

**Identification of the complete
sequence of SWP1, a spore wall
protein in the microsporidia
*Encephalitozoon hellem***

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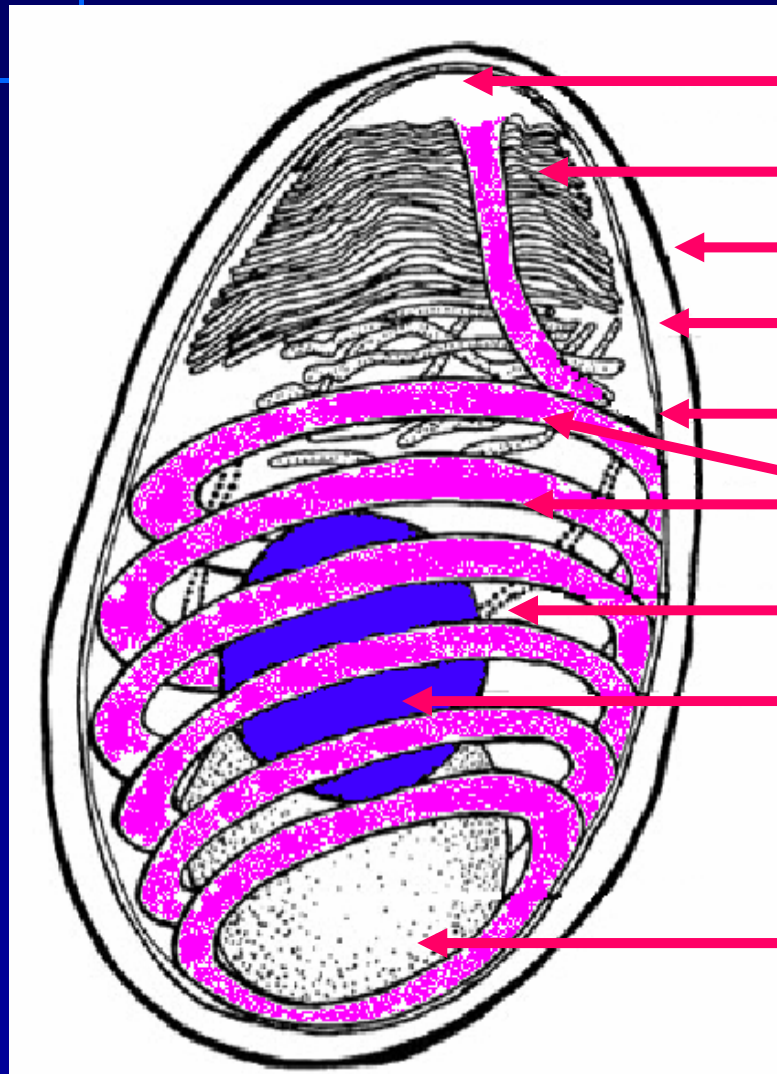
Microsporidia

- ✓ Unicellular eukaryotes
- ✓ Obligate intracellular parasites
- ✓ Opportunistic infections in human
- ✓ Environmental-resistant spores with a unique invasive apparatus (polar tube)

Encephalitozoon hellem

- ✓ Human: the unique known mammalian host
- ✓ Zoonotic opportunistic infection

Structure of the spore



Anchorage disk

Polaroplast

Exospore

Endospore

Plasma membrane

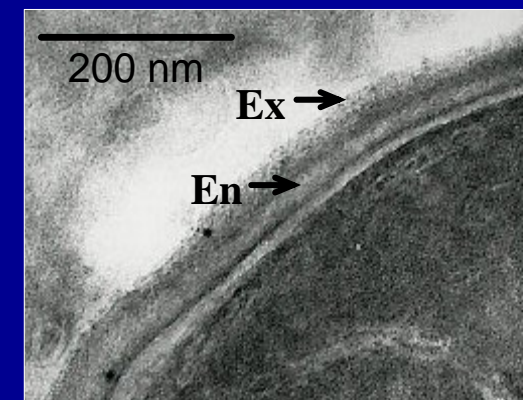
Polar tube

Ribosomes

Nucleus

Posterior vacuole

Cell Wall



Cell wall proteins identified in *Encephalitozoon* spp

In *E. cuniculi*

(Bohne et al., 2000)

1 gene encoding

✓ EcSWP1 (450 aa, 46 kDa)

In *E. intestinalis*

(Hayman et al., 2001)

