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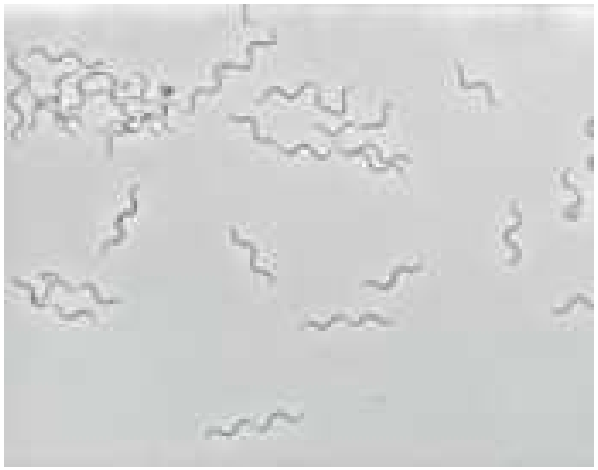
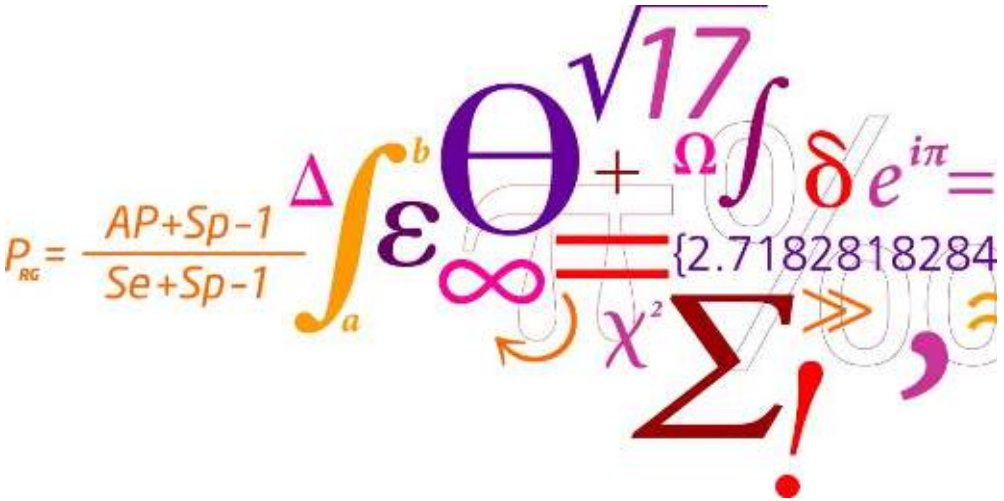
Danmarks Tekniske Universitet DTU

Brachyspira hyodysenteriae - diagnostiske udfordringer

Øystein Angen
Seniorforsker, dyrlæge





DVHS 2. november 2012

- Dyrlæge fra Norges Veterinærhøgskole 1989
- 2 års klinisk praksis i Norge
- 2 års klinisk praksis i Danmark
- Phd i bakteriologi KVL, København
- Nu seniorforsker i bakteriologi, DTU-VET

- Hovedarbejdsfelt:

  Forskning og testudvikling vedrørende bakterielle sygdomme hos produktionsdyr

Disposition

- Speciesidentifikation
- Detektion i fæces

Brachyspira hyodysenteriae

- *Treponema hyodysenteriae* 1972
- *Serpula* 1991
- *Serpulina* 1992
- *Brachyspira hyodysenteriae*1998

Species	Patogen	Nonpatogen
B. alvinipulli	Kylling	
B. aalborgii	Menneske	
B. hyodysenteriae	Gris, rotte, kylling (challenge)	
B. innocens		Gris
B. intermedia	Kylling	Gris (patogen?)
B. murdochii		Gris (colitis?)
B. pilosicoli	Kylling, menneske, gris	
"B. suanatina"	Gris, gråand	
"B. hampsonii"	Gris	

- Ikke valide species: "B. pulli", "canis", "ibaraki", "carvi", "rattus", "muridarium", "muris",
- B. "hampsonii" (indol negative, kraftigt haemolyserende spirochaeter, USA)

Speciesidentification ved biotypning

- Gruppe I *Brachyspira hyodysenteriae*
- Gruppe II *B. intermedia*
- Gruppe III *B. innocens/B. murdochi*
- Gruppe IV *B. pilosicoli*

Table 3
Biochemical classification of porcine intestinal *Brachyspira* spp.

Biochemical group	Indicated species	Haemolysis	Indole production	Hippurate hydrolysis	Beta-glucosidase activity
I	*B. hyodysenteriae*, "*B. suanatina*"	Strong	+/− [a]	−	+
II	*B. intermedia*	Weak	+	−	+
III	*B. murdochii*, *B. innocens*	Weak	−	−	+
IV	*B. pilosicoli*	Weak	−	+/− [a]	−

[a] The large majority of the isolates reacts positive.

