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1 **Expression by *Lawsonia intracellularis* of type III secretion system components**
2 **during infection**

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21

22

23 **Abstract**

24 Contact-dependent secretion systems, such as the type III secretion system (T3SS), have
25 been shown to play significant roles in the pathogenicity of many Gram-negative
26 bacterial pathogens. *L. intracellularis* is a novel, obligate intracellular Gram-negative
27 bacterium, which has been identified as the etiological agent of proliferative
28 enteropathies in numerous animal species. Analysis of the genome sequence of the
29 *Lawsonia intracellularis* strain PHE/MN1-00 has revealed the presence of a T3SS
30 secretion system in this bacterium. In this study we aimed to determine whether this
31 important virulence mechanism is also present in our *L. intracellularis* strain
32 LR189/5/83. Using bacteria PCR-based approach, we verified the presence of a
33 genomic region encoding a T3SS. Specifically, a gene highly homologous to the *yscN*
34 energiser component of the prototypic T3SS of *Yersinia* spp. was identified and termed
35 *lscN*. Two further open reading frames (ORFs) contiguous with *lscN* were also
36 identified: *lscO* and *lscQ*, which are also homologues of ORFs within the T3SS of
37 *Yersinia* spp. To establish whether this T3SS may be functional, expression was
38 monitored directly by RT-PCR and indirectly by detection of serological responses in
39 vaccinated and infected animals. Transcripts for *lscN* and *lscQ* were detected and
40 purified rLscQ was recognized by antiserum from infected pigs, indicating expression
41 *in vivo* during infection. By analogy to other bacteria, this T3SS may be crucial for
42 intracellular development and is likely to play a significant role in the virulence of this
43 unusual pathogen.

44

45 **Keywords:** *Lawsonia intracellularis*; Obligate intracellular; Pig; Proliferative
46 enteropathy; Type III secretion system; Pathogenesis

47

48 **Introduction**

49 *Lawsonia intracellularis* is an obligate intracellular Gram-negative bacterium which
50 causes proliferative enteropathy (PE), a highly important disease in pigs. The disease is
51 characterised by proliferation of the small intestinal epithelium leading to hyperplasia
52 thereby causing poor feed conversion, weight loss and diarrhoea with fatalities
53 occurring in some animals (Smith and Lawson, 2001). Only rudimentary understanding
54 of the molecular basis of *L. intracellularis* physiology and pathogenicity has been
55 gained to date due to its genetic intractability.

56 Sequencing of the *Lawsonia intracellularis* genome has identified a T3SS with
57 similarity to that of *Yersinia* species as well as flagellar type III secretion system, which
58 is considered ancestral to T3SS (Hueck, 1998). Contact-dependent secretion systems
59 such as the type III system (T3SS) are found in many Gram-negative including
60 enteroinvasive pathogens such as *Shigella*, *Salmonella* and *Yersinia* (Hueck, 1998;
61 Coburn et al., 2007) as well as the other obligate intracellular bacteria such as
62 *Chlamydia/Chlamydophila* (Peters et al., 2007). This is a common secretion system
63 through which bacterial proteins (effectors) are translocated into the host cell thereby
64 dysregulating various cellular processes (Espinosa and Alfano, 2004; Cornelis, 2006).

65 In all of these pathogens, T3SS contribute essential functions to pathogenicity including
66 roles in cellular invasion, promotion or prohibition of apoptosis, and suppression of
67 innate defences. Consequently, we postulate that secretion systems such as T3SS could
68 play a significant role in the pathogenicity of *Lawsonia intracellularis*.

69 In this study we have detected for the first time that T3SS components of *L.*
70 *intracellularis* are expressed during infection. This is a very important development
71 towards achieving better understanding of the molecular mechanisms underlying the
72 pathogenicity and to further develop effective control measures for *L. intracellularis*.

