

Research Article



Correlations Between Melioidosis Seroprevalence in Livestock and Meteorological Factors in Peninsular Malaysia

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Abstract | Melioidosis is an emerging saproozoonosis which constitutes animal and public health problems in the endemic areas. This study examined the effects of meteorological factors on distribution, trends and patterns of melioidosis seroprevalence in livestock in Peninsular Malaysia. Data on melioidosis surveillance and climatic factors were obtained from the Department of Veterinary Services (DVS) and Malaysian Meteorological Department (MMD). Seroprevalence of the disease in different animal species were calculated and the relationships between the prevalence and climatic factors were examined. A total of 72,941 animals were sampled from 1,765 livestock farms across all the states and tested, out of which 4,516 (6.20%, 95% CI 6.02-6.37) were serologically positive for melioidosis. Correlation analysis of seroprevalence and the climatic factors showed strong, positive and statistically significant correlation ($r = 0.58$, 95% CI, 0.12-0.87, $p = 0.047$) between the disease prevalence and average monthly rainfall. There was a moderate but non-significant positive correlation ($r = 0.47$, 95% CI, 0.13-0.82, $p = 0.11$) between disease prevalence and average monthly rainy days and weak and non-significant correlation ($r = -0.23$, 95% CI, 0.71-0.39, $p = 0.47$) with the average monthly wind speed. Animal melioidosis is widespread within Peninsular Malaysia where the disease appears to occur more in the domestic compared to wild animals. The study showed that more resources and attention appeared to have been placed on small ruminants compared to other livestock species. Amount of rainfall recorded during the previous decade may have contributed to the occurrence of the disease in livestock over the years.

Keywords | Livestock, Melioidosis, Meteorological factors, Rainfall, Seroprevalence

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INTRODUCTION

Melioidosis is a saproozoonosis caused by soil saprophytic bacterium, *Burkholderia pseudomallei*. The disease is endemic in Southeast Asia and northern Australia, mostly in areas within latitudes 20°N and 20°S (Rachlin et al., 2020) where it causes significant public and animal health problems (Ryan et al., 2018; Inglis and Sousa, 2009). In Malaysia, serological presence of the disease

has been reported in different animal species in different parts of the peninsula (Hambali et al., 2018; Masrin et al., 2018; Musa et al., 2012). Human melioidosis cases have been previously shown to be significantly associated with rainfall and flooding in different parts of Peninsular Malaysia (Hassan et al., 2010; Sam and Puthuchear, 2007). Occurrence of the disease has also been observed to be related to wind speed and direction as well as the amount of soil water content (Pongmala et al., 2022). A

high concentrations of *B. pseudomallei* were found in the atmosphere during typhoon season (Chen et al., 2014) and at particular depth of the soil during rainy season in endemic areas (Pongmala et al., 2022) thus increasing possibility of more infections during such a period. A recent 16 years longitudinal study in the endemic areas have observed a higher risk of infections during humid and windy conditions (Bulterys et al., 2018).

In Malaysia, despite availability of relatively sizeable number of published information on the disease in humans (Adib et al., 2021; Nathan et al., 2018; Kingsley et al., 2016), the situation cannot be said to be the same with information on the disease in animal populations in the country. The available information on the disease in animals in the country (Hambali et al., 2018; Masrin et al., 2018; Musa et al., 2012, 2015) shows that adequate attention has not been given to the disease in animals. Studies among humans in selected states showed that the disease is a major socio-ecological and socio-economic problems (Abu Hassan et al., 2019; Hassan et al., 2010) with the disease incidence, risk factors and clinical manifestation in the country recently reviewed by Nathan et al. (2018). The environmental factors such as increased rainfall and severe weather events among others have been reported to be the drivers of endemicity and occurrence of the diseases (Birnie et al., 2022; Mardhiah et al., 2021).

This study describes retrospectively the distribution, trends and patterns of melioidosis in livestock and its relationships with rainfall and wind speed in Peninsular Malaysia between 2000 and 2009. It is believed that the information from the study would enhance understanding of the epidemiology of melioidosis as well as to appreciate the historical perspective of the disease among the local animal population. It may serve as a useful input in planning of control measures against the disease in animals in the country.

MATERIALS AND METHODS

STUDY AREA AND LIVESTOCK POPULATION

Malaysia is located in Southeast Asian region and is comprised of two main parts, the West Malaysia (Peninsular Malaysia) and East Malaysia (Sabah and Sarawak, both located on the Borneo Island). The Peninsular Malaysia is made up of 11 states and two federal territories (Wilayah Persekutuan and Putrajaya). The two parts of Malaysia are separated by the South China Sea (MNRE, 2004). Peninsular Malaysia covers an area of 131,598 square kilometers sharing common borders with Thailand in the north and Singapore in the south. The 11 states in Peninsular Malaysia include Johor, Kedah, Kelantan, Melaka, Negeri Sembilan, Pahang, Perak, Perlis, Pulau Pinang, Selangor

and Terengganu (Weightman, 2011). A map of Peninsular Malaysia showing its administrative divisions into states is shown in Figure 1A. The country lies entirely in the equatorial zone and is situated in the northern latitudes between 1 and 6° N and the eastern longitude from 100 to 103°E. The climate of Peninsular Malaysia is influenced by two monsoon seasons, the northeast monsoon, which starts in November and ends in March and the southwest monsoon, which starts in May and ends in September. The northeast monsoon is usually characterized by prolonged heavy rainfall in northern and eastern regions of Peninsular Malaysia causing severe floods in low-lying areas especially the east coastal states of Kelantan, Terengganu, Pahang and east Johor. On the other hand, the southwest monsoon is characterized by drier conditions with less amount of rainfall throughout the Peninsular (Deni et al., 2008; Shakirah et al., 2016; Weightman, 2011). According to the Malaysian Meteorological Department (MMD), Peninsular Malaysia has an average rainfall of 2,400 mm with hot and humid weather throughout the year (Shakirah et al., 2016).