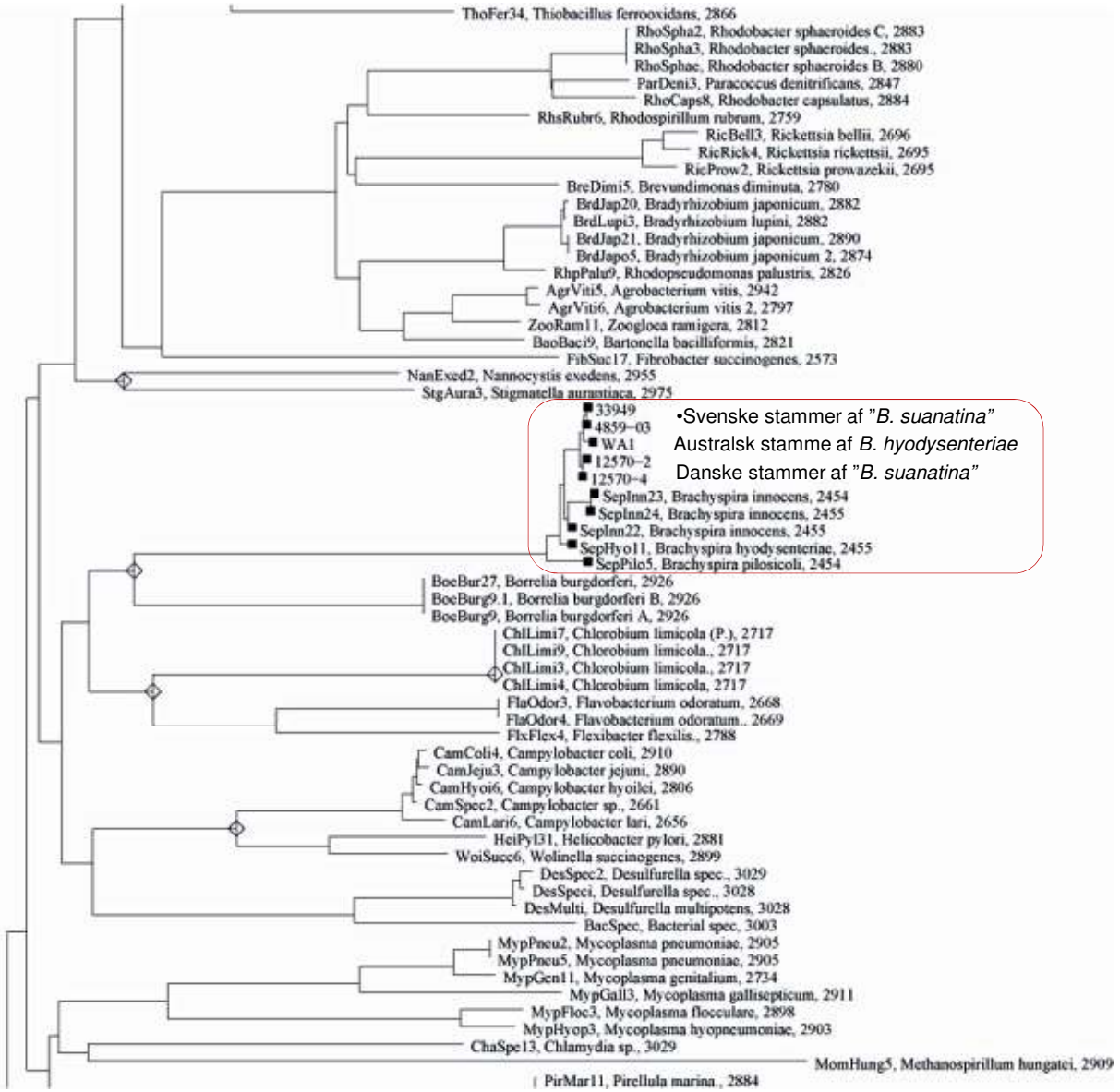
Journal of Microbiological Methods 72 (2008) 133 – 140

Problemer i diagnostik

- Haemolyse er afhængig af medium og inkubation
- Kraftigt haemolyserende isolater af *B. intermedia*?
- Påvisning af kraftigt haemolyserende spirochaeter fra gråand. Lignende typer også fundet i grise.
  - "*Brachyspira suanatina*" (Råsbäck et al., 2007)
- Kraftigt hamolyserende indol negative spirochaeter i USA
  - *"Brachyspira hampsonii"*
- Sådanne isolater giver problem for artsidentifikationen.
  - Biotypning skal bekræftes af PCR
  - Varierende resultater i PCR test, vanskeligt at stole på kun en test!
    - *nox* (NADH oxidase), *tlyA* (hæmolysin), 16S, 23S
    - Der findes mange forskellige test baseret på det samme gen!

23S

Meget få stammer sekvenseret



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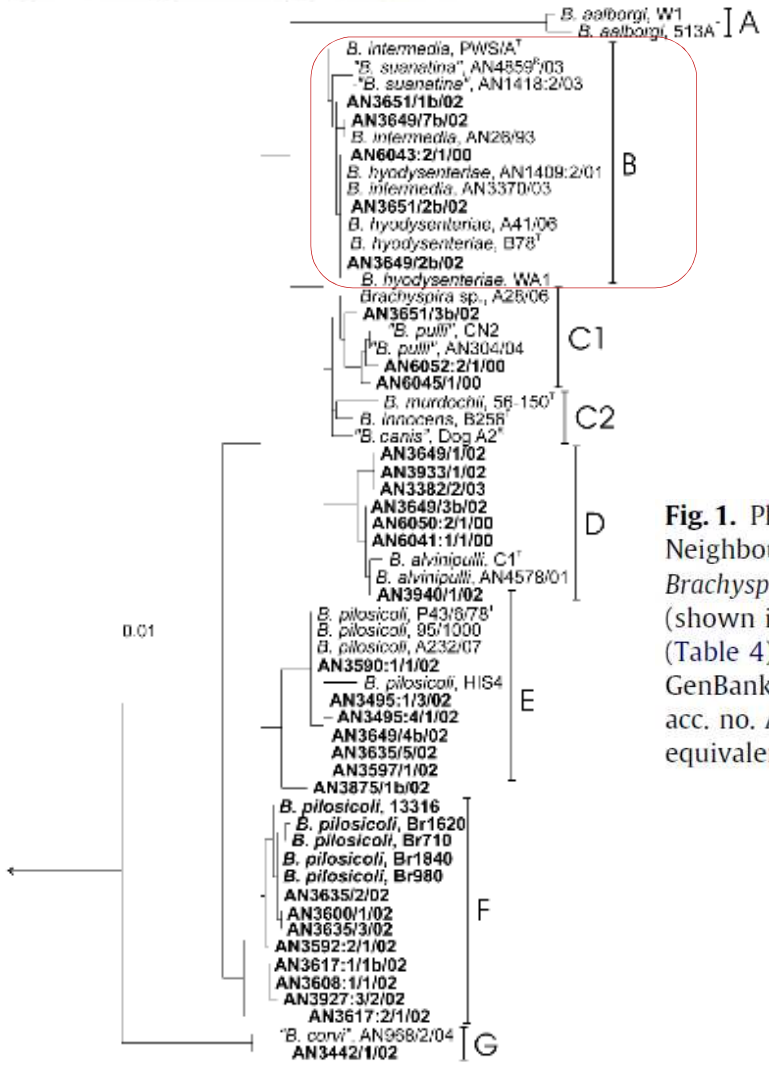
Phenotypic and genetic diversity among intestinal spirochaetes (genus *Brachyspira*) in free-living wild mallards (*Anas platyrhynchos*) sampled in southern Sweden

Désirée S. Jansson [a,b,*], Marianne Persson [c], Ulla Zimmerman [c], Karl-Erik Johansson [a,c]

Systematic and Applied Microbiology 34 (2011) 566–575



Fylogeni baseret på 16S-genet,

Fig. 1. Phylogenetic tree based on the 16S rRNA gene (1433 positions) computed by Neighbour Joining showing the evolutionary relationships among 35 characterized *Brachyspira* spp. isolates from free-living wild mallards, Finnish pigs and a chicken (shown in bold) (Table 3), and strains and isolates used for comparative analysis (Table 4). Main clusters are designated A–G. Strains of *Borrelia burgdorferi* (B31^T, GenBank acc. no. AE000783) and *Treponema denticola* (ex Flügge 1886) (a^T, GenBank acc. no. AF139203) were used as outgroup. The scale bar represents the distance equivalent to 1 nucleotide substitution per 100 positions.