2 genes encoding

✓ EiSWP1 (388 aa, 41 kDa)

✓ EiSWP2 (1002 aa, 107 kDa)

Labelling restricted to the electron-dense exospore

Signal peptide: 18 aa

Conserved N-terminal regions

Specific C-terminal parts

EcSWP1

5 repeats Gly and Ser-rich of 16 aa

EiSWP1

34 aa

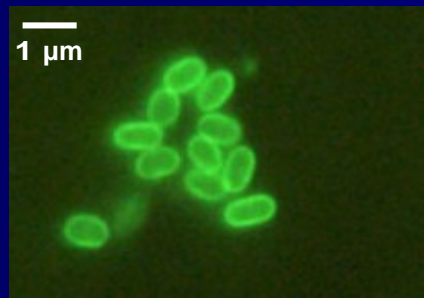
EiSWP2

50 repeats Glu and Asp-rich of 12-15 aa

Identification of a *swp1* gene in *Encephalitozoon hellem*

Southern blot experiments suggested *E. hellem* contains a *swp1* homologous gene (Bohne et al., 2000)

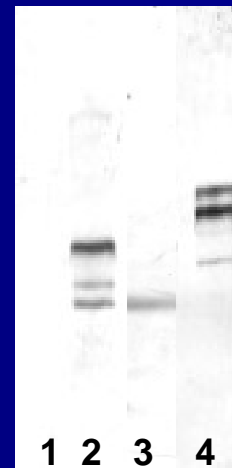
Cross reactivity with antibodies raised against *Encephalitozoon intestinalis* exospore (Mab1E4)



