Identification of Immunogenic and Virulence associated 

Campylobacter jejuni Proteins.

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Running title: Campylobacter jejuni antigens and virulence factors
Abstract
With the aim of identifying proteins important for host interaction and virulence, we have screened an expression library of NCTC 11168 *C. jejuni* genes for highly immunogenic proteins. A commercial *C. jejuni* ORF library consisting of more than 1600 genes was transformed into the *E. coli* expression strain BL21 (DE3) resulting in 2304 clones. This library was subsequently screened for immunogenic proteins using antibodies raised in rabbit against a clinical isolate of *C. jejuni*; this resulted in 52 highly reactive clones representing 25 different genes after sequencing. Selected candidate genes were inactivated in *C. jejuni* NCTC 11168 and the virulence was examined using INT 407 epithelial cell line and motility-, biofilm-, autoagglutination and serum resistance assays. These investigations revealed Cj0034c to be a novel virulence factor and support the usefulness of the method. Further, several antigens were tested as vaccine candidates in two mouse models, in which Cj0034c, Cj0404 and Cj0525c resulted in a reduction of invasion in spleen and liver after challenge.
The food-borne pathogen, *Campylobacter jejuni* is a gram-negative, microaerophilic, spiral-shaped and motile bacterium. It is the most common cause of food- and water-born gastroenteritis worldwide causing approximately 500 million human infections every year (10,29). Infection is often associated with consumption and handling of undercooked poultry meat, but water and other food sources also play a great role in the transmission of *C. jejuni* (10). The symptoms of campylobacteriosis range from mild non-inflammatory, watery, self-limiting diarrhoea to severe abdominal cramps and bloody diarrhoea with fever and vomiting. Also post-infectious complications such as reactive arthritis and Guillain-Barré syndrome are found to be associated with *C. jejuni* (3).

To colonize hosts, microorganisms require adherence factors, which are often surface structures such as pili that are expressed by many bacteria. However, genome annotations of *C. jejuni* strains have not revealed obvious pilus or pilus-like open reading frames (23). Other bacterial surface structures can also interact with host tissue and they are likely responsible for the ability of *C. jejuni* to colonize the gastrointestinal tract of humans, which is believed to be essential for infection. A study has shown that *C. jejuni* isolated from patients with fever and diarrhoea revealed greater binding to epithelial cells compared to isolates from patients without fever and diarrhoea (8). Several mechanisms involved in the survival and persistence of the bacteria in the gut are known. Colonization of the gut is promoted by flagellar-mediated motility and binding to host tissue such as fibronectin mediated by CadF and FlpA (9,17). Furthermore, several other outer membrane proteins (OMP) are implicated in colonisation including Major OMP (MOMP) (22), PEB1 (19), Omp50 (5), lipoproteins Omp18 (6,18), JlpA (13) and Cia proteins (28). In addition, some of the surface exposed proteins are found to be immunogenic (6,26), which opens the possibility of vaccine
development. Humoral immune response to a number of C. jejuni antigens is developed in most people upon an infection and epidemiological studies indicate that the immunity is crucial for the development of protection against Campylobacter disease (31).

The purpose of this study was to identify novel C. jejuni antigens and potential new virulence factors by screening a C. jejuni ORF expression library (25) with serum from rabbits immunised with a clinical C. jejuni human isolate. Selected candidates of the identified genes were examined for their role in virulence and tested as potential vaccines by subcutaneous immunization followed by oral challenge with C. jejuni in mouse colonisation and invasion models.
Material and methods

Bacterial strains and plasmid

The bacterial strains used in this study included *E. coli* SURE (Stratagene) and *E. coli* BL21 (DE3) (Stratagene) and the plasmid was pTLJ03. Strains and plasmid originates from a NCTC 11168 *C. jejuni* ORF library (25) available from Geneservice. The expression clone set comprises of >1,600 *C. jejuni* ORF's and the expression vector pTLJ03 generates N-terminal GST-His-tagged fusion proteins. Strains were grown in LB media or the expression media MagicMedia (Invitrogen) at 37°C. pTLJ03-containing strains were grown in media containing 50 µg/mL ampicillin unless otherwise specified. The *C. jejuni* strains used in this study included *C. jejuni* NCTC 11168, *C. jejuni* NCTC 11168H, *C. jejuni* 81116 and *C. jejuni* 72Dz/92 (33). *C. jejuni* NCTC 11168H is a stable hypermotile variant of the reference strain *C. jejuni* NCTC 11168 (16). *C. jejuni* strains were grown at 37°C microaerobically on blood plates (BaseII and 5% blood), in Brucella broth, BHI broth or biphasic (blood agar overlayed with BHI- or Brucella broth) with antibiotic when needed (30 µg/mL kanamycin or/and 50 µg/mL streptomycin).