73

74 Materials and Methods**75 Bacterial isolates and growth conditions**

76 Bacterial isolates and plasmids used in this study are listed in Table 1. *L. intracellularis*
77 isolates LR189/5/83, LI916/91 and LI963/93 were co-cultured as previously described
78 (Lawson et al., 1993) in IEC 18 cells at 37°C under microaerophilic conditions (8.8%
79 CO₂; 8.0% O₂). *Escherichia coli* TOP10 (Invitrogen) and AAEC189 strains (Blomfield
80 et al., 1991), used for maintaining recombinant plasmids, were routinely cultured on
81 Luria Bertani (LB) medium containing ampicillin (100 µg/ml). *E. coli* BL21 and
82 BL21(DE3)pLysS were host strains for expression of recombinant fusion proteins and
83 were grown on Luria-Bertani medium containing ampicillin (50 µg/ml) alone or in
84 combination with chloramphenicol (35 µg/ml).

85

86 Bacterial transformation procedures

87 *E. coli* TOP10 and AAEC189 chemically competent cells were transformed with 10 ng
88 plasmid DNA and selected with the appropriate antibiotics at a concentration of 100
89 µg/ml for ampicillin or 50 µg/ml for chloramphenicol. Transformants were confirmed
90 by plasmid isolation using the QIAprep Spin Miniprep Kit (Qiagen Valencia, CA) and
91 restriction endonuclease digestion with enzymes purchased from New England Biolabs
92 following standard protocols.

93

94 Screen for T3SS genes in *L. intracellularis* and genome walking

95 We designed degenerate primers based on the T3SS components which are highly
96 conserved in Gram-negative pathogens. DOP-PCR was performed as previously
97 described (Wren et al., 1992; McCluskey et al., 2002) with primers listed in Table 1 and

98 100 ng of *Lawsonia intracellularis* LR189/5/83 DNA. Amplified products were
99 analysed directly by agarose gel electrophoresis. We used the DNA Walking
100 SpeedUp™ kit (SeeGene, Del Mar, CA) to acquire sequences upstream and
101 downstream adjacent sequences to PCR-DOP-amplified T3SS sequences. This
102 approach confirmed the presence of a non-flagellar T3SS region in the *L. intracellularis*
103 LR189/5/83 and was verified in an independent isolate (strain PHE/MN1-00) when a
104 full genome sequence was made available (accession numbers AM_180252,
105 AM_180253, AM_180254, and AM_180255)

106

107 **Southern blotting**

108 Total genomic DNA from three different *L. intracellularis* isolates was digested with
109 restriction endonucleases EcoRI, HindIII, KpnI and PstI (New England Biolabs),
110 submitted to electrophoresis, then blotted onto nitrocellulose membranes (Hybond), and
111 probed with a digoxigenin (DIG; Boehringer Mannheim)-labeled fragment specific for
112 the *lscN* gene under low stringency conditions. After hybridisation, blots were washed
113 with SSC (0.15 M NaCl, 0.015 M sodium citrate) then developed following
114 manufacturer's protocols.

115

116 **DNA manipulation, cloning and sequencing**

117 Methods were carried out following standard protocols (Sambrook, 1989). PCR
118 products were cloned into pCR 4-TOPO (Invitrogen) for sequencing using an ABI
119 PRISM DYE Terminator Cycle Sequencing Kit (Perkin-Elmer) on a model 373A
120 Sequence System (Applied Biosystems). Sequence analyses and comparisons were
121 carried out using programs BLASTN, BLASTX
122 (<http://www.ncbi.nlm.nih.gov/BLAST/>), InterProScan (Apweiler et al., 2001), and

123 CLUSTALW (Larkin et al., 2007). A partial gene library of *L. intracellularis* isolate
124 LR189/5/83 was constructed from agarose gel-purified EcoRI-HindIII digested
125 fragments cloned into vector pUC18. Recombinant plasmids were transformed into *E.*
126 *coli* TOP10 cells (Invitrogen), and positive clones were screened by colony blotting
127 using a 567 bp long fragment of the *lscN* gene DIG-labeled as a probe. Plasmids were
128 prepared from positive clones with the QIAprep Spin Mini Kit following
129 manufacturer's protocols. The same probe was used to screen total DNA from *L.*
130 *intracellularis*, extracted by means of a QIAGEN DNA tissue kit, on a dot blot. T4
131 DNA ligase and alkaline phosphatase, were purchased from New England Biolabs and
132 used following manufacturer's protocols.