E. hellem spores

66 kDa →

45 kDa →



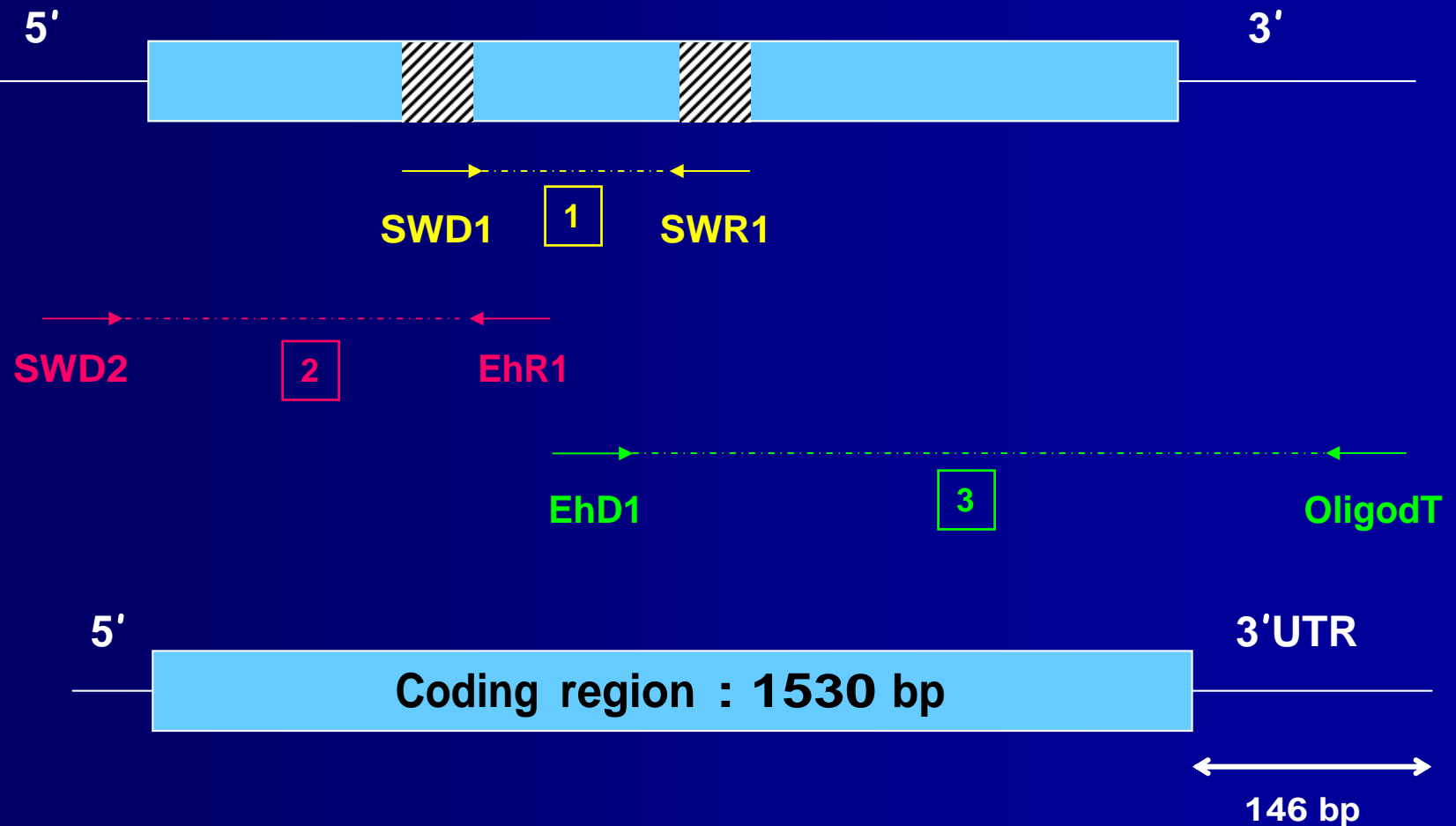
1. non- infested
HFF cells

2. HFF- Ec

3. HFF- Ei

4. HFF- Eh

Strategy used to amplify the *EhSWP1* gene

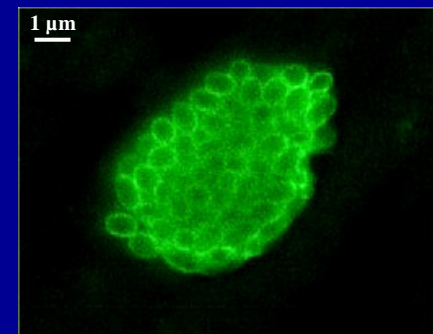
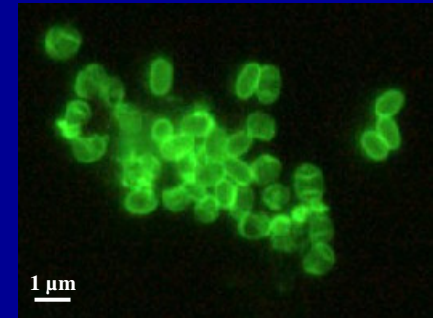
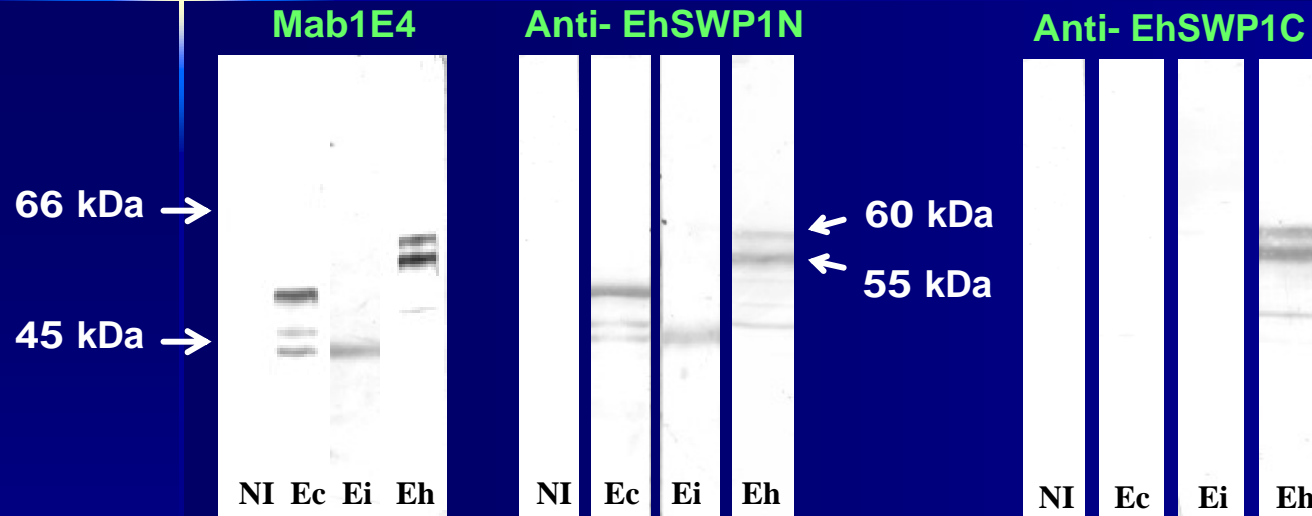