Expression library

The library was originally created in *E. coli* SURE for optimal storage. The strain does not contain the T7 polymerase and for that reason the *E. coli* BL21 (DE3) expression strain was used. The clones were grown separately in microtiter plates in 200 µl LB media containing ampicillin overnight and subsequently the plasmids were purified as a pool. The chemical competent *E. coli* BL21 (DE3) strain was then transformed with the pool of vectors and plated on selective plates. This revealed an expression library consisting of 2304 clones (24 microtiter plates).

Immunoblot assay
Individual clones were grown 16-20 hrs in microtiter plates in MagicMedia for optimal expression. 2 μl of the culture was spotted on nitrocellulose membranes. The membranes were blocked in blocking buffer 30 min., washed in PBS tween and then incubated in *C. jejuni* primary antibody raised in rabbits (1:1000) at 4°C for 16-20 hrs. The membranes were then washed in PBS tween and incubated in secondary antibody (Polyclonal goat anti rabbit immunoglobulins/HRP, Dako) for 1 hr. The reaction was visualised by chemoluminescence (chemoluminescent substrate, Invitrogen).

The *C. jejuni* primary antibody was raised in a rabbit immunised with a heat-killed (100°C for 1 h) *C. jejuni* Penner serotype 2 isolated from a human patient (30). This serotype was chosen since it corresponds to that used for creating the commercial library (NCTC 11168). The serum was preincubated with *E. coli* BL21 (DE3) before use to minimise background reaction. To verify that the antigens also reacted against human serum, a dot blot with 10 selected clones expressing antigens and serum isolated from a patient infected with *C. jejuni* Penner serotype 2 (30) was carried out as described above.

**Clone sequencing**

Plasmid DNA was isolated from 100 ml *E. coli* BL21 (DE3) cultures using MidiPrep (Qiagen).

Sequencing was conducted by Macrogen Inc. and the primer 5´GCT ATC CCA CAA ATT GAT AA 3´.

**Recombinant DNA techniques**

*C. jejuni* 11168H knock-out mutants were kindly provided by Brendan Wren from the London School of Hygiene and Tropical Medicine, University of London. Mutants were constructed via insertion of the Km cassette into unique sites present in pUC18-based recombinant plasmids containing random 1-2 kb fragments from the *C. jejuni* NCTC 11168 genome library (24).
NCTC 11168H knock-out mutants provided for this study were: Cj0034c, Cj0645, Cj0917 and Cj1371c.

Subsequently the gene knock-outs were transferred from *C. jejuni* 11168H strain to *C. jejuni* 11168 strain to restore motility and spiral morphology. Natural transformation was performed as described previously (32) with some modifications. *C. jejuni* cultures grown overnight on BHI agar plates were collected and resuspended in 12 ml BHI broth to OD$_{600}$ of 0.001. Bacterial suspensions in three dilutions were transferred to sterilized Petri dishes, incubated at 37°C with no shaking under micro-aerobic conditions over night. Cultures (200 μl) with OD$_{600}$ 0.2-0.3 were transferred to sterilized tube containing 1 ml BHI and incubated at 37°C with shaking under micro-aerobic conditions for 2 h. Then 10 ng of genomic DNA (purified with Qiagen blood and tissue kit) of the mutants, was added to each tube. After additional incubation for 3 h, bacterial cultures were serially diluted and plated on BaseII agar plates with antibiotics (50 mg/l kanamycin). The agar plates were incubated at 37°C under microaerobic conditions 3 days. The mutants were checked for curved shape and motility before being tested in assays.

**INT407 adhesion assay**

INT407 cells (ATCC CCL6 [derived from human embryonic jejunum and ileum]) were grown in Minimum Essential Media (MEM) (+ GlutaMAX) (Invitrogen) containing 25 μg/ml gentamycin and 10% heat-inactivated foetal bovine serum in 5 % CO$_2$. Cells were seeded at 2.5 x 10$^5$ pr well in 24 well plates, incubated overnight and checked for 100 % confluent monolayer. The *E. coli* clones were grown overnight in MagicMedia broth (Invitrogen) at 37°C and *C. jejuni* on blood agar plates microaerobically at 37°C. Immediately before testing, the OD$_{600}$ of the bacteria was adjusted to 1 in PBS and 1 ml bacteria culture was added to the INT407 cells and which were then incubated for 2
hours at 37°C. The cells were then resuspended and diluted in PBS and spotted on agar plates with appropriate antibiotics.