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Fylogeni baseret på nox-genet

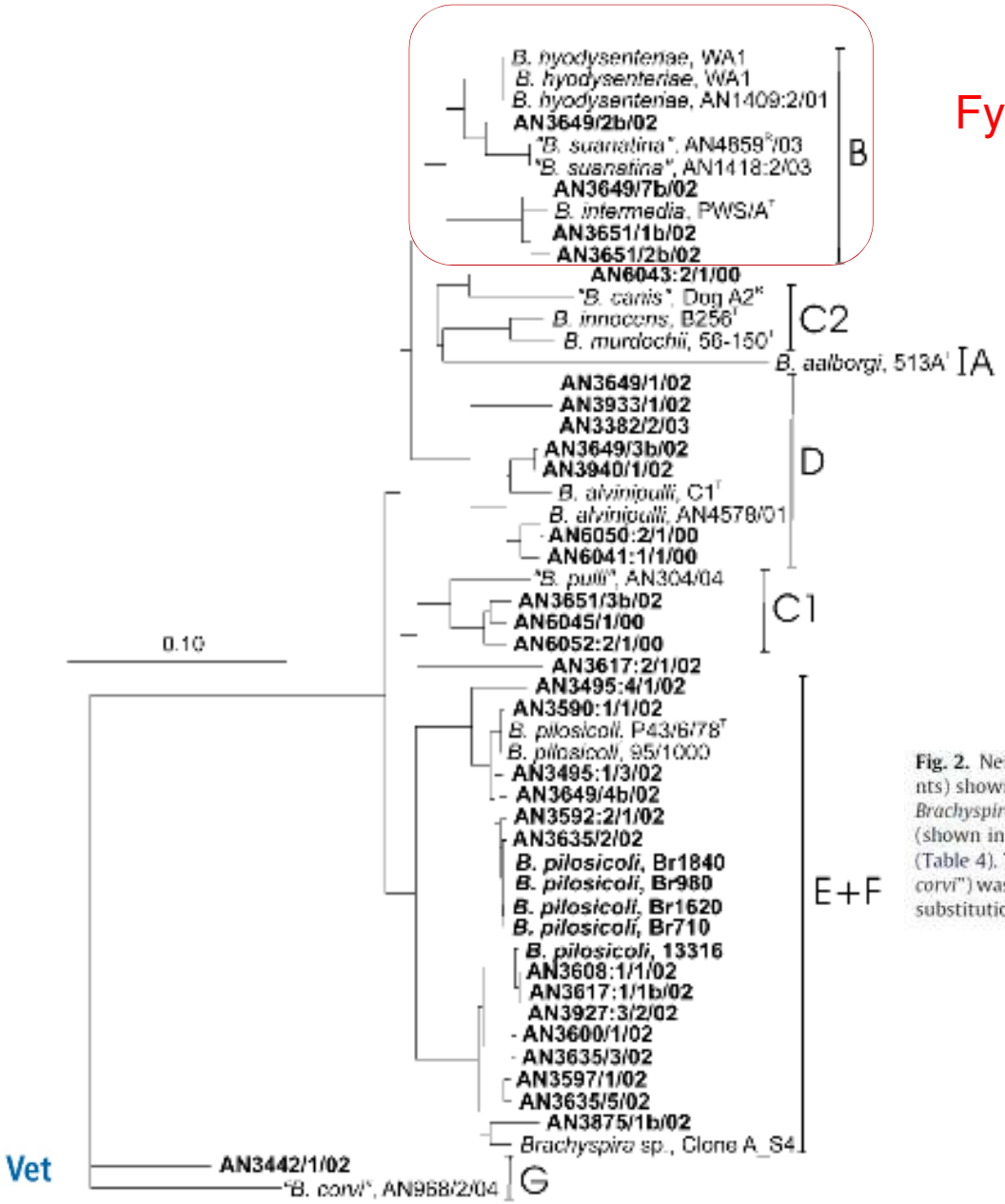


Fig. 2. Neighbour Joining tree based on NADX oxidase (*nox*) gene sequences (829 nts) showing genetic relationships among *Brachyspira* spp. from 35 characterized *Brachyspira* spp. isolates from free-living wild mallards, Finnish pigs and a chicken (shown in bold) (Table 3), and strains and isolates used for comparative analysis (Table 4). The main clusters are designed A–G as in Fig. 1. Isolate AN968/2/04 ("*B. corvi*") was used as outgroup. The scale bar represents the distance equivalent to 10 substitutions per 100 nucleotide positions.

A novel enteropathogenic, strongly haemolytic spirochaete isolated from pig and mallard, provisionally designated '*Brachyspira suanatina*' sp. nov.

Environmental Microbiology (2007) 9(4), 983–991

Thérése Råsbäck,[1,2]* Désirée S. Jansson,[1,3] Karl-Erik Johansson[2,4] and Claes Fellström[1]

Konventionel PCR: tlyA, nox, 23S

Table 1. Designation, species origin and PCR results of strongly β-haemolytic strains and field isolates of *Brachyspira* spp. used in this study.

		B. hyodysenteriae[a]				*B. pilosicoli*[a]	*B. intermedia*[a]		*B. spp*[a]		GenBank accession no. of	
Strain (ATCC)	Species, origin	*tlyA*	*nox*[b]	*nox*[c]	23S rRNA	16S rRNA	*nox*	23S rRNA	*nox*	Reference	16S rRNA genes	*nox* genes
B78[T] (27164)	*B. hyodysenteriae*, Pig, USA	+	+	+	+	–	–	–	+	Harris *et al.* (1972)	U14930	AF060800
B204[R] (31212)	*B. hyodysenteriae*, Pig, USA	+	+	+	+	–	–	–	+	Kinyon and Harris (1979)	U14932	U19610
AN 174/92	*B. hyodysenteriae*, Pig	+	+	+	+	–	–	–	+	Fellström and Gunnarsson (1995)	U14931	DQ487117
AN 2420/97	*B. hyodysenteriae*, Pig	+	+	+	+	–	–	–	+	Råsbäck *et al.* (2005)	DQ473574	DQ487118
AN 4859/03	'*B. suanatina*', Pig, fattening herd A	–	–	–	–	–	–	–	+	Råsbäck *et al.* (2006)	DQ473575	DQ487119
AN 1681:1/04	'*B. suanatina*', Pig, fattening herd B	–	–	–	–	–	–	–	+	This study	DQ473576	DQ487120
AN 2384/04	'*B. suanatina*', Pig, piglet-producing herd	–	–	–	–	–	–	–	+	This study	DQ473577	DQ487121
Dk 12570-2	'*B. suanatina*', Danish piglet-producing herd	–	–	–	–	–	–	+	+	This study	DQ473578	DQ487122
AN 3949:2/02	'*B. suanatina*', Mallard, wild migrating	–	–	–	–	–	–	–	+	Jansson *et al.* (2004)	AY352290	DQ487123
AN 1418:2/01	'*B. suanatina*', Mallard, public park	–	–	–	–	–	–	–	+	Jansson *et al.* (2004)	AY352282	DQ487124
AN 1409:2/01	*B. hyodysenteriae*, Mallard, public park	+	+	+	+	–	–	–	+	Jansson *et al.* (2004)	AY352281	DQ487115
AN 383:2/00	*B. hyodysenteriae*, Mallard, farmed	+	+	+	+	–	–	–	+	Jansson *et al.* (2004)	AY352291	DQ487116
AN 3930:2/02	*Brachyspira*. sp., Mallard, wild migrating	–	–	–	–	–	–	–	+/–[d]	Jansson *et al.* (2004)	AY352288	DQ487125