EcSWP1	MMKLSLLLGLVFSFAVLASENRRGN-CQMPEG-TRYFEKNNLLGRRFKNDVRKLGDGM	58
EiSWP1	MIKLSLLLGLASFTAVLANQRPR---CQRPVSSSKYFQQNNLLGSRFQNEVQRLARRV	57
EhSWP1	MLKLSLLLGLANFTAILAGGERRRSGLLQSANGSRYFREQNLLGIKYKDDVKRLGERP	60
	* * ***** * ** * * * * * * * * * * * * * * *	
EcSWP1	SSDVHDESSSHSSSSSDCKPRRRRHRRYEDSCSFGSSDDDSSTYSSCVSSECSPPCRP	118
EiSWP1	REESSSESSSSSSSE-DCSRRRRRPHREWEDSCSSSYSSS-----STDCSSSAPCPPP	111
EhSWP1	EGPHPSESSSCSSSCSEECGRRRFPGIRDDCEYSSWDACSS----SWDECTDSTPCTTP	116
	***** ** *	
EcSWP1	VPLNDYELKTPIIINMGERIFEFLKNYEDQYKKAUVLFLTRILSQIDGFAPSYPSADYEP	178
EiSWP1	VAQRDIELKTPIIILMGERIYEFLKNYEDQYKKAUVLLFLTNILSQISGFNPVFPGGDYDA	171
EhSWP1	TPLRDAELRVPIINMGERIYEFLKNYEDQYESAVTLALNNILSQVSGFNPIFAGADYAA	176
	* **** ***** *	
EcSWP1	LIEQLETLGVTVPNSMAADLAALDAEATSLAGTIRANAQKVIGDLLARVNTMAYLDLMS	238
EiSWP1	LIEQLKTLGVTVPANTAAELAAIDAESSALTRAIQANAQKVISDLLTRVSAMAYLDIMS	231
EhSWP1	LVEQLETLGVNVPANTAAELASIDSSESAALSRIIQGNAQKIIISDLFARVGSMAAYSDITS	236
	***** *	
EcSWP1	LVTSGLFASQVTSAFSNTQPIIITIAGNDLFTKQMAVFQRLPGTLPAAITAITNALQANK	298
EiSWP1	LVNSGLLASQVSSVFNNIQPIIITITGNDLFAKQMAVFQKIPGTLPSAAITAITNALQGNR	291
EhSWP1	LINSGLFASQISSAFSNTQPVITIASNDLYAKQMAVFQRIPGTLPAAAVTAITNALQTNR	296
	***** *	
EcSWP1	NNFVTFFTTQTTNLQTDVQNALITALITLTLTSTTSTEFQFANSEIGALTGRIFGSSG	358
EiSWP1	TNFVTFFTTQTSNLQTSVQNSLTTLISELEKLATDTETAFTAFANAELISALISRIFPTST	351
EhSWP1	NNFATFFTTQATTLQTNVQNILTALTTALTTLTNSTSTEFQFANSEIAAALAAARIFPATT	356
	***** *	
EcSWP1	SGSGGSSGGSSGSGSDGSGSGGSSGGSSGSGSDGSGSGGSSGGSSGSGSDGSGSGGSSGG	418
EiSWP1	TSSGSGD-----STGSGSTGDAGDAGDTGEDGGDDEGTEGTG-----	388
EhSWP1	ASGDNNSGSTGGDDTGDEGDGGEETPEEETPEEETPEEETDPVTALLNTIKEEGEKLGAAM	416
	** *	
EcSWP1	SSGSGSDGSGSGGSSGGSSGSGSGGESGGSS-----	450
EiSWP1	-----	388
EhSWP1	EEVMTAIKEQGTQGAQAVQEMMDAVKNEADTKKTETVDEILSKVKDEADTKKTETVTEI	476
EcSWP1	-----	450
EiSWP1	-----	388
EhSWP1	MDKVKTEADTKKTDTVTEIMEKAKETWEAGKSS	509

