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SHORT PAPER

Serological titres to *Leptospira fainei* serovar hurstbridge in human sera in Australia

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SUMMARY

A set of 723 diagnostic sera from human patients, submitted for the microscopic agglutination test (MAT) for antibodies to a group of 6 leptospiral serovars, was also tested by MAT for antibodies to the recently-discovered *Leptospira fainei* serovar hurstbridge. MAT titres of $\geqslant 128$ to serovar hurstbridge were detected in 13·4% of these sera, and titres of $\geqslant 512$ in 7·2%. In contrast, none of 62 sera obtained from a control population of laboratory staff gave titres of $\geqslant 128$. The difference between the number of titres of $\geqslant 128$ given by the two groups of sera was highly significant ($P < 0{\cdot}01$). The titres observed may have been due to cross-reactions with other leptospiral serovars, but this could not be demonstrated. An alternative explanation is that serovar hurstbridge is present in the human population.

Human leptospirosis in its milder forms may present with symptoms that resemble those of severe influenza. In severe or untreated cases it can lead to complications or to prolonged disability [1]. It is almost invariably either a zoonosis, acquired occupationally by contact with infected livestock, or an environmental disease contracted from contaminated water. In southern Australia most human leptospirosis is found in dairy farmers, exposed in the milking shed to the urine of infected cows, and in abattoir workers in contact with slaughtered pigs. *Leptospira borgpetersenii* serovar hardjobovis, derived from cattle, is currently recognized as the most common agent of human leptospirosis in Australia. *Leptospira fainei* serovar hurstbridge was isolated from culled sows in Australia in 1994, and represents both a new species and a previously unknown serogroup (Hurstbridge) [2, 3]. Since then, antibodies to serovar hurstbridge have been shown to be widespread in Australian pigs and cattle [2, 4].

A sample of 723 sera submitted to Monash University for diagnostic leptospirosis serology was also tested by MAT for antibodies to serovar hurstbridge. These sera were received between 1993 and 1996, predominantly 1995–6, and 568 (79%) were from males. Most were thought to have come from patients with symptoms consistent with leptospirosis, but frequently little or no clinical history accompanied serum samples.

The MAT [5, 6] as used at Monash University included the following 6 serovars as antigens: *L. borgpetersenii* serovars ballum (strain Su73/11), hardjobovis, (strain 08/1) and tarassovi (strain Perepelicin), *L. interrogans* serovars australis (strain Ballico), copenhageni (strain H45), and pomona (strain MP1). Agglutination was detected microscopically after transferring a loop of suspension from each

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Table 1. *MAT titres to serovar hurstbridge in diagnostic sera submitted for leptospirosis testing, compared with a control population*

MAT titre (serovar hurstbridge)	Test group (Diagnostic sera)			Control group (VIAS staff)		
	Male	Female	Total	Male	Female	Total
< 32	393	116	509	28	34	61
32	55	13	68	0	1	1
64	37	12	49	0	0	0
128–256	38	7	45	0	0	0
⩾ 512	45	7	52	0	0	0
Total	568	155	723	28	35	62

well of a microtitre plate onto a microscope slide. Titres were expressed as the reciprocal of the final serum dilution (including the volume of antigen) at which agglutination of 50% or more was seen. The first serum dilution in the dilution series was 1/50. Rabbit antiserum to each serovar tested was included on each microtitre plate as a positive control. The MAT for serovar hurstbridge (strain WKID) was performed at the Victorian Institute of Animal Science (VIAS) in a similar manner. However, the starting dilution used was 1/32 and agglutination was assessed in the well of the microtitre tray using a dark-field microscope.

A control group of sera from 62 staff at VIAS was also tested by the MAT for serovar hurstbridge antibodies. With permission of the subjects these sera were obtained from a bank of samples collected over many years. The 62 sera came from 27 males and 35 females, bled between 1975 and 1995 (predominantly 1991 and 1994–5).