Electron microscopy
To investigate, whether the *C. jejuni* mutant strain differed morphologically from the wild type strain, transmission electron microscopy was conducted. Initially, the bacterial cultures were fixated in 1% glutaraldehyde (EMS, Hatfield, USA) for 30 minutes. To improve the adhesion of the bacteria, formvar coated 400-mesh copper grids were treated for 5 minutes with alcian blue (Sigma-Aldrich). Grids were placed on top of cultures of *C. jejuni* NCTC 11168 and *C. jejuni* NCTC 11168ΔCj0034c, respectively, and after 5 minutes of incubation, most of the suspensions were removed from the grids with filter paper and the grids were stained for 30 seconds with phosphotungstic acid (BDH Chemicals). The grids were allowed to air-dry, and then viewed in a Morgagni 268D transmission electron microscope, and pictures taken using a Mega-view III digital camera.

Motility assay
Motility assay was carried out to ensure no altered motility for the NCTC 11168ΔCj0034c mutant. To 0.25 % soft agar plates was added 1 μl bacterial culture (OD600 adjusted to 0.1) in the middle of the plate and diameter was measured over a time period of 24 h.

Serum resistance assay
Serum sensitivity assays were performed by modification of the method of Blaser *et al* (4). *C. jejuni* NCTC 11168 and *C. jejuni* NCTC 11168ΔCj0034c were grown overnight in Brucella biphasic cultures at 37°C, washed in PBS, pH 7.4, and adjusted to a concentration of $10^3$ CFU/ml. *C. jejuni*
cells (10-μl aliquots) were incubated in 240μl pools of whole human blood (venous blood), human serum (whole blood incubated at 25°C 30 min, centrifuged 1000xg 10 min at 4°C and supernatant isolated) and heat inactivated human serum (56°C for 30 min) respectively for 30, 60, 90 and 120 min. Following the incubation period, CFU were enumerated on BHI agar.

Biofilm and autoagglutination

Cell-to-cell autoagglutination was assayed in PBS as described by Misawa and Blaser (20). Biofilm assays were performed in 50 ml centrifuge tubes containing 25 ml inoculated Brucella broth with C. jejuni NCTC 11168 and the knock-out mutant in Cj0034c. A glass slide was added to each tube and incubated micro-aerobically for 48 h. Then the slides were stained with crystal violet and biofilm formation visualised.

Predictions of protein localization

Prediction of protein localization and amount of transmembrane helices was made by TMHMM 2.0 server (21). The SignalP 3.0 server predicts the presence and location of signal peptide cleavage sites in amino acid sequences. The method incorporates a prediction of cleavage sites and a signal peptide/non-signal peptide prediction based on a combination of several artificial neural networks and hidden Markov models. The LipoP 1.0 server produces predictions of lipoproteins and discriminates between lipoprotein signal peptides, other signal peptides and N-terminal membrane helices in Gram-negative bacteria (14). All three servers are available at http://www.cbs.dtu.dk/services/.

Protein purification

His-tag purification was made with the already GST-His-tagged constructed vector from
Geneservice. An overnight pre-culture of *E. coli* BL21(DE3) containing the vectors was 50-fold diluted to inoculate 1000 ml LB medium containing appropriate antibiotics. The cultures were incubated with shaking at 37 °C to an OD_{600} of 0.5, then induced with 10 mM IPTG and incubated with shaking for 16 hours at 30 °C. After induction, cells were lysed on ice in 20 ml lysis buffer (50 mM NaH_{2}PO_{4}, 300 mM NaCl, 10 mM imidazole, 10 % glycerol) by addition of 1 mg/ml lysozyme followed by sonication. Lysates were cleared by centrifugation at 15,000xg for 30 min. Proteins were purified by nickel affinity chromatography using the Ni-NTA resin (Qiagen) equilibrated with lysis buffer and eluted with 250 mM imidazole. Eluted proteins were concentrated and dialyzed against 25 mM HEPES pH 7.5, 50 mM NaCl, 10 % glycerol.