133

134 **RNA methods**

135 Total RNA was extracted following QIAGEN protocols from *in vitro* grown *L.*
136 *intracellularis* LR189/5/83 cultures, and pig intestine samples submitted to the Scottish
137 Agricultural College, Veterinary Sciences Division, for routine diagnostic procedures
138 and previously confirmed as positive for *L. Intracellularis* by PCR for 16S rDNA. The
139 resulting RNA was thoroughly digested with RNase-free DNase (QIAGEN) for 15 min
140 at 37°C to exclude genomic DNA contamination. Random hexamer primers were used
141 to reverse transcribe RNA. Ten microliters of the cDNA was then used for the PCRs
142 with primers *lscNF-lscNR* (Table 1) for *lscN* transcripts. Nested PCR was performed
143 with primers *lscNNF-lscNNR* for the nested step using the PCR product from the first
144 round of amplification as template. PCRs were carried out under standard conditions
145 with Taq polymerase, and products were analyzed using ethidium bromide stained
146 agarose gels.

147

148 Expression and purification of recombinant LscQ protein

149 Using primers listed in Table 1, we amplified by PCR a fragment of the *L.*
150 *intracellularis* genome corresponding to the *lscQ* gene. PCR products were digested
151 using KpnI-EcoRI restriction enzymes (Promega) to construct an in-frame clone in the
152 pRSETA vector (Invitrogen). This clone was designated pMPAV6 (Table 1) and used
153 to transform *E. coli* BL21 (DE3) pLysS cells. The expression product was purified after
154 induction from 50 ml of Luria Broth containing ampicillin (50 µg/ml) and
155 chloramphenicol (35 µg/ml) that was inoculated with 5 ml of an overnight culture of
156 freshly transformed expression isolate and incubated at 37°C with vigorous shaking.
157 Fusion protein carrying an N-terminal six-histidine (6xHis) tag was purified from the
158 whole-cell lysates using a nickel column (Probond Purification System, Invitrogen)
159 under denaturing conditions following manufacturer's instructions. The protein content
160 of the elution fraction was analysed by SDS-PAGE as previously described (Laemmli,
161 1970). Expression of rLscQ in *E. coli* resulted in a protein of approximately 26 kDa and
162 the identity of rLscQ was confirmed by mass spectrometry at Moredun Proteomics
163 Facility.

164

165 Immunoblotting

166 The antigen, recombinant LscQ–His fusion protein (rLscQ), purified from *E. coli* BL21
167 (DE3) pLysS, was electrophoresed on a 12.5% acrylamide gel over the whole gel width
168 and then transferred to nitrocellulose membranes using an Ancos Semidry Blotter.
169 Molecular size standards were included routinely on gels. Membranes were incubated
170 with primary antibody (pig serum samples dilution optimised at 1:5000), washed with
171 PBS-0.3% Tween 20, and then incubated with the secondary HRP conjugated anti-
172 species specific antibody (Sigma) at a 1:10000 dilution. After further washes, the

173 membranes were developed using ECL (Amersham), X-rayed and scanned. Sera from
174 *L. intracellularis* vaccinated, infected and uninfected pigs (kindly donated by
175 Boehringer Ingelheim) were used, serological status of sera were established by the
176 providers using BioScreen ELISA prior to dispatch to our laboratories.

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180 **Results**

181 **Identification of *L. intracellularis* T3SS components**

182 The PCR-DOP and chromosome walking approach identified approximately 2kb of
183 sequence comprising three open reading frames showing significant identity to three
184 T3SS gene products (Hueck, 1998). The subsequent availability of the complete
185 genome for another *L. intracellularis* isolate provided verification and the sequence of
186 this region was >98% identical between these two isolates of disparate origins.
187 Alignment of deduced amino acid sequences of gene products showed closest similarity
188 for each gene product with orthologues in *Desulfovibrio* species; in *D. vulgaris* these
189 proteins are termed DvATPase (shown in Fig. 1), DvYscO and DvYscQ respectively,
190 with identities of approximately 82%, 30% and 24%. These belong to the *Yersinia*
191 T3SS family thus we have designated these *L. intracellularis* genes as *lscN*, *lscO* and
192 *lscQ* following the nomenclature in use for T3SS genes in *Yersinia* spp, *lsc* indicating *L.*
193 *intracellularis* secretion component.

