

## Prevalence of *Leptospira*-agglutinating Antibodies in Abattoir Workers and Slaughtered Animals in Selected Slaughterhouses in Cavite, Philippines

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**Leptospirosis is known to be endemic in the Philippines with high incidence during rainy season or after heavy rains in flood-prone areas. The aim of this study was to determine the prevalence of antibodies against *Leptospira* in abattoir workers and slaughtered animals in selected slaughterhouses in Cavite, Philippines. Serum samples obtained from 46 abattoir workers and 69 slaughtered animals were subjected to microscopic agglutination test. Results showed that 15.2% of abattoir workers in the study sites were positive for *Leptospira*-agglutinating antibodies against *L. interrogans* serovar (sv) Canicola, Losbanos, and Ratnapura; *L. fainei* sv Hurtsbridge; and *L. borgpetersenii* sv Poi. On the other hand, the overall *Leptospira*-seropositivity in slaughtered animals was 58.0%, 61.7% of which was in pigs and 33.3% in cows. The most frequently occurring serovar in pigs was Poi with 38.3%, followed by *L. interrogans* sv Icterohaemorrhagiae strain Ictero No. 1 (18.3%), Copenhageni (16.7%), and Icterohaemorrhagiae strain RGA (8.3%). On the other hand, the most frequently occurring serovar in cows was Poi (22.2%). The presence of common serovars in abattoir worker-slaughtered animal interface could indicate continual source of leptospires and could pose problems on human health. These serovars could be potential candidates for the development of vaccines and diagnostic tests.**

Key words: abattoir workers, *Leptospira*-agglutinating antibodies, microscopic agglutination test, prevalence, slaughtered animals

### INTRODUCTION

Leptospirosis has been recognized as an emerging global public health problem because of its increasing incidence in both developing and developed countries (WHO & ILS 2003). It is highly endemic in the Philippines with disease incidence estimated at 4.8 per million population (Pappas et al. 2008). In a four-year period from 2011 to 2014, an average of 786 leptospirosis/leptospirosis-like illnesses was observed (DOH 2014). Most of the cases peak during

rainy months or after typhoons due to flooding and have been a continuing problem in the country (Victoriano et al. 2009). However, during summer months occupationally at-risk groups such as farmers, abattoir workers, and garbage collectors are affected (DOH 2014).

Cavite is the smallest province in Region IV-A, occupying a land area of 8.72% of the region and 0.48% of the Philippines. In spite of rapid urbanization in the province, Cavite continues to have agricultural land used in livestock farming with support facility such as dressing plants,

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slaughterhouses, and meat processing plants (PPDO 2014). Rampant urbanization of municipalities can lead to improper garbage management, creating a favorable condition for the animal carrier in which the most important animals associated with human leptospirosis are rodents.

Leptospire can be classified as either pathogenic or saprophytic. Pathogenic leptospire include *Leptospira interrogans*, *L. borgpetersenii*, *L. fainei*, etc. These leptospire are maintained in nature in the renal tubules of certain animals. Rodents, especially rats, are considered to be the most important reservoir hosts of leptospire (Faine et al. 1999; Villanueva et al. 2010). The organisms are shed by these hosts into the environment via urination. Humans and other animals acquire this infection through direct contact with urine of infected rats or indirectly through contact with contaminated environments (WHO & ILS 2003). In humans, leptospirosis manifests as mild to severe illness that can result in hospitalization and even deaths of susceptible hosts (WHO & ILS 2003; Yanagihara et al. 2007). Leptospirosis has also been reported in many domestic animals such as water buffaloes (Carlos et al. 1971; Basaca-Sevilla et al. 1986), pigs (Aragon & Famatiga 1965; Basaca-Sevilla et al. 1986), horses (Carlos et al. 1971), cows (Basaca-Sevilla et al. 1986), and dogs (Carlos et al. 1971). Although the impact on growth and reproduction performance in cow and pig has not been quantified at industry level in the Philippines, leptospirosis in livestock can become a problem in animal farming and can be source of leptospire to humans (WHO & ILS 2003; Dreyfus et al. 2014). Saprophytic leptospire (e.g., *L. biflexa*) are found in many types of wet or humid environments causing no harm to humans and animals (WHO & ILS 2003).

