

**Prevalence, risk factors and molecular epidemiology
of *Brachyspira pilosicoli* in humans and animals**

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ABSTRACT

The work described in this thesis was concerned with identifying the prevalence and risk factors associated with colonisation by the intestinal spirochaete *Brachyspira pilosicoli* in:

humans: long term residents of Perth, Western Australia (WA) and Indonesians either living temporarily in Perth or as long term residents in urban and rural areas of Bali, Indonesia,

animals: domestic animals including alpacas, birds, cattle, cats, chickens, dogs, doves, ducks, goats, horses, pigs, and sheep (housed at a wide variety of places around Perth), and a range of wild animals housed in various Zoos and wildlife centres in WA.

This study shows that for humans:

- *Brachyspira pilosicoli* was significantly more prevalent in Indonesians of all sub groups, be they temporary residents of Perth (9.4% - 216 faecal samples from 180 individuals), or long term residents of Indonesia (12.6% - 992 faecal samples from 617 individuals) compared with long term residents of Australia living mainly in Perth (0.2% of 766 sampled), even in those with gastrointestinal complaints. This suggests a relationship between a high prevalence of *B. pilosicoli* and living in Indonesia;
- In Bali, *B. pilosicoli* was significantly more prevalent in the impoverished urban area of Sesetan (20.3-23.4%) where the husbandry of pigs is poor and effluent treatment is non-existent compared to four traditional farming villages (Badung, Karang Suwung, Melinggih, Payangan Desa) (3.3-22.6%). In the latter villages

effluent and drainage is better and there is less likely to be contamination of drinking water

- There was no significant association between the presence of *B. pilosicoli* and the presence of clinical symptoms including headaches, abdominal pains, diarrhoea, joint/muscular pain and constipation.
- Amongst Indonesians living in Indonesia, there was no significant difference in the prevalence of *B. pilosicoli* between people with and without contact with animals and between farmers and other occupational groups.
- Indonesians visiting Perth who were positive for *B. pilosicoli* originated from nine cities and five main islands in Indonesia. This suggests that *B. pilosicoli* is endemic throughout Indonesia.
- Strain typing of isolates of *B. pilosicoli* showed that they were genetically heterogenous and did not show any consistent pattern with respect to geographical location, family of origin or disease status. Isolates from the same individual were sometimes unrelated, suggesting the probability of re-infection with another strain between the samplings.
- Some households (~7%) had more than one member positive for *B. pilosicoli*. Strain analysis suggested transmission between family members, and this could be due to either faecal-oral transmission, or from a common external source, such as contaminated water.

- *B. pilosicoli* was cultured from only 0.2% of Australians. This low prevalence may be a result of little or no exposure to *B. pilosicoli* due to good personal hygiene and environmental sanitation.
- *B. pilosicoli* strain H1b and H171 that were isolated from healthy Indonesians were able to colonise mice and day-old chickens, and induced clinical signs of pasty faeces in the latter. Histological sections showed mild typhlitis and typical end-on attachment of *B. pilosicoli* to the caecal epithelial mucosa of the chickens. This finding suggests that the human isolates had pathogenic potential.

This study showed that for animals investigated:

- Intestinal spirochaetes were cultured from 46.4% (13/28) of bilbies with 14.3% (4/28) positive for *B. pilosicoli*. Spirochaetes were also cultured from the faeces of two Western Barred bandicoots and one (1.2%) kangaroo.
- Intestinal spirochaetes were not isolated from any alpacas, cattle, goats, horses, pigs, and sheep but were detected in 40.5% of ducks, 14.3% of chickens, 14.9% of ostriches and 1.5% of cats.
- Few pets that are commonly kept in households (dogs, cats and aviary birds) were colonised, suggesting that they are not an important focus of *B. pilosicoli* infection in Australia.

DECLARATION

I declare that this thesis is my own account of my research and its main content is work which has not previously been submitted for a degree at any tertiary education institution.

Signed:

Kusuma Rini Margawani

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I thank you God, for your help and all the blessing during the study and writing this thesis. It was more than a decade ago since I started this study and at one stage I thought I would be not being able to make it. During this time, I experienced a lot of support, help, and love from you God.

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PUBLICATIONS AND CONFERENCE PROCEEDINGS

As a result of the work carried out in this thesis, the following papers and conference proceedings were published:

- Margawani KR, Robertson ID and Hampson DJ. 1998. Intestinal Spirochaetes in people in Western Australia. Proceedings of the Combined Biological Science Meeting, Perth, Western Australia 21 August.
- Brooke CJ, Margawani KR, Pearson AK, Riley TV, Robertson ID and Hampson DJ. 2000. Evaluation of blood culture systems for detection of the intestinal spirochaete *Brachyspira (Serpulina) pilosicoli* in human blood. *Journal of Medical Microbiology* 49:1031-36.
- Mikosza ASJ, La T, Margawani KR, Brooke CJ and Hampson DJ. 2001. PCR detection of *Brachyspira aalborgi* and *Brachyspira pilosicoli* in human faeces. *FEMS Microbiology Letters* 197: 167-70.
- Margawani KR, Robertson ID, Brooke CJ and Hampson DJ. 2003. Prevalence, risk factors and molecular epidemiology of *Brachyspira pilosicoli* in humans on the island of Bali, Indonesia. Proceedings of the International Conference on Colonic Spirochaetal Infections in Animals and Humans. Scotland, UK. p 38.
- Margawani KR, Robertson ID, Brooke CJ and Hampson DJ. 2004. Prevalence, risk factors and molecular epidemiology of *Brachyspira pilosicoli* in humans on the island of Bali, Indonesia. *Journal of Medical Microbiology* 53: 325-32.
- Munshi MA, Margawani KR, Robertson ID and Hampson DJ. 2008. An unexpectedly high prevalence of colonisation with *Brachyspira aalborgi* amongst residents on the Indonesian island of Bali. *Journal of Medical Microbiology* 57: 1234-37.
- Margawani KR, Robertson ID and Hampson DJ. 2009. Isolation of the anaerobic intestinal spirochaete *Brachyspira pilosicoli* from long-term residents and Indonesians visitors to Perth, Western Australia. *Journal of Medical Microbiology* 58: 248-52.

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