Characteristics of *Encephalitozoon* SWPs

Protein	Length (amino acids)		pI	%					Nb of Cys	Nb of potential O- glycosylation sites
	Precursor	Mature protein		S	G	A	T	E		
EcSWP1	450	432	5.2	18.9	14.1	6.5	7.6	3.9	11	41
EiSWP1	388	370	5	14	6.2	8.6	8.9	5.6	10	19
EiSWP2	1002	984	4	5	17.2	3	7.8	25	10	3
EhSWP1	509	491	4.6	8.9	6.3	10.1	11	10.6	12	2

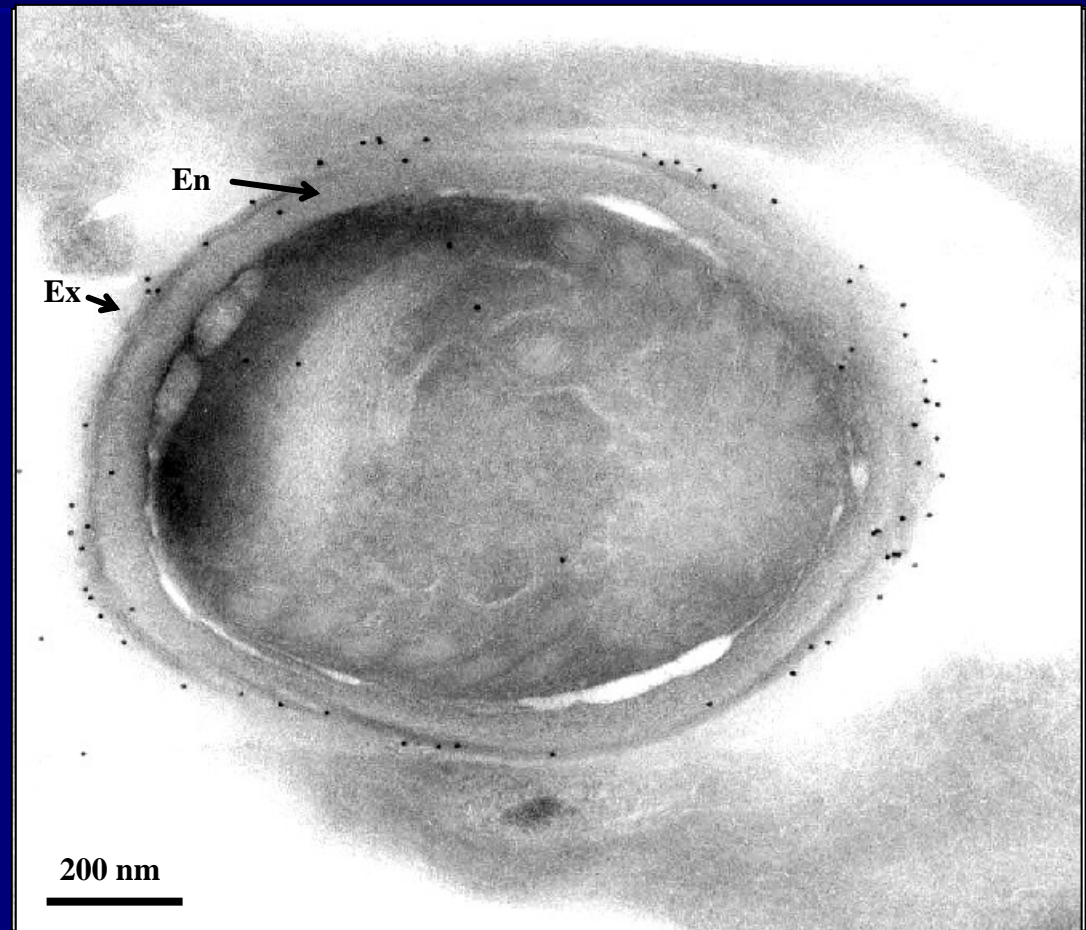
Immunolabelling with antisera raised against recombinant proteins



- ✓ Identification of 2 major bands (60 and 55 kDa) in *E. hellem*
- ✓ Anti- EhSWP1N cross react with EcSWP1 and EiSWP1
- ✓ EhSWP1 is localized in the spore wall