Increasing cases of leptospirosis in Cavite province from 2009 to 2013 was observed with a case fatality rate of 0.1% (PIDS 2014). Although clusters of cases were observed in the lowest lowland areas, other cases were seen in central hilly and upland areas. The population at-risk in these central hilly and upland areas includes abattoir workers, garbage collectors, and street sweepers. Strategies to prevent and control leptospirosis have been done for years yet the number of cases continues to be high. Transmission of leptospire from reservoir hosts to humans and animals was already identified (Villanueva et al. 2010). However, much has yet to be elucidated regarding *Leptospira* serovars common in both abattoir workers and slaughtered animals. This could add more information on the source of leptospirosis and improvement of vaccine designs and diagnostic kits.

This study aimed to determine the prevalence of leptospiral antibodies in abattoir workers and slaughtered animals in selected slaughterhouses in Cavite, Philippines. Further, *Leptospira* serovars were examined in the abattoir worker-slaughtered animal interface.

## MATERIALS AND METHODS

### Sampling Design, Duration, and Study Population

Stratified sampling design was utilized in this study. From the 23 cities/municipalities of Cavite, Philippines, six were selected based on the number of leptospirosis cases for the past five years (PIDS 2014). The cities of Bacoor, Cavite, Dasmariñas, and Imus plus the municipalities of Noveleta and Tanza comprised the strata. From these strata, public and private abattoirs were identified and a minimum number of 44 abattoir workers who volunteered to participate were included in the study. The inclusion criteria were apparently healthy abattoir workers, 18 years old and above, and employed in the identified abattoirs. The exclusion criterion was defined as abattoir workers who were not residents of the study area or had a high blood pressure of >180/100 mg/dL before blood extraction. As advised by the physician, hypertensive abattoir workers might bruise at the site where blood was taken. These conditions might scare respondents in participating the research study.

A simple random sampling of animals slaughtered in each of the abattoirs was employed until a minimum number of 52 were achieved.

Blood samples from slaughtered animals were collected from Apr 2015 to May 2015, while blood samples from abattoir workers were collected from Jul 2015 to Aug 2015.

### Collection of Samples

Blood samples were extracted from abattoir workers by a registered nurse. Blood samples from animals were obtained after they were stabbed in the neck region by the abattoir worker. Ten milliliters (10 mL) of blood were collected in BD Vacutainer® Plus tubes. The samples were stored in a cooler and transported to the UP College of Public Health (UP CPH) Leptospirosis Laboratory for processing. Blood samples were centrifuged immediately at 2,800 x g for 10 min. Afterwards, the sera were transferred to cryovials and stored at -20°C until use.

### Microscopic Agglutination Test (MAT)

The reference method for serological diagnosis of leptospirosis is the microscopic agglutination test (MAT), in which the human and animal sera are made to react with live antigens of different leptospiral serovars (WHO & ILS 2003). The panel of leptospiral serovars that was used in this study is composed of 39 strains belonging to 32 serovars, seven of which are local isolates i.e., strains LT 398 (Kmety & Dikken 1993), LT 101-69 (Kmety & Dikken 1993), K5, K6, K37, K64, and UP-BLFR13 (Villanueva et al. 2010) (Table 1).

