The *Campylobacter jejuni* general glycosylation system is important for attachment to human epithelial cells and in the colonization of chicks

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It has recently been shown that the enteropathogen *Campylobacter jejuni* has an *N*-linked general protein glycosylation pathway (Pgl) that modifies many of the organism’s proteins. To determine the role of the *N*-linked general glycosylation in *C. jejuni*, the authors studied the *pglH* gene, which shows high similarity to a family of sugar transferases. *pglH* mutants were constructed in strains 81116 and 11168H. Both mutants were shown to be deficient in their ability to glycosylate a number of *C. jejuni* proteins, but their lipooligosaccharide and capsule were unaffected. The *pglH* mutants had significantly reduced ability to adhere to and invade human epithelial Caco-2 cells. Additionally, the 81116 *pglH* mutant was severely affected in its ability to colonize chicks. These results suggest that glycosylation is important for the attachment of *C. jejuni* to human and chicken host cells and imply a role for glycoproteins in the pathogenesis of *C. jejuni*.

INTRODUCTION

*Campylobacter jejuni* is the major cause of gastrointestinal diseases in many countries (Bereswill & Kist, 2003). A number of virulence-related properties of this important pathogen, including motility, attachment, colonization and invasion, have been described (Wassenaar & Blaser, 1999). Bacterial cell attachment is known to be assisted by a fibronectin-binding protein (Konkel et al., 1987), flagella (Newell et al., 1985), PEB1 (Pei et al., 1998) and the lipoprotein JlpA (Jin et al., 2001). However, the mechanism of binding and the nature of the host receptors involved in such attachment remain unknown. A distinctive feature of *C. jejuni* is that many proteins, including the flagellum, are glycosylated (Szymanski et al., 2003a).

The genome sequence of *C. jejuni* NCTC 11168 has revealed a number of genes that may be important in the biosynthesis and transport of polysaccharide structures (Parkhill et al., 2000). One locus was found to be dedicated to the formation of a polysaccharide capsule (Karlyshev et al., 2000), while other gene clusters are involved in lipooligosaccharide (LOS) biosynthesis (Fry et al., 1998; Linton et al., 2000a; Wood et al., 1999). In *C. jejuni* strain 81116 a locus comprising 11 genes related to sugar biosynthesis and transport, designated *wlaB* through to *wlaM*, has been cloned and sequenced (Fry et al., 1998). It was suggested that the genes from this locus were involved in both LOS and O-antigen biosynthesis, as insertional inactivation of *orfF* (or *wlaK*) and deletional inactivation of *orfA–orfF* genes (equivalent to *cj1120–cj1126* in NCTC 11168, or *wlaF–wlaK* in strain 81116) resulted in altered LOS immunoreactivity (Wood et al., 1999). In contrast, mutations in the genes *wlaF–wlaL*, named *pglA–pglF*, had no effect on the LOS in *C. jejuni* 81-176, but resulted in reduced levels of protein glycosylation (Szymanski et al., 1999, 2002). The *pgl* gene cluster was found to be fully functional when transferred into *Escherichia coli* as monitored by glycosylation of a co-expressed protein, AcrA (Wacker et al., 2002). These genes were found to be involved in *N*-linked protein glycosylation, in contrast to some other genes, including *ptmA* and *ptmb*, that are involved in *O*-linked glycosylation of flagella (Guerry et al., 1996; Szymanski et al., 2003a).

The biological significance of protein glycosylation in *C. jejuni* remains unclear. Until recently this post-translational modification was considered uncommon in prokaryotes (Messner, 1997; Moens & Vanderleyden, 1997) but it is now recognized in many archaea and bacteria (Schaffer et al., 2001). It is hypothesized that some bacterial glycoproteins may interact with host cell receptors, potentially...
generating mechanisms of adherence (Jennings et al., 1998; Marceau & Nassif, 1999; Marceau et al., 1998). In this report we demonstrate that the pglH gene is involved in protein glycosylation. No effect on LOS or capsular polysaccharide (CPS) was detected in pglH::kan<sup>r</sup> mutants. However, these mutants revealed significant reductions in both adhesion and colonization. These results suggest that C. jejuni glycoproteins may act as adhesins promoting the colonization process.