194 To confirm the presence of the *lsc* region in different isolates of *L. intracellularis*, we
195 used primers based on the LR189/5/83 sequence (Table 1), which resulted in
196 amplification of the desired products from isolates LR189/5/83, LI916/91 and LI963/93
197 (data not shown). For confirmation, Southern hybridisation of chromosomal DNA with
198 a probe containing regions of *lscN* and *lscO* (see below) of *L. intracellularis* was used
199 to screen total DNA from *L. intracellularis* isolates LR189/5/83, LI916/91 and
200 LI963/93 digested with endonuclease EcoRI, HindIII, KpnI and PstI. Southern
201 hybridisation revealed a strong signal with the *lscN-lscO* probe, thus confirming the
202 genes are present in three representative *L. intracellularis* isolates (data not shown).

203

204

205 **Transcription of T3SS component *in vivo***

206 Transcription of *L. intracellularis* T3SS components *lscN* and *lscQ* was detected *in*
207 *vitro* using *L. intracellularis* LR189/5/83 cultures (not shown). To determine whether
208 the *L. intracellularis* *lscN* locus was also transcribed during infection *in vivo*, RT-PCR
209 was performed on total RNA extracted from *L. intracellularis*-infected pig intestinal
210 tissue. To confirm the presence of *L. intracellularis*, RNA was extracted from *L.*
211 *intracellularis*-infected tissues and used as a template in RT-PCR with 16S rDNA
212 specific primers. RT-PCR with *lscN* and *lscQ* primers revealed products of the
213 predicted size (Fig. 2) indicating transcription of these genes. No products were
214 detected in controls, which were not reverse transcribed (representative control shown).
215

216 **Antibody response of naturally infected and vaccinated animals against rLscQ of**
217 **the *L. intracellularis* T3SS**

218 As a correlate of expression in animals during infection and to assess immunogenicity,
219 seroreactivity to purified, rLscQ was monitored. rLscQ was blotted and probed with
220 serum from vaccinated, naturally-infected and uninfected animals in immunoblotting
221 studies. Sera from five of the nine pigs known to be infected with *L. intracellularis*
222 showed strong recognition (Fig. 3). All five sera from pigs vaccinated with Enterisol
223 Ileitis™ (Boehringer Ingelheim) reacted strongly with rLscQ whereas none of the five
224 sera from animals confirmed as seronegative reacted.

225

226

227 **Discussion**

228 T3SSs perform pivotal roles in the pathogenicity of many Gram-negative bacteria
229 including enteroinvasive pathogens such as *Salmonella* spp. (Ochman et al., 1996),
230 *Shigella* spp. (Buchrieser et al., 2000) and *Yersinia* spp. (Cornelis and Wolf-Watz,
231 1997). Furthermore, T3SSs are important components in the pathogenicity of invasive,
232 obligately intracellular pathogens of the Family *Chlamydiaceae* (Peters et al., 2007).
233 T3SSs secrete bacterial effector proteins into the intracellular milieu of host cells to
234 alter cellular functions such as phagocytosis/endocytosis, inflammation and apoptosis
235 (Tran Van Nhieu et al., 2000; Espinosa and Alfano, 2004; Guiney and Lesnick, 2005),
236 thus promoting bacterial pathogenicity. The presence of a T3SS in the novel bacterium
237 *L. intracellularis* suggests a role in pathogenicity although much further investigation
238 will be required to define what role, if any, this T3SS plays.

239 Typically, T3SSs are present on pathogenicity islands that may comprise 30 or more
240 genes grouped into operons encoding functionally distinct elements: basal apparatus,
241 “needle” complex (secretory apparatus) and effector proteins (Hueck, 1998) plus
242 accessory components such as regulators and chaperones. Proteins of the basal
243 apparatus are highly conserved among diverse bacterial genera and genes representing
244 these were detected in three different *L. intracellularis* isolates. Specifically, we
245 detected genes designated as orthologous to *lscN*, *lscO* and *lscQ* which represent
246 energizing, core element and structural components of the T3SS respectively.

