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Occupational Exposure of Animal Handlers to Rodent-borne Pathogens

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Abstract

Objective: With nearly two-thirds of human infectious diseases of zoonotic origin, zoonotic diseases are a major threat to the global health and economy. Rodent-borne pathogens are of great concern as rodents are often found in close proximity to humans. Individuals involved in rodent control activities are likely to face a higher occupational risk of acquiring zoonotic diseases. This study aims to identify occupational risk factors associated with increased exposure to various rodent-borne pathogens.

Methods: A total of 77 volunteers participated in this study and their IgG antibody profile against common pathogens present in the local rodent population such as Hantaviruses, Leptospira and *Rickettsia typhi* were studied.

Results: Generally, seropositivity rates of any disease tested were higher in animal handlers compared to non-animal handlers, and the prevalence of specific IgG among participants that handle animals were 6.8% for Hantavirus, 32.2% for Leptospira and 13.6% for *Rickettsia typhi*. Interestingly, the handling of rodents was associated with a decreased risk of Leptospira infection (OR: 0.09, 95% CI: 0.02, 0.31, p<0.001). Gender was also found to influence the risk of rodent-borne diseases.

Conclusion: Our findings highlight the presence of appropriate personal protective equipment (PPE) could serve as an effective mitigation measure, as well as the importance of accounting for risk factors when designing occupational mitigation measures against such diseases.

Keywords: Animal handlers; Hantavirus; Leptospira; Occupational exposure; *Rickettsia typhi*; Rodents; Serology

Abbreviations: PPE: Personal Protective Equipment; HFRS: Haemorrhagic Fever Renal Syndrome; NEA: National Environment Agency; IFA: Immunofluorescence Assay; FBS: Fetal Bovine Serum; PBS: Phosphate Buffer Solution; ELISA: Enzyme Linked Immunosorbent Assay; MAT: Microscopic Agglutination Test

Introduction

Zoonotic infections represent an important global health and economic burden [1,2]. Globally, it is estimated that 320,000 employees die from occupational infectious diseases, where more than 70% of such infections are zoonotic [1,3]. Individuals with occupations that exposes them to different wild animals are known to be susceptible to zoonotic infections [4]. Since the 19th century, zoonotic pathogens have been found to be associated with occupational risks [5]. Despite the well documentation of zoonotic diseases transmission as an occupational hazard, there are still many reported cases of zoonotic diseases acquired through work [6]. For example, a biologist acquired pneumonic plague and died one week after a post-mortem examination of an infected mountain lion [7]. Workers handling animals such as abattoir workers, pest control and veterinarians have been previously reported to be seropositive against Leptospira, typhus and multiple zoonotic diseases respectively [1,8-10]. Likewise, field workers in the forestry industry developed antibodies against Hantaviruses [11]. These previous studies and surveys conducted reflect the substantial occupational risk of zoonotic infections.

One potential source of zoonotic infections are urban rodents, due to their capability to host a multitude of pathogens [12]. Furthermore, their close proximity to the dense human population contributes to their rising public health concern. A previous study has reported the presence of two genetically different strains of Hantaviruses circulating amongst the rodents of Singapore [13]. Leptospira and *Rickettsia typhi* have also been previously shown to exist in the local rat population [unpublished data]. While there has been no report of Hantavirus infections among humans in Singapore, leptospirosis has been reported in both human and animal cases in Singapore [14].

Hantaviruses

The genus Hantavirus, which belongs to the family Bunyaviridae, typically infects rodents with no apparent disease [15]. Although Hantavirus is known to manifest symptoms when transmitted to humans; most Hantavirus infections are mild and asymptomatic. While aerosol inhalation or contact with the excretions of infected rodents are main modes of transmission, rodent bites and scratches are other possible sources of infection. Occupational risk for Hantavirus infection, such as haemorrhagic fever with renal syndrome (HFRS), is described as a dominant factor. Occupations involving rodent trapping and working in forested areas were reported to be associated with elevated risk of Hantavirus infections [16].