**Table 1.** *Leptospira* strains that were used in the study for MAT.

Species	Serogroup	Serovar	Strain	
<i>Leptospira borgpetersenii</i>	Javanica	Javanica	K6	
		Javanica	Veldrat Batavia 46	
		Poi	Poi	
	Tarassovi	Tarassovi	Perepelitsin	
	Autumnalis	Ballum	Fort Bragg	
	Celledoni	Anhoa	LT90-68	
	Mini	Mini	Sari	
	Sejroe	Sejroe	M84	
	<i>L. fainei</i>	Hurtsbridge	Hurtsbridge	BUT 6T
	<i>L. noguchii</i>	Louisiana	Louisiana	LSU 1945
Panama		Panama	CZ 214 K	
<i>L. alexanderi</i>	Manhao	Manhao 3	L60	
<i>L. weilii</i>	Sarmin	Sarmin	Sarmin	
<i>L. santarosai</i>	Shermani	Shermani	1342 K	
<i>L. interrogans</i>	Pyrogenes	Manilae	LT 398	
		Manilae	K64	
		Pyrogenes	Salinem	
	Canicola	Canicola	Hond Utrecht IV	
	Autumnalis	Autumnalis	Akiyami A	
		Bataviae	Losbanos	LT 101-69
	Hebdomadis	Losbanos	K37	
		Hebdomadis	Akiyami B	
		Hebdomadis	Hebdomadis	
	Australis	Australis	Akiyami C	
		Australis	Ballico	
		Copenhageni	M20	
		Icterohaemorrhagiae	Icterohaemorrhagiae	RGa
		Icterohaemorrhagiae	Icterohaemorrhagiae	Ictero No.1
	Pomona	Pomona	Pomona	
	Sejroe	Hardjo	Hardjoprajitno	
	Djasiman	Djasiman	Djasiman	
	Grippotyphosa	Grippotyphosa	K5	
		Ratnapura	UP-BL-FR13	
		Grippotyphosa	Grippotyphosa	Moskva V
<i>L. kirschneri</i>	Cynopteri	Cynopteri	3522 C	
<i>L. meyeri</i>	Semaranga	Semaranga	Veldrat Semarang 173	
<i>L. biflexa</i>	Semaranga	Patoc	Patoc 1	
	Andamana	Andamana	CH 11	
<i>L. yanagawae</i>	Semaranga	Saopaulo	Sao Paulo	

MAT screening was performed first followed by quantitative MAT for both human and animal sera (WHO & ILS 2003). In MAT screening, human serum samples were diluted to 1:25 in phosphate buffered saline (PBS) prior to mixing with the antigens. The 39 antigens were added individually into diluted serum samples in the microtiter plates to have a final dilution of 1:50. The plates were incubated at 30°C for 2-4 h. After incubation, the serum-antigen mixtures were checked for agglutination under darkfield microscope. The serum samples giving  $\geq 50\%$  agglutination were considered MAT-positive. The

samples that tested positive were subjected to quantitative MAT. In quantitative MAT, human serum samples were serially diluted from 1:50 to 1:51,200. PBS with antigen served as a negative control. The antigens that tested positive to MAT screening were added individually into the diluted serum samples in the microtiter plates. The plates were incubated at 30°C for 2-4 h. After incubation, the serum-antigen mixtures were checked for agglutination under a darkfield microscope. The highest serum dilution that had greater than 50% agglutination or less than or equal to 50% free leptospire – as compared

to negative control – was considered the endpoint titer of quantitative MAT. The same procedure was followed in animals; however, the serum sample was diluted to 1:10 in PBS. The final dilution of the sera was 1:20 (Villanueva et al. 2010). In humans, a titer of  $\geq 400$  in a single serum sample was considered MAT-positive (Masuzawa et al. 2001). In animals on the other hand, a cut-off titer of  $\geq 20$  was used (Villanueva et al. 2010).

### Ethical Clearance

The study protocol was approved by the UP Manila Research Ethics Board for the conduct of research involving human participation (UPMREB 2015-078-01) and the UP Manila Institutional Animal Care and Use Committee (UPM IACUC 2015-009) for the conduct of animal experimentation.

## RESULTS

A total of 46 (31.1%) abattoir workers participated in the study (Table 2). The abattoir workers came from private abattoirs in Bacoor City (n=4), Dasmariñas City (n=4), and Noveleta (n=4), plus public abattoirs in Cavite City abattoir (n=5), Imus City (n=19), and Tanza (n=10). The oldest abattoir workers were observed in Cavite City with an average age of 48.8 years (95% CI 32.48-65.12), while the youngest workers were observed in Bacoor City with an average age of 28.0 years (95% CI 21.25-34.75). On the other hand, the well-experienced abattoir workers were observed in Dasmariñas City with an average service period of 14 years (95% CI -4.69-32.69), while the least-experienced were observed also in Bacoor City with 4.3 years (95% CI -2.16-10.66).

**Table 2.** Geographic elevation of six abattoirs of Cavite province with the mean age and average number of years as abattoir worker, and their 95% confidence interval.