247 Unsurprisingly, highest identity was apparent with orthologues of *D. vulgaris*, the
248 nearest relative of *L. intracellularis* in which T3SS is present (Heidelberg et al., 2004).
249 LscN/YscN proteins are associated with the bacterial cytoplasmic membrane
250 functioning as ATPases which energise secretion and essential for the secretion
251 (Eichelberg et al., 1994; Woestyn et al., 1994; Kubori et al., 1998; Tamano et al., 2000)

252 and virulence (Tomich et al., 2003). Genes *lscO* and *lscQ* also encode orthologues of
253 key components of the T3SS export machinery. Firstly, *lscO* (homologous to *yscO* of
254 *Yersinia* spp.) is required for high level expression and secretion of effectors (Payne and
255 Straley, 1998) and secondly, *lscQ* is homologous to *yscQ*, which encodes a structural
256 component of the T3SS apparatus (Hueck, 1998; Cornelis, 2006). The genome sequence
257 of *L. intracellularis* PHE/MN1-00 carries a second copy of *lscQ* within a second T3SS
258 locus (showing approximately 25% identity at the amino acid level). Whether these are
259 expressed concurrently and whether they confer similar, overlapping or distinct
260 functions remains to be established. The gene order is conserved between *L.*
261 *intracellularis* (*lscNOQ*) and *Yersinia* spp. (*yscNOPQ*) although the former lacks a
262 homologue of *yscP* in this contiguous region. Even though *lscP* is not contiguous with
263 *lscNOQ*, its gene product LscP shows sequence similarity to YscP, another T3SS
264 component required for secretion (Journet et al., 2003; Stainier et al., 2000). The *L.*
265 *intracellularis* PHE/MN1-00 genome sequence shows that the *lscNOQ* T3SS locus is
266 incomplete and that a second T3SS region is located approximately 800 kbp distant.
267 Together these loci possess genes that encode an entire T3SS apparatus.
268 Sophisticated expression analyses are not routinely feasible in *L. intracellularis* since
269 there are no convenient methods available for genetic manipulation so we have studied
270 the genes encoding basal (*lscN*) and needle assembly (*lscO*, *lscQ*) components using
271 methods similar to those we have employed previously (McCluskey et al., 2002). Firstly
272 we employed RT-PCR to detect expression of *lscN* & *lscQ* using RNA isolated from
273 infected pig intestinal tissue selected from animals confirmed as either negative or
274 positive by immunohistochemistry and PCR for *L. intracellularis* 16S rDNA. Among
275 the known positive animals, products indicating expression of *lscN-lscQ* were detected
276 whilst no products were obtained from PCR-negative animals. The detection of

277 transcript in infected animals is a not only strong indicator that T3SS is expressed
278 during infection but also that T3SS is conserved in different field isolates of *L.*
279 *intracellularis*. To further corroborate T3SS expression, recognition of purified rLscQ
280 by pig sera was assessed. Sera from all vaccinated animals and the majority of pigs
281 naturally infected with *L. intracellularis* showed strong recognition of rLscQ whilst
282 none of the uninfected animals did so. Thus LscQ (a key T3SS component) is expressed
283 by *L. intracellularis* in animals previously exposed to *L. intracellularis*. As with the
284 transcript monitoring, this survey of field material has indicated that different animals
285 recognise this T3SS component, indicating consistent presence and expression of this
286 determinant among *L. intracellularis* isolates. Since LscN, LscO and LscQ represent
287 major structural components of bacterial T3SS, the confirmation of their expression is
288 highly suggestive that T3SS is functional in *L. intracellularis* and is thus likely to play a
289 significant role in pathogenesis.

290

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300

301

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414 Table 1. Oligonucleotide primers, bacterial isolates and plasmids used in this study.

