Epidemiol. Infect. (1999), **123**, 133–138. Printed in the United Kingdom

Analysis of *Serpulina hyodysenteriae* strain variation and its molecular epidemiology using pulsed-field gel electrophoresis

R. F. ATYEO, S. L. OXBERRY AND D. J. HAMPSON*

Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia

(*Accepted 17 March 1999*)

SUMMARY

Pulsed-field gel electrophoresis (PFGE) was applied as a molecular typing tool for the spirochaete *Serpulina hyodysenteriae*, the agent of swine dysentery. Analysis of a collection of 40 mainly Australian isolates, previously characterized by other methods, divided these into 23 PFGE types. This confirmed that there are many strains of the spirochaete in Australia. PFGE was more discriminatory for strain typing than both multilocus enzyme electrophoresis and serotyping. It had similar discriminatory power to restriction endonuclease analysis, but the results of PFGE were easier to interpret. When applied to 29 isolates collected from 4 farms over periods of up to 8 years, 2 PFGE patterns were found on 3 farms, and a single pattern on the other. In each case a new strain had apparently emerged as a variant of an original parent strain. PFGE was found to be a powerful technique for investigating the molecular epidemiology of swine dysentery outbreaks.

INTRODUCTION

The intestinal spirochaete *Serpulina hyodysenteriae* is the agent of swine dysentery (SD), a severe muco-haemorrhagic diarrhoeal disease of weaner and grower/finisher pigs [1]. A number of methods have been developed for differentiating strains of the organism, including serotyping [2, 3], restriction endonuclease analysis (REA) [2, 4–6], multilocus enzyme electrophoresis (MLEE) [7, 8], ribotyping [6], and random amplification of polymorphic DNA [9]. Recently, pulsed field gel electrophoresis (PFGE) has been used to analyse variation amongst small numbers of *S. hyodysenteriae* isolates [10, 11]. The purpose of the current study was to develop PFGE as a strain-typing technique for *S. hyodysenteriae* and then apply it to investigate the molecular epidemiology of swine dysentery on infected piggeries.

* Author for correspondence.

METHODS

Spirochaete strains

Sixty-nine strains of *S. hyodysenteriae* were analysed by PFGE. Forty of these, which had previously been examined by MLEE, REA and/or serotyping [2, 7], were obtained from the collection held at the Australian Reference Laboratory for Intestinal Spirochaetes, Murdoch University. These include 39 Australian field isolates from 5 Australian states (Queensland, New South Wales, Victoria, South Australia and Western Australia), and the type strain B78^T from the USA. These isolates had previously been assigned to 20 REA types (1 of the 40 isolates not typed), 12

https://doi.org/10.1017/S0950268899002691 Published online by Cambridge University Press

Table 1. *Comparison of results of typing 40 strains of* S. hyodysenteriae *by pulsed field gel electrophoresis, restriction endonuclease analysis, multilocus enzyme electrophoresis and serotyping*

Isolate	PFGE type	REA type†	ET‡	Serogroup†§
NSW3	A1	H2	3	B
WA1	A1	H1	3	B
WA4	A1	H1	3	B
WA8	A1	H1	3	B
Vic35	A2	H1	3	B
WA2	A2	H1	3	B
WA9	A2	H1	3	B
Vic36	B1	H5	4	B
WA26	B2	H3	4	B
Vic31	C1	H9	7	B
Q14	C1	H9	7	G
Q17	C2	H10	7	B
Q18	C3	H10	7	NT
Q22	C3	H11	7	G
Vic30	D1	H8	8	B
Vic32	D1	H8	8	B
Vic40	D2	NT	8	NT
B78^T	E1	A	9	A
Vic2	E2	H6	9	H
WA28	F1	H4	11	A
Q10	F1	H9	11	A
Q11	F1	H9	11	B
WA3	G1	I1	14	E
WA5	G1	I1	14	E
WA6	G1	I2	14	E
Vic23	H1	L5	18	D
Vic24	H2	L5	18	D
Vic25	H3	L5	18	D
Vic33	H3	L5	18	NT
WA14	I1	J	19	A
WA27	I2	J	19	A
Q1	J1	L2	21	D
Q2	J1	L2	21	D
Q3	J1	L2	21	D
Q8	J2	L3	21	D
Q9	J2	L3	21	D
SA1	K1	L8	22	D
SA2	K1	L8	22	D
NSW2	K2	L9	22	D
Vic38	L1	M	26	B

* Results of restriction endonuclease analysis [2].
† NT, not typed.
‡ Results of multilocus enzyme electrophoresis [7]. ET, electrophoretic type.
§ Results of serogrouping [2, 3].

electrophoretic types (ETs) in MLEE, and 6 serogroups (3 isolates not typed) (Table 1). Another 29 isolates, which were obtained from pigs during outbreaks of SD in four Australian pig herds over the period 1987–96, were evaluated separately just by PFGE (Table 2). These herds were located in New South Wales, Queensland, Victoria and Western Australia respectively.

