MOLECULAR EPIDEMIOLOGY OF MULTIPLE ISOLATES OF *Brachyspira pilosicoli* USING AP-PCR

Corona BE[1*], Smith DGE[1], Murray B[2] and Thomson JR[2]
[1]Edinburgh University, Zoonotic and Animal Pathogens Research Laboraty, Edinburgh EH8 9AG, Scotland, UK. [2]Scottish Agricultural College-Veterinary Investigation Centre Bush Estate Penicuik EH26, Scotland, UK.

Introduction. The genus *Brachyspira* (Serpulina) includes commensal and pathogenic species among which *B. pilosicoli* and *B. hyodysenteriae* are pathogenic for pigs. *B. pilosicoli* infection in pigs has been referred as spirochaetal diarrhoea (5), and is characterised by greyish diarrhoea and reduced growth rates in pigs of 6 to 12 weeks of age (2). Genetic typing of intestinal spirochaetes has been done by various molecular methods. Arbitrarily primed PCR (AP-PCR) is a molecular method that generates reproducible DNA fingerprints to distinguish genetic variation between strains (8). The aim of this study was to assess the genetic variation of multiple isolates of *B. pilosicoli* by AP-PCR for epidemiological purposes.

Material and Methods. Forty three field isolates of *B. pilosicoli* from pigs, two from dogs, one from human, the type strain (ATCC 51139), and one field isolate of *B. hyodysenteriae* were included in this study. Genomic DNA was extracted from bacterial cultures of the isolates using Qiagen columns (QIAamp-DNA Mini Kit, Qiagen-UK). Four arbitrary primers (Sigma-Genosys, UK) were used individually for the AP-PCR. Each PCR reaction was performed with 3 mM of $MgCl_2$, 200μM of each dNTPs, 2 U of Taq polymerase, 1X of PCR buffer (Life Technologies- Gibco BRL, UK), 62.5 ng/μl DNA template and 62.5 pmol of primer per reaction in 50 μl final volume. The resulting PCR products were electrophoretically displayed on 12 % polyacrylamide gels and stained with silver nitrate. All bands generated by each primer were transformed to a binary format (1,0), where 1= band present and 0= band absent. Further, a data set including all the fragment data of the four primers was created and designated as pooled data. Data as (1,0) format was used to generate distance matrices on RAPDDIST version 2.0 computer programme based on Nei's genetic distance method, using a 1000 bootstrap value. Phylogenetic trees were generated by Neighbor-Joining method using NEIGHBOR computer programme and a consensus tree was also generated using the CONSENSE computer programme, both from PHYLIP computer programme version 3.5. Phylogenetic trees were visualised on the TREEVIEW computer programme version 1.5 as phylograms for each primer and as a cladogram for the pooled data set. Further, the pooled data set was also analysed by parsimony method on the PAUP version 4.0 computer programme.

Results. The total number of fragments generated by the individual primers (37+ 45 + 37 + 44= 163) gave a pooled data set with a total of 163 DNA fragments. Although a grade of consistency in the cluster forming of some sets of multiple isolates was observed on phylograms by individual primers, intra-specific genetic variation was also observed on other sets of the multiple isolates of *B. pilosicoli*. Pooled data (four primers = 163 fragments) confirmed the consistency observed on the cluster forming by each primer and gave better definition of the genetic relatedness of the multiple isolates (Fig. 1). Overall, the mean genetic distance of the multiple isolates by both methods (distance method and parsimony method) ranked the multiple isolates from farm P676 as the most closely related. Those isolates had a mean genetic distance at the level of (d= 0.032) followed by

the isolates from farm P657 (d=0.052), whereas the most distantly related were the isolates from farm P249 (d=0.152) (Table 1.)

