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Research paper

Multiple locus variable number tandem repeat analysis (MLVA) of the pathogenic intestinal spirochaete *Brachyspira pilosicoli*

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ABSTRACT

Brachyspira pilosicoli is an anaerobic intestinal spirochaete that colonizes the large intestine of various host species, in which it may induce diarrhoea, poor growth rates and a localized colitis known as intestinal (or colonic) spirochaetosis. The spirochaete is considered to be potentially zoonotic. The purpose of the current study was to develop a multiple-locus variable number tandem repeat analysis (MLVA) method as a simple and rapid tool to investigate the molecular epidemiology of *B. pilosicoli*. The genomic sequence of *B. pilosicoli* strain 95/1000 was analyzed for potential tandem repeats using the default parameters of the Tandem Repeat Finder program. A total of 22 repeat loci were identified and tested for their presence and variability on a set of 10 *B. pilosicoli* isolates. Five loci that were present in most isolates and that showed evidence of allelic variation were selected and used with a collection of 119 isolates from different host species and geographical locations. Not all the isolates amplified at all loci, but using the available data a total of 103 VNTR profiles were generated. The discriminatory power of this method was 0.976. A phylogenetic tree constructed from the allelic profiles confirmed the diversity of *B. pilosicoli*, and the general lack of clustering of strains based on species of origin or geographic origin. Some isolates with known epidemiological links were found to be identical or highly similar. The MLVA method was simple and easy to use, and could readily differentiate between strains of *B. pilosicoli*. MLVA should prove to be a useful tool for rapid identification of relationships between *B. pilosicoli* isolates in epidemiological investigations.

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1. Introduction

The genus *Brachyspira* includes species of anaerobic spirochaetes that colonize the large intestine of animals and birds (Stanton, 2006). The two most important pathogenic species are *Brachyspira hyodysenteriae*, the agent of swine dysentery, and *Brachyspira pilosicoli*, the agent of intestinal spirochaetosis (Hampson, 2012). *B. pilosicoli* has a broader host range than *B. hyodysenteriae*, colonizing various species of animals and birds, as well as human beings (Hampson et al., 2006). Colonized individuals may develop focal colitis, with chronic

diarrhoea. Infections with *B. pilosicoli* are particularly common in intensively housed pigs and chickens, in which rates of growth and production may be depressed. Colonization also is common amongst humans living in crowded and unhygienic conditions (Trott et al., 1997a; Nelson et al., 2009). Spirochaetaemia with *B. pilosicoli* has been recorded in immunocompromised or debilitated human beings (Trott et al., 1997b), but not yet in animals. Transmission may be by direct exposure to infected faeces, or by indirect routes; for example, carcasses of spent hens in supermarkets may be contaminated with *B. pilosicoli* (Verlinden et al., 2012), while accessing drinking water from wells has been linked to transmission in humans (Margawani et al., 2004).

To help answer questions about transmission routes of *B. pilosicoli*, a simple, rapid and discriminating typing

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system that gives information about genetic relatedness of isolates is needed. Pulsed field gel electrophoresis has been used for this purpose (Atyeo et al., 1996), but it is quite time consuming and requires large quantities of pure DNA. Multiple-locus variable number tandem repeat analysis (MLVA) is a method that uses non-coding regions of genomic DNA, consisting of direct repeats of the same sequence of bases, referred to as tandem repeats. The number of repeats in the sequence is determined genetically and can vary between different strains. These regions are known as variable number tandem repeats (VNTR) loci. Recently, an MLVA scheme had been developed and used for *B. hyodysenteriae* (Hidalgo et al., 2010). The purpose of the current study was to develop an MLVA scheme for *B. pilosicoli*, and to use it as an epidemiological tool to compare isolates from different sources.

2. Materials and methods

2.1. *Spirochaete* isolates

A total of 119 *B. pilosicoli* isolates and strains recovered from different host species and geographical areas were obtained from the culture collection at the Reference Centre for Intestinal Spirochaetes at Murdoch University (Table 1). These consisted of 45 isolates from pigs, 42 from human beings, 25 from chickens, 5 from dogs, and 2 from

Table 1

Names of the 119 isolates, the host species from which they were isolated, location where they were isolated, their position in Fig. 1 and their VNTR profile.