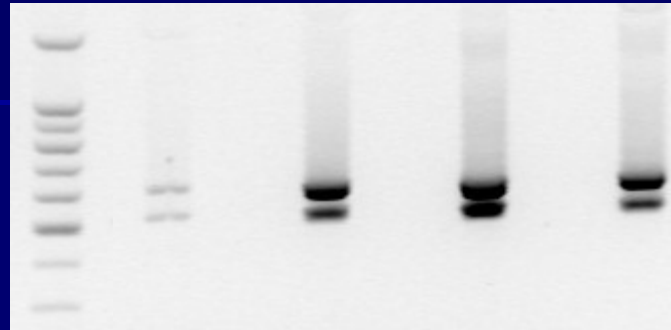
Immunolocalization in Transmission Electron Microscopy

Sporoblast
Sporoblast



EhSWP1 is localized in the exospore

Genotyping *E. hellem* isolates by analysis of the region encoding the C-terminal end of SWP1



EhD EhV213 EhV242 EhV257

- ✓ PCR amplification from genomic DNA : 2 bands for each isolate
- ✓ By RT-PCR, 2 transcripts are obtained for each isolate

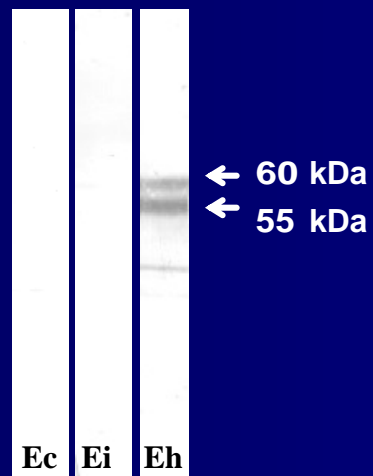
1. Intra-isolate variability

Eh-Didier-s	TTASGDNNSGSTGGDDTGDEGDGEEEEETPEEEEGDEEEEDP-----
Eh-Didier-l	TTASGDNNSGSTGGDDTGDEGDGEEEEESPEEEESRKQRSIQPKKGLRNKRQTLPVYP
	*****:***** ..:..
Eh-Didier-s	-----VTALLNTIKEEGEKLGAAMFEVMTAIKEQGTQGAEAVQEMMDAVKNEADT
Eh-Didier-l	DRESPKAILTALLNTIKEEGEKLGAAMKEVMDAVKSEADTKKTETVEDILNKVKDEVDT
	:*****:*** *:*. . *: :*:*:*:*:*:*:*:*:*:*:*:*
Eh-Didier-s	KKTETVDEILSKVKDEADTKKTETVTEIMDKVKTEADTKKTDVTEIMEKAKETWEAGKS
Eh-Didier-l	KKTETVTEIMDKVKTEADGKKADTVTEIMNEVKTEGETEVDKIVKEFIEEAKTAYEKE-
	***** **:*.*** ** **:*:*****:*****:*. . *.*:*:*:*:*:*:*:*
Eh-Didier-s	S
Eh-Didier-l	-

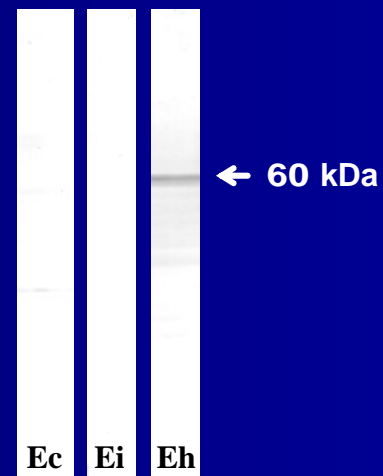
54.7% of identity

The 60 kDa band is recognized by Ab raised against the 26 AA insertion

Anti-EhSWP1C



Anti-EhSWP1C (26 AA)



2. Inter-isolate variability

EhV242-S	TTASGDNNSGSTGGDDTGDEGDGGEETPEEEEGDEEEEDPVTALLNTIKEEGEKLGA
EhV257-S	TTASGDNNSGSTGGDDTGDEGDGGEETPEEEEGDEEEEDPVTALLNTIKEEGEKLGA
Eh-Didier-S	TTASGDNNSGSTGGDDTGDEGDGGEETPEEEEGDEEEEDPVTALLNTIKEEGEKLGA
EhV213-S	TTASGDNNSGSTGGDDTGGE---GEEEDGGEEESPEEEEDLLTALLNTIKEEGEKLGA