Leptospira

Leptospira, a genus comprising of leptospires, are gram-negative spirochetes bacteria [17] that cause leptospirosis, a re-emerging zoonotic disease. Its transmission is often related with recreational or occupational events [18,19] and flooding [20], and its prevalence

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is often underestimated. A review conducted in 2015 estimated that leptospirosis causes about 1 million cases globally every year, making it one of the leading zoonotic diseases with high morbidity and mortality [21]. Mammalian species, especially rodents, serve as main reservoirs. Humans can be infected upon exposure with contaminated body fluids, either through direct contact or mucosal membranes. Leptospira also resides in environmental soil and water, especially when conditions are made favourable by tropical climates [22].

Rickettsia typhi

Rickettsia species are obligate intracellular gram-negative bacteria that cause arthropod-borne zoonotic infections worldwide. *Rickettsia typhi*, which belongs to the typhus group of Rickettsia, is known as the etiological agent for the endemic murine typhus. Murine typhus has a global distribution with higher incidence rates in areas where rodents are common, because rat species such as *Rattus rattus* and *Rattus norvegicus* are the main reservoirs of *Rickettsia typhi* [23]. Contaminated bites and excretions of the oriental rat flea, *Xenopsylla cheopis*, are responsible for the transmission of the bacteria between rodents and from rodents to humans [24]. Murine typhus cases have been sporadically reported in Singapore over the years, with majority of the cases from migrant workers living in unsanitary conditions [25-27]. With the presence of *Rickettsia typhi* transmission and the rodent reservoirs commonly found in Singapore, there is a risk of murine typhus infection during occupational contact with rats.

The global pest control services industry is estimated to have an increase in annual revenue of $18 billion to $27 billion from 2017 to 2025 [28]. This reflects the growth of pest control industry and the increasing demand of pest control operators worldwide. Despite the occupational risk of zoonotic pathogens to pest control workers, no study has assessed the prevalence of reported zoonotic infections among them in Singapore. In this study, we examined the serology profile against Hantavirus, Leptospira and *Rickettsia typhi* infections in animal handlers of Singapore, with the aim to identify associated risk factors for such zoonotic infections to inform the implementation of appropriate interventions to reduce disease.

Materials and Methods

Ethics statement

This study was reviewed and approved by National Environment Agency's Environmental Health Institute management committee with bioethics consideration (IRB014). A consent form was provided and signed by participants to indicate their voluntary participation in the study before answering the questionnaire and blood collection. All participants' identifiers were removed prior to the processing of blood samples and analysis of the answered questionnaires.

Survey data collection

From July 2015 to March 2016, 77 participants were recruited into our cross-sectional study based on convenience sampling. Participants with occupations commonly known to handle or be in close contact with wild animals were recruited. Participant characteristics such as age, gender, job profession and years of experience, pet ownership and the types of animals they handled during work were collected using a standardized self-administered questionnaire.

Blood collection and processing

Using EDTA tubes (Vacuette), 3 mL of blood from each participant was collected through venepuncture. Tubes were centrifuged at 2500 rpm for 10 minutes at 4°C, before freezing the separated plasma and red blood cells at -80°C.

Detection of α-*Rickettsia typhi* IgG using Immunofluorescence Assay (IFA)

IgG antibodies against *Rickettsia typhi* were identified using in-house IFA slides. Vero cell cultures with Medium 199 (HyClone™) containing 2% fetal bovine serum (FBS) were infected with *Rickettsia typhi* in a biosafety level 3 (BSL3) laboratory. The infected Vero cells were incubated at 35°C with 5% CO_2 and harvested upon observing 80-90% infection. Infected cells were centrifuged at 3,000 rpm for 10 minutes, followed by washing and eventually re-suspended in sterile 1x phosphate buffer solution (PBS). Re-suspended cells were spotted onto each well of the 30 well Teflon coated glass slide (Electron Microscopy Science, Cat No. 63434-02) and left to air dry at room temperature, before fixation through soaking in 80% chilled acetone for 10 minutes. All fixated slides were air dried and kept at -80°C prior to utilization.