a. Primers, see Table 3.
b. Atyeo and colleagues (1999a).
c. La and colleagues (2003).
d. Positive three of seven times.
Unless otherwise indicated, all field isolates originated from Sweden.

Table 3
Phenotypic and molecular results of *Brachyspira* spp. isolates from free-living wild mallards sampled in southern Sweden, from pigs and a chicken that were used for characterization in the present study.

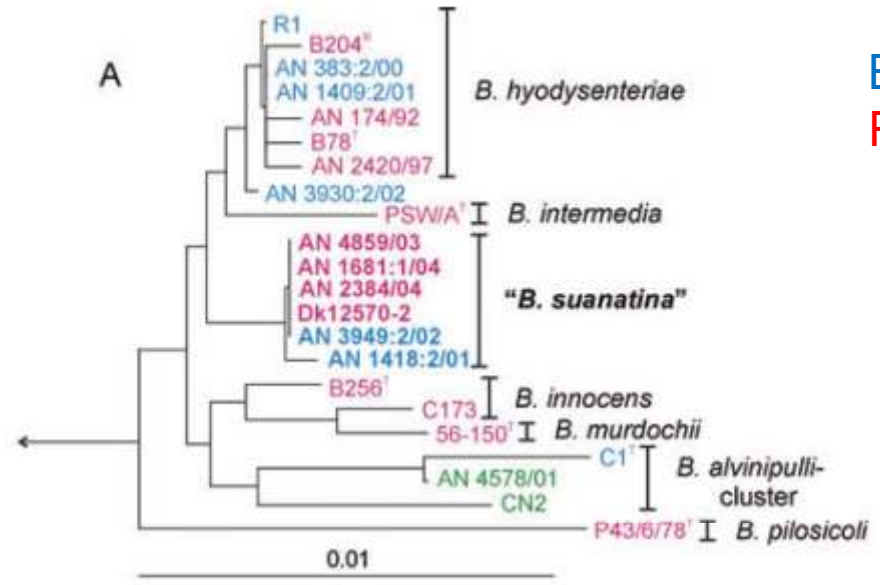
No.	Isolate	Country of origin	Species of origin	Penotype[a]	PCR									GenBank acc. nos.		Proposed species
					Bhy			*Bim*			*Bpi*		*Bin/Bmu*	16S rRNA gene	*nox*gene	
					*tly*A [25]	23S [19]	*nox* [1]	23S [19]	*nox* [1]	*nox* [13,22]	16S [25]	16S [22]	*nox* [1]			
1	AN6043:2/1/00	Sweden	Mallard	12001[b]	–	+	–	–	–	–	–	–	–	JF430695	JF430734	*Brachyspira* sp.
2	AN3649/2b/02	Sweden	Mallard	11001[b]	–	+	+	–	–	–	–	–	–	JF430696	JF430735	*Brachyspira* sp.
3	AN3649/7b/02	Sweden	Mallard	11001[b]	–	–	–	(+)	+	+	–	–	–	JF430697	JF430736	*B. intermedia*
4	AN3651/1b/02	Sweden	Mallard	11001[b]	–	–	–	+	(+)	+	–	–	–	JF430698	JF430737	*B. intermedia*
5	AN3651/2b/02	Sweden	Mallard	12001[b]	–	–	–	+	–	–	–	–	–	JF430699	JF430738	*B. intermedia*
6	AN6045/1/00	Sweden	Mallard	10011[c]	–	–	–	–	–	–	–	–	–	JF430700	JF430739	"*B. pulli*"
7	AN6052:2/1/00	Sweden	Mallard	10001[d]	–	–	–	–	–	–	–	–	–	JF430701	JF430740	"*B. pulli*"
8	AN3651/3b/02	Sweden	Mallard	10011[c]	–	–	–	(+)	–	–	–	–	–	JF430702	JF430741	"*B. pulli*"
9	AN3649/1/02	Sweden	Mallard	10101[e]	–	–	+	–	–	–	–	–	–	JF430703	JF430742	*B. alvinipulli*
10	AN3933/1/02	Sweden	Mallard	10201[e]	(+)	–	+	–	–	–	–	–	–	JF430704	JF430743	*B. alvinipulli*
11	AN6041:1/1/00	Sweden	Mallard	10201[e]	–	–	+	–	–	–	–	–	–	JF430705	JF430744	*B. alvinipulli*
12	AN6050:2/1/00	Sweden	Mallard	10201[e]	–	–	–	–	–	–	–	–	–	EF371459 [27]	JF430745	*B. alvinipulli*
13	AN3649/3b/02	Sweden	Mallard	10201[e]	–	–	–	–	–	–	–	–	–	JF430706	JF430746	*B. alvinipulli*
14	AN3940/1/02	Sweden	Mallard	10201[e]	–	–	–	–	–	–	–	–	–	JF430707	JF430747	*B. alvinipulli*
15	AN3590:1/1/02	Sweden	Mallard	10210[f]	–	–	–	–	–	–	+	+	–	JF430708	JF430748	*B. pilosicoli*
16	AN3495:4/1/02	Sweden	Mallard	10201[e]	–	–	–	–	–	–	+	+	–	JF430709	JF430749	*B. pilosicoli*
17	AN3597/1/02	Sweden	Mallard	1020v	–	–	–	–	–	–	+	+	–	JF430710	JF430750	*B. pilosicoli*
18	AN3635/5/02	Sweden	Mallard	1020v	–	–	–	–	–	–	+	+	–	JF430711	JF430751	*B. pilosicoli*
19	AN3875/1b/02	Sweden	Mallard	10100	–	–	–	–	–	–	+	+	–	JF430712	JF430752	*B. pilosicoli*
20	AN3649/4b/02	Sweden	Mallard	10201[e]	–	–	–	–	–	–	+	+	–	JF430713	JF430753	*B. pilosicoli*
21	AN3495:1/3/02	Sweden	Mallard	10201[e]	–	–	–	–	–	–	+	+	–	JF430714	JF430754	*B. pilosicoli*
22	AN3600/1/02	Sweden	Mallard	10111	–	–	–	–	–	–	+	+	–	JF430715	JF430755	*B. pilosicoli*
23	AN3635/3/02	Sweden	Mallard	10111	–	–	–	–	–	–	+	+	–	JF430716	JF430756	*B. pilosicoli*
24	AN3592:2/1/02	Sweden	Mallard	10001[d]	–	–	–	–	–	–	+	+	–	JF430717	JF430757	*B. pilosicoli*
25	AN3617:2/1/02	Sweden	Mallard	10000	–	–	–	–	–	–	+	+	–	JF430718	JF430758	*Brachyspira* sp.
26	AN3635/2/02	Sweden	Mallard	10001[d]	–	–	–	–	–	–	+	+	–	JF430719	JF430759	*B. pilosicoli*
27	Br1620	Finland	Pig	10001[d]	–	–	–	–	–	–	+	+	–	JF430720	JF430760	*B. pilosicoli*
28	Br1840	Finland	Pig	10001[d]	–	–	–	–	–	–	+	+	–	JF430721	JF430761	*B. pilosicoli*
29	Br710	Finland	Pig	10001[d]	–	–	–	–	–	–	+	+	–	JF430722[g]	JF430762	*B. pilosicoli*
30	Br980	Finland	Pig	10001[d]	–	–	–	–	–	–	+	+	–	JF430723	JF430763	*B. pilosicoli*
31	AN3927:3/2/02	Sweden	Mallard	20211	–	–	–	–	–	–	+	+	–	EF371460 [27]	JF430764	*B. pilosicoli*
32	AN3608:1/1/02	Sweden	Mallard	20211	–	–	–	–	–	–	+	+	–	JF430724	JF430765	*B. pilosicoli*
33	AN3617:1/1b/02	Sweden	Mallard	20211	–	–	–	–	–	–	+	+	–	JF430725	JF430766	*B. pilosicoli*
34	13316	The Netherlands	Chicken	20211	–	–	–	–	–	–	+	+	–	JF430726	JF430767	*B. pilosicoli*
35	AN3442/1/02	Sweden	Mallard	10011[c]	–	–	–	–	–	–	+	+	–	JF430727	JF430768	*Brachyspira* sp.