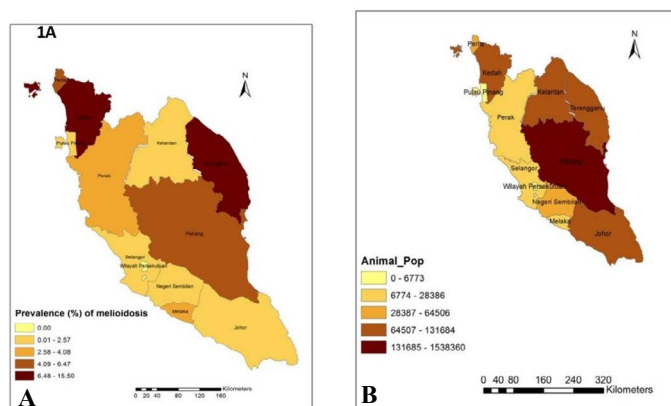


Figure 1: (A) Seroprevalence of melioidosis in livestock in Peninsular Malaysia from 2000-2009 while (B) annual populations of livestock in Peninsular Malaysia from 2006-2009.

According to the Department of Veterinary Services, Malaysia, the average annual population of cattle on the Peninsular between the year 2006 and 2009 was 763,727 heads. For buffalo, the average annual population for the same period was 78,654 while those for goats, sheep and swine were 384,076, 122,446 and 1,440,898 animals respectively. The four years (2006-2009) annual average recorded slaughter figures for cattle, buffalo, goats, sheep and swine were 102,627, 10,713, 16,456, 3,640 and 1,359,537 animals respectively (DVS, 2011a).

DATA SOURCES

In Malaysia, animal melioidosis is monitored under an animal surveillance program run by states and federal veterinary services department as a multispecies disease. The surveillance program is coordinated by the headquarters of the Department of Veterinary Services

(DVS) in Putrajaya, which maintains database on the surveillance exercise. The database serves as official record for the national animal diseases surveillance program and contained information on farm location, number of animals sampled and their respective seroprevalence rates as well as demographics such as age, sex and breeds. The program helps in monitoring levels of endemic livestock diseases and in detecting outbreak of new ones. The program recommended regular serological screening of livestock population against specific number of animal diseases in the country according to standard operations procedure prescribed in Animal Disease Surveillance Protocol (Document No. APTVM 22 (g): 1/2011) (DVS, 2011b). The state veterinary departments and the regional veterinary laboratories located in states across the peninsula perform the sampling and serological tests in the program. The recommended serological test for monitoring the levels of antibodies against *B. pseudomallei* was the complement fixation test (CFT) as described in the OIE Manual (OIE, 2004) while confirmatory test for melioidosis was isolation and identification of the agent from biological samples from suspected animals. For the serological test, an animal was considered to be positive if it had antibody titer of 1:80 or more. The CFT was the preferred as screening test because of its high specificity in serodiagnosis of melioidosis (Thomas et al., 1988), while the culture and identification method was the preferred confirmatory test since it remains the gold standard in the diagnosis of melioidosis (Wongsuvan et al., 2018). Both tests were performed at the regional veterinary laboratories located in states and at a reference laboratory at the Veterinary Research Institute (VRI). Results of the tests are sent to the Epidemiology Section of the Animal Diseases Information Center (ADIC) of the DVS, which manages the database. Records for a period of 10 years from the year 2000 to 2009 were obtained from the Epidemiology and Surveillance Unit of the ADIC of DVS, Putrajaya, and also from VRI, Ipoh. The data were checked for duplicate entries and errors and collated. Information in the database included names of farms and their addresses, date of sampling, location and state, species, breed, age range, number of animals tested and seroprevalence in the tested animals.

Meteorological data on monthly amount of rainfall, number of rainy days in a month and the average daily wind speed were obtained from Malaysian Meteorological Department (MMD). Retrospective records for a period of 10 years (2000-2009) were obtained from 27 weather stations located across the peninsula. The meteorological data were summarized based on month, year and state. Data from both the surveillance and meteorology were managed and stored in a Microsoft Excel® 2011 for Mac OS X (Microsoft Corporation) as spreadsheet files.

SPATIAL DISTRIBUTION

Spatial distributions of melioidosis seroprevalence in livestock during the study period were presented as choropleth maps using ArcGIS (Version 10.2, ESRI, Inc., Redlands, CA, US). Similarly, choropleth maps of the distribution of the populations of respective livestock species were also drawn. When plotting the maps, results of the seroprevalence of melioidosis in all livestock species were aggregated according to states, so as to maintain confidentiality of farms and also due to non-availability of exact coordinates of some of the study farms. Digital base maps of states and that form the peninsula were obtained from the Department of Survey and Mapping Malaysia (JUPEM). Data for wild and zoo animals were too scanty and so were excluded from the spatial maps. Digital data on spatial attributes such as geology, soil type, elevation, cadastral division and population density could not be obtained so as to enable the performance of spatial analysis.

DATA ANALYSIS

Seroprevalence rates were calculated as the number of seropositive animals divided by total number sampled using EpiTools epidemiological calculators (Sergent, 2014). The estimates and their respective 95% confidence intervals were calculated using the Wilson (score) method as described by Brown et al. (2001). The differences between proportions were tested using the Chi-square or Fisher's exact test as the case may be. Relationships between the proportions of animals that were serologically positive for melioidosis and weather elements (rainfall, rainy days and wind speed) were examined according to states, years and months of the year using correlation analysis. To examine the patterns, averages of total monthly rainfall, number of rainy days and monthly wind speed were calculated for each state, month and year using data from the weather stations located in the respective states. Proportions of positive animals were determined for each state, year and month from the surveillance data. Correlations between proportions of seropositive animals and amount of rainfall, number of rainy days and wind speed were examined using correlational analysis.