Plasma samples were diluted 100x in 5% skim milk (Sigma-Alrich) consisting of 0.1% Tween20 (Sigma-Alrich), and 2µl of diluted plasma was loaded onto their respective well on the previously spotted glass slides, followed by an incubation at 37°C for 30 minutes. The glass slides were washed twice with 1x PBS and left to air dry. Subsequently, another 2 µl of 1:50 anti-human IgG (Chemicon, Cat No. AP316F) diluted in 0.01% Evan's blue was loaded onto the wells and the slides were further incubated for another 15 to 30 minutes at 37°C. After incubation, glass slides were rinsed twice with 1x PBS and air dried in the dark. Lastly, a coverslip was mounted over the glass slides using a fluoroshield mounting medium (Abcam) and viewed under ultraviolet light using a fluorescent microscope (Olympus).

Detection of IgG antibodies against Hantavirus and Leptospira using Enzyme-linked immunosorbent assay (ELISA)

ELISA was used to detect seropositivity towards Hantavirus and Leptospira. Participants' plasma samples were tested according to the instructions of Euroimmun α-Hantavirus pool 1 "Eurasia" ELISA kit (Cat No. EI 278h-9601-1 G) to identify presence of α-hantavirus IgG. Also, SERION ELISA classic Leptospira IgG kit (Cat No. ESR125G) was used to test for Leptospira antibodies, with protein-AG horseradish peroxidase (HRP) and tetramethylbenzidine (TMB) as secondary antibody and substrate substitutes respectively. Plasma samples positive for Leptospira IgG were shipped to Centers for Disease Control and Prevention (CDC) for further serovar identification through microscopic agglutination test (MAT).

Statistical analysis

The outcome measures were dichotomous variables and hence separate logistic regression models were used to examine their respective associations with the independent variables collected in the participant surveys. Odds ratio (OR), 95% confidence intervals and p-values were calculated and presented for each independent variable. A 5% level of significance was established for all statistical tests. All analyses were performed using R software version 3.0.2.

Results

Characteristics of respondents

A total of 77 participants were successfully recruited for this study, with a higher number of male participants (76.6%). The majority of the participants were pest control operators (64.9%), followed by wildlife biologists (24.7%) and researchers (10.4%). Only a subset of each category handles animals. Demographic characteristics of participants are as shown in Table 1. The ages of all study participants range from 21 to 60 years old, with a mean of 33.8 years old. Animal exposure at home was reflected to be lower (n=35/77, 45.5%) as compared to during work (n=59/77, 76.6%). Among the animal handlers in this study, 64.4% of the participants were involved in the handling of rodents during work.

Seropositivity of participants and risk factors towards rodent-borne pathogens

Presence of any previous infection was defined by the detection of IgG in the plasma samples. Among the three occupational groups, wildlife biologists were observed to have the highest seropositivity rates for all three rodent-borne pathogens tested in this study (Table 2). Because only a subset of each occupational group handle animals, we analyse the participants according to animal handling. Participants that handle animals had higher seropositive rates for all three rodent-borne pathogens, when compared with those that do not handle animals (Table 3). Leptospira seropositive rate was the highest for both participants that handle (32.2%) and do not handle animals (22.2%). However, rodent handling at work was associated with decreased odds of Leptospira infections (OR: 0.09, 95% CI: 0.02, 0.31, p<0.001) (Table 4).

Hantavirus seropositive rates for both groups were about the same (Animal handlers (6.8%) and non-animal handlers (5.6%)). Regression analysis showed that male participants were at higher risk of acquiring Hantavirus infections compared to females (Table 4). The same analysis showed that participants who handled animals at work were at higher risk of murine typhus infection (Table 4), with 13.6% seropositive rates for *Rickettsia typhi*. None was detected among non-animal handlers (Table 2).

