Antibodies to *Leptospira* among blood donors in higher-risk areas of Australia: possible implications for transfusion safety

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Background. *Leptospirosis* is one of the most common bacterial zoonoses worldwide, and clinical manifestations range from asymptomatic infection to acute febrile illness, multi-organ failure and death. Asymptomatic, acute bacteraemia in a blood donor provides a potential for transfusion-transmission, although only a single such case from India has been recorded. Human leptospirosis is uncommon in developed countries; however, the state of Queensland in Australia has one of the highest rates among developed countries, especially after increased rainfall. This study examined the prevalence of antibodies to *Leptospira spp.* in blood donors residing in higher-risk areas of Australia, to evaluate the appropriateness of current blood safety guidelines.

Materials and methods. Plasma samples collected from blood donors residing in higher-risk areas of Australia during 2009 and 2011 were included in the study. All samples were tested for the presence of antibodies to 22 leptospiral serovars using the microscopic agglutination test.

Result. No sample had antibody titres suggestive of a current or recent infection, however, seven samples (1.44%, 95% CI: 0.38-2.50%) had titres suggestive of a past infection.

Discussion. This study provides data that may support the appropriateness of current relevant donor selection policies in Australia. Given that the risk profile for leptospirosis is expanding and that the infection is likely to become more prevalent with climate change, this disease may become more of a concern for transfusion safety in the future.

Keywords: emerging pathogen, climate, rainfall.

Introduction

Leptospirosis is one of the most common bacterial zoonoses worldwide; its incidence and outbreak frequency are likely to increase with the greater occurrence of extreme weather events associated with climate change[1]. In developed countries, the risk profile for infection is changing. Traditionally, infection was associated with occupational exposure (farming and livestock industries), but in recent years, international travel and recreational activities (swimming, canoeing or caving) have become increasingly important sources of exposure to infection[2].

The causative agents are spirochaetes of the genus *Leptospira*, of which there are 20 different species and over 200 serovars, many of which can be pathogenic to humans[3]. A range of mammals such as rodents, livestock and domestic pets can act as reservoir hosts and humans may become infected through direct contact or through an environment contaminated by animal urine[3]. Infection can be asymptomatic or present as a mild influenza-like illness that resolves spontaneously[3]. Common symptoms include fever, chills, headache, myalgia, conjunctival suffusion and jaundice; however, none of the presenting features is specific for *leptospirosis*[3]. Clinically the disease is often poorly recognised and is commonly misdiagnosed because symptoms and signs are often similar to those of other febrile illnesses, such as the tropical infections dengue and malaria[2,3]. A more serious illness (known as Weil's disease), manifested by multi-organ failure, may develop in some individuals[3].

Leptospirosis is found throughout the world, but is more common in tropical and subtropical areas such as India, Thailand, Vietnam, the Seychelles, and various Pacific Islands[4-6]. The global burden of *leptospirosis*

is not accurately known. Annual incidence estimates range from 0.1-1 (temperate areas) to >100 (tropical areas during epidemics) cases per 100,000[3]. In Australia, *leptospirosis* is a nationally notifiable disease. The north-eastern state of Queensland, spanning both the tropics and sub-tropics, accounts for over half of all notifications and has one of the highest rates among developed countries[3,7].

Heavy rainfall and flooding increases the risk of *leptospirosis* by bringing bacteria and their animal hosts into closer contact with humans[1]. Numerous outbreaks have been reported after flooding in various countries, including Indonesia, Italy and the United States of America (USA)[4,8,9]. In northern Queensland most cases occur during the warm and wet summer months, and outbreaks have occurred after flooding[7]. Much of Queensland experienced extensive rainfall and flooding in the summer of 2010/2011, and a cluster of patients with *leptospirosis* was reported following exposure to floodwater in Central Queensland in early 2011[10,11].

Many emerging pathogens pose a potential risk to transfusion safety[12-14]. While transmission of *leptospirosis* via a transfusion is possible due to asymptomatic leptospiraemia[3,15], only a single case, in India, has been reported[16]. The rarity of such cases could imply that transfusion is not a major route of transmission. Nevertheless, the majority of transfusion recipients are immunocompromised, which may leave them more vulnerable to developing severe disease from potential transfusion-transmitted *leptospirosis*. The management of the risk of transfusion-transmitted *leptospirosis* in Australia includes deferral from donation for 3 months following recovery from infection, or in the case of potential occupational exposure, fresh product restrictions while working in an abattoir and for 12 months afterwards. The Australian Red Cross Blood Service (Blood Service) also routinely screens all platelet products with the BacT/ALERT blood culture system to detect products contaminated with bacteria. This system can support viable leptospires[17], but can be associated with high false negative rates due to the high sampling error that is associated with the test[18]. Given that asymptomatic leptospiraemia can occur[3] and that leptospires may not always be detected by routine bacterial culturing[18], it is possible that this bacterium could pose a risk to transfusion safety, especially in higher-risk regions following extreme flooding.