City/Municipal abattoir	Geographic elevation (feet above sea level)	Total number of abattoir workers (number of respondents)	Mean age of respondents	95% Confidence Interval	Average number of years as abattoir worker	95% Confidence Interval
Bacoor City	55	8 (4)	28	21.25 - 34.75	4.3	-2.16 – 10.66
Cavite City	23	15 (5)	48.8	32.48 - 65.12	8.4	-5.99 – 22.79
Dasmariñas City	304	8 (4)	37.5	12.12 - 62.88	14	-4.69 – 32.69
Imus City	48	75 (19)	34.4	29.11 - 39.73	10.3	5.33 – 15.20
Noveleta	27	7 (4)	30.5	25.73 - 35.27	6	-1.46 – 13.46
Tanza	47	35 (10)	31.4	27.55 - 35.25	5.7	2.80 – 8.60
Total		148 (46)	34.7	31.45 - 37.94	8.5	5.90 – 11.10

**Table 3.** Number of abattoir workers found to have antibodies to any of the *Leptospira* panel of antigens and the titer of the corresponding *Leptospira* serovars.

City/Municipal Slaughterhouse	Abattoir workers <sup>a</sup>	Abattoir workers with antibody to <i>Leptospira</i> (%)	<i>Leptospira</i> species serovars (strain)	Titer
Bacoor City	4 (8) (assigned to pigs)	1(25.0%)	<i>L. interrogans</i> sv Ratnapura (UP-BL-FR13)	800
Cavite City	5 (15) (assigned to pigs)	1(20.0%)	<i>L. borgpetersenii</i> sv Poi (Poi)	400
Dasmariñas City	4 (8) (assigned to pigs)	0		
Imus City	14 (25) (assigned to cows)	5 (35.7%)	<i>L. interrogans</i> sv Canicola (Hond Utrecht IV)	400
			<i>L. fainei</i> sv Hurtsbridge (BUT 6T)	800
			<i>L. interrogans</i> sv Losbanos (K37)	800
			<i>L. interrogans</i> sv Losbanos (K37)	400
			<i>L. interrogans</i> sv Ratnapura (UP-BL-FR13)	800
	5 (50) (assigned to pigs)	0		
Noveleta	4 (7) (assigned to pigs)	0		
Tanza	0 (5) (assigned to cows)	0		
	10 (30) (assigned to pigs)	0		
Total	46 (148)	7 (15.2%)		

<sup>a</sup>Number of abattoir workers who participated in the study (total number of abattoir workers)

Blood samples were obtained from a total of 69 slaughtered animals with nine cows and 60 pigs. The distribution of sampled pigs by city/municipality were as follows: Bacoor City (n=6), Cavite City (n=8), Dasmariñas City (n=6), Imus City (n=21), Noveleta (n=3), and Tanza (n=16). Whereas, cows were sampled only in Imus City (n=6) and Tanza (n=3). These slaughtered animals were obtained from the farms in the same city/municipality.

### Microscopic Agglutination Test

MAT was used in detecting leptospiral antibodies in the serum samples of abattoir workers and slaughtered animals. Seven of the 46 (15.2%) abattoir workers were seropositive (Table 3) while 40 of the 69 (58.0%) slaughtered animals had antibodies to *Leptospira*, of which 37 (61.7%) were obtained from pigs and three (33.3%) were obtained from cows (Table 4). These results indicate that the seven abattoir workers and 40 slaughtered animals had previous exposure to *Leptospira* serovars.

In abattoir workers, *Leptospira* serovars Hurtsbridge, Losbanos, and Ratnapura were observed with a titer of 800 each. In slaughtered animals, the most frequently occurring serovar in pigs was Poi (41.7%), followed by Icterohaemorrhagiae strain Ictero No. 1 (18.3%), Copenhageni (16.7%), Semarang (10.0%), and Icterohaemorrhagiae strain RGA (8.3%). Serovar Poi was also observed to be frequently occurring in cows (22.2%). Serovars Canicola and Copenhageni had a titer of 1,280 each. The high titer observed suggested that the slaughtered pigs might have recent exposure to these serovars.