Serovar identification of Leptospira seropositive samples using MAT

Only two out of the 23 Leptospira seropositive plasma samples have their serovar identified *via* MAT, due to loss of sample integrity during shipping. They were identified as L. interrogans serovar Bataviae Van Tienen and L. interrogans serovar Djasiman.

Discussion

This study aimed to compare the exposure of Hantavirus, Leptospira and *Rickettsia typhi* infections among three occupational groups that are typically exposed to animals. It is of no surprise that animal handling gave rise to higher seropositive rates for all three rodent-borne pathogens, as handling of animals potentially brings an animal worker in close proximity to an infected animal, including those harboring an infected flea. However, the finding of decreased risk of leptospiral infections with rodent handling was unexpected, as people handling rodents, or working in rodent urine contaminated

Characteristics	Animal handlers (*n*= 59)	Non-animal handlers (*n*=18)	Total (*n*=77)
Age			
21-30	28 (47.5%)	4 (22.2%)	32 (41.5%)
31-40	20 (33.9%)	9 (50.0%)	29 (37.7%)
41-50	9 (15.2%)	4 (22.2)	13 (16.9%)
>50	2 (3.4%)	1 (5.6%)	3 (3.9%)
Gender			
Male	48 (81.4%)	11 (61.1%)	59 (76.6%)
Occupation			
Pest control operators	43 (72.9%)	7 (38.9%)	50 (64.9%)
Wildlife biologists	14 (23.7%)	5 (27.8%)	19 (24.7%)
Researchers	2 (3.4%)	6 (33.3%)	8 (10.4%)
Pets			
Yes	30 (50.8%)	5 (27.8%)	35 (45.5%)
Handling of rodents			
Yes	38 (64.4%)	-	-

Table 1: Demographic information of study participants (n=77).

Serology	Pest control operators (*n*=50)		Wildlife biologists (*n*=19)		Researchers (*n*=8)	
	n_{pos}	%	n_{pos}	%	n_{pos}	%
Hantavirus	3	6	2	10.5	0	0
Leptospira	8	16	13	68.4	2	25
Rickettsia typhi	4	8	4	21.1	0	0

Table 2: Serology results of study participants across various occupational groups.

Serology	*Hantavirus*		*Leptospira*		*Rickettsia typhi*	
	Animal handlers	Non-animal handlers	Animal handlers	Non-animal handlers	Animal handlers	Non-animal handlers
Pest control officers (*n*=50)	3	0	7	1	4	0
Wildlife biologists (*n*=19)	1	1	11	2	4	0
Researchers (*n*=8)	0	0	1	1	0	0
Total	4 (6.8%)	1 (5.6%)	19 (32.2%)	4 (22.2%)	8 (13.6%)	0 (0.0%)

Table 3: Distribution of seropositive plasma samples between animal handlers and non-animal handlers in different occupational groups.

Physical Parameters	*Hantavirus*			*Leptospira*			*Rickettsia typhi*		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Age									
<30 years	Reference			Reference			Reference		
31 to 40 years	0.72	(0.11, 4.62)	0.725	0.56	(0.19, 1.64)	0.287	0.81	(0.16, 3.96)	0.792
41 to 50 years	0	0	0	0.27	(0.05, 1.40)	0.118	0.58	(0.06,5.78)	0.645
Gender									
Female	Reference			Reference			Reference		
Male	∞	0	0	0.31	(0.10, 0.94)	0.038	0.46	(0.10,2.16)	0.327
Pets									
No	Reference			Reference			Reference		
Yes	0.79	(0.12, 5.00)	0.8	1.89	(0.70, 5.07)	0.206	0.36	(0.07,1.93)	0.235
Animal handling at work									
No	Reference			Reference			Reference		
Yes	1.24	(0.13, 11.82)	0.854	1.66	(0.48, 5.73)	0.421	∞	0	0
Rodent handling at work									
No	Reference			Reference			Reference		
Yes	1.8	(0.18, 18.44)	0.621	0.09	(0.02, 0.31)	<0.001*	0.29	(0.06, 1.37)	0.118