Abbreviations: *Bhy* – *Brachyspira hyodysenteriae*; *Bim* – *B. intermedia*; *Bpi* – *B. pilosicoli*; *Bin* – *B. innocens*; *Bmu* – *B. murdochii*.
PCR results: + – positive; – – negative; (+) – weakly positive or variable amplification.

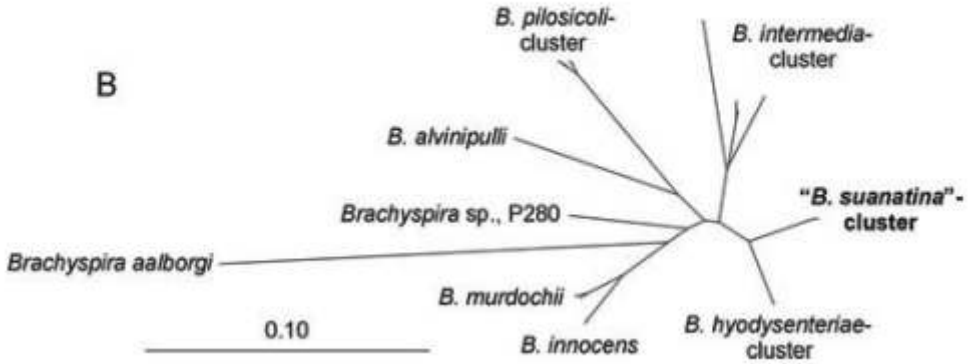
[a] Phenotypic results given in the following order: intensity of β-haemolysis, tryptophanase, hippuricase, α-galactosidase, and β-glucosidase activities; 1 – positive; 2 – strongly positive, 0 – negative; v – variable).
[b] Phenotype consistent with type strain of *B. intermedia* (PWS/A^T, ATCC 51140).
[c] Phenotype consistent with type strain of *B. innocens* (B256^T, ATCC 29796).
[d] Phenotype consistent with type strain of *B. murdochii* (56-150^T, ATCC 51284).
[e] Phenotype consistent with type strain of *B. alvinipulli* (C1^T, ATCC 51933).
[f] Phenotype consistent with type strain of *B. pilosicoli* (P43/6/78^T, ATCC 51139).
[g] Previously published 16S rRNA sequence GenBank acc. no. AY514025 [7].

Jansson et al., Syst Appl Microbiol 2011

16S



nox



Blå: isolater fra fugle
Rød: isolater fra svin

Fig. 2. A. Evolutionary tree based on 16S rDNA sequences comprising 1393 nucleotide positions showing the phylogenetic relations between different *Brachyspira* spp. Porcine isolates are indicated in red, avian isolates in blue and canine isolates in green. *Brachyspira aalborgi* was used as out-group. The scale bar shows the distance equivalent to one substitution per 100 nucleotide positions, corresponding to approximately 14 substitutions. Superscripts T and R indicate type and reference strains respectively.
B. Radial representation of a phylogram based on the *nox* gene sequences comprising 893 nucleotide positions showing relationship of 29 *Brachyspira* isolates/strains, including the atypical isolates designated as '*Brachyspira suanatina*'. A cluster was defined as a monophyletic group comprising three or more isolates of the same species. *Brachyspira aalborgi* was used as out-group. The scale bar shows the distance equivalent to 10 substitutions per 100 positions, corresponding to approximately 89 substitutions.