Table 2. Serpulina hyodysenteriae *isolates from four farms characterized by PFGE*

Isolate	Month/year isolated	Farm	PFGE pattern
NSW2	1990	M	M1
NSW6	1991	M	M1
NSW9	1992	M	M1
NSW7	1991	M	M2
NSW10	1992	M	M2
NSW11	June 1995	M	M2
NSW12	June 1995	M	M2
NSW13	June 1995	M	M2
NSW14	June 1995	M	M2
Q33a	1991	N	N1
Q47	1991	N	N2
Q34	1991	N	N2
Q35	1992	N	N2
Q48	1994	N	N2
Q49	1994	N	N2
Vic10	1987	O	O1
Vic48	1991	O	O1
Vic57	1991	O	O1
Vic58	1991	O	O1
Vic59	1991	O	O1
Vic62	1991	O	O1
Vic63	1991	O	O1
Vic64	1991	O	O1
Vic60	May 1995	O	O2
Vic61	May 1995	O	O2
WA34	Oct 1995	P	P1
WA35	Oct 1995	P	P1
WA36	May 1996	P	P1
WA37	May 1996	P	P1

Pulsed field gel electrophoresis (PFGE)

All strains were cultured in pre-reduced Trypticase Soy broth (BBL), harvested, and stored in 10% sucrose buffer pH 8·0, as previously described by us for *Serpulina pilosicoli* [12]. Preparation of DNA from cells suspended in gel plugs, and DNA restriction digests using the enzyme *Mlu*I (Boehringer–Mannheim, Germany) were also carried out as previously described [12]. Where isolates had the same banding pattern, their DNA was subsequently digested with *Bgl*I (Boehringer–Mannheim) in an attempt to differentiate them further. Gels were loaded onto a contour-clamped homogenous electric field-DR 11 system

https://doi.org/10.1017/S0950268899002691 Published online by Cambridge University Press

(Biorad Laboratories, USA), and were subjected to electrophoresis at 180 V for 18 h at 14 °C, with an initial switch time of 1 sec and a final switch time of 50 s, and a linear ramp. The gels were stained with a fresh ethidium bromide solution (0·625 μg/ml) and photographed over a UV transilluminator. Each strain was analysed at least twice to confirm the stability of its banding pattern.

Analysis of PFGE banding patterns

Analysis of banding patterns was carried out by a combination of two methods; patterns were initially categorized by eye, and the predominant PFGE types were then analysed by scanning photographs (Ofoto 2.0) into the Molecular Analyst program, version 1.0. (BioRad Laboratories). This programme created a dendrogram from a matrix of band matching coefficients (Fuzzy Logic) by the UPMGA clustering fusion strategy. A lambda ladder PFG marker (New England Biolabs, USA) was used in order to normalize all isolates represented in the dendrogram. PFGE banding patterns generated by isolates in the herd studies were analysed visually, with isolates having similar patterns being electrophoresed again in adjacent wells.

RESULTS

The results of the analysis with the first 40 strains are summarized in Table 1. These were divided into 23 PFGE types. Each isolate was assigned an upper case letter (A–L), corresponding to the 12 ETs to which they had previously been assigned [7]. Isolates in the same ET which had distinct banding patterns were then given a different numeral after the letter (e.g. patterns A1 and A2). An average of 8–10 bands was obtained per isolate with *Mlu*I, and examples of profiles generated are shown in Figure 1. Although some problems were experienced with wide banding patterns, DNA shearing and DNA degradation, this enzyme gave consistent and repeatable results. Digestion with *Bgl*I gave 12 or more bands, but band separation tended to be poorer, and no further strain differentiation was achieved using this enzyme (Fig. 2).