## DISCUSSION

MAT was used to determine the presence of anti-*Leptospira* antibodies in apparently healthy abattoir workers. The presence of these antibodies can indicate previous exposure to the pathogen or recent infection of the person. Ideally, MAT should be performed on paired sera collected during acute and convalescent stage of the disease to determine sero-conversion or four-fold rise in titer, which is the evidence of current or recent infection (WHO & ILS 2003). However, since the research focused on apparently healthy abattoir workers, only single serum samples were collected. In areas where leptospirosis is endemic, a single titer of  $\geq 800$  in symptomatic patients is generally indicative of leptospirosis (Faine 1988), but titers as high as  $\geq 1,600$  have been recommended (Alexander 1986). For this study, a titer of  $\geq 400$  was used to indicate a significant MAT titer, which was based on the study by Masuzawa et al. (2001). The overall prevalence of *Leptospira*-

seropositivity obtained from apparently healthy abattoir workers in selected slaughterhouses in Cavite province was 15.2%. This prevalence was comparable to the results obtained from the study of Arambulo and colleagues (1972) in the Philippines with 10.4%, and Dreyfus and colleagues (2014) in New Zealand with 10.9% among abattoir workers. On the other hand, the prevalence obtained was lower compared to the study of Abiayi and colleagues (2015) in Nigeria, which was 87.8%, 76.5% in India (Natarajaseenivasan et al. 2010) and 34.7% in Iran (Majd et al. 2012). The different prevalences obtained from these studies can be attributed to the different cut-off titers used. Majd and colleagues (2012) used a cut-off titer of 200 while Abiayi and colleagues (2015), Natarajaseenivasan and colleagues (2010), and Dreyfus and colleagues (2014) used a cut-off titer of 160, 80, and 48, respectively. The use of lower cut-off titer can over-estimate the prevalence of *Leptospira*-antibody positivity, and hence would have higher seroprevalence.

The presence of *Leptospira*-agglutinating antibodies among abattoir workers might be attributed to constant exposure of these workers to body fluids of slaughtered animals or contaminated abattoir environment. Abattoir workers who tested positive for leptospiral antibodies worked for an average of three years as abattoir workers in Bacoor and Cavite City. On the other hand, workers in the Imus City abattoir who tested positive for *Leptospira* serovars worked for an average of 11 years as abattoir workers. In relation to work positions in the slaughter line of pigs, workers are assigned to stunning and stabbing, scalding and dehairing, opening of carcass and gut removal, and gut cleaning. Stunning is used as a form of restriction. However, in Bacoor City, Cavite City, Dasmariñas City, and Noveleta abattoirs, workers used either bare hands and feet or by tying the front and hind limbs of pigs before slaughtering. Abattoir workers assigned to this task might have higher probability of being exposed to mud stuck onto the body of slaughtered animals or to urine and blood during stabbing. In Imus and Tanza abattoirs, workers suspend the carcasses in an overhead rail after stabbing, scalding and dehairing for carcass opening, and evisceration. In the study of Dreyfus and colleagues (2014), workers assigned to yards, stunning, and sticking were 17.4 times more likely to be infected with leptospires than those assigned to boning, chiller, or office. However, the results of the present study showed that only one of the eight abattoir workers assigned to stunning or stabbing had antibodies against *Leptospira*. This worker had a titer of 800, which is indicative of past exposure or the worker was continuously exposed to infected pigs. The exposure may not have been significant enough to trigger the onset of disease, but were able