**METHODS**

**Strains and growth conditions.** The following C. jejuni strains were used: 11168H (HS:2), which is a hypermotile derivative of strain NCTC 11168 (Karlyshev et al., 2001), and 81116 (NCTC 11828, HS:6). E. coli strain XL2-MRF® (Stratagene) was used in cloning experiments. C. jejuni was grown in microaerobic conditions in an incubator (85% N<sub>2</sub>/10% CO<sub>2</sub>/5% O<sub>2</sub>) for 2 days at 37°C, on Columbia base agar (Oxoid) supplemented with 5% horse blood. E. coli was grown on Luria–Bertani agar. Where necessary, kanamycin or ampicillin was added to a concentration of 50 μg ml<sup>-1</sup> or 100 μg ml<sup>-1</sup> respectively.

**Construction of pglH::kan<sup>r</sup> mutants.** Plasmid cam169d1, from the NCTC 11168 genomic sequencing library, was used as a source of the pglH gene. The plasmid contains a 2037 bp insert comprising 61% of wlaB and 91% of pglH. A unique MluI site was used for insertion of a kan<sup>r</sup> cassette consisting of a blunt-ended BamHI fragment of pMK30 (van Vliet et al., 1998). Clones containing the kan<sup>r</sup> gene transcribed in the same direction as pglH were selected by PCR with kan<sup>r</sup>- and pglH-specific primers DL3 (5′-ACCCAGCG-GAACCATTGGAGG-3′) and AK2 (5′-GAGCCGGAATAAAGATTCTC-ACACGG-3′) respectively. The resultant recombinant plasmid cam169d1K was transformed into the appropriate C. jejuni strain by electroporation as described previously (Wassenaar et al., 1993a). Kanamycin-resistant colonies were selected and recombination was confirmed by PCR with kan<sup>r</sup>- and pglH-specific primers DL3 and AK62 (5′-GCAAGCTCTAATATCCCAAATAGCACC-3′) respectively.

**Complementation studies.** Insertional vector pRRC (A. V. Karlyshev and others, unpublished) was used for integration of the pglH gene into one of the three of rRNA gene clusters in the pglH::kan<sup>r</sup> mutant of 11168H. The vector contains a constitutively expressed cam<sup>r</sup> gene derived from plasmid pAV35 (van Vliet et al., 1999) with a unique Xhol site located immediately downstream from the antibiotic-resistance gene. The pglH gene was PCR amplified using primers AK248 (5′-GCTCTAGACTTAAAGGAGAAATGA-GCTCTAGACTTAAAGGAGAAATGA-3′) (dir) and AK249 (5′-GCTCTAGATCATTAG-GCTCTAGATCATTAG-3′) (rev). The AK249 primer included an sd sequence. Plasmid pRPGHLH generated by cloning of the Xhol-digested PCR product into the Xhol site of pRRC vector in a proper orientation was used in natural transformation of 11168H/pglH::kan<sup>r</sup> mutant and Kan<sup>r</sup> Cam<sup>r</sup> clones were selected. Integration of cam<sup>r</sup>-pglH fusion into the rRNA gene cluster via allelic replacement was confirmed by PCR using a cam<sup>r</sup>-specific primer AK237 (5′-TCTCTGAACCTTCTCATGCAGTGG-3′) and primers AK233 (5′-GCAAGATGGTTTGTATGTGAGC-3′), AK234 (5′-GAATTTCCGGAAGTTATCTCCG-3′) and AK235 (5′-GTG-CGGAATGTTTGTGCTG-3′) complementary to three potential integration sites. Extracts from the transformants were analysed by Western blotting and tested in attachment and invasion assays as described in the respective sections.

**Glycolipid analysis.** Glycolipid fractions, containing both LOS and CPS, were prepared by solubilizing bacteria from 2-day blood agar plates in 100 μl lysis buffer containing 31-25 mM Tris/HCl (pH 6-8), 4% SDS, 0-025% bromophenol blue and 20% (v/v) glycerol. Samples were heated at 100°C for 5 min followed by proteinase K treatment (100 μg ml<sup>-1</sup>) at 50°C for 1 h. The samples were analysed by electrophoresis on Tricine-buffered 12.5% polyacrylamide gels, which were stained with either Alcan Blue dye or silver, or alternatively Western blotted onto PVDF membranes (Millipore) as described previously (Karlyshev & Wren, 2001). For high-resolution analysis of LOS, Tricine-buffered 16% polyacrylamide gel and longer electrophoresis time were used (4 h instead of 1 h in the case of 12.5% gels) followed by silver staining (Tsai & Frasch, 1982). Blots were probed with Penner O:6 typing antiserum (1:100 dilution) followed by treatment with anti-rabbit IgG peroxidase conjugate (Sigma, 1:1000 dilution) as a secondary antibody. All antibody dilutions were made using TBST buffer (Tris-buffered saline, containing 0-01% Tween 20) supplemented with BSA (Sigma, 1%). Prestained protein markers (New England Biolabs) were used. The blots were developed using the dianinobenzidine staining kit with nickel enhancement according to the manufacturer’s instructions (Vector Laboratories).