Stratification of most livestock species from the database based on the breeds, age and districts were not possible because information on these variables were either missing or ambiguous in some farms. For comparison of cattle, the local Indian dairy cattle and Kedah-Kelantan were categorized as "local breeds" whereas crossbreeds between local and foreign breed were categorized hybrids. On the other hand, exotic breeds of cattle imported from outside the country were categorized, as foreign breeds for the purpose of this analysis because some of the exotic breeds sampled were too few to be placed into categories. For buffalo, the two breeds available breed were the Sawah and

Murah breeds. In goats, exotic breeds such as the Anglo Nubian, British Alpine, Feral, Tonggenburg and Saanen were all categorized as foreign breeds whereas hybrids, Jamnapari, and Katjang were considered as 'local breeds'. Comparisons according to age categories of livestock species were not feasible because ages were not consistently recorded and sometimes presented as aggregates as adults, young, mixed etc. Statistical analyses were done using SPSS (version 21.0 for Mac OS X; SPSS Inc., Chicago, IL, USA) at the significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

DESCRIPTIVE STATISTICS

During the period of the study, a total of 72,941 animals were sampled from 1,765 farms from all the states were tested out of which 4,516 (6.20%, 95% CI 6.02-6.37) were serologically positive for melioidosis. A total of 620 cattle from eight states were sampled out of which 46 (7.42%, 95% CI; 5.61-9.75) were seropositive. For buffalo, out of a total of 71 animals sampled from three states of Johor, Perak and Selangor, only 20 (28.18%, 95% CI; 19.04-39.54) tested positive. For goats, a total of 49,109 animals from all the states on the peninsula except Kuala Lumpur were sampled and 1,073 (2.19%, 95% CI; % 2.06-2.37) tested positive. For sheep, a total of 18,949 animals were sampled from all the states on the peninsula with the exceptions of Melaka, Perlis and Pulau Pinang out of which 3,136 (16.55%, 95% CI; 16.03 – 17.09) tested positive. A total of 434 deer were sampled from four states of Negeri Sembilan, Pahang, Perak, Perlis and Selangor out of which 28 (6.45%, 95% CI; 4.50-9.17) were positive. Only 17 pigs from Penang were tested during the study period and only 1 pig (5.88%, 95% CI; 1.05-26.98) tested positive of melioidosis. From the total 1,336 rabbits sampled from the state of Johor, 52 (3.90%, 95% CI 2.90-4.90) were positive. However, the 7 dogs and the only horse tested during the study period were all negative.

Results of the screening test in wild animal sera showed out of the 5 monkeys tested from three states of Melaka, Perak and Selangor, only 1 (20.0%, 95% CI; 3.62-62.45) monkey from Perak tested positive. However, none of the 4 elephants, 11 primates (species not stated), 3 orangutans, 1 lion and 6 tigers sampled yielded positive results. The big cats (tigers and lions) tested were from states of Johor, Perak, Selangor and the federal territory of Kuala Lumpur.

SEROPREVALENCE OF MELIOIDOSIS IN ANIMALS ACCORDING TO SPECIES

Table 1 shows prevalence of antibodies against *B. pseudomallei* in animals during the study period. The seroprevalence of the disease in cattle according to breed varies from 6.25% in the local breed to 7.87% in the hybrids.

Table 1 also shows of comparisons of the seroprevalence of the disease in cattle in the study based on breeds, year and states. There was no significant difference ($p > 0.05$) in terms of seroprevalence of the disease when foreign and hybrids were compared with local breeds. Comparisons of the rates in cattle based on state and year were not possible due to scanty cell numbers. Similarly, comparison of the rates in buffaloes was not possible due empty cells.

The seroprevalence in goats varied from a low of 0.56% in the year 2000 to a high of 6.97% in 2003. Comparison of the seroprevalence in goats according to the year of the study showed significant differences ($p < 0.05$) between the proportions of goats tested positive in the base year (2000) compared to 2002, 2003, 2005 and 2007. Similarly, comparison proportions according to state show significant differences ($p < 0.05$) in terms of the prevalence between goats from Johor compared to those from Kedah, Kelantan and Pahang states. The comparison of the seropositivity of the disease based on breeds of goats sampled in the study showed a significant difference ($p > 0.05$) between foreign and the local Katjang breed with the latter less likely to be tested positive when compared to the former. The seroprevalence in sheep based on year of sampling revealed a significant difference ($p < 0.05$) between the base year and the year 2004. No significant difference ($p > 0.05$) was observed between the sheep sampled from Johor compared to those from other states.

Table 2 shows prevalence of antibodies against *B. pseudomallei* in wild animals in Peninsular Malaysia during the years under review. In deer, the seroprevalence of the disease according to breeds varied between 4.53% in Jawa compared to 10.48% in Sika breed. In rabbits, the seroprevalence of the disease according to breeds varied between 2.05% in New Zealand white to 6.80% in hybrids. In other animals such as dogs, the big cats (lion and tigers), and the elephants the tests were negative while data for crocodiles and horses could not be compared because only one animal each was tested.

TEMPORAL DISTRIBUTION OF SEROPREVALENCE OF MELIOIDOSIS IN ANIMALS IN PENINSULAR MALAYSIA 2000-2009

Table 3 shows the yearly seroprevalence of melioidosis in animals in Peninsular Malaysia during the period under review. The seroprevalence rate varies from a low 3.14% (95% CI, 2.84-3.46) in 2008 to high of 11.79% (95% CI, 10.60-13.09) in 2003. The seroprevalence of the disease in animals for the duration of the study was 6.20% (95% CI 6.02-6.37%). The prevalence rate increased between 2001 and 2003 during which it almost doubled the average yearly rate. The prevalence then slightly declined in 2004 after which it fluctuates between 2005 and 2009.