**Mouse vaccination and challenge studies**

Five antigens Cj0525c, Cj0404, Cj1371, Cj0034c and Cj1382c were tested for their ability to protect against *C. jejuni* infection in 2 mouse models: a) prevention of invasion of internal organs (liver and spleen) in CH3/HeN mice, and b) prevention of intestinal colonization in Balb/c mice. Mice (10 in each group) were subcutaneously immunized with 5 µg protein/dose, except Cj1371c with 1.6 µg protein/dose, along with adjuvant GNE (Intervet, NL). Four weeks later, the C3H/HeN mice were treated for three days with streptomycin (5 g/l in drinking water) and challenged orally one day later with strain 72Dz/92 (33) \(5\times10^7\) CFU). The strain was selected for its virulence in our model because invasion in organs was better compared to *C. jejuni* 81-176 and *C. jejuni* RM1221 (data not shown). Six days later mice were euthanized and approx. 1/10 of liver and spleen were homogenized in 0.75 ml physiological salt solution in the Percellys® homogenizer and used to determine the number of viable Campylobacters. In the study of prevention against colonization strain *C. jejuni* 81116 \(6\times10^5\) CFU was orally inoculated in Balb/c mice. Subsequently, one fresh faecal dropping was collected regularly from each mouse, resuspended in medium and dilutions
were made in order to determine CFU/gram faeces. Statistical significance (P<0.05) was calculated using the Student t-test (2-tailed with equal variance).

Results

Identification of C. jejuni antigens

With the aim of identifying immuno-reactive C. jejuni proteins, plasmid DNA was isolated from a pooled mixture of commercial library clones expressing C. jejuni NCTC11168 ORFs in the plasmid pTLJ03 (21) and transformed E. coli BL21 to allow expression from the T7 promoter. The resulting transformants were individually spotted on a nitrocellulose membrane and reacted with serum isolated from a rabbit immunised with a C. jejuni human clinical isolate (serotype 2). The screening revealed several immunogenic E. coli clones that selectively reacted with the serum as shown in figure 1. Inserts in plasmids isolated from the transformants that repeatedly proved as highly immunogenic were selected for sequencing and from a total of 2304 clones, 52 inserts were sequenced representing 25 genes encoding potential antigens (table 1). The identified C. jejuni genes were classified according to their predicted function (20) as shown in table 1.

Prediction of localization of the proteins and other structural features was performed by THHMM 2.0 server (21) and results are shown in table 2. 14 out of 25 proteins were predicted to contain one or more membrane helixes, two of the further with a signal peptide (predicted with The SignalP 3.0 server). Ten of the proteins were predicted to be located externally, where three of these harbor a signal peptide. One protein was predicted to be located inside the cell. None of the proteins were predicted to contain a lipoprotein signal peptidase.

Antigens are antigenic in humans
To confirm that the identified antigens are also recognised in human infection we selected 10 clones for Western blot analysis with human antiserum obtained from a patient infected with a *C. jejuni* Penner serotype 2. The clones were chosen to cover various cellular predicted localizations and functions and were found in all cases to react with the antiserum (figure 2).

Several antigens support host cell adhesion

Adhesion of *C. jejuni* to host cells forms the first important step in the infection process. With the aim of addressing if the identified antigens contribute to host cell adhesion, the clones reacting with the human antiserum were investigated for their ability to adhere to the intestinal epithelial cell line, INT407 (figure 3). Interestingly, expression of three of the *C. jejuni* antigens enhanced the ability of *E. coli* BL21 to adhere to INT407 cells, namely Cj0034c, Cj0404 and Cj1371. Subsequently, the gene-specific *C. jejuni* mutants, available from Campylobacter Resource Facility, London School of Hygiene & Tropical medicine, were examined in the same cell adhesion assay. While the absence of Cj0404 and Cj1371 did not affect the ability of *C. jejuni* to adhere to INT407 cells inactivation of Cj0034c dramatically reduced adhesion suggesting that Cj0034c may contribute to establishment of *C. jejuni* in host organisms (figure 4). Thus, Cj0034c may encode a novel *C. jejuni* adhesion.

Characterization Cj0034c, a new *C. jejuni* virulence factor

Since Cj0034c reduces the ability of *C. jejuni* to adhere to host cells we attempted to obtain more information about the gene product using the structural prediction and homology search tool, HHpred (http://toolkit.tuebingen.mpg.de/hhpred/). This search predicted homology to the *E. coli* YggE that plays a role in the defence of oxidative stress imposed by the toxic salt potassium tellurite. With the anticipated location and the potential adhesive properties of Cj0034c inactivation of the corresponding gene might influence the cell surface. This was examined by electron
microscopy and the result revealed that the mutation does not result in major structural changes to bacterial cell morphology (figure 5). Also, the inactivation of Cj0034c did not influence other properties associated with cell surface composition such as serum resistance, motility, auto-agglutination and biofilm formation, when compared to wild type strain (data not shown).