**Protein analysis and lectin blotting.** Bacteria were resuspended in sample buffer, incubated at 100°C for 10 min and the lysate was analysed by SDS-PAGE in 12.5% precast polyacrylamide gels (Invitrogen). Gels were blotted onto PVDF membrane (Millipore), blocked in phosphate buffered saline (PBS) containing 0.5% Tween 20 (PBST) for at least 30 min and incubated with biotinylated soybean agglutinin (SBA) (Vector Laboratories) at a concentration of 10–20 μg ml<sup>-1</sup> in PBST for 1 h. Following three brief washes in PBST, blots were incubated in Extravidin peroxidase (Sigma) diluted 1 in 1000 in PBST for 30 min. Following a further three brief washes in PBST, blots were developed using the DAB staining kit with nickel enhancement according to the manufacturer’s instructions (Vector Laboratories). Broad-range molecular mass standards from New England Biolabs were used.

**Adhesion and invasion assays.** C. jejuni 81116, and the isogenic 81116 pglH::kan<sup>r</sup> mutant, were grown in Mueller–Hinton broth and on Mueller–Hinton agar (Oxoid) under microaerophilic conditions (10% O<sub>2</sub>/5% CO<sub>2</sub>/85%N<sub>2</sub>) in a variable-atmosphere incubator (VAIN; Don Whitley) at 37°C. Human colon cancer cells (Caco-2) were stored in liquid nitrogen and cultivated in minimal essential medium (MEM; Merck) with 10% heat-inactivated fetal bovine serum (Gibco), 0.2 mM l-glutamine, 0.1 mM non-essential amino acids and 1 mM sodium pyruvate. Monolayers were split and grown until confluence and differentiation, which was indicated by the presence of microvilli on the cell surface – approximately 20 days post confluence. Microvilli were detected by scanning electron microscopy. To a confluent monolayer of approximately 10<sup>6</sup> epithelial cells per well of a six-well plate, bacteria were added at an m.o.i. of either 10 or 100 bacteria to one epithelial cell. Infected monolayers were incubated, for 3 h at 37°C in a 5% CO<sub>2</sub>/95% air atmosphere, to allow adhesion and invasion to occur. The monolayers were then washed three times with sterile PBS and incubated for 2 h with MEM containing 200 μg gentamicin ml<sup>-1</sup> to kill extracellular bacteria, enabling enumeration of internalized bacteria. Other monolayers had MEM without gentamicin added. These control wells gave the total numbers of C. jejuni both adhering to and invading the epithelial cell monolayers. After this period the monolayer was washed as described above and lysed with 0.1% Triton X-100 in PBS for 15 min at room temperature on an orbital shaker. Following serial dilution in PBS, adherent and invaded bacteria were enumerated by colony counting on MH agar (Oxoid) cultured under microaerophilic conditions. Each assay was performed simultaneously in three separate wells. The assays were triplicates in a single assay repeated on three independent occasions on different days. Results are presented as the mean ± standard error.
Colonization studies using a chick colonization model. The colonization potential of the 81116 pglH::kan' mutant was tested in an orally dosed, 1-day-old chick model as previously described (Wassenaar et al., 1993b). Briefly, groups of ten 1-day-old white Leghorn chicks, housed in isolators, were orally dosed with a suspension containing between 10^2 and 10^5 c.f.u. Two experiments were performed. In the first experiment, doses of 10^2 and 10^4 c.f.u. were administered. In the second experiment the doses were 10^5, 10^7 and 10^9 c.f.u. The dose-response of the parent strain controls of the parent strain 81116 were administered at doses of 10^2 and 10^8 c.f.u. The birds were killed 5 days post-infection and the bacterial counts per g caecal contents determined. In each experiment controls of the parent strain 81116 were administered at doses of 10^5 and 10^7 c.f.u. The dose-response of the parent strain 81116 has been previously described (Wassenaar et al., 1993b).