Table 1: Seroprevalence of *Burkholderia pseudomallei* in livestock and comparisons of seroprevalence based on breeds, year and states in Peninsular Malaysia between the year 2000 and 2009.

Species	Variable	Category	N	+ve	Prevalence (95% CI)	OR	95% CI	p-value	
Cattle	Breed	Local	64	4	6.25, (2.40-15.0)	1.00	-	-	
		Foreign	200	14	7.00, (4.22-11.41)	0.89	0.28-2.74	0.65	
		Hybrids	356	28	7.87, (5.5-11.13)	0.78	0.26-2.30	0.80	
Goats	Year	2000	708	4	0.56, (0.22-1.44)	1.00	-	-	
		2001	2,734	66	2.41, (1.90-3.06)	2.07	0.62-6.98	0.23	
		2002	1,122	42	3.74, (2.78-5.02)	4.53	1.23-16.58	0.017*	
		2003	1,780	124	6.97, (5.87-8.24)	5.36	1.54-18.63	0.005*	
		2004	3,109	137	4.41, (3.74-5.19)	2.46	0.76-8.06	0.12	
		2005	5,186	117	2.26, (1.89-2.70)	3.41	1.09-10.99	0.027*	
		2006	11,110	88	0.79, (0.64-0.97)	1.59	0.51-4.93	0.14	
		2007	7,420	226	3.05, (2.68-3.46)	3.50	1.13-10.83	0.021*	
		2008	9,668	159	1.64, (1.41-1.92)	2.04	0.66-6.25	0.20	
		2009	6,972	120	1.72, (1.44-2.05)	1.91	0.61-5.89	0.25	
	State	Johor	6,091	48	0.79, (0.59-1.04)	1.00	-	-	
		Kedah	1,203	81	6.73, (5.45-8.29)	4.03	1.73-9.39	0.008*	
		Kelantan	981	4	0.41, (0.16-1.04)	0.23	0.07-0.72	0.007*	
		Melaka	206	8	3.88, (1.98-7.47)	1.10	0.19-6.37	0.90	
		N.Sembilan	7,259	115	1.58, (1.32-1.90)	1.30	0.77-2.19	0.32	
		P. Pinang	535	12	2.24, (1.29-3.88)	0.79	0.26-2.38	0.67	
		Pahang	7,584	334	4.4, (3.96-4.89)	1.68	1.02-2.75	0.037*	
		Perak	10,703	240	2.24, (1.98-2.54)	1.06	0.66-1.70	0.80	
		Perlis	584	31	5.31, (3.76-7.44)	2.21	0.71-6.85	0.15	
		Selangor	12,264	157	1.28, (1.10-1.49)	1.18	0.70-1.97	0.52	
Terengganu	2,396	53	2.17, (1.70-2.88)	1.37	0.69-2.72	0.36			
	Breed	Foreign	1,180	52	4.41, (3.38-5.73)	1.00	-	-	
		Hybrids	20159	399	1.98, (1.80-2.18)	0.90	0.48-1.65	0.73	
		Jamnapari	13,451	154	1.14, (0.98-1.34)	0.71	.37-1.35	0.30	
		Katjang	3,186	80	2.51, (2.02-3.11)	0.46	0.22-0.95	0.03*	
Sheep	Year	2000	1,769	150	8.48, (7.27-9.87)	1.00	-	-	
		2001	3,804	291	7.65, (6.85-8.54)	2.27	0.75-6.82	0.13	
		2002	1,266	227	17.93, (15.92-20.14)	2.65	0.64-11.00	0.16	
		2003	610	158	25.9, (22.58-29.52)	4.15	0.47-36.60	0.17	
		2004	1,094	193	18.1, (15.93-20.49)	0.31	0.11-0.83	0.018*	
		2005	2,009	561	27.92, (26.01-29.93)	1.25	0.42-3.73	0.68	
		2006	3,190	552	17.3, (16.03-18.66)	1.18	0.45-3.11	0.72	
		2007	2,579	718	27.84, (26.14-29.6)	1.61	0.56-4.59	0.36	
		2008	2327	219	9.41, (8.29-10.67)	0.61	0.23-1.59	0.31	
		2009	2,006	214	10.67, (9.39-12.09)	1.09	0.40-2.97	0.85	
	State	Johor	4,962	333	6.71,(6.05-7.44)	1.00	-	-	
		Kedah	1,857	365	19.66, (17.91-21.52)	2.62	0.82-8.33	0.09	
		Kelantan	114	24	21.05, (14.58-29.48)	0.70	0.11-4.10	0.69	
		N.Sembilan	251	17	6.77, (4.27-10.58)	0.61	0.16-2.30	0.46	
		Pahang	1,221	217	17.77,(15.73-20.02)	0.82	0.34-1.95	0.66	
		Perak	1,190	139	11.68, (9.98-13.63)	0.78	0.34-1.77	0.55	
		Selangor	1,392	59	4.24, (3.30-5.44)	0.58	0.22-1.53	0.27	
		Terengganu	9,625	2129	22.12, (21.30-22.96)	1.35	0.73-2.50	0.33	
		Breed	Hybrids	11,802	2356	19.96, (19.25-20.69)	1.00	-	-
			Malin	1,809	331	18.30, (16.58-20.15)	0.53	0.23-1.20	0.12
Swine	State	Penang	17	1	5.88, (1.05-26.98)	-	-	-	

N=Total sampled, +ve=number tested positive, OR=Odds ratio, CI=Confidence Interval, *=significantly different.

Table 2: Seroprevalence of *Burkholderia pseudomallei* in wild and domesticated-wild animals in Peninsular Malaysia between the year 2000 and 2009.