The 39 isolates which were typed by both PFGE and REA [2] were divided into 22 PFGE types and 20 REA types (Table 1). In most cases the two techniques gave identical strain discrimination, but in four cases isolates with the same REA pattern could be divided further using PFGE, with the opposite occurring in four other instances. As with REA, PFGE typing gave much better strain discrimination than did both MLEE (12 ETs) and serotyping (6 serogroups).

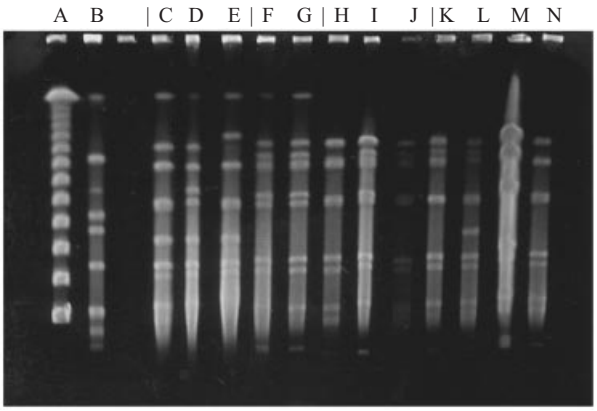

Fig. 1. PFGE patterns generated from strains of *S. hyodysenteriae* using *Mlu*I. Lanes; A, lambda ladder PFG marker; B, internal control strain; C, Vic25 (pattern H3); D, Vic24 (pattern H2); E, Vic23 (pattern H1); F, WA6 (pattern G1); G, WA6 (pattern G1); H, Vic40 (pattern D2); I, Vic32 (pattern D1); J, Vic30 (pattern D1); K, Q18 (pattern C3); L, Q17 (pattern C2); M, Q14 (pattern C1, smeared); N, Vic31 (pattern C1).

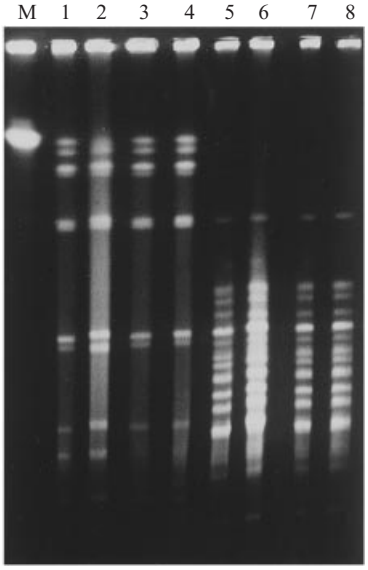

Fig. 2. PFGE patterns generated from isolates of *S. hyodysenteriae* from farm M. Lanes; M, lambda ladder; Lanes 1 through 4, and 5 through 8, contain isolates NSW11, NSW12, NSW6 and NSW9 respectively. Lanes 1–4 DNA restricted with *Mlu*I and lanes 5–8 with *Bgl*I. Isolates NSW11 and NSW12 have PFGE pattern M2 and isolates NSW6 and NSW9 have pattern M1. Pattern M1 lacks a band of low molecular mass at the bottom of lanes 3 and 4, compared to pattern M2 shown in lanes 1 and 2.

The dendrogram prepared by scanning the PFGE gels is shown as Figure 3. The percentage similarity of

https://doi.org/10.1017/S0950268899002691 Published online by Cambridge University Press

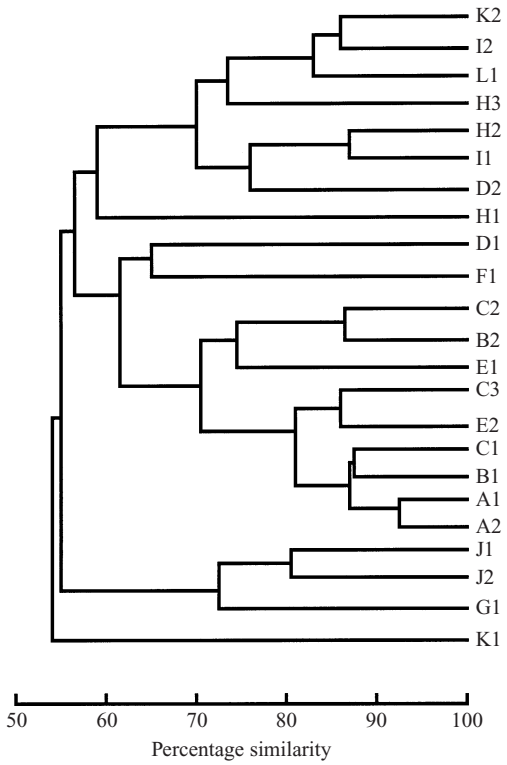

Fig. 3. Dendrogram created by Molecular Analyst Version 1.0, showing relationships of *S. hyodysenteriae* strains studied by PFGE. Isolates and their corresponding PFGE type are listed in Table 1.