RESULTS

The pgl locus

The pglH gene (cj1129) is a part of a gene cluster containing other sugar-biosynthesis-related genes (Fig. 1), consisting of cj1131 (annotated as galE) and cj1119–cj1130, which correspond to the wlaM–wlaB genes according to the nomenclature reported by Fry et al. (1998). cj1122 (wlaJ) is absent from strain 81116. The genes from this locus in strain 81116 show significant similarities to polysaccharide-biosynthesis-related genes and were found to be involved in LOS biosynthesis (Fry et al., 1998). However, the same genes were also reported to be essential for protein glycosylation (Linton et al., 2002; Szymanski et al., 2003a, b). The dual nomenclature of genes in this region (wla or pgl) reflects the controversy as to their function in C. jejuni. Since our findings showed that the genes in these loci are involved in protein glycosylation in strains 81116 and 11168H (Linton et al., 2002; and see below), we have used the pgl (protein glycosylation) gene designation (Szymanski et al., 1999).

Three genes from the locus (pglH–pglJ) partially overlap with each other and all encode putative glycosyltransferases. We chose the first sugar-transferase-encoding gene from this area, pglH, for further analysis.

Construction and characterization of mutants

To determine the role of the pglH gene product in strains 81116 and 11168H, we constructed three independent pglH::kan' mutants. Glycolipids of the wild-type strain and the respective pglH::kan' mutant were analysed using silver and Alcian Blue staining, as well as by Western blotting with Penner O:6 antiserum (Fig. 2). Glycolipids of C. jejuni consist of LOS and CPS (Karlyshev et al., 2000). Whereas LOS can be stained with silver, in many strains CPS can only be detected by either Western blotting and/or Alcian Blue staining (Karlyshev & Wren, 2001). For strain 81116 there was no detectable difference in LOS production by the pglH::kan' mutants and the wild-type strains (Fig. 2A–C). Similarly, the qualitative amount of CPS remained unchanged using both Alcian Blue staining (Fig. 2B) and Western blotting with Penner O:6 antiserum (Fig. 2C). These results suggest that in strain 81116, LOS and CPS are unaffected by pglH mutation.

Similarly to pgl mutants of strain 81116, there were no changes in LOS extracted from pgl mutants of strain 11168H. Fig. 3 demonstrates identical sizes of LOS molecules on a high-resolution Tricine-buffered 16% polyacrylamide gel. A difference of just one sugar residue can easily be detected using a control LOS extracted from mutant cj1139::kan' (lane 2). Gene cj1139 was found to encode a terminal Gal-transferase, so that the size difference between the wild-type strain and this mutant would correspond to about 0.2 kDa (Fig. 3). These results confirm that pglH mutation does not affect LOS.

In order to check a role of pglH in protein glycosylation, proteins from whole-cell lysates were analysed by Western blotting with biotinylated lectin SBA, specific to terminal N-/O-linked N-acetylgalactosamine. This lectin was found to interact with a number of C. jejuni glycoproteins produced by strain 11168H (Linton et al., 2002). The

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Fig. 1. Region of the C. jejuni 8116 genome containing the pgl gene cluster. A, gene designation according to Szymanski et al. (1999), including genes renamed in this paper (in bold); B, previous gene designation (Fry et al., 1998); C, gene designation according to the NCTC 11168 genome sequence (http://www.sanger.ac.uk/Projects/C_jejuni/). The insert in plasmid cam169d1 and the location of the MluI site are also shown. The open box indicates the genes shown to be involved in protein glycosylation in C. jejuni 811-176 (Szymanski et al., 1999).
results demonstrated a dramatic effect of pglH mutation on glycoproteins (Fig. 4). Since SBA lectin is specific for terminal GalNAc residues, these data indicate a loss of this residue in a conserved heptasacharide glycan found in many C. jejuni glycoproteins (Young et al., 2002; Szymanski et al., 2003b). It has recently been suggested that the PglH is a GalNAc α-1-4 glycosyltransferase (ref. 30 in Szymanski et al., 2003a) Together the data suggest that the pglH gene product is involved in protein glycosylation, rather than the biosynthesis of glycolipids.