Animals	Variable	Category	N	+ve	Prevalence (95%CI)	OR	95% CI	p-value
Deer	Breed	Jawa	287	13	4.53, (2.59-7.67)	Ref	-	-
		Sika	124	13	10.48, (6.23-17.11)	1.85	0.10-34.43	0.67
		Hybrids	23	2	8.70, (2.42-26.80)	1.07	0.16-7.42	0.94
	State	N. Sembilan	43	2	4.65, (1.28-15.46)	Ref	-	-
		Perak	348	17	4.89, (3.07-7.68)	1.60	0.25-10.13	0.16
		Selangor	43	9	20.93, (11.43-35.21)	0.41	0.28-6.06	0.51
Rabbit	Breed	Hybrids	397	27	6.80, (4.72-9.71)	Ref	-	-
		N. Zealand W	342	7	2.05, (0.99-4.14)	0.44	0.03-5.58	0.52
		Carolina	142	7	4.93, (2.41-9.83)	-	-	-
Monkey	Monkey	Monkey	5	1	20.0, (3.63-62.45)	-	-	-

Ref= Reference group; OR= Odds Ratio; N= Total number sampled; +ve= number tested positive; Prev= prevalence; CI= Confidence Interval.

Table 3: Comparisons of seroprevalence melioidosis in animals according to year of sampling.

Variable	Categories	N	Prevalence % (95%CI)	OR	95% CI	p-value
Sampling year	2000	154	6.11 (5.24-7.11)	Ref	-	-
	2001	377	5.63 (5.10-6.21)	1.14	0.60-2.13	0.67
	2002	277	10.43 (9.32-11.65)	1.30	0.64-2.63	0.45
	2003	303	11.79 (10.6-13.09)	1.29	0.64-2.57	0.46
	2004	339	7.46 (6.73-8.26)	0.52	0.28-0.98	0.042*
	2005	701	8.89 (8.29-9.54)	0.95	0.52-1.74	0.88
	2006	661	4.56 (4.24-4.92)	0.57	0.32-1.02	0.05
	2007	987	9.68 (9.12-10.27)	1.05	0.59-1.88	0.84
	2008	381	3.14 (2.84-3.46)	0.52	0.29-0.93	0.26*
	2009	336	3.64 (3.27-4.04)	0.53	0.30-0.95	0.032*

N= number of animals positive; Prev= Prevalence; OR= Odds Ratio; CI= confidence interval *= significantly different.

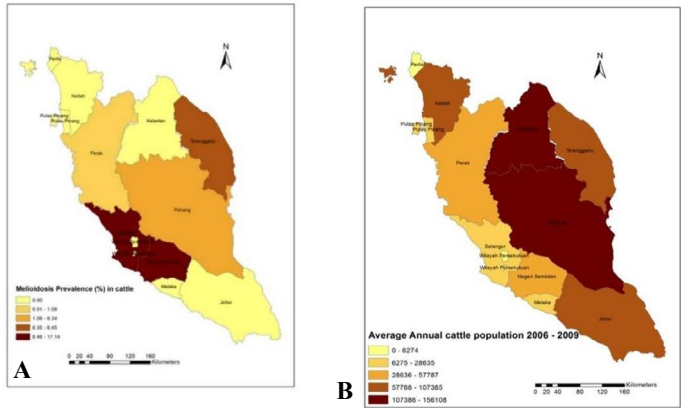


Figure 2: A: Choropleth map of seroprevalence of melioidosis in cattle in Peninsular Malaysia from 2000-2009 while B shows Choropleth map of average annual cattle population in Peninsular Malaysia between 2006 and 2009.

Spatial distribution of melioidosis seroprevalence among livestock in Peninsular Malaysia between 2000 and 2009 The choropleth maps in Figures 1A, B show spatial

distributions of the seroprevalence of melioidosis in animal population and the average annual populations respectively for the study area during the period of study. Figure 2A, B show the seroprevalence of melioidosis in cattle in the study and annual average cattle population in 2006-2009. Figures 3A, B, 4A, B as well as Figure 5A, B show the seroprevalence and average annual populations of goats, sheep and buffaloes respectively during similar periods. There were no apparent patterns that indicated that the seroprevalence rates in animals are influenced by the population size (Figures 1A, B, 2A, B).

CORRELATIONS BETWEEN SEROPREVALENCE OF MELIOIDOSIS IN ANIMALS AND RAINFALL AND WIND SPEED IN PENINSULAR MALAYSIA BETWEEN 2000-2009

Table 4 summarizes the correlations between melioidosis seroprevalence and meteorological elements according to months, year and state in Peninsular Malaysia during the period under review. Figure 6 shows the trends of seroprevalence of melioidosis, average monthly rainfall, average monthly rainy days and wind speed in Peninsular

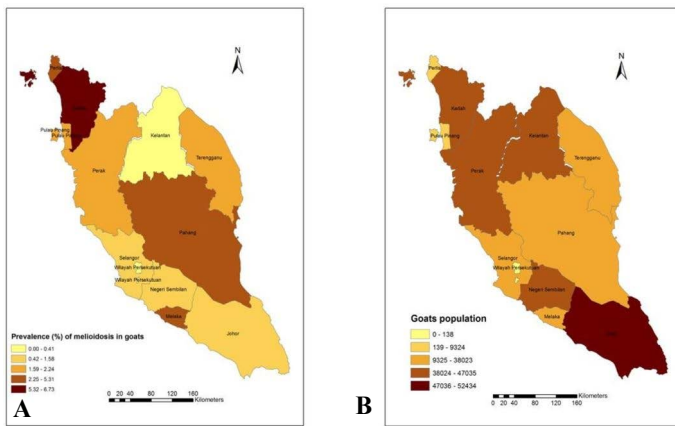


Figure 3: A: Choropleth map of seroprevalence melioidosis in goats in Peninsular Malaysia between 2000 and 2009. B: Choropleth map of annual average goat population in Peninsular Malaysia between 2006 and 2009.

Table 4: Correlations between seroprevalence of melioidosis and meteorological elements according to months, year and state in Peninsular Malaysia between 2000-2009.