EhV242-S	AMEEVMTAIKEQGTQGAEAVQEMMDAVKNEADTKKETVDEILSKVKDEADTKKETVT
EhV257-S	AMEEVMTAIKEQGTQGAEAVQEMMDAVKNEADTKKETVDEILSKVKDEADTKKETVT
Eh-Didier-S	AMEEVMTAIKEQGTQGAEAVQEMMDAVKNEADTKKETVDEILSKVKDEADTKKETVT
EhV213-S	AMEEVMTAIKEQGTAQGTEAVEEVMKAVKNEADTKKTTAQEIMDAVKNEVDTKKTTAQ

EhV242-S	EIMDKVKTEADTKKTDTVTEIMEKAKETWEAGKSS
EhV257-S	EIMDKVKTEADTKKTDTVTEIMEKAKETWEAGKSS
Eh-Didier-S	EIMDKVKTEADTKKTDTVTEIMEKAKETWEAGKSS
EhV213-S	EIMDAVKTEAEGKKTETVTEIMNKAKETWGAGKSG

500 bp band

EhV242-L	TTASGDNNSGSTGGDDTGDEGD--GEEEEESPEEEEEESRKQRSIQPKKGLRNKRQTLPV
EhV257-L	TTASGDNNSGSTGGDDTGDEGD--GEEEEESPEEEEEESRKQRSIQPKKGLRNKRQTLPV
Eh-Didier-L	TTASGDNNSGSTGGDDTGDEGD--GEEEEESPEEEEEESRKQRSIQPKKGLRNKRQTLPV
EhV213-L	TTASGDNNSGSTGGDDTGDEGEEEDGGEEESPEEEEEESRKQRSIQPKKGLRNKRQTLPV

EhV242-L	YPDRESPKAILTALLNTIKEEGEKLGAAMKEVMDAVKSEADTKKTETVEDILNKVKDEV
EhV257-L	YPDRESPKAILTALLNTIKEEGEKLGAAMKEVMDAVKSEADTKKTETVEDILNKVKDEV
Eh-Didier-L	YPDRESPKAILTALLNTIKEEGEKLGAAMKEVMDAVKSEADTKKTETVEDILNKVKDEV
EhV213-L	YPDRESPKAILTALLNTIKEEGEKLGAAMKEVMDAVKSEADTKKTETVEDILNKVKDEV

EhV242-L	DTKKTETVTEIMDKVKTEADGKKADTVTEIMNEVKTEGETEVDKIVKEFIEEAKTAYEEKE
EhV257-L	DTKKTETVTEIMDKVKTEADGKKADTVTEIMNEVKTEGETEVDKIVKEFIEEAKTAYEEKE
Eh-Didier-L	DTKKTETVTEIMDKVKTEADGKKADTVTEIMNEVKTEGETEVDKIVKEFIEEAKTAYEEKE
EhV213-L	DTKKTETVTEIMDKVKTEADGKKADTVTEIMNEVKTEGETEVDKIVKEFIEEAKTAYEEKE

600 bp band

EhSWP1 is a good target for genotype analysis

Marker	E. hellem isolate			
	EhDidier	EhV242	EhV257	EhV213
ITS	1A	1A	1A	1A
SSU rRNA	1A	1A	1A	1A
PTP1	1A	1A	1A	1B
SWP1	1A	1A	1A	1B

SWP1 as PTP1 is more discriminating than ITS or SSU rDNA

In prospect

1. Southern blot analysis (2 genes?)
2. Use of EhC-terminal specific recombinant proteins in serodiagnostic studies
3. Involvement of SWP1 in cell invasion ?