Inactivation of the pglH gene affects attachment and invasion

Mutation of pglH in C. jejuni strains 11168H and 81116 resulted in the reduced attachment to and invasion of Caco-2 cells. Reduction in attachment and invasion for strain 81116 were 23-fold (P value 5 × 10^{-2}) and 15-fold (P value 1 × 10^{-2}) respectively, whereas the corresponding figures for strain 11168H were sixfold (P value 0.026) and 5.4-fold (P value 0.08) respectively. Such reductions suggest a direct or indirect biological function for glycosylation in C. jejuni. The reduced invasion in both strains is likely to be a consequence of the reduced attachment.

In order to confirm that the observed effect of pglH mutation on the adhesion and invasion is not attributable to genetic variation in other genes we used the chromosomal DNA extracted from 11168H/pglH::kanR mutant to transform the recipient strain 11168H in triplicate. The three independent pglH mutants demonstrated significant reduction in both adhesion and invasion efficiency (data not shown), confirming that the observed phenotype was unlikely to be the result of secondary mutations on the chromosome and further validating the data presented above.

In addition, SBA reactivity (Fig. 5), and both adhesion and invasion properties (Fig. 6) were fully restored after complementing of the pglH mutation in strain 11168H.
Together, these data demonstrate that the observed phenotypic changes are attributable to mutation in a single gene, rather than being a result of independent mutations in other genes or of a polar effect.

**pglH**:kan¹ mutant of strain 81116 has reduced colonization efficiency in a chick model

Since the ability of the bacteria to bind to epithelial cells may be linked to the ability to colonize host tissues, we investigated the effect of pglH mutation on colonization potential in chicks. Strain 81116 was selected for these experiments, as it shows a good dose-response and has been used routinely for such tests, producing consistent and reproducible results in over 20 experiments, and has previously been used to demonstrate reduction in colonization potential with defined mutants of other genes (Cawthraw et al., 1996; Wassenaar et al., 1993b).

In comparison with the parent strain, the mutant had a substantially reduced colonization potential (Fig. 7). The minimum dose of *C. jejuni* strain 81116 which colonizes the caecum of the 1-day-old chick is approximately 10⁵ c.f.u., and maximal colonization of about 10⁹ c.f.u. per g caecal contents occurs at a dose of about 10⁵ c.f.u. No colonization by the mutant strain was found at the dose of 10⁴ c.f.u.,
whereas at the dose of $10^5$ c.f.u. colonization efficiency was over 8000-fold lower than that of the wild-type strain. Even at a high dose of $10^7$ c.f.u. the pglH mutant colonized 4.6-fold less efficiently than the wild-type strain. The data clearly suggest that the pglH gene product is important for colonization.

**DISCUSSION**

In this study we have demonstrated that the pglH gene of strains 81116 is not involved either in the biosynthesis of LOS, as has been suggested from previous data on characterization of this locus (Fry et al., 1998; Wood et al., 1999), or in the biosynthesis of CPS. In contrast, insertional inactivation of this gene has a dramatic effect on the ability of strain 81116 proteins to bind SBA lectin, indicating a general effect on protein glycosylation. The specificity of lectin SBA to $\alpha$- or $\beta$-linked N-acetylgalactosamine residues suggests that many proteins in strain 81116 may also carry these sugar residues. These results are consistent with the previously demonstrated effects of other genes from the same operon, namely pglA–F, on protein glycosylation (Szymanski et al., 1999) (Fig. 1). Products of several genes in the operon, including pglH, have the highest similarity to a number of glycosyltransferases involved in the biosynthesis of glycolipids, including lipopolysaccharides. This previously led to the assumption that these genes were involved in LPS/LOS biosynthesis and hence their designation as wla according to the nomenclature of LPS-related genes (Reeves et al., 1996). Such anomalies demonstrate the limitations of in silico function prediction; i.e. the highest similarity by e-value does not necessarily relate to the predicted functional similarity.

The biological role of protein glycosylation in bacteria remains unclear (Messner, 1997). Although some glycoproteins may play a role in bacterial attachment (Muthukumar & Nickerson, 1987), glycosylation of other cell-surface proteins is not important for adhesion (Marceau et al., 1998). Other functions of protein glycosylation in prokaryotes, such as increased thermal and proteolytic stability of proteins, have been suggested (Moens & Vanderleyden, 1997).