Variable	Meteorological element	r	95% CI	p value
Months (January -December)	Rainfall	0.58	0.12-0.87	0.047*
	Number of rainy days	0.47	-0.13-0.82	0.11
	Wind speed	-0.23	-0.71-0.39	0.47
Year (2000-2009)	Rainfall	-0.52	-0.86-0.15	0.11
	Number of rainy days	-0.41	-0.82-0.28	0.23
	Wind speed	0.41	-0.28-0.82	0.23
States (Johor-Terengganu)	Rainfall	0.31	-0.35-0.76	0.35
	Number of rainy days	0.08	-0.53-0.65	0.79
	Wind speed	0.23	-0.43-0.73	0.47

CI= confidence interval; * = significantly different; r = correlation coefficient.

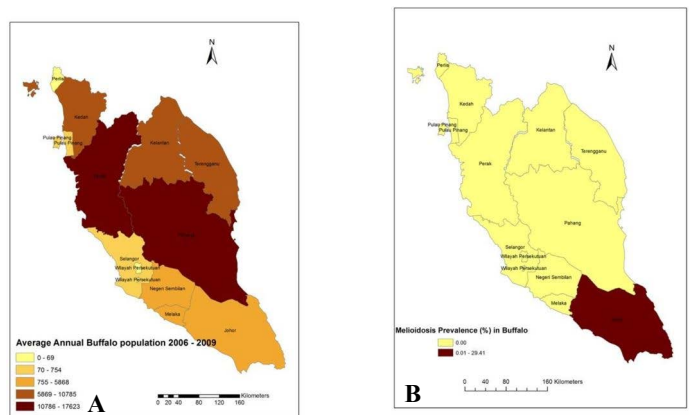


Figure 5: A: Average annual buffalo population in Peninsular Malaysia between 2006 and 2009. B: Choropleth map of seroprevalence of melioidosis in buffalo in Peninsula Malaysia between 2000 and 2009.

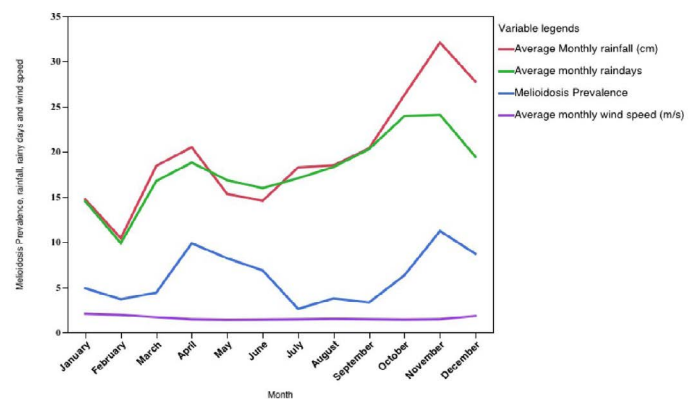


Figure 6: Trends of melioidosis seroprevalence, average monthly rainfall, average monthly rainy days and wind speed according to months in Peninsular Malaysia between 2000-2009.

Malaysia according to months of the years during the study period. Correlation analysis of the seroprevalence and weather elements showed strong, positive and statistically significant correlation ($r = 0.58$, 95%, CI 0.12-0.87, $p = 0.047$) between the prevalence and average monthly rainfall, a moderate but non-significant positive correlation ($r = 0.47$, 95%, CI -0.13-0.82, $p = 0.11$) with average monthly rainy days and weak and non-significant correlation ($r = -0.23$, 95%, CI -0.71-0.39, $p = 0.47$) with average monthly wind speed.

Figure 7 shows the trends of seroprevalence of melioidosis, average monthly rainfall, average number of rainy days and average monthly wind speed according the year in Peninsular Malaysia between 2000-2009. The yearly trends showed moderate, non-significant negative correlation ($r = -0.52$, 95% CI -0.86-0.15, $p = 0.11$) between melioidosis prevalence and average monthly rain and moderate, negative non-significant correlation ($r = -0.41$, 95% CI -0.82-0.28, $p = 0.23$) with the average monthly rain and positive non-significant correlation ($r = 0.41$, 95% CI

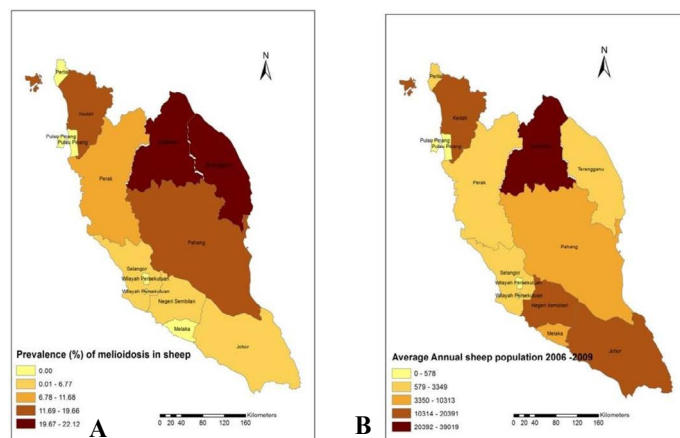


Figure 4: A: Choropleth map of seroprevalence of melioidosis in sheep in Peninsula Malaysia between 2000 and 2009. B: Choropleth map of average annual sheep population in Peninsular Malaysia between 2006 and 2009.

-0.28-0.82, $p=0.23$) with the average monthly wind speed.