Råsbäck et al., Environ Microbiol 2007

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Brachyspira intermedia strain diversity and relationships to the other indole-positive *Brachyspira* species

Nyree D. Phillips, Tom La, Maswati Mat Amin, David J. Hampson*

Animal Research Institute, School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia

Veterinary Microbiology 143 (2010) 246–254

MLST

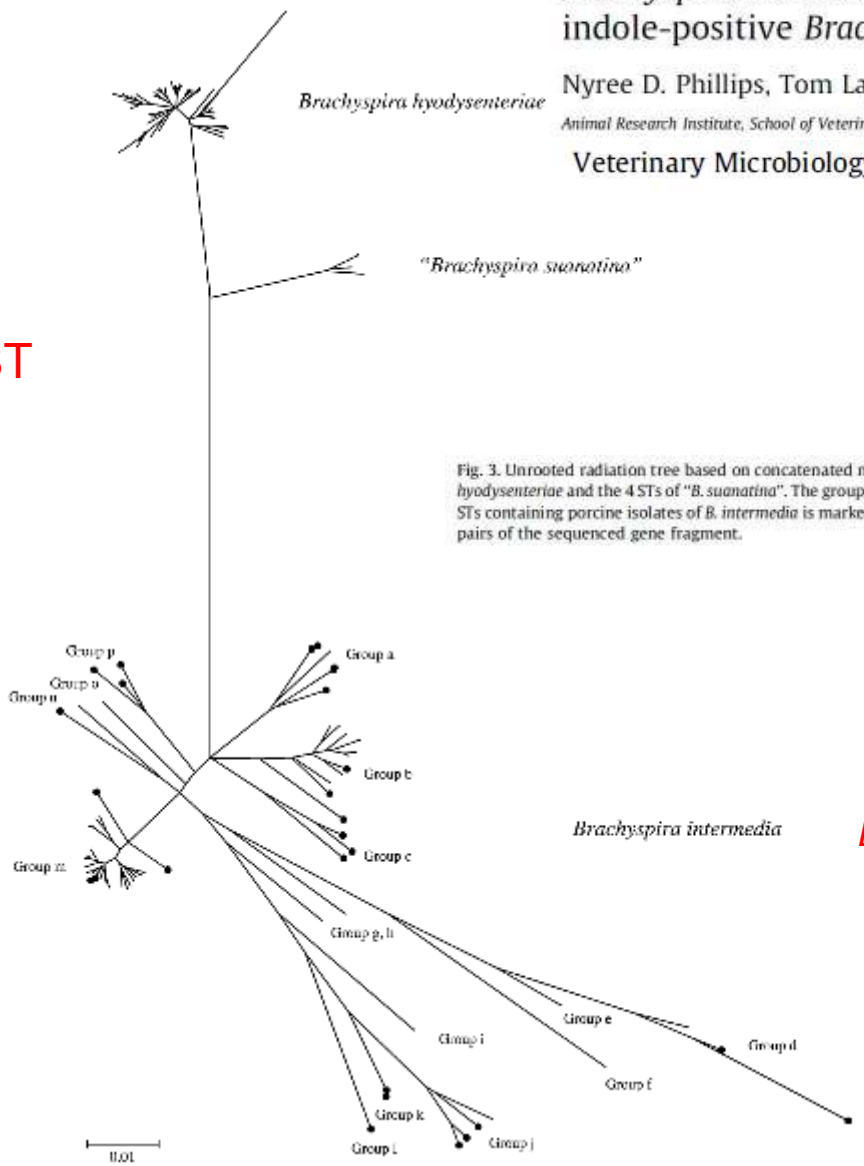

Fig. 3. Unrooted radiation tree based on concatenated nucleotide sequences showing the relationships of the 71 STs of *B. intermedia*, and the 67 STs of *B. hyodysenteriae* and the 4 STs of "*B. suanatina*". The groups of *B. intermedia* STs marked a through p correspond to the groups marked in Fig. 2. The location of STs containing porcine isolates of *B. intermedia* is marked with filled circles. The length of the scale bar represents one nucleotide substitution in 100 base pairs of the sequenced gene fragment.

B. intermedia meget heterogen

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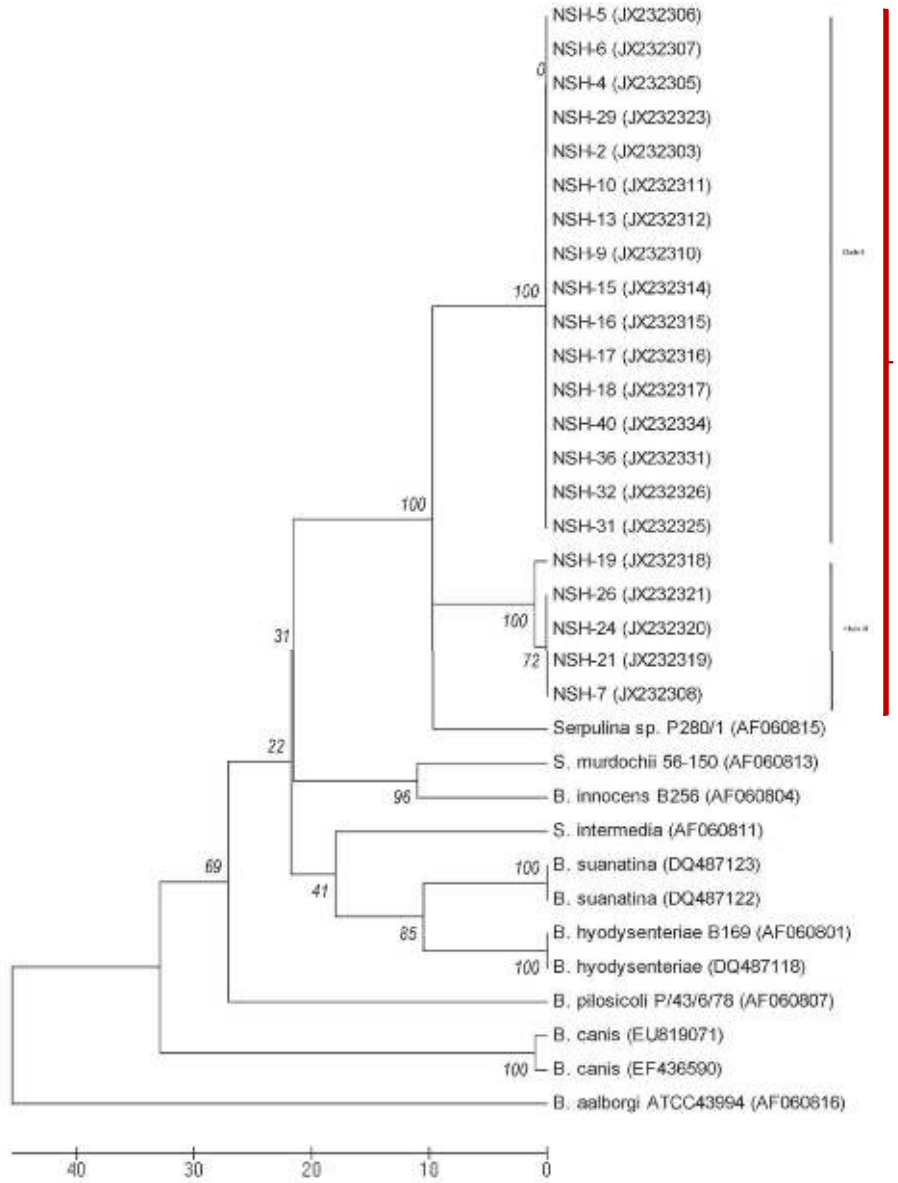

B. ”hampsonii”

Figure 1. Phylogenetic analysis of the *nox* gene of *Brachyspira* sp. inferred using the neighbor-joining method and 100 bootstrap replicates (values shown on tree). The evolutionary distances were computed using the number of differences method, and evolutionary analyses were done in MEGA 5.[2] Sequences starting with NSH are from the novel strongly hemolytic (NSH) *Brachyspira* sp., provisionally designated "*Brachyspira hampsonii*", isolated in the present study, and the other sequences were obtained from GenBank and are identified with accession numbers.