the isolates ranged from approximately 53–100%. In no case were isolates from different ETs assigned to the same PFGE type. Where isolates from the same ET (i.e. having the same upper case letter) were differentiated by PFGE, these were more usually allocated to different areas of the dendrogram than in adjacent closely-related branches of the tree. For example, whilst A1 and A2 were only separated by a distance of approximately 8%, K1 and K2 were separated by a distance of approximately 46%.

Only 7 PFGE types were identified amongst 29 isolates from 4 farms experiencing problems with SD (Table 2). Two PFGE types were found on each of three farms, with single PFGE type identified on the other (farm P). Where different patterns were found amongst isolates from a farm, these differences were slight, involving only one or two bands (e.g. see Fig. 2).

DISCUSSION

Analysis of the Australian field isolates of *S. hyodysenteriae* by PFGE confirmed previous REA studies demonstrating the existence of a large number of strains [2]. PFGE was shown to have a similar capacity to discriminate between strains as REA, but it had other advantages over this technique, particularly where large numbers of isolates were to be analysed. In previous work in which REA was used to compare *S. hyodysenteriae* strains, the large number of fragments produced by the frequent base cutting enzymes used sometimes created patterns which were difficult to distinguish, particularly where the quality of DNA was sub-optimal, or if isolates were not run on neighbouring lanes on the gel [2, 4]. In contrast, PFGE produced only a small number of bands, which were spaced further apart and were therefore very much easier to distinguish and record. Results were also highly reproducable. Both techniques were more discriminatory for strain typing than serotyping and MLEE, and hence were more useful for epidemiological studies.

The dendrogram created from the PFGE data presented some unexpected results. It has generally been assumed that a set of isolates belonging to a given ET in MLEE studies represent a bacterial clone, or single line of recent descent [13], however the current results suggest that this is not always the case. Thus, by PFGE, some isolates in the same ET were apparently more distantly related to other isolates in that ET than they were to isolates in less related ETs. This finding has implications for interpretation of MLEE data, particularly in the context of deducing the population genetics of *S. hyodysenteriae* [8]. Nevertheless, the computer-generated dendrogram produced here from scanned PFGE gels may not necessarily give a more accurate representation of genetic relationships than a phenogram prepared from MLEE data. All that can be said is that the results of the two techniques show differences, and that additional confirmatory techniques are needed to clarify the situation.

Where isolates with the same PFGE pattern were recovered from different farms, it was presumed that these represented the same strain. Consistent with previous reports [2, 7, 8], some strains of *S. hyodysenteriae* were present in piggeries in different States of Australia, and these piggeries did not always have obvious epidemiological links. The majority of isolates from different farms, however, had large variations in their PFGE patterns (see Fig. 1 and Table 1). Interestingly, however, where multiple isolates from each of 4 farms were examined either no (farm P) or only minor (farms M, N and O) pattern differences were found. The occurrence of minor differences in

https://doi.org/10.1017/S0950268899002691 Published online by Cambridge University Press

PFGE banding patterns (one to three bands) between bacterial isolates is thought to imply that these are closely related, particularly when they are recovered from the same source [14]. That these differences on the 3 farms were in this category suggests that they had arisen from small genetic changes to a strain that was originally present on the farm. The alternative possibility would be that they represented newly-introduced strains, but this is unlikely given that strains from unrelated farms usually were quite distinct. The interpretation of genetic change in an original strain is supported by the data from the farms, where the 'new' pattern either only first predominated (farms M and N) or became apparent (farm O) a number of years after the original isolations were made. Farm O had on-going problems with SD in the period 1986–91, and this was eventually controlled by the use of antimicrobial drugs and restocking from a disease-free source. Swine dysentery re-emerged in 1995, and the PFGE results indicate that this was associated with a strain of *S. hyodysenteriae* that was only slightly different from the original strain.