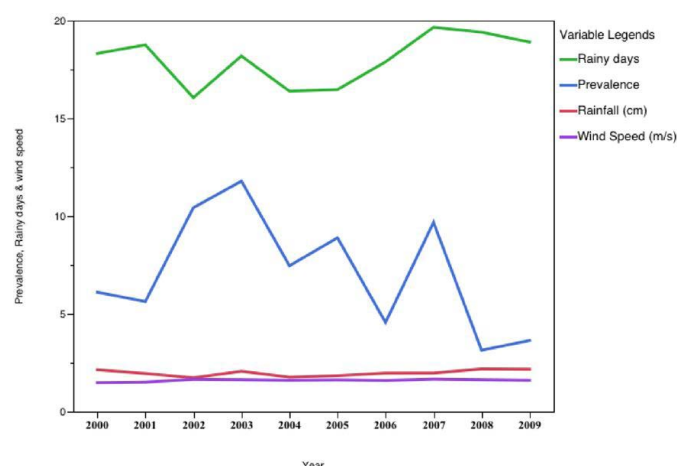


Figure 7: Trends of seroprevalence of melioidosis, average monthly rainfall, average number of rainy days and average monthly wind speed according the year in Peninsular Malaysia between 2000-2009.

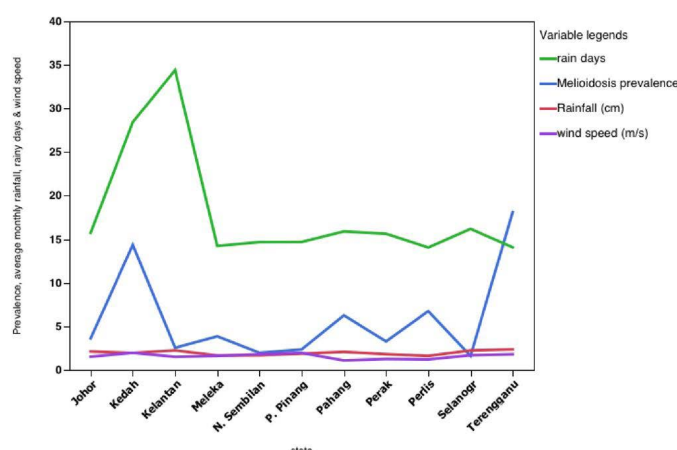


Figure 8: Trends of seroprevalence of melioidosis, average monthly rainfall, average number of rainy days and average monthly wind speed according the states in Peninsular Malaysia between 2000-2009.

Figure 8 shows the relationship between seroprevalence of melioidosis, average monthly rainfall, average number of rainy days and wind speed according to states in Peninsular Malaysia during the period of the study. There was a weak non-significant positive correlation ($r=0.31$, 95% CI -0.35-0.76, $p=0.35$) between seroprevalence and average monthly rainfall, the average monthly rainy days ($r=0.08$, 95% CI -0.53-0.65, $p=0.79$) and the average monthly wind speed ($r=0.23$, 95% CI -0.43-0.73, $p=0.47$).

The nationwide sero-surveillance for melioidosis in animals was observed not to have been carried out evenly across states and among the different animal or livestock species. Evidently, more resources and concentrations were expended on small ruminants particularly goats and sheep. The reasons for this are not clear. However, it is possible that

higher concentration for the disease in these two species was due to the higher probability of clinical manifestation of melioidosis in these species especially in goats that are shown to be highly susceptible in both clinical and experimental settings (Soffler et al., 2014; Tonpitak et al., 2014; Yi et al., 2019). According to Choy et al. (2000) small ruminants are particularly more susceptible to melioidosis and succumbed to the infection more frequently compared to other animal species such cattle, dogs, cats and birds. However, the reasons behind this susceptibility have not yet been understood. Small ruminants are usually kept under extensive management systems thereby exposing them to higher risks of contracting infection from the major reservoirs (soil and water) of the causative organism, *B. pseudomallei* whose presence is in turn influenced by the physicochemical properties of such a reservoir (Manivanh et al., 2017; Musa et al., 2015, 2016, 2018; Pongmala et al., 2022). The reported prevalence of the disease in goats (2.19%) is observed to slightly above the 1.05% reported from goats in southern Thailand (Kongkaew et al., 2017). This suggests that the goats' exposure to the agent is very low in both countries.

Evidence of exposure to the *B. pseudomallei* was observed in most of livestock species in Malaysia regardless of the size of the species population and breed. The overall prevalence of 6.2% in this study is observed to be similar to an earlier reported prevalence of 5.7% for livestock for West and East Malaysia (Musa et al., 2012). This may be due the fact that the data collection for both studies carried around same period. It is evident that some states such Kedah and Pahang have higher seroprevalence compared to others. However, bearing in mind that the data were not consistently recorded, surveillance intensity varies between states and some variables were scanty or missing, the study cannot conclude with confidence that the findings were unbiased and the study free from sampling error.

The apparently low seroprevalence rates observed among pigs and goats may partly be explained by the mainly intensive farm management system practiced for the earlier species in the country where they had less contact with soil and therefore at a lower risk of contracting the disease agent. This is supported by recent observation from a study in Vietnam where grazing pigs were reported to have significantly higher seropositivity compared to farmed pigs (Norris et al., 2020; Trinh et al., 2018). However, only few pigs were tested in our study so the finding may not accurately represent the situation among pig population in the country bearing in mind that pigs have been reported to be highly susceptible to the disease (Trinh et al., 2018) evident from the recent outbreak of the disease in pigs in an endemic area (Kwanhian et al., 2020). On the other hand, the relatively high seroprevalence in buffaloes (28.2%) was observed to lower than the 48.2% reported for