Merci

EcSWP1	MMKLSLLLGLVFSFAVLASENRRGN-CQM C PEG-TRYFEKNNLLGRRFKNDVRKL C GDGM	58	
EiSWP1	MIKLSLLLGLASFTAVLANQRPR---CQR C PVSSSKYFQQNNLLGSRFQNEVQRL C ARRV	57	
EhSWP1	MLKLSLLLGLANFTAILAGGERRRSGLLQ C SANGSRYFREQNLLGIKYKDDVKRL C GERP * * ***** * ** * * * ***** ***** * * *	60	
EcSWP1	SSDVHDESSSHSSSSSDCKPRRRRHRRYEDSCSFGSSD C DDSSTYSSCVSSECSPPCRP	118	
EiSWP1	REESSSESSSSSSSE-DCSRRRRRPHREWEDSCSSSYSS C S-----STDCSSSAPCPPP	111	
EhSWP1	EGPHPSSESSSCSSSCSEECGRRRFPGIRDCEYSSWDAC C SS----SWDECTDSTPCTTP ***** ** *	116	
EcSWP1	VPLN C DYELKTPIIINMGERIFEF L KNYEDQYKKA V VLFLTRILSQIDGFAPSYPSADYEP	178	SWD1
EiSWP1	VAQR C DIELKTPIIILMGERIYEF L KNYEDQYKKA V LLFLTNILSQISGFNPVFPGGDYDA	171	
EhSWP1	TPLR C DAELRVPIINMGERIYEF L KNYEDQY E SA V TLAL N ILSQVSGFNPIFAGADYAA * **** ***** ***** ***** ***** * *	176	EhR1
EcSWP1	LIEQLETLGVTVPNSMAADLAALDAAEATSLAGTIRANAQKVIGDLLARVNTM C YLDLMS	238	
EiSWP1	LIEQLKTLGVTVPANTAAELAAIDAESSALTRAIQANAQVISEDLLTRVSAM C YLDIMS	231	
EhSWP1	LVEQLETLGVNVPANTAAELASIDSSESAALSRIIQGNAQKIIISDLFARVGS M CYSDITS ***** ***** ***** ***** ***** * * * ***** ***** *****	236	
EcSWP1	LVTSGLFASQVTSAFSNTQPIITIAGNDL F TKQMA V FQ R LPGTLPSAAITAITNALQANK	298	SWR1
EiSWP1	LVNSGLLASQVSSVFNNIQPIITITGNDL F AKQMA V FQ K IPGTLPSAAITAITNALQGNR	291	EhD1
EhSWP1	LINSGLFASQISSAFSNTQ P VITIAS N DLYAKQMA V FQ R IPGTLPAAAVTAITNALQTNR ***** ***** ***** ***** ***** ***** ***** ***** *	296	
EcSWP1	NNFVTFFTTQTTNLQTDVQNALTALITALTTLTSTTSTEF T QFANSEIGALTGRIFGSSG	358	
EiSWP1	TNFVTFFTTQTSNLQTSVQNSLTTLLISELEKLATDTETAFTAFANA E ISALISRIFPTST	351	
EhSWP1	NNFATFFTTQATTLQTNVQNILTALTTALTTLT N STSTEF T TFANSEIAALAA R IFPATT ***** ***** * * * ***** ***** ***** ***** * * * * * *	356	
EcSWP1	SGSGSGSGSGSGSGSDGSGSGSGSGSGSGSDGSGSGSGSGSGSGSGSDGSGSGSGSGSGSG	418	
EiSWP1	TSSGSGD-----STGSGSTGDAGDAGDTGEDGGDDEGTEGTG-----	388	
EhSWP1	ASGDNNSGSTGGDDTGDEGDG G EE F TE P EE E EGDE E ED P VTALL N TIKEEGEKLGKAAM *	416	
EcSWP1	SSGSGSDGSG	450	
EiSWP1	-----	388	
EhSWP1	EEVMTAIKEQ G TTQ G AEAVQ E MMDA V KNEAD T KK T ET V DE I L S K V KDEAD T KK T ET V TE I	476	
EcSWP1	-----	450	
EiSWP1	-----	388	
EhSWP1	MDK V KTEAD T KK T DT V TE I MEK A K E T W E A G K S S	509	

Eh-Didier-s

TTASGDNNSGSTGGDDTGDEGDGEEEEETPEEEEGDEEEEDP-----

Eh-Didier-l

TTASGDNNSGSTGGDDTGDEGDGEEEEESPEEEESRKQRSIQPKKGLRNKRQTLPVYP
*****:***** ..:..

Eh-Didier-s

-----VTALLNTIKEEGEKLGAAMEEVMTAIKEQGTQGAEVQEMMDAVKNEADT

Eh-Didier-l

DRESPKAILTALLNTIKEEGEKLGAAMKEVMDAVKSEADTKKTETVEDILNKVKDEVDT
:*****:*** *:*. . *: :*:*:*:*:*:*:*:*:*:*:*:*:*.**

Eh-Didier-s

KKTETVDEILSKVKDEADTKKTETVTEIMDKVKTEADTKKTDVTEIMEKAKETWEAGKS

Eh-Didier-l

KKTETVTEIMDKVKTEADGKKADTVTEIMNEVKTEGETEVDKIVKEFIEEAKTAYEKE-
***** *:*.** ** *:*:*****:*****.:*:. *.*:*.** :*: :

Eh-Didier-s

S

Eh-Didier-l

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