Recently, it has been determined that the Pgl pathway $N$-glycosylates many of C. jejuni proteins and that the glycan component consists of a heptasaccharide structure (Linton et al., 2002; Wacker et al., 2002; Young et al., 2002). In this study we found that the lack of protein glycosylation in strains 81116 and 11168H pglH::kanR mutants resulted in a dramatic reduction of bacterial cell adhesion in vitro. It is possible, therefore, that for at least some of the proteins, the heptasaccharide modification is important for the attachment of C. jejuni to human host cells, possibly via host cell-surface structures, such as lectins. We have shown that mutations affecting the adjacent genes pglI and pglJ also affect both protein glycosylation and attachment efficiency (data not shown). A similar effect on colonization and invasion of mutations in other pgl genes in a different strain of C. jejuni (81-176) has been reported (Szymanski et al., 2002). Some of the glycoproteins of C. jejuni may be cell-surface exposed and perform the role of adhesins. For example, one of the glycoproteins, PEB3 (Linton et al., 2002), contains an N-terminal leader peptide and is annotated as 'major antigenic peptide' with similarity to Vibrio cholerae accessory colonization factor AcIC (Sanger Institute, UK). The PEB3 glycoprotein is found to be immunodominant and is known to cross-react with convalescent patient antiserum (Pei et al., 1991).

**In vivo models of pathogenicity for C. jejuni are fraught with difficulties (Newell, 2001). The avian gut appears to be the natural environment for C. jejuni and provides a sensitive model to assess colonization. For most strains low oral doses can achieve very high caecal colonization levels. With strain C. jejuni 81116 a dose-response of colonization
is obtainable (Wassenaar et al., 1993b), which provides an opportunity to determine the effects of gene ablation on colonization (Cawthraw et al., 1996). In our study, the pglH mutation in strain 81116 resulted in a significantly reduced ability to colonize chicks, suggesting that the proteins modified by the general glycosylation pathway in C. jejuni are important in the colonization of the natural host. The difference in colonization between the mutant and wild-type strain was observed at relatively low infection doses. It is possible that at lower dose some bacteria are required to stick to host cells first via glycosylated proteins and the other bacteria stick to those bacteria already attached, i.e. like a biofilm or microcolony effect. Whether colonization of the avian gut is dependent on adherence is debatable. There is no evidence that campylobacters attach to intestinal epithelial cells during colonization of the avian gut (Beery et al., 1988). Nevertheless, some of these bacteria can traverse the avian intestinal epithelium and are recovered from the liver and spleen (Newell & Wagenaar, 2000). It is, therefore, to be expected that such invasive events would require a close interaction between bacterial and host cells, suggesting a possible role for adhesins.

One of the genes in the pgl cluster is galE (Fig. 1), which appears to be important for LOS biosynthesis in strain 81116 (Fry et al., 2000). Mutation of the galE gene affected LOS biosynthesis and attachment to human small intestine cells INT407, but not colonization potential in chicks (Fry et al., 2000). In this study we demonstrated that in the 81116 pglH:: kan" mutant, reduction in the attachment to Caco-2 cells also coincided with the reduced ability of the mutants to colonize chicks.

In strain 11168H, adjacent to galE there is also another large gene cluster (cj1133–cj1151) containing a number sugar transferases, as well as the genes involved in sugar biosynthesis. It is likely that this cluster is dedicated to LOS biosynthesis. We have recently found that two genes from this region (Fig. 1), neuB1 (Linton et al., 2000a) and cj1139 (Linton et al., 2000b), affect LOS production. Gene cj1148 of 11168H and its homologue in NCTC 11828 (Oldfield et al., 2002), as well as homologues of genes cj1140–cj1148 in strain MCS57360 (HS : 1) (Guerry et al., 2000), were also found to be involved in the biosynthesis of LOS. Our data support the hypothesis that the pgl locus is independent and probably dedicated to protein glycosylation in this micro-organism.

As evidence suggests that both LOS and proteins are associated with the adhesion of C. jejuni bacteria to cellular and mucous substrates (McSweegan & Walker, 1986), understanding the mechanisms involved may enable the development of novel approaches to control foodborne campylobacteriosis, based on the specific inhibition of bacterial attachment. Our results complement previous studies (Szymanski et al., 2002) and suggest that glycoproteins are important contributors to adherence and are therefore potential future targets for such approaches.

NOTE ADDED IN PROOF

Hendrixson & DiRita (2004) have recently shown using signature-tagged mutagenesis that a pglH mutant does not colonize chicks.

ACKNOWLEDGEMENTS

The work was supported by the BBSRC and the Department for Environment, Food & Rural Affairs (DEFRA), GB.

REFERENCES