There are no published human sero-epidemiological studies of *leptospirosis* in Australia. This study therefore examined *leptospirosis* seroprevalence rates among blood donors in areas of Queensland where higher numbers of cases are reported, which allowed for an evaluation of the appropriateness of current Blood Service guidelines for the management of *leptospirosis*.

Materials and methods

Sample collection

Whole blood samples collected in 2009 and 2011 from donors in areas of Queensland in which public health notifications of *leptospirosis* were increased were made available for this study. Areas targeted for the study included: Brisbane, Cairns, Ingham, Innisfail, Mareeba, Townsville and Tully[19]. These samples represent a "convenience sample", with a total of 485 from individual donors included in the study, with 274 collected during 2009 (January-October) and 211 collected during 2011 (February-July). Samples were collected into Plasma Preparation Tubes (BD Vacutainer PPT tubes®, BD®, Franklin Lakes, NJ, USA) centrifuged and stored at −20 °C. Demographic data were obtained for all donations. All samples were then de-identified prior to testing. This study was carried out under approval from the Blood Service Human Research Ethics Committee.

Antibody detection

All samples (n=485) were tested for the presence of anti-*Leptospira* antibodies by the microscopic agglutination test[3] at the WHO/FAO/OIE Collaborating Centre for Reference and Research on *leptospirosis* at Coopers Plains, Queensland. Samples were tested against 22 serovars that the laboratory routinely uses for Australian samples (Table I), starting at a dilution of 1:50. Following an approach similar to that described by Lau *et al.*[6], a titre of 1:400 or higher was used to define current or recent infection, while titres equal to or greater than 1:50 but less than 1:400 were taken to indicate a past infection.

Statistical analysis

The proportion of donations/donors with antibody present was determined and a 95% confidence interval (CI) calculated. Logistic regression modelling was used to assess the relationship between gender, age, location and year with the presence of anti-*Leptospira* antibodies. The antibody response (reactive or non-reactive) was the dependent variable, with gender, location, year and age group as factors. The Statistical Package for the Social Sciences (SPSS; IMB Australia Ltd., St. Leonards, Australia) was used for analyses. P values of 0.05 or less were considered statistically significant.

Results

A total of 485 specimens were included in the study: 274 collected in 2009 and 211 in 2011 (Table II). Overall, 52% of samples were from male donors, whose median age was 51 years (interquartile range, 39-58 years).

Of the 485 plasma samples tested, none had antibody titres suggestive of a current/recent infection. However, seven donors (1.44%, 95% CI: 0.38-2.50%) had titres

Table I - Organisms used in the microscopic agglutination test.

Genus	Species	Serogroup	Serovar	Strain
Leptospira	*interrogans*	Pomona	Pomona	Pomona
Leptospira	*interrogans*	Sejroe	Hardjo	Hardjoprajitno
Leptospira	*borgpetersenii*	Tarassovi	Tarassovi	Perepelitsin
Leptospira	*kirschneri*	Grippotyphosa	Grippotyphosa	Moskva V
Leptospira	*weilii*	Celledoni	Celledoni	Celledoni
Leptospira	*interrogans*	Icterohaemorrhagiae	Copenhageni	M20
Leptospira	*interrogans*	Australis	Australis	Ballico
Leptospira	*interrogans*	Pyrogenes	Zanoni	Zanoni
Leptospira	*interrogans*	Pyrogenes	Robinsoni	Robinson
Leptospira	*interrogans*	Canicola	Canicola,	Hond Utrecht IV
Leptospira	*interrogans*	Hebdomadis	Kremastos	Kremastos
Leptospira	*interrogans*	Mini	Szwajizak	Szwajizak
Leptospira	*interrogans*	Sejroe	Medanensis	Hond HC
Leptospira	*kirschneri*	Autumnalis	Bulgarica	Nicolaevo
Leptospira	*kirschneri*	Cynopteri	Cynopteri	3522C
Leptospira	*borgpetersenii*	Ballum	Arborea	Arborea
Leptospira	*interrogans*	Bataviae	Bataviae	Swart
Leptospira	*interrogans*	Djasiman	Djasiman	Djasiman
Leptospira	*borgpetersenii*	Javanica	Javanica	Veldrat Batavia 46
Leptospira	*noguchii*	Panama	Panama	CZ214
Leptospira	*santarosai*	Shermani	Shermani	1342K
Leptospira	*weilii*	Tarassovi	Topaz	94-79940/3

Table II - Characteristics of the study population.