Analysis of allelic distribution amongst a large collection of *S. hyodysenteriae* strains has been interpreted to suggest that the species has the capacity to undergo genetic recombination [8], perhaps associated with a generalized transducing bacteriophage [15]. The current findings support the existence of relatively rapid genetic change, which has been termed microevolution [16], and suggests that it has occurred naturally in 3 herds over a 3–8 year period. Clearly this occurrence is of considerable importance for a proper understanding of the epidemiology of SD, and for development of appropriate long-term control measures. The emergence and subsequent predominance of an altered strain amongst animals in a piggery implies that the new strain has some biological advantage over the parent strain. Advantageous changes might include altered virulence and/or greater antimicrobial drug resistance. Further work is required to analyse phenotypic and genotypic differences between the parent and variant strains identified on these piggeries.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Australian Pig Research and Development Corporation (PRDC). RFA was in receipt of a postgraduate scholarship from the PRDC.

REFERENCES

1. Hampson DJ, Atyeo RF, Combs BG. Swine dysentery. In: Hampson DJ, Stanton TB, eds. Intestinal spirochaetes in domestic animals and humans. Wallingford, England: CAB International, 1997; 175–209.
2. Combs BG, Hampson DJ, Harders SJ. Typing of Australian isolates of *Treponema hyodysenteriae* by serology and by DNA restriction endonuclease analysis. Vet Microbiol 1992; **31**: 273–85.
3. Hampson DJ, Mhoma JRL, Combs BG, Buddle JR. Proposed revisions to the serological typing system for *Treponema hyodysenteriae*. Epidemiol Infect 1989; **102**: 75–84.
4. Combs BG, Hampson DJ, Mhoma JRL, Buddle JR. Typing of *Treponema hyodysenteriae* by restriction endonuclease analysis. Vet Microbiol 1989; **19**: 351–9.
5. Harel J, Belanger M, Forget C, Jacques M. Characterisation of *Serpulina hyodysenteriae* isolates of serotype 8 and 9 from Quebec by restriction endonuclease fingerprinting and ribotyping. Can J Vet Res 1994; **58**: 302–5.
6. ter Huurne AA.H M, van Houten M, Koopman MBH, van der Zeijst BAM, Gastra W. Characterization of Dutch porcine *Serpulina* (*Treponema*) isolates by restriction endonuclease analysis and DNA hybridization. J Gen Microbiol 1992; **138**: 1929–34.
7. Lee JI, Hampson DJ, Combs BG, Lymbery AJ. Genetic relationships between isolates of *Serpulina* (*Treponema*) *hyodysenteriae*, and comparison of methods for their subspecific differentiation. Vet Microbiol 1993; **34**: 35–46.
8. Trott DJ, Oxberry SL, Hampson DJ. Evidence for *Serpulina hyodysenteriae* being recombinant, with an epidemic population structure. Microbiol 1997; **143**: 3357–65.
9. Dugourd D, Jacques M, Bigras-Poulin M, Harel J. Characterisation of *Serpulina hyodysenteriae* isolates of serotypes 8 and 9 by random amplification of polymorphic DNA analysis. Vet Microbiol 1996; **48**: 305–14.
10. Rayment SJ, Barrett SP, Livesley MA. Sub-specific differentiation of intestinal spirochaete isolates by macrorestriction fragment profiling. Microbiol 1997; **143**: 2923–9.
11. Trott DJ, Atyeo RF, Lee JI, Swayne DE, Stoutenburg JW, Hampson DJ. Genetic relatedness amongst intestinal spirochaetes isolated from rats and birds. Lett Appl Microbiol 1996; **23**: 431–6.
12. Atyeo RF, Oxberry SL, Hampson DJ. Pulsed-field gel electrophoresis for sub-specific differentiation of *Serpulina pilosicoli* (formerly '*Anguillina coli*'). FEMS Microbiol Letts 1996; **141**: 77–81.
13. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol 1986; **51**: 873–84.
14. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. Interpreting

chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; **33**: 2233–9.
15. Humphrey SB, Stanton TB, Jensen NS, Zuerner RL. Purification and characterisation of VSH-1, a generalised transducing bacteriophage of *Serpulina hyodysenteriae*. J Bacteriol 1997; **179**: 323–9.
16. Achtman M. Clonal spread of serogroup A meningococci: a paradigm for the analysis of microevolution in bacteria. Mol Microbiol 1994; **11**: 15–22.