269 Antigens as vaccine candidates

With the aim examining the identified antigens as vaccine candidates we attempted to express and purify the gene products from the 10 characterized clones. However, after several attempts it was only possible to obtain sufficient quantities of five of the antigens. These were tested in two Campylobacter oral challenge mouse models; one in C3H/HeN mice in which invasion of strain 72Dz/92 in liver and spleen was measured and the other in Balb7c mice in which shedding of strain 81116 in faeces was measured. The combination of mice and Campylobacter challenge strain was chosen based on trial experiments and those were selected that gave the most reproducible results (data not shown). The challenge study (figure 6) showed the absence of invasion in spleen for at least two of the proteins; Cj0525c and Cj1382, however this was not statistically significant. Invasion in the liver was only significantly reduced in the group immunized with Cj0404. When invasion in both liver and spleen were combined as one parameter, antigens Cj0525c, Cj0404 and Cj0034c showed significant protection against invasion. Challenge colonization results are shown in figure 7, but no significant protection against colonization by any of the proteins was observed.
In this study we have successfully identified immuno-reactive proteins of the important human pathogen, *C. jejuni*. An *E. coli* library expressing single *C. jejuni* open reading frames was screened using specific *C. jejuni* antibodies resulting in the identification of immuno-reactive *C. jejuni* proteins. Of the 25 identified antigens, 14 were predicted to include one or more membrane helixes of which three carry a predicted signal peptide. Another three proteins were predicted to express a signal peptide and only one candidate was proposed to be located in the cytoplasm. In a previous study, Cordwell *et al* (7) examined surface located *C. jejuni* proteins and described several proteins also identified in our study eg. Cj0034c, Cj0404, Cj0917c, Cj0774c, Cj1092, and Cj1094. Thus, our data suggest that *E. coli* is able to express and translocate *C. jejuni* cell surface proteins and that the *E. coli* library is suitable for the identification of *C. jejuni* antigens. Surprisingly, we did not detect the most immunodominant and well characterized antigens described for *C. jejuni* namely FlaA, Peb1A and PorA. This may be a consequence of the library construction, as a pool of library clones was used for transforming the T7-expressing BL21 cells and this pool may be biased with some genes being present in more copies than others. Alternatively, some *C. jejuni* extracellular proteins may be less well expressed and secreted by *E. coli* and if so they will not be detected in the Western blot analysis.

One of the interesting genes identified in our study, Cj0034c, may encode a new adhesion factor in *C. jejuni* as deletion of gene resulted in reduced adhesion to epithelial cells, and conversely over-expression of Cj0034c in *E. coli* enhanced the ability of the bacteria to adhere to INT407 epithelial cells. The gene product is grouped within the 3.C.5 Miscellaneous periplasmic proteins (table 1) together with other important antigen and virulence genes, such as PEB2, 3 and 4 (23). A homology search using [http://toolkit.tuebingen.mpg.de/hhpred](http://toolkit.tuebingen.mpg.de/hhpred), predicted the secondary structure of Cj0034c.
to be homologous of YggE in *E. coli*. YggE defends *E. coli* against oxidative stress caused by the toxic salt tellulite but the mechanism remains obscure (1). In general other studies of *C. jejuni*, have suggested a link between oxidative stress survival and virulence. Haddad *et al* (12) discovered an increase of bound *C. jejuni* to epithelial cells when exposed to oxidative stress and Gundogdu *et al* (11) found Cj1556 to be involved in resistance to oxidative stress and adhere to epithelial cells. We examined if Cj0034c similarly influences oxidative stress tolerance in *C. jejuni* but preliminary results indicate that Cj0034c mutant and wildtype cells survive oxidative stress equally well (data not shown). Cj0034c was also predicted to contain one helix and one signal peptide, suggesting that it is surface exposed and therefore an accessible target for the immune system. A surface location may also stimulate interactions with host cells by adhering to epithelial cells, for example. Despite the lack of identifiable pili and other adherence organelles, several proteins promote adherence of *C. jejuni* to eukaryotic cells. These include CadF that binds to fibronectin and is required for maximal binding and invasion (17) and JlpA and CapA needed for adherence to epithelial cells (2,13).