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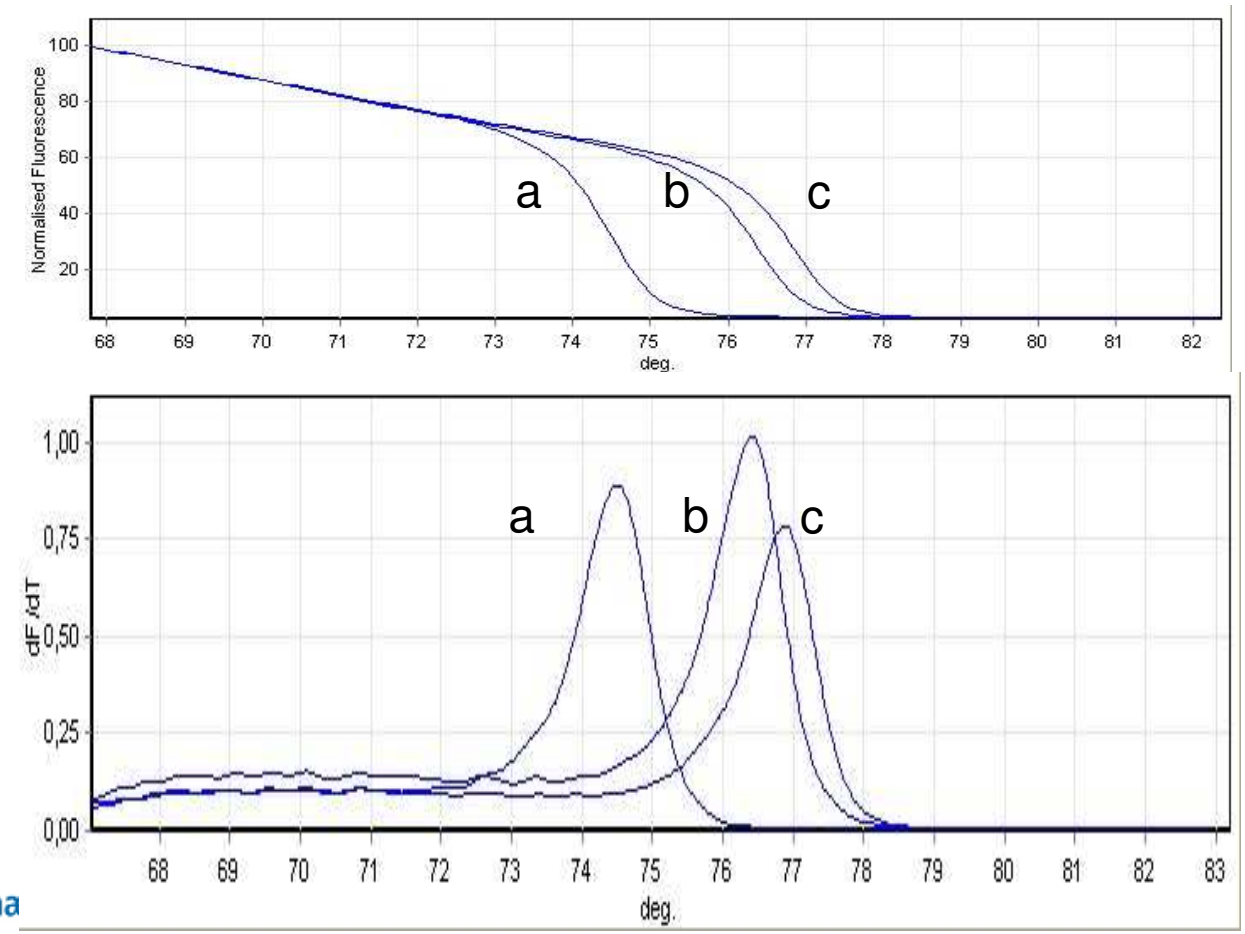
High resolution melt (HRM) – 23S



a: *B. pilosicoli*
b: *B. hyodysenteriae*
c: *B. intermedia*

Også et problem i Danmark

- 2009 blev der isoleret på VET en kraftigt hæmolyserende *Brachyspira* gruppe I (der var resistent overfor tiamulin, valnemulin, oxytetracyclin og tylosin).
- FISH: Negativ for *B. hyodysenteriae* og positiv for *B. intermedia*
- PCR på 23S*: B. hyodysenteriae*
- HRM på *nox*-genet*:* Gruppe II eller III
- VET har forsøgt at designe en FISH-test for *"B. suanatina"*, men denne har vist sig at krydsreagere med *B. hyodysenteriae*
- For tiden er diagnostikken på VET baseret på FISH

PCR afprøvning på VET

- Konventionelle PCR test baseret på *nox*-genet brugt indtil 2006.
- Real-time PCR (VET) baseret på *nox*-genet:
  - Krydsreagerer med høje mængder DNA fra *B. intermedia*
- HRM-test baseret på *nox* kan skille *B. hyodysenteriae* fra de øvrige *Brachyspira spp.*
- Real-time PCR (Akase et al., 2008) baseret på *nox*-genet:
  - Fundet at være species-specifik og anvendelig til kvantitativ diagnostik (*kan* køres sammen med diarrepakken)

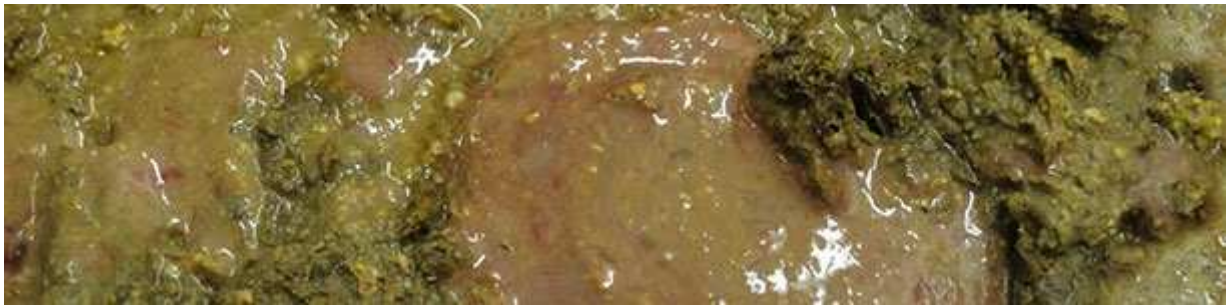
Brachyspira hyodysenteriae påvist på laboratorium for Svinesygdomme i perioden 2005 til 31.06.2012