**Table 4.** MAT titers of *Leptospira* serovars in seropositive slaughtered animals.

<i>Leptospira</i> serovars	Strains	Bacoor City Pigs			Cavite City Pigs				Dasmariñas City Pigs						
		BP1	BP2	BP4	CP4	CP5	CP6	CP7	CP8	DP1	DP2	DP3	DP4	DP5	DP6
Poi	Poi	-	-	-	-	-	-	320	40	-	40	-	640	320	40
Tarassovi	Perepelitsin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mini	Sari	-	-	-	-	-	-	-	-	-	160	-	-	-	-
Hurtsbridge	BUT 6T	-	-	-	-	-	-	-	-	-	-	-	-	-	40
Louisiana	LSU 1945	-	-	-	-	80	-	-	-	-	-	-	-	-	-
Manilae	K64	-	-	-	-	-	-	-	-	-	-	-	-	-	40
Canicola	Hond Utrecht IV	-	-	40	80	1280	-	-	-	-	-	-	-	-	-
Losbanos	K37	-	-	-	-	-	-	-	-	40	-	-	-	-	-
Hebdomadis	Hebdomadis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Copenhageni	M20	-	-	80	-	40	-	-	40	-	80	320	-	1280	-
Icterohaemorrhagiae	RGA	-	-	-	40	-	-	-	80	-	-	-	-	-	-
	Ictero No.1	-	-	80	-	320	160	320	-	-	-	-	-	-	-
Djasiman	Djasiman	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grippotyphosa	Moskva V	-	20	-	-	-	-	-	640	-	-	-	-	-	-
Semarang	Veldrat Semarang 173	-	-	-	-	160	-	-	-	-	160	160	160	-	-
Patoc	Patoc 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Andamana	CH 11	40	-	-	-	-	-	-	-	-	-	40	-	-	20
Saopaulo	Sao Paulo	-	-	-	-	-	-	40	-	-	-	-	-	-	-

to induce antibody response. In the slaughter line of cows, workers are assigned to knocking or stabbing, hide removal, opening of carcass and gut removal, cutting, and gut cleaning. Of the 15 abattoir workers assigned in slaughtering of cows, five were positive for *Leptospira*-agglutinating antibody with a titer range of 400-800. These workers were assigned to stabbing, hide removal, gut removal, and butchering, and were more likely to be exposed to leptospires. During sampling, cows excessively urinate before knocking. This condition might have introduced the leptospires to the workers in the slaughter line or to the next cow to be slaughtered. Exposure to body fluids increases the likelihood of getting infected.

The abattoir workers in this study were found to have antibodies against *Leptospira* serovars Canicola, Hurtsbridge, Losbanos, Poi, and Ratnapura. In a recent study, only serovar Poi was observed among the abattoir workers in Manila (Gloriani et al. 2016b). However, in the study of Arambulo and colleagues (1972), antibodies against *Leptospira* serovars Pyrogenes, Bataviae, Pomona, Grippotyphosa, Manilae, and Javanica were observed among abattoir workers but not in the present study. The results may indicate that the circulating serovars in Cavite might probably

be different from those in Manila. The absence of *Leptospira*-agglutinating antibodies (84.8% of the abattoir workers) indicates that either the worker was not exposed to the pathogen, or the leptospires used in the panel of antigens were still incomplete. *Leptospira*-agglutinating antibodies were seen only in workers working in abattoirs of Bacoor City, Cavite City, and Imus City. These areas also have cases of death due to leptospirosis (PIDS 2014). These cities are also classified as the lowest lowland areas of Cavite province with geographical elevation of 23-55 ft above sea level and a history of prolonged flooding.

MAT was also used to determine the *Leptospira*-seropositivity in slaughtered animals. However, since there was no cut-off titer set in animals, any sample that exhibited a titer of 20 for any serovar was considered positive in this study. This titer was set at this level because in the study of Villanueva and colleagues (2010) with specific pathogen-free rats, no antibodies against the panel of antigens were detected at this titer.

The overall *Leptospira*-seropositivity in slaughtered animals in the present study was 58.0%. The prevalence proportion observed in pigs was 61.7%. This result was significantly lower compared to the study of Gloriani