both West and East Malaysia in an earlier study (Musa et al., 2012). The disparity may be as result of sampling error or a disproportionately higher infection rates in the East Malaysia. The apparently high exposure in buffaloes may partly be as a result of the extensive management system in which they had more extensive contacts with contaminated soil and water thereby exposing them to a higher risk of exposure to the disease agent in the environment (Musa et al., 2018; Nathan et al., 2018; Palasatien et al., 2008). In Malaysia, almost all buffaloes are raised extensively. As mentioned earlier, the number of buffaloes tested during the study period is also low and the finding may not accurately represent the situation on ground. An earlier study conducted covering the entire country found a prevalence of 54.0% in goats 37.2% in sheep, 8.1% in cattle and 0.63% in buffaloes (Masrin et al., 2018). This finding may suggest that goats are relatively more susceptible to *B. pseudomallei* infection leading to higher fatality as compared to cattle (Tonpitak et al., 2014). Cattle apparently may be frequently exposed to the organism (leading to higher seroprevalence) but may be less likely to succumb to the infection as compared to goats. The significantly lower likelihood of antibody evidence among the local breed of goats (Katjang) as compared to the foreign breeds could signify some level of resistance towards this endemic agent. The non-detection of antibodies in dogs and horses may suggest low level of exposure of the disease agent in these species. However, this needs to be interpreted with caution bearing in mind that very few animals were tested in this study and fact that clinical disease has been reported in dogs species (Ryan et al., 2018).

Investigation of incidence of melioidosis among animals in neighboring Thailand between the year 2006 and 2010 showed that the incidence was highest among goats (1.63:100,000/year) followed by pigs (0.02:100,000/year) and then in cattle (0.01:100,000/year) (Limmathurtsakul et al., 2012). Another study among dairy cattle in Chiang Mai Province in northern Thailand found a prevalence rate of 2.0% (95% CI, 0.3-3.7%) using IHA at a cut-off point 1:40 (Srikitjakarn et al., 2002). Another study across 18 provinces in Thailand found prevalence rates of 2.56% among cattle, 0.33% in goats, 6.83% in sheep and 7.23% pigs using IHA at a cut-off point of 1:160 (Srikawkheaw and Lawhavinit, 2007). A comparison of the prevalence rates among cattle from Thailand (2.56%) and the present study showed relatively higher rate (6.6%) in Malaysia compared to Thailand. However, this comparison should be interpreted with caution since the serological tests and cut-off points utilized were different. The sera for this study were tested using CFT using a cut-off point of 1:80 while the one from Thailand used IHA at a cut-off point of 1:160. As for the disease in sheep, a prevalence of 10.00% was reported in Sabah (Masrin et al., 2018) which

is observed to slightly below the 16.55% reported in the present study. Investigations of the disease among sheep from small ruminants farms in Selangor state did not detect antibodies in all the 100 sheep serum samples examined (Hambali et al., 2018). The disparity may be accounted for by the differences in the risk of exposure between the two study locations.

The relatively higher level of seroprevalence between 2000-2004 may be as a result increased importation of animals and occurrence of unfavorable weather conditions during the respective years (Sam and Puthucheary, 2007). Malaysia imported large number of live animals from the neighboring countries especially Thailand in the late 1990's so as to enrich the breeding pool of local animals. Previous studies have implicated various environmental factors such as temperature, rainfall, soil type and composition as factors influencing survival of *B. pseudomallei* in the environment (Inglis and Sagripanti, 2006; Palasatien et al., 2008). These factors may partly account for the variations in seroprevalence in space and time observed in this study. The intensity of rainfall during the respective years may also have played some roles since it has been reported to correlate with increase in melioidosis cases in humans here (Birnie et al., 2022; Currie and Jacups, 2003; Liu et al., 2015; Smith et al., 2021). Evidently, the correlation analysis between melioidosis prevalence and weather elements showed only rainfall has a positive, significant correlation with melioidosis prevalence during the study years. This is in agreement with findings from Cambodia and Laos where windspeed, humidity, number of rainy days and low visibility were found to be significantly associated with the number of cases of melioidosis (Bulterys et al., 2018). Similarly, studies in other endemic areas showed strong wind increases the amount of the agent in the atmosphere result in an increased risk of infection (Chen et al., 2014; Currie and Jacups, 2003; Mu et al., 2014). The power of the study may not be large enough to achieve statistical significance in the correlational analysis between the occurrence of the disease and wind speed, number of rainy days and visibility as reported elsewhere (Liu et al., 2015; Smith et al., 2018) which was not included in the present study. However, wind speed has recently been shown to positively correlate with incidence of melioidosis as it was shown to accelerate spread of infection (Zhu et al., 2020). Other factors might have influenced the seropositivity of animals during the study period may be present in the individual farms and may be not be captured in this study.

CONCLUSIONS AND RECOMMENDATIONS

This study highlighted the epidemiological features of melioidosis in animals through the examination of

serological evidence for the presence of the infection among different species of animals in the study area. It also revealed that some meteorological factors correlate with the seroprevalence of melioidosis in animals. Animal melioidosis is widespread within Peninsular Malaysia where the disease appears to occur more in the domestic compared to wild animals. Even though the surveillance and data recording were not consistent between states, the study gave some clues on the distribution and trend of the disease in animals on the peninsula. It is recommended that investigations of the farm environments should be conducted to reveal some the factors influencing distributions of the bacteria in the farm environment and thus the distributions of the disease in the farm animals.

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NOVELTY STATEMENT

This study provides insights by establishing a strong correlation between rainfall and melioidosis seroprevalence in livestock in Peninsular Malaysia, based on large-scale surveillance data. It highlights the role of meteorological factors, particularly rainfall, in influencing disease patterns, offering valuable guidance for future public health strategies and in melioidosis-endemic regions.

AUTHOR'S CONTRIBUTION

HIM and LH conceptualization the research. HIM and CP extracted the data from databases. Data analyses and investigation were carried out by HIM and LH. Writing of the original draft, reviewing, correction and editing were by HIM, LH, ZZ, CP and SAA. Supervision of the research was by LH, ZZ and SAA. Funding acquisition was by LH. All authors read and approved the final manuscript.

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ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This is an observational study. The Universiti Putra Malaysia Animal Use and Ethics Committee has confirmed that no ethical approval is required.

DATA AVAILABILITY

The datasets used and analysed in this study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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