Strain name	Position on Figure 1	Species of origin	Location of isolation ^a	VNTR profile
V1 H 106	1	Human	PNG (village 1)	70
GP 6	2	Pig	PNG (piggery 1)	70
MC F1	3	Human	Australia (WA)	75
D17	4	Dog	Australia	16
V1 H 11	5	Human	PNG (village 1)	65
W7	6	Pig	Australia	51
IMR 48	7	Human	PNG	14
Q98.0026.12	8	Chicken	Australia	15
Q98.0027.36	9	Chicken	Australia	18
V1 H 103	10	Human	PNG (village 1)	12
16242-94	11	Dog	USA	82
Q97.2224.3.1	12	Chicken	Australia	92
IMR 2	13	Human	PNG	73
IMR 39	14	Human	PNG	77
2152	15	Horse	Australia	102
Q98.000.6.1	16	Chicken	Australia	56
*H21	17	Human	Australia	61
*OF 15	18	Pig	Australia	68
Q98.0228.5.2	19	Chicken	Australia	95
V1 H 126	20	Human	PNG (village 1)	80
GP 24	21	Pig	PNG (piggery 1)	89
HRM7	22	Human	Italy	27
*HRM7A	23	Human	Italy	62
GP 17	24	Pig	PNG (piggery 1)	81
Q94.0354.0.6	25	Chicken	Australia	59
GP 20	26	Pig	PNG (piggery 1)	76
V1 H 116	27	Human	PNG (village 1)	6
V1 D 1	28	Dog	PNG (village 1)	7
95/1000	29	Pig	Australia	88
GP 28	30	Pig	PNG (piggery 1)	72

Table 1 (Continued)

GP 36	31	Pig	PNG (piggery 1)	71
Gap 418	32	Human	Australia	1
V1 H 120	33	Human	PNG (village 1)	8
Q98.0228.5.7	34	Chicken	Australia	74
EM1	35	Human	Australia (WA)	83
H4-2	36	Human	Australia	83
Gap 51.2	37	Human	Australia	83
V1 H 141	38	Human	PNG (village 1)	84
SC1	39	Human	Australia (WA)	63
JF2	40	Human	Australia (WA)	63
B1	41	Human	Australia (WA)	64
IMR 81	42	Human	PNG	11
NZ 91/31349	43	Pig	NZ	87
TH NF	44	Human	Australia (WA)	3
Q97.000.6.10	45	Chicken	Australia	38
*K1	46	Human	Australia (WA)	66
H43-2	47	Human	Australia	67
GP 32	48	Pig	PNG (piggery 1)	9
IMR49	49	Human	PNG	10
*GP 14	50	Pig	PNG (piggery 1)	5
V1 H 12	51	Human	PNG (village 1)	20
*QF1	52	Human	Australia (WA)	22
N1	53	Human	Australia (WA)	24
Q96.1037.0	54	Chicken	Australia	45
*WIA8	56	Pig	Australia	50
WW25	55	Pig	Australia	46
OF 11	57	Pig	Australia	39
89/1069	58	Pig	Canada	40
*P43/6/78 ^T	59	Pig	UK	29
GP 49	60	Pig	PNG	35
Q97.3008.4.2	61	Chicken	Australia	37
D9201243	62	Pig	USA	37
*W015/C138	63	Pig	Australia	44
JJ1	64	Human	Australia (WA)	25
M1	65	Human	Australia (WA)	36
PWS/B	66	Pig	UK	26
RV1	67	Human	Australia (WA)	26
HRM 4B	68	Human	Italy	26
HRM 2B	69	Human	Italy	26
L72	70	Pig	Australia	49
GP 35	71	Pig	PNG (piggery 1)	57
Q98.0072.37	72	Chicken	Australia	69
WG6	73	Pig	Australia	30
Q95.3281.0	74	Chicken	Australia	85
GP 3	75	Pig	PNG (piggery 1)	53
Q98.0033.72	76	Chicken	Australia	55
WesB	77	Human	Australia (WA)	54
Q98.0072.08	78	Chicken	Australia	54
89-2005A	79	Pig	Canada	60
KP5	80	Pig	Australia	41
KP1	81	Pig	Australia	90
Q97.000.6.2	82	Chicken	Australia	91
89-2005B	83	Pig	Canada	13
9803.1	84	Pig	Australia	21
Meyers K-9	85	Dog	USA	94
12				
WF1	86	Pig	Australia	58
Q1588.5	87	Pig	Australia	32
Q98.0028.3	88	Chicken	Australia	32
Cof 10	89	Pig	Australia	34
WG	90	Pig	Australia	34
OF 2	91	Pig	Australia	33
Q98.0062.14	92	Chicken	Australia	47
Q97.000.6.8	93	Chicken	Australia	48

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