Target	Primer	Sequence	
<i>L. intracellularis lscN</i>	yli1	5' -CCTTGAGGTGAGTGAATTGAG	
	yli1R	5' -TAGCATCATCCGCAGCAGGATCAT	
	YscNF	5' -TCTGGAGCAGAAGGTTTAA	
	YscNR1	5' -TGCTGCAGCTAACTTCCTTG	
	YscNR2	5' -ATACAACAAGTACAGACGCT	
	YscNR3	5' -TGCTGTATAAGCTGATTTAA	
	TSPF1	5' -AGCGAGCAGGAAACTCAGAT	
	TSPF2	5' -GACAGAGCCAATTGCTGACGAAA	
	TSPF3	5' -CAAGGAAGTTAGCTGCAGCAAATC	
	TSPR1	5' -CCTATTTCACGTAATGCACGA	
	TSPR2	5' -GTCGCTCCATAGAAGAACGTCAG	
	TSPR3	5' -CTCTGCTCCCAAGATCATGCTC	
	*DW-ACP1 TM	5' -ACP-AGGTC	
	*DW-ACP2 TM	5' -ACP-TGGTC	
	*DW-ACP3 TM	5' -ACP-GGGTC	
	*DW-ACP4 TM	5' -ACP-CGGTC	
	*DW-ACPN	5' -ACP-NGGTC	
	*Uni-primer	5' -TCACAGAAGTATGCCAAGCGA	
	lscNF	5' -TGAAATAGGCTTGCACTGAG	
	lscNNF	5' -CATCTAGGCCCTGCTGTAAAAA	
	lscNR	5' -GTTCCACCTTCTGTCTTTTCT	
	lscNNR	5' -ATTTCCCTGCATTTCTTTAT	
	<i>L. intracellularis lscQ</i>	yli3	5' -GAGCTATTAAGAATCTTACAGAAT
		yli3R	5' -AGTCCCGTATATGATTTATTCTCT
		TSPF4	5' -TCCAGCTTATCTTCTGTAAGAT
		TSPF5	5' -TGCCCCATTAAGCATAAGTTACAGA
		TSPF6	5' -GAACCATTAGTAGATTATCAGTTTGGATG
TSPR4		5' -GCTAAAATACAAACATCAGCAGTTGTG	
TSPR5		5' -TTCTCCAAGTCCATTAATACACGAC	
<i>L. intracellularis</i> 16S rRNA		16SF	5' -CGCCGCGTGAGGGATGAA
	16SR	5' -CACGGCACGAGCTGACGACA	
	16SNF	5' -ACGGTACCCCGAGAGGAAGAACAC	
	16SNR	5' -AGCGTACGGCACCGAAGATAACTC	
pRSETA	lscQK	5' -CGGGTACCAGATGTCTAATCTGGGG	
	lscQE	5' -CGGAATTCTTAGTCCCGTATATGATT	
Isolate or plasmid	Description	Source or reference	
pRSETA		Invitrogen	
pMPAV6	pRSETA::lscQ	This study	
TOP10	<i>E. coli</i> cloning isolate	Invitrogen	
AAEC189	<i>E. coli</i> cloning isolate, $\Delta lac recA endA$	(3)	
LR189/83	<i>L. intracellularis</i> isolate	UK isolate	
LI916/91	<i>L. intracellularis</i> isolate	UK isolate	
LI963/93	<i>L. intracellularis</i> isolate	UK isolate	

415 * primers used for DOP-PCR analysis

416 **Figure legends**

417

418 **Fig.1**

419 **Amino acid sequence alignment of the predicted *L. intracellularis* LscN gene**
420 **product with its *D. vulgaris* orthologue using CLUSTALW**

421 A. LiLscN, *L. intracellularis* PHE MN-01 LscN; DvATPase, *D. vulgaris*, Type III
422 secretion system ATPase. "*" indicates amino acid identity; ":" indicates residue
423 substitutions are conserved; "." indicates semi-conserved substitutions. We compared *L.*
424 *intracellularis* LR189/5/83 UK isolate LscN sequence to that of the publicly available
425 PHE/MN1-00 US isolate, and since they appeared to be more than 99% identical it was
426 not included in these alignments.