	Brach. hyodysenteriae påvist, Indsendelser	Brach. hyodysenteriae påvist, CHR-numre	Total undersøgte, indsendelser	Procent Brach. hyodysenteriae påvist, Indsendelser	Procent, Brach. hyodysenteriae påvist, CHR-numre
2005	14	12	244	5,7	4,9
2006	6	5	178	3,4	2,8
2007	9	8	199	4,5	4,0
2008	7	5	107	6,5	4,7
2009	2	2	112	1,8	1,8
2010	4	4	163	2,5	2,5
2011	6	6	135	4,4	4,4
2012	7	5	73	9,6	6,8
Total	**55**	**47**	**1211**	**4,5**	**3,9**

	Brach. hyodysenteriae påvist, Indsendelser	Total undersøgte, indsendelser	Total undersøgte, prøver	Procent Brach. hyodysenteriae påvist, Indsendelser	Procent Brach. hyodysenteriae positive indsendelser i forhold til antal prøver.
Fæces	33	615	7518	5,4	0,4
Tarme	22	596	1342	3,7	1,6

Detektion fra fæces

- Dyrkning - standardmetode
- PCR
  - Der findes mange forskellige test i brug baseret på:
  - Forskellige gener
  - Forskellige multiplex-formater
  - Almindelig PCR
  - Real-time PCR



Dyrkning - PCR

- Råsbäck et al. 2006:
  - PCR på DNA ekstraheret fra fæces reducerer sensitiviteten med en faktor 10^3 – 10^5 i forhold til dyrkning (konventionel PCR på *tlyA*-genet)
- Philips et al., 2009:
  - 128 samples, 18 positive i PCR, 1 positiv ved dyrkning

  (multiplex PCR baseret på *nox*-genet)
- Song and Hampson, 2009:
  - Højere sensitivitet af real-time PCR end konventionel PCR
  - Højere sensitivitet af PCR på agarpladekultur end på DNA oprenset fra fæces

Table 2
Results of a comparison of qPCR and nPCR applied to DNA extracts from faecal samples and/or primary plate cultures from pigs and chickens.

Animal species	Health status	No. of samples	Species tested for	Culture-nPCR	Culture-qPCR	Faecal-nPCR	Faecal-qPCR
Pigs	Farms negative for swine dysentery	100	*B. hyodysenteriae*	0	0	0	0
			B. pilosicoli	0	0	0	0
			B. intermedia	0	0	0	0
Pigs	Farms with swine dysentery diagnosed	112	*B. hyodysenteriae*	12	12	7	9
			B. pilosicoli	8	8	5	5
			B. intermedia	0	0	0	0
Pigs	Experimentally infected with *B. hyodysenteriae*	39	*B. hyodysenteriae*	35	35	24	32
			B. pilosicoli	0	0	2	2
			B. intermedia	8	8	6	14
Laying chickens	Unknown	100	*B. hyodysenteriae*	NT	NT	0	0
			B. pilosicoli	NT	NT	43	46
			B. intermedia	NT	NT	30	34
% Positive pigs		251	*B. hyodysenteriae*	18.7%	18.7%	12.4%	16.3%
			B. pilosicoli	3.2%	3.2%	2.0%	2.0%
			B. intermedia	3.2%	3.2%	2.4%	5.6%
% Positive chickens		100	*B. hyodysenteriae*	NT	NT	0%	0%
			B. pilosicoli	NT	NT	43.0%	46.0%
			B. intermedia	NT	NT	30.0%	34.0%

Song and Hampson 2009, realtime PCR baseret på *nox*. nPCR=normal PCR

Table 2. Detection of *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli* and *Lawsonia intracellularis* in porcine faeces and tissue samples by multiplex-PCR. Agreement between multiplex-PCR and microbiological isolation, multiplex-PCR and uniplex nested-PCR (nPCR) or multiplex-PCR and immunofluorescence (IFT) are expressed according to kappa index considering positive (+) and negative (−) samples

		Multiplex-PCR (+)	Multiplex-PCR (−)	Kappa index
Brachyspira hyodysenteriae				
Isolation	(+)	19	2	0.84
	(−)	3	49	
nPCR	(+)	6	1	0.73
	(−)	1	7	
Brachyspira pilosicoli				
Isolation	(+)	6	1	0.92
	(−)	0	67	
nPCR	(+)	3	0	1.00
	(−)	0	13	
Lawsonia intracellularis				
IFT	(+)	12	10	0.49
	(−)	2	25	
nPCR	(+)	7	4	0.69
	(−)	0	18	

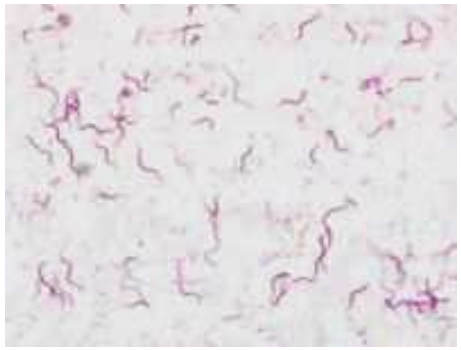
Nathues et al., 2007. baseret på ukendt gen. nPCR = nested PCR

Table 1. Positive numbers for each method, Ct values and quantities according to real time PCR

Real time PCR	Ordinary PCR	Isolation	Sample number	Ct values of real time PCR
+	+	+	47	26.3 ± 1.8
+	–	+	2	32.5 ± 0.5
+	+	–	2	36.9 ± 2.2
+	–	–	3	40.0 ± 4.9
–	–	–	13	–

Akase et al., 2008, realtime PCR baseret på *nox*-genet

Opsummering



- For at kunne vurdere et laboratorieresultat skal vi kende:
  - Hvilket gen er testen baseret på
  - Konventionel PCR, nested PCR, realtime PCR
  - Testens specificitet
  - Testens sensitivitet
- Der findes stammer i svin og ænder, der ikke lader sig nemt identificere og tilordnes en species
- Potentielt problem i SPF-systemet
- Veterinærinstituttet kan sætte op en realtime PCR, men dette vil kræve en grundig validering af testens specificitet i SPF-besætninger.