Collection site and date	Number of samples	Age group (years)						Male (%)
		>24	25-34	35-44	45-54	55-64	>65	
2009								
Brisbane	40	0	0	1	18	13	8	75%
Cairns	69	3	10	15	18	14	9	57%
Ingham	19	7	4	0	6	1	1	37%
Innisfail	39	8	2	8	7	10	4	54%
Mareeba	50	3	3	3	14	16	11	30%
Townsville	42	8	7	7	12	7	1	52%
Tully	15	4	0	3	0	7	1	73%
Total	***274***	***33***	***26***	***37***	***75***	***68***	***35***	***53%***
2011								
Brisbane	39	4	3	11	10	9	2	31%
Cairns	36	3	7	5	13	5	3	61%
Ingham	76	6	4	10	21	24	11	53%
Innisfail	18	1	5	2	3	5	2	39%
Mareeba	11	0	1	1	4	2	3	64%
Townsville	30	6	2	2	3	6	11	57%
Tully	1	0	0	0	0	1	0	0%
Total	***211***	***20***	***22***	***31***	***54***	***52***	***32***	***50%***
Overall Total	**485**	**53**	**48**	**68**	**129**	**120**	**67**	**52%**

suggestive of past infection, three of which were collected in 2009 with the remaining four were collected during 2011 (Table III). The average age of donors with past exposure to *Leptospira spp.* was 45 years (range, 22-67 years) and four of the donors were male (Table IV). Three of the donors with serological evidence of past infection were from Ingham, two were from Brisbane, and the remaining two were from Mareeba and Innisfail. There was no significant effect ($p>0.05$) of age, gender, region or year on rates of past infection.

Table III - Serological evidence of previous exposure to *Leptospira spp.* in Queensland blood donors.

Year	Number tested	Number with titre >1:50	Proportion reactive	
			%	95% CI
2009	274	3	1.09	0.00-2.33
2011	211	4	1.90	0.06-3.74
Total	**485**	**7**	**1.44**	**0.38-2.50**

Table IV - Characteristics of donors with previous exposure to *Leptospira spp.*

Sample	Age	Sex	Year collected	City/ town	Serovar
1	69	M	2009	Ingham	Canicola
2	55	F	2009	Mareeba	Javanica
3	32	M	2009	Innisfail	Arborea, Canicola
4	47	F	2011	Brisbane	Copenhageni
5	22	F	2011	Brisbane	Canicola
6	33	M	2011	Ingham	Grippotyphosa
7	55	M	2011	Ingham	Hardjo, Medanensis

Discussion

We found no evidence of recent exposure to *Leptospira spp.* in a convenience sample of Queensland blood donors residing in higher-risk areas. However, in this same group, we did find serological evidence of past exposure to *Leptospira spp.* These findings provide support to the appropriateness and effectiveness of current relevant Australian donor selection policies and suggest that, even in areas with a relatively high incidence of *leptospirosis*, this bacterium does not currently seem to be a major concern for blood services in the developed world. Climate changes are expected to result in an increased frequency of extreme weather events including rainfall, flooding and high temperatures, all of which can increase *Leptospira* transmission[1]. This disease, as well as other transfusion-transmissible infections that are predicted to increase with climate changes[13], may become more of a concern for transfusion safety in the future, especially in regions with a high incidence of infection. Additional localised flood-related restrictions, particularly after extreme rainfall in high-risk areas and/or during outbreaks, may be warranted in the future.

Our study demonstrates that the rate of past *Leptospira spp.* infection in areas of Queensland with increased notification numbers was 1.44%. We did not see a significant difference in past exposures between 2009 and 2011, despite there being high levels of rainfall and flooding across much of Queensland at the end of 2010 and early 2011[10,11]. It is possible that small changes may have occurred and been missed, which would be difficult to detect through such studies. In addition, we used a blood donor population as a proxy for the general population, and this donor population may not have been representative of the general population, or the population at high risk of *leptospirosis*.

This study has a number of limitations. First, the results are based on testing against 22 serovars that were deemed the most appropriate for Australia. Although not likely, it is possible that individuals could have been exposed to other serovars. Second, testing was performed on small numbers of samples; although not possible in this study due to clinical testing requirements during the study period, increasing the number of samples would have improved the precision of our estimates.

Conclusion

The risk profile for *leptospirosis* in developed countries has expanded to include recreational exposures. For example, *leptospirosis* has become associated with adventurous outdoor activities, including ecotourism, rafting, kayaking, canoeing, caving and trail biking, especially in remote areas[20]. Indeed, outbreaks of *leptopsirosis* have occurred during international outdoor adventure events around the world[20-22]. This highlights the importance for the international transfusion medicine community to continually revise risk profiles for relevant diseases, and update risk modelling accordingly. Globally, high-risk areas are concentrated in developing countries where there might be less stringent screening of blood donors/samples, and risk assessment of transfusion-acquired *leptospirosis* in these areas is therefore warranted. It is important for the international transfusion medicine community to be aware of the changing epidemiology of *leptospirosis*, and update risk models and guidelines accordingly.

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Authorship contribution

Helen Faddy, Clive Seed, Colleen Lau, Robert Flower, Lee Smythe and Philip Weinstein designed the study; Mary-Anne Burns, Michael Dohnt and Scott Craig performed the experiments; Helen Faddy, Colleen Lau and Vanessa Racloz performed analyses;

Lee Smythe, Mary-Anne Burns, Michael Dohnt and Scott Craig contributed reagents/materials/analysis tools; Helen Faddy prepared the manuscript; all authors contributed to conceiving the project, interpreting results and manuscript development and review.

The Authors declare no conflicts of interest.

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