A study by Prokhorova *et al* (27) used proteomics for the identification of *Campylobacter* surface proteins and demonstrated that some of them were also protective in a *C. jejuni* challenge study. Flavodoxin (Cj1382c) was among the identified proteins but it was not examined in a mouse vaccination-challenge experiment. Flavodoxin and Cj1292 (deoxycytidine triphosphate deaminase) were identified as being among the proteins expressed in an efficient chicken colonizing strain when compared to a poor colonizer Kaakoush *et al* (15). Furthermore, this study determined that Cj0203 was over-expressed along with important surface adhesins such as CadF and FlaA (9,15).
Vaccination challenge experiments showed that Cj0525c, Cj0404 and Cj0034c offered the best protection against invasion of spleen and liver. Cj0525c is a large antigen, not likely to be expressed by a vaccine strain (Salmonella typhimurium ΔrecA:ΔhtrA (not published)). Cj0034c is a smaller antigen that might be suitable for expression in a vaccine strain as is Cj0404 and both were easily expressed by E. coli BL21 (DE3) and purified. Interestingly, of the examined antigens Cj0404 gave the best protection against invasion of C. jejuni. The protein Cj0404 is grouped with membrane- and lipoproteins and porins and is predicted to contain one transmembrane helix and most likely be surface exposed. Therefore, we propose that Cj0404 is a vaccine candidate against C. jejuni.

Our screen for immune-reactive proteins resulted in identification of a new protein important for C. jejuni virulence; Cj0034c and a possible new vaccine candidate; Cj00404.

Acknowledgements

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Figure 1. Immunoblot assay screen. The figure show a representative immunoblot assay with 96 E. coli clones each expressing a C. jejuni gene reacting against rabbit serum. Dots with a strong reaction where isolated and the genes sequenced. A total of 52 clones were sequenced representing 25 genes. Lower left dot is the BL21 expression strain with no insert and the right a media control.

Figure 2. Immunoblot assay with human serum. The clones Cj0034c (1), Cj0203 (2), Cj0404 (3), Cj0525c (4), Cj0645 (5), Cj0917c (6), Cj1094c (7), Cj1371 (8), Cj1382c (9), Cj1632c (10), from left to right, were tested for its reactivity against the antiserum isolated from a human patient infected with a C. jejuni serotype 2. All the clones reacted more or less with the serum. Lower left is a medium control (MC) and lower right a negative BL21 (DE3) control (NC).

Figure 3. INT407 cell line adhesion assay. The clones Cj0034c, Cj0203, Cj0404, Cj0525c, Cj0645, Cj0917c, Cj1094c, Cj1371, Cj1382c, Cj1632c were examined for their ability to adhere to INT407 epithelial cell. BL21 is used as negative control and standard bars indicate standard deviations.

Figure 4. INT407 cell line assay. Knock out mutants of C. jejuni 11168 ΔCj0917, ΔCj0034c, ΔCj1371, and ΔCj0645 were tested for their ability to adhere to INT407 epithelial cells compared to the NCTC 11168 wildtype. Standard bars indicate standard deviations.

Figure 5. Electron micrographs of C. jejuni NCTC 11168 (A) and C. jejuni NCTC11168ΔCj0034 (B).

Figure 6. Challenge invasion study. Invasion of C. jejuni/coli 72Dz/92 in spleen and liver of
C3H/He mice (N=10), 6 days after challenge. Values determined individually, log-transformed (log
1+CFU) and calculated as averages per group. Error bars indicate standard deviation. The mice
were immunized with the indicated antigens before challenge. Statistical significance (P < 0.05)
was found for invasion in liver for Cj0404 (filled star) and for combined invasion in liver and
spleen for Cj0525, Cj0404, and Cj0034 (open star).

Figure 7. Challenge colonization study. Colonization of Balb/c with C. jejuni strain 81116 in days
after challenge. Individual colonization was determined per faecal pellet, log-transformed (log
1+CFU) and calculated as average per group. The mice were immunized with buffer, Cj0525,
Cj0404, Cj1371c, Cj0034c and Cj1382c before challenge. No statistical significance differences
found.
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TMH: Transmembrane helixes, SpI: Signal peptidase I, SpII: Lipoprotein signal peptidase II, CYT: cytoplasmic or all the rest.


Figure 1. Dot blot
Figure 6. Invasion spleen and liver