Discussion. There are numerous methods of typing bacterial strains for epidemiological purposes. AP-PCR has been used successfully for characterising isolates from disease outbreaks in other studies and has been reported as more sensitive than MEE (6). The primers used in this study produced similar information and the analysis of a total of 163 fragments (characters) as pooled data set gave robustness to the genetic analysis of multiple isolates of *B. pilosicoli,* and confirmed what was observed on phylograms by individual primers. This is important as the probability of regaining or losing a band is unknown and is not expected to be equal so, scoring data as (0,1) format is an approximation (7). A study on field isolates of *B. hyodysenteriae, B. innocens* and *B. pilosicoli* using AP-PCR with two primers used independently showed that *B. innocens* and *B. pilosicoli* were more heterogeneous than *B. hyodysenteriae* (1). This is in accordance with the findings in the current study where the multiple isolates from farms P93, P249 and P595 had higher mean genetic distances than that of isolates (unrelated) between farms, indicating high intra-specific variation, this strongly suggested that more than one clone might be infecting a single herd. In fact, it has been suggested that more than one type of intestinal spirochaete might be isolated from the same area, or pig unit (3,4). On the other hand, the current study found a degree of genetic homogeneity among some of the multiple isolates, particularly multiple isolates from farms P676, P657, P152, and P126. Clear determination of the genetic variation of multiple isolates of *B. pilosicoli* from the same herd needs further investigation over long periods of time to monitor changes in genotype. In the present study only two dog isolates and one human isolate were included, the phylograms (distance method and parsimony) of pooled data showed that the dog isolates paired together but were in the same cluster as the reference strain ATCC-51139, indicating a possible epidemiological link with *B. pilosicoli* of pig origin.
In conclusion, AP-PCR was used successfully to differentiate multiple isolates of *B. pilosicoli*. *B. pilosicoli* was shown to be highly heterogeneous at the genetic level examined in this study. Distribution of isolates from other origins (dog and human) among porcine isolates suggests that genotypes are not host-specific and cross-species transmission may occur. As none of the multiple isolates were found to be identical suggests that infections by *B. pilosicoli* in UK farms may not be caused by a single genotype (clone), this might have implications for controlling infections in farms caused by *B. pilosicoli*.

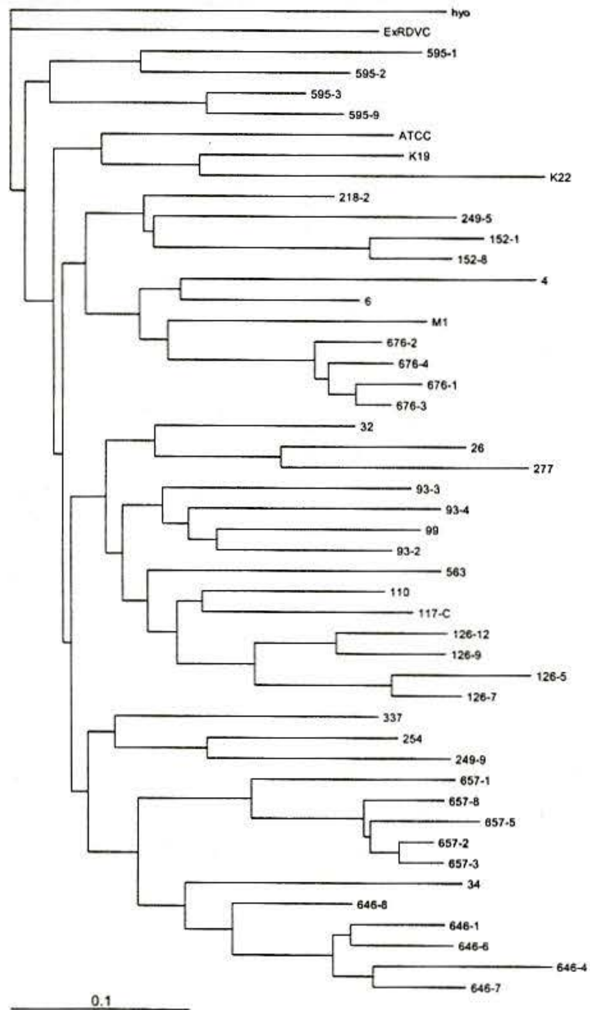

Fig. 1. Phylogram (distance method) showing genetic distances and cluster forming of multiple isolates of *B. pilosicoli*.

Table 1. Genetic distances by distance and parsimony methods of multiple and single isolates of *B. pilosicoli*.

Farm (no. isolates)	Mean genetic distance	
	Distance method	Parsimony method
P676 (4)	0.032	4.50
P657 (5)	0.052	7.20
P152 (2)	0.056	8.50
P126 (4)	0.060	9.25
P646 (5)	0.065	9.40
P595 (4)	0.100	14.75
P93 (3)	0.125	18.33
P249 (2)	0.152	20.50
K19- K22	0.152	15.50
13 farms (13 single isolates)	0.127	14.53

References

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