In vitro studies of *Brachyspira pilosicoli* pathogenesis

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This thesis is dedicated to

My Late Father (Appa)

A Man of Moral Values

You proved that an illiterate father
and farmer can educate his son very well
You were always reluctant to send me away from home
But you never stopped me and I kept on crossing seas
You were my best teacher to introduce me in this world
You taught me at the very beginning to be respectful and kind
I am still living very happily on your philosophies
You were a man of big heart, no one ever saw you in tears or crying
Sorry I was not around at the last day of your life
Though you are immortal father

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Great Animal Lover

You were the best animal lover I could ever see in my life,
I still remember those broken leg dogs you used to bring home
You treated them as an ancient veterinarian
You fought several people for animal cruelties
You were skilled to communicate with animals
I always feel that I became a veterinarian to represent
your traditional skills through university education
You were more than a mum for many children in the community
Perhaps you knew my future and asked me to learn cooking
I laughed at you. I learned cooking during my Australian PhD!
You were not a quitter in any situation

My lovely daughters

Chelsi (Anvesha) and **Khushi** (Aduesha)
Little puppies you were my rare emotional
strength in Australia during this PhD
You both sacrificed in many ways
Love You Both

Declaration

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

Ram Naresh

Abstract

Brachyspira pilosicoli is an intestinal spirochaete that colonizes the large intestine of a variety of species of birds and animals, including human beings. Colonization can lead to local inflammation and to diarrhoea in a condition known as “intestinal spirochaetosis”. This infection has been described in many countries throughout the world. In the colonization process the bacterium must cross the thick mucus blanket overlaying the colonic epithelium. Characteristically, *B. pilosicoli* then attaches by one cell end to the underlying epithelium, forming a dense “false brush border”. The mechanisms involved in moving through the mucus layer, attaching to enterocytes and inducing local cellular damage are poorly understood. The lack of *in vitro* models to study these events has been a major constraint to understanding the pathogenesis of *B. pilosicoli* infections.

The work described in this thesis deals with i) the development of an *in vitro* model of spirochaete attachment by using cells in suspension (erythrocytes) and cell monolayers (Caco-2), ii) the attraction of *B. pilosicoli* to mucin, and iii) the effects of norepinephrine exposure on expression of virulence traits by *B. pilosicoli*.

Attachment assays conducted with erythrocytes from different species at different ratios and time intervals identified one human isolate (WesB) that

adhered to goose and chicken erythrocyte at a 1:1000 ratio. This same strain, and an isolate from a pig (95/1000) also attached to Caco-2 cells. Transmission and scanning electron microscopy confirmed that the attachment resembled the *in vivo* situation. Exposure of the Caco-2 cells to *B. pilosicoli* resulted in actin rearrangements, damaged cell junctions and apoptosis. Caco-2 cells that were colonized with *B. pilosicoli* also demonstrated a significant up-regulation of interleukin-1ß (IL-1ß) and IL-8 expression, helping to confirm that the spirochaetes were inducing pathological changes in the cultured cells. Treatment of the monolayers with *B. pilosicoli* sonicates caused significant up-regulation of IL-1ß, TNF-α, and IL-6, but culture supernatants and non-pathogenic *Brachyspira innocens* did not altered cytokine expression. Hence Il-8 expression was specifically associated with exposure to live *B. pilosicoli* cells.

For mucin attraction, 15 *B. pilosicoli* strains isolated from humans, pigs, chickens and dogs, and a control strain of *Brachyspira hyodysenteriae*, were analysed for their ability to enter solutions of hog gastric mucin in an *in vitro* capillary tube assay. Attraction started in a 2 % mucin solution, and then increased with increasing concentrations to peak at around 6 - 8 % mucin. Attraction varied from strain to strain. *B. pilosicoli* strain 95/1000 and *B. hyodysenteriae* strain B204 also were attracted to viscous solutions of polyvinylpyrillodone (PVP), in a manner mirroring the response to mucin. This suggested that as well as chemotaxis to mucin components, “viscotaxis” is involved in the attraction to mucin.

Finally, exposure of *B. pilosicoli* to norepinephrine enhanced the attachment to Caco-2 cells, chemotactic response to mucin, and spirochaete growth. Taken together, these *in vitro* studies have shed new light onto the pathogenic processes that are involved in intestinal spirochaetosis caused by *B. pilosicoli.*

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Awards and publications from thesis work

Awards

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2. **Best Poster Award. Pathogens and Parasite.** Attraction of *Brachyspira pilosicoli* chemotaxis to mucin. Research Poster Day 2009, School of Veterinary and Biomedical Sciences, Murdoch University – 2009. Sponsored by Gene Works Pty Ltd.

Publications

1. **Ram Naresh** and David J. Hampson. 2010. Attraction of *Brachyspira pilosicoli* to mucin. *Microbiology (SGM)* 156:191-197
2. **Ram Naresh**, Yong Song and David J. Hampson. 2009. The intestinal spirochete *Brachyspira pilosicoli* attaches to cultured Caco-2 cells and induces pathological changes. *PLoS ONE* 4 (12): e8352.

Presentations

1. **Ram Naresh**, Yong Song and David Hampson. 2009. Interactions between *Brachyspira pilosicoli* and Caco2 cells. Annual Meeting of the Australian Society of Microbiology, Perth. July 6-10, 2009.
2. **Ram Naresh** and David Hampson. 2009. *Brachyspira pilosicoli* chemotaxis to mucin. 5th International Conference on Colonic Spirochetal Infections in Animals and Humans, 8^{th}-10^{th} June, Leon, Spian.
3. **Ram Naresh** and David Hampson. 2009. Chemotaxis to mucin by *Brachyspira pilosicoli*. Annual Meeting of Australian Society of Microbiology, Perth. July 6-10, 2009.
4. **Ram Naresh** and David Hampson. 2008. Development of an *in vitro* attachment assay for the intestinal spirochaete *Brachyspira pilosicoli*. XII International Congress of Bacteriology and Applied Microbiology 5-9 August 2008, Istanbul, Turkey.
5. **Ram Naresh** and David Hampson. 2008. Development of an *in vitro* attachment model for the intestinal spirochaete *Brachyspira pilosicoli*. Combined Biological Science Meeting, 29 August 2008, Perth, Western Australia.

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