427

428 **Fig. 2**

429 **Transcriptional analysis of *L. intracellularis* 16S rRNA, *lscN* and *lscQ* during**
430 **infection *in vivo*.**

431 RNA was extracted from the intestine of pigs infected with *L. intracellularis* as
432 confirmed by PCR and immunohistochemistry. Products were separated by
433 electrophoresis through 1% agarose gels and stained with ethidium bromide. A & B
434 Transcriptional analysis of *L. intracellularis* 16S rRNA (A) and *lscN* (B) carried out
435 using RNA isolated from 15 infected animals. C. Transcription of *lscQ* was assessed on
436 a separate occasion using RNA from 13 animals (lanes 1-13); lane 14 of gel (C)
437 represents an RT-PCR reaction without reverse transcriptase (negative control) and lane
438 15 of gel (C) is an RT-PCR reaction containing reverse transcriptase and RNA from an
439 LR189/5/83 *in vitro* infected intestinal epithelial cell (positive control).

440

441

442 **Fig. 3**

443 **Reactivity of rLscQ with sera from infected animals.**

444 Lanes 1 to 5 and 11: sera from seronegative pigs; lanes 6 to 10: sera from pigs
445 vaccinated with Enterosol Ileitis™ (Boehringer Ingelheim); Lanes 12 to 19: sera from
446 naturally-infected pigs. Sera from infected and vaccinated animals reacted specifically
447 with rLscQ, whereas sera from uninfected animals did not. Induction of specific
448 antibodies indicates that LscQ was expressed during infection with *L. intracellularis*.

```

LiLscN      MALEYIASLLEEAVQNTSPVEVRGRVEQVIGTIIIRAVVPGVKIGELCELRNPWDNWTLQA 60
DvATPase    MAFEYIGPLLEEAVNSGPSVEVRGRVEQVVGTTIIIRAVVPGVKVGECLLRNPWDDWNLRA 60
            **:***.*****:..*****:*****:*** ***:*. *.*
LiLscN      EVVGFVKHVALLTPLGDLQGISPSTEVIPTGMVHAI PVGRELLGRVNLGLGEPIDGKAPL 120
DvATPase    EVVGFVKHVALLTPLGNLQGISPATEVIPTGEILSI PVGEDLLGRVLDGLGDPIDGGPPL 120
            *****:*****:***** : :***.:*****:***:*** .**
LiLscN      RLRTYYPVYADPPNPLRRIIEKPISLGLRVLGDILTCEGQRMGIFAAAGGKSTLLSS 180
DvATPase    KPRTRYYPVYADPPNPMTRRIIDRPISLGLRVLGDVLTCEGQRMGIFAAAGGKSTLLSS 180
            : ** ***** ***: :*****:*****:*****
LiLscN      IIKGTADVCILALIGERGREFIEHDLGAEGLKRSVLVVSTSDRSSMERLKSAYTAT 240
DvATPase    IIKGSADVCVLALIGERGREFIEHDLGPEGRKAVLVVSTSDRSSMERLKAAYTAT 240
            **** :***:*****:***** . ** *.:*****:*****:*****
LiLscN      AIAEYFRDQGKKVLLMDSVTRFGRALREI GLASGEPPTRRGFPPSVFSSLPKLMERAGN 300
DvATPase    AIAEYFRDQRRSVLLMDSVTRFGRQREI GLAAGEPPTRRGFPPSVFSTLPRLMERAGN 300
            ***** :.***:***** *****:*****:*****.***.*****
LiLscN      SDKGSITALYTVLVEGDDMTEPIADETRSILDGHIILSRKLAANHYPADVVLASVSRVM 360
DvATPase    SDRGSITALYTVLVEGDDMTEPIADETRSILDGHIVLSRKLAANHYPADVQASVSRVM 360
            **:*****:*****:*****.*****:*****.*** ***:***
LiLscN      NAIVTKEHKENAGLRKYLAKYAEVELLVQI GEYKAGNDPAADDAIKHNKGINAFLQOGL 420
DvATPase    NAIVGKEHGAAQKLRKILAKYAEVELLVQI GEYKGSDEADDALARVGAVNAFLRQGL 420
            **** ** * **** ***** *.* ***: : .:***:***
LiLscN      DEKSTFEQTLQALKKAVA 438
DvATPase    DERSTFDETLAALYKATE 438
            **:***:*. ** ** *.

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