensive herds. Lilenbaum & Santos (1996) reported that there was a positive association between herd size and the presence of cattle infected with serovar Hardjo. However, there was limited understanding regarding seroprevalence and transmission of pathogenic *Leptospira* in herds comprised of both dairy and beef cattle (Martins et al., 2011).

Moreover, in accordance with other studies, L. Hebdomadis (30.26%) and L. Hardjo hardjo bovis (31.37%) were also the most popular leptospires in cattle in Ho Chi Minh City (Table 3) and the ratios were 23.10% and 18.05%, respectively (Tran, 2016). In Northern Ireland, Ellis and others made the remarkable observation that serovar hardjo was present in 41.6% of 245 randomly selected aborted bovine fetuses (Ellis et

Serovars tested	Number of cases	Frequency $(\%)$
L. Autumnalis	6	2.21
L. Bataviae	3	1.10
L. Hardjo Hardjo-bovis	85	31.37
L. Hardjo Hardjoprajitno	51	18.82
L. Hebdomadis	82	30.26
L. Tarassovi	35	12.92
L. Vughia	9	3.32

**Table 3.** Serovar distribution among 131 seropositive cattle determined by positive MAT (titer  $\geq 1:100$ )

Table 4. The percentage of positive samples reacting with one or more serovars

Number of positive serovars	Seropositive samples (a titer $\geq 1/100$ for any serovars)	Ratio (%)
1	59	42.45
2	40	28.78
3	28	20.14
4	12	8.63
Total	139	100.00

Table 5. Antibody titers rates are agglutinated to Leptospira

Antibody titer mean value	1/100	1/200	1/400	1/800	Total
Number of MAT positive	42	145	72	11	270
%	15.56	53.70	26.67	4.00	100.00

al., 1982). According to Radostits et al., (2000) reported that serovar Hardjo is usually the mostprevalent in the cattle world widely and is considered the most adapted to cattle. This result was in agreement with the study of Bahlibi (2015), the prevalence was significantly highest in cattle with greater than 6 year's age group (29.7%), followed by 3 to 6 years age group (23.2%); while the lowest rate was found in cattle less than three years old (6.7%). It was likely that the older animals were not at greater risk of infection by this organism, but this may be a reflection of the long duration and persistence of antibodies in the animals and a longer period of exposure.

Table 4 showed the majority (42.45%) of the cattle which was tested positive for MAT results showed a reaction to only one serovar, and the rest reacted multiple serovars. The highest numbers of serovars detected were four serovars in 12 animals (8.63%) (Table 4). According to Cousins et al. (1989), each serovar is adapted to a particular maintenance host, although they may cause disease in any mammalian species. Cattle are maintenance hosts for *L. borgpetersenii* serovar Hardjo. Cu Chi district was large and adjacent to many neighboring provinces such as Tay

Ninh, Binh Duong and Long An, this leads to the transportation and trade of cattle occurred more frequently and created favorable conditions for pathogens to spread and infect easily (Tran, 2016).

Antibody titers in this study ranged from 1:100 to 1:800. With 270 *Leptospira*-positive serum samples, there were 145 (53.70%) positive cattle for agglutination test at 1/200 dilution and 11 cattle showed samples with an antibody titer against serovar L. hardjo bovis and L. hebdomatic at of 1/800 dilution (Table 5).

As mentioned above, samples are considered to be positive if agglutination occurred at a titer of 1/100 or more (OIE, 2014). However, a titer of  $\geq$ 100 is often used as evidence of past exposure (Faine & WHO, 1982). According to the Centers for Disease Control and Prevention (CDCP, 1997), a titer of  $\geq$  200 is used to define a probable case with a clinically compatible illness and this defining case occurred in a population in which exposure to leptospirosis is uncommon; but, a higher cut-off titer is necessary for defining probable cases of leptospirosis in most tropical countries. According to the guidelines of the Veterinary Department of Ho Chi Minh City, cattle infected with Leptospira spp. at antibody titer rates of 1/200 or higher must be treated with antibiotics. All cases of positive cattle at 1/200 to 1/800 titer should be isolated from their herds and treated with antibiotics. After this, the second serum samples would be collected at an interval of 10 to 14 days between samples (repeated sampling) to check if treatment was effective or infected cattle should be culled. In areas where leptospirosis is endemic, a single titer of  $\geq$  800 in symptomatic patients is generally indicative of leptospirosis (Faine, 1988).

### 4. Conclusions

In conclusion, of 446 cattle serum samples assayed for anti-leptospiral antibodies by microscopic agglutination testing, 85 (31.37%) reacted with serovar Hardjo hardjo bovis and 82 (30.26%) with serovar Hebdomadis. Results obtained in this study raised our concerns about the spread of infection of leptospirosis in cattle in Cu Chi district and will be used for the development of leptospirosis control program in Cu Chi district.

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