MAT titres to serovar hurstbridge in the two groups of sera are shown in Table 1. Of 723 diagnostic sera, 7·2% had titres of ⩾ 512 and 13·4% had titres of ⩾ 128. In contrast, all 62 sera in the control group (VIAS staff) had titres of 32 or less. The difference in titres of ⩾ 128 between the groups was highly significant ($\chi^2 = 9{\cdot}55$; 1 d.f.; $P < 0{\cdot}01$). Analysis of source postal area codes for the diagnostic sera showed that patients with titres to serovar hurstbridge came predominantly from dairying and pig-producing areas of Victoria.

The prevalence of high titres to each serovar in the diagnostic sera is shown in Table 2. About 7% of sera gave titres of ⩾ 400 to serovar hardjobovis, and a similar percentage gave titres of ⩾ 512 to serovar hurstbridge, whereas there were far fewer titres of ⩾ 400 to the other serovars. The results for serovars hurstbridge and hardjobovis should be compared with caution, as testing was done in different laboratories with slightly different methods.

Table 2. *MAT titres of ⩾ 400 to different leptospiral serovars in 723 sera submitted for leptospirosis testing*

Serovar	No. (%) sera with titres ⩾ 400
australis	1 (0·1)
ballum	2 (0·3)
copenhageni	1 (0·1)
hardjobovis	49 (6·8)
hurstbridge (⩾ 512)	52 (7·2)
pomona	0 (0)
tarassovi	3 (0·4)

Table 3. *Relationship between high MAT titres to leptospiral serovars hurstbridge and hardjobovis in 723 sera submitted for leptospirosis testing*

MAT titre, hurstbridge	MAT titre, hardjobovis	
	< 400	⩾ 400
< 512	650	21
⩾ 512	24	28

There was a tendency for sera with high MAT titres to one serovar to have titres to one or more of the other serovars. Table 3 illustrates an association between high MAT titres to serovars hardjobovis and hurstbridge, suggesting that some observed titres to serovar hurstbridge could be cross-reactions, attributable to serovar hardjobovis. Conversely, 20 titres of ⩾ 128 to serovar hurstbridge were obtained in sera that were negative (< 50) to all other serovars. In some cases, in the absence of comparable titres to

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Table 4. *Patients with high titres to leptospiral serovar hurstbridge and with clinical histories suggesting possible leptospirosis*

Age of patients	Clinical history	MAT titre hurstbridge	MAT titres other serovars*
28	Possible recent leptospirosis	4096	hardjobovis 100
46	Possible leptospirosis	1024	All < 50
43	Illness resembling influenza	512	tarassovi 50
21	Abattoir worker, fever (8 weeks), headache, myalgia	1024	All < 50

* The MAT was performed using serovars australis, ballum, copenhageni, hardjobovis, pomona and tarassovi as antigens. Titres to all serovars not listed in this column were < 50.

other serovars, high titres to serovar hurstbridge were anecdotally associated with symptoms consistent with leptospirosis. Examples are shown in Table 4.

Cross-reactions between serovars in the MAT are well recognized in human leptospirosis [7] and associations such as those illustrated in Table 3 are not unexpected. However, it is possible for individuals who are at occupational risk, such as dairy farmers, to be infected simultaneously or at different times with 2 different serovars.

There are at least 2 possible explanations for the high prevalence of high titres to serovar hurstbridge in the diagnostic sera. High titres to serovar hurstbridge may have been due to cross-reaction with one or more other leptospiral serovars. Our results do not support this explanation, despite the association between high titres to serovars hurstbridge and hardjobovis (Table 3). Nevertheless, cross-reaction as an explanation for high titres to serovar hurstbridge cannot be excluded. Paradoxical MAT reactions to other serovars have been recorded in some human patients who were serologically negative to the infecting serovar [7]. Furthermore, titres to serovar hurstbridge might be caused by another leptospiral serovar that is not currently recognized in Australia.

A second possibility is that serovar hurstbridge is present in the human population in Australia, perhaps at levels comparable to those of serovar hardjobovis. The diagnostic sera tested for serovar hurstbridge in this study came predominantly from dairying and pig producing areas of Victoria, and there is serological evidence that both cattle and pigs are infected [2].

A prospective controlled study of serovar hurstbridge as a possible human pathogen is clearly warranted.

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