Samples positive for *Leptospira* antibody

Samples positive for <i>Leptospira</i> antibody																									
Imus City													Noveleta					Tanza							
Pigs													Cows		Pigs			Pigs					Cows		
IP2	IP3	IP4	IP8	IP12	IP13	IP14	IP15	IP16	IP17	IP18	IP19	IC1	IC3	NP1	NP2	NP3	TP2	TP3	TP4	TP5	TP6	TP7	TP9	TP12	TC1
80	80	-	40	-	-	160	40	160	80	160	80	80	80	80	80	80	-	160	160	-	-	160	40	80	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40
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and colleagues (2016a), which was 91.7% but higher compared to the study of Wasiński and co-workers (2012) in Poland, which was 34.4% among pigs exposed to flooding. During the time of sampling of blood of slaughtered animals, rats were sighted inside the piggens and around the abattoir. There is a high possibility that the pigs were exposed to rat excreta as well as mud. Although the presence of *Leptospira* antibodies in slaughtered animals in this study is most likely from its prior exposure, it is also possible that holding of pigs in piggens may increase the likelihood of being infected. *Leptospira interrogans* (serovars Bratislava, Canicola, Hardjo, Icterohaemorrhagiae, and Pomona), *Leptospira borgpetersenii* (serovars Sejroe and Tarassovi), and *Leptospira kirschneri* (serovar Grippotyphosa) were all reported to infect pigs (Bolin 2000). Serovar Pomona causes abortions, fetal deaths, and the birth of feeble piglets while Serovar Bratislava is linked to stillbirths (Schollum & Blackmore 1982). Serovars Icterohaemorrhagiae and Grippotyphosa observed from the present study may have caused incidental infections in pigs. It is surprising that serovars Pomona and Bratislava adapted to pigs were not observed in the study. Serovars Poi, Manilae, and Copenhageni were also observed from the study of

Gloriani and colleagues (2016a). This means that some serovars obtained from slaughtered pigs in Manila could also be observed from slaughtered pigs in Cavite. Most of the pigs slaughtered in Manila were bought from farms in the provinces of Cavite, Laguna, Batangas, Rizal, and Quezon (CALABARZON); hence, animals might have been exposed to the same pathogenic serovars. Some of the pigs had relatively high titers of antibodies (up to 1,280), which indicate fairly recent exposure.

On the other hand, the prevalence proportion observed in cows was 33.3%, which was significantly lower compared to the study of Gloriani and colleagues (2016a) in the Philippines with 92.0%. However, the figure is higher compared to the study of Ngbede and colleagues (2012) in Nigeria with 3.5% and Wasiński and co-workers (2012) in Poland with 26.8% among cows exposed to flooding. The prevailing *Leptospira* serovars identified in the present study were Canicola, Copenhageni, Poi, and Tarassovi. The most commonly documented cause of leptospirosis among cows worldwide is serovar Hardjo for which they are the maintenance host (Levett 2001). Infection with this serovar can persist in the reproductive tract resulting to infections leading to infertility (Ellis et al. 1981).

This is the most economically challenging aspect of leptospirosis in cows. Infecting serovars (e.g., Pomona and Icterohaemorrhagiae) can also be observed although less common. However, these serovars were not observed in the present study. Leptospirosis with non-host-adapted *Leptospira* serovars affects pregnant cows causing embryonic death, abortions, stillbirths, retained placenta, and the birth of weak calves. Serovars Poi and Tarassovi were also observed in the study of Gloriani and colleagues (2016a) in cows. This means that some serovars obtained from slaughtered cows in Manila could likewise be observed from slaughtered cows in Cavite. The risk factors for leptospirosis in cows have been reported to include open herd, access to contaminated water sources, co-grazing with other animals (Radostits et al. 2006), and the presence of field rats. These risk factors were also observed in the cow farms in Cavite province.

As for the limitations of the study, first, the antiserum standard (positive control) for each of the serovars tested was not performed due to high cost and maintenance of the antiserum. Second, abattoir workers who volunteered to participate in the study were not representative of the workers assigned in blood-related task or work positions. There is a possibility that the workers who experienced manifestations of leptospirosis did not participate for fear of losing their jobs. Third, work positions or task of abattoir workers assigned in pigs change from time to time depending on the number of pigs to be slaughtered. Workers assigned in stabbing can be transferred to offal room. Finally, because of the difficulty in restricting cows during stabbing, the number of blood samples collected was limited.

## CONCLUSION

This study demonstrated that abattoir workers and slaughtered animals in selected slaughterhouses in Cavite province were exposed to pathogenic *Leptospira*. The detection of antibodies common against serovars (e.g., *L. borgpetersenii* sv Poi, *L. fainei* sv Hurtsbridge, and *L. interrogans* sv Losbanos) in abattoir worker-slaughtered animal interface could indicate continual source of leptospires and could pose problems on human health. These serovars could be potential candidates for the development of vaccines and diagnostic tests.

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