Secretory IgA N-glycans contribute to the protection against *E. coli* O55 infection of germ-free piglets.

Leona Raskova Kafkova, Diana Brokesova, Michal Krupka, Zuzana Stehlikova, Jiri Dvorak, Stepan Coufal, Alena Fajstova, Dagmar Srutkova, Katerina Stepanova, Petra Hermanova, Renata Stepankova, Ivo Uberall, Jozef Skarda, Zdenek Novak, Luca Vannucci, Helena Tlaskalova Hogenova, Zuzana Jiraskova Zakostelska, Marek Sinkora, Jiri Mestecky, Milan Raska
Supplementary Table 1. Scoring of clinical status of individual piglets.

| Piglets       | Temperature | Volume per feeding | Stool | Viability |
|---------------|-------------|--------------------|-------|-----------|
| Normal        | 1           | 1                  | 1     | 1         |
|               | 1           | 2                  | 1     | 1         |
| O55           | 4           | 2                  | 2     | 3         |
|               | 4           | 2                  | 2     | 2         |
|               | 3           | 2                  | 2     | 2         |
|               | 3           | 2                  | 2     | 4         |
| O55 + Fc-SC   | 2           | 1                  | 2     | 1         |
|               | 3           | 3                  | 2     | 4         |
|               | 2           | 1                  | 2     | 1         |
|               | 2           | 3                  | 2     | 3         |
|               | 2           | 1                  | 2     | 3         |
| O55 + SlgA    | 3           | 1                  | 1     | 2         |
|               | 3           | 1                  | 1     | 2         |
|               | 3           | 3                  | 1     | 3         |
|               | 3           | 1                  | 1     | 1         |
|               | 3           | 1                  | 1     | 2         |
| O55 + Fab     | 2           | 2                  | 1     | 1         |
|               | 2           | 2                  | 1     | 1         |
|               | 3           | 2                  | 1     | 3         |
|               | 2           | 2                  | 1     | 1         |
|               | 2           | 2                  | 1     | 2         |
| O55 + dg-SlgA | 4           | 2                  | 1     | 4         |
|               | 4           | 1                  | 1     | 4         |
|               | 4           | 2                  | 2     | 4         |
|               | 5           | 3                  | 2     | 5         |
|               | 4           | 3                  | 2     | 5         |

Individual parameters scaling is detailed in Table 2. Parameters were used for calculation of weighted mean for each group as summarized in Figure 2.
Supplementary Table 2. Genotyping of *E. coli* O55 used in experiments.

### F antigen - *E. coli*

| Fimtype | %Identity | Query/HS P length | Contig | Position in contig | Acc. No. |
|---------|-----------|-------------------|--------|-------------------|---------|
| *fimH39* | 100.00 | 489 / 489 | NODE_11_length_166574_cov_29.24 2607 | 34278..3476 6 | KJ096308.1 |
| F4      | No hit found | | | | |
| F5      | No hit found | | | | |
| F6      | No hit found | | | | |
| F18     | No hit found | | | | |
| F41     | No hit found | | | | |
| aeaA    | No hit found | | | | |

### O (LPS) type genes

| Gene | Serotype | Identity | Template / HSP length | Contig | Position in contig | Acc. No. |
|------|----------|----------|-----------------------|--------|-------------------|---------|
| wzy  | O55      | 97.65    | 978 / 978             | NODE_263_length_156 576_cov_28.013201 | 32640..3 3617 | AB353132 |
| wzy  | O55      | 97.65    | 978 / 978             | NODE_263_length_156 576_cov_28.013201 | 32640..3 3617 | CP003109 |
| wzx  | O55      | 98.59    | 1278 / 1278           | NODE_263_length_156 576_cov_28.013201 | 31345..3 2622 | JH958641 |

### H type genes

| Gene | Serotype | Identity | Template / HSP length | Contig | Position in contig | Acc. No. |
|------|----------|----------|-----------------------|--------|-------------------|---------|
| fliC | H4       | 100      | 1050 / 1050           | NODE_7_length_110642_cov_28.547831 | 31034..32 083 | AJ6057 64 |

### Toxin genes

| Virulence factor | Identity | Query / Template length | Contig | Position in contig | Protein function | Acc. No. |
|------------------|----------|-------------------------|--------|-------------------|------------------|---------|
| STa              | No hit found | | | | | |
| STb              | No hit found | | | | | |
| LT               | No hit found | | | | | |
| stb1             | No hit found | | | | | |
| stb2             | No hit found | | | | | |
| hlyA             | No hit found | | | | | |
### Virulence genes for Escherichia coli

| Virulence factor | Identity | Query / Template length | Contig | Position in contig | Protein function | Acc. No. |
|------------------|----------|--------------------------|--------|--------------------|------------------|----------|
| CNF              | 100      |                          |        |                    | Cytotoxic necrotizing factor 1 |          |
| cdtB             | 100      | 810 / 810                | NODE_65_length_423 7_cov_21.189993 | 1338..2147      | Cytolethal distending toxin B | AY365044 |
| f17A             | 100      | 546 / 546                | NODE_48_length_935 0_cov_38.190052 | 1098..1643      |                                | L43373   |
| f17G             | 100      | 1032 / 1032              | NODE_48_length_935 0_cov_38.190052 | 4988..6019      | Enterobactin siderophore receptor protein | CP001162 |
| iroN             | 99.95    | 2178 / 2178              | NODE_370_length_39 709_cov_34.301922 | 10819..12996    | Increased serum survival | CP001509 |
| iss              | 98.98    | 294 / 294                | NODE_58_length_236 2_cov_71.282387 | 1758..2051      |                                |          |
| lpfA             | 100      | 573 / 573                | NODE_2_length_1742 38_cov_29.314823 | 14375..8144330  | Long polar fimbriae | AY646923 |
| mchB             | 100      | 294 / 294                | NODE_370_length_39 709_cov_34.301922 | 34109..34402    | Microcin H47 part of colicin H | AE014075 |
| mchC             | 100      | 1551 / 1551              | NODE_370_length_39 709_cov_34.301922 | 32287..33837    | MchC protein | AE014075 |
| mchF             | 100      | 2115 / 2115              | NODE_370_length_39 709_cov_34.301922 | 28293..30407    | ABC transporter protein MchF | AE014075 |
| mcmA             | 100      | 279 / 279                | NODE_370_length_39 709_cov_34.301922 | 27761..28039    | Microcin M part of colicin H | AJ586887 |

### Enterobacteriaceae plasmids

| Plasmid          | Identity | Query / Template length | Contig | Position in contig | Note | Acc. No. |
|------------------|----------|--------------------------|--------|--------------------|------|----------|
| IncFIB(AP001918) | 99.56    | 682 / 