Table 4: Odds Ratios (OR), 95% confidence intervals (CI) and p-values of exposure and development of IgG antibody to selected rodent-borne.

environment are perceived to have a higher risk of leptospiral infection [29,30]. The decreased risk among these pest control workers, as compared to the others who are largely wild life biologists, could be attributed to the general opinion of rodents being filthy, and thus the awareness and compliance to basic personal protection equipment (PPE) such as gloves and boots. Basic PPE are able to protect against any entry of pathogens through exposure of mucosal membranes or open wounds to contaminated fluid - the main mode of transmission for Leptospira. Such an awareness of risk for wildlife contact may not be as high. This is supported by another observation from this study that wildlife biologists had the highest rate of seropositivity against all three rodent-borne pathogens, higher than that of pest control operators.

Though the seroprevalence of Hantavirus is low at 5.6-6.8%, we observed that those who were seropositive against Hantavirus were either pest control operators or wildlife biologists. Although animal handling was not identified as a risk factor for Hantavirus infections, gender was associated with an increased risk, as only male participants in this study were seropositive against Hantavirus. This is consistent with reports indicating men being overrepresented in Hantavirus infections studies [31]. However, in this study, this could be due to the larger number of male participants (76.6%), skewing the positive results towards the male group.

The presence of rodent-borne infection risks indicates the importance of proper mitigation measures. Workers can greatly benefit from educational programmes that provide more information about the various pathogens they are potentially exposed to and also their route of transmission [6]. Rodent borne disease training and education sessions for wildlife biologists should be further reinforced and based on the requirements of an individual due to their professional diversity. Generally, education and training sessions should also be readily available in multiple platforms so as to provide convenience for animal handlers who are usually working outfield.

Increased awareness regarding the route of transmission for potential infections would facilitate the selection of proper PPE for maximum protection. PPE should be readily available and regulations should be in place to ensure worker compliance, since they serve as an effective protective barrier as shown in this study. The use of PPE could be further encouraged by supervisors as this has been shown to increase PPE compliance in workers [32-35].

Application of repellents is one crucial PPE which is often disregarded [36]. Although effective, exposure to such chemicals is another potential occupational health hazard as certain active ingredients are reported to be associated with several health implications [37]. Due to job requirements, animal handlers would have to put on such repellents for extended periods of time, especially with the current increasing rodent sightings. Consequently, the health effects of repellents may contribute to delayed recovery times when integrated with rodent-borne pathogen infections.

With animal handlers' increased risk of exposure to such rodent-borne pathogens and chemicals, healthcare systems should be aware and prompt in the detection and treatment of such health implications. Companies are also advised to provide at risk workers with annual health checks and insurance so as to safeguard the welfare of their workers.

This is a first study to identify the risk of exposure to rodent-borne pathogen infections in Singapore contributed by animal handling. One limitation of this study is that this study relies on convenience sampling which may not be representative of the entire population of occupational workers. Furthermore, the use of self-administered questionnaires could possibly introduce respondent bias into the study [38,39].

Conclusion

The findings of our study show that Hantavirus, Leptospira and *Rickettsia typhi* infections are potential occupational infections for workers handling pest or wildlife animals. It highlights the need for general education and PPE to protect workers from the potential exposure.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

KLYM led the writing of the manuscript, conducted the experiments and interpreted the analysed data. GYSL conceptualized the study and designed the survey. YPSG, LXF, DM and MH designed the survey and conducted the experiments. NLC provided feedback and revised the drafts of the article. All authors critically revised the article for important intellectual content and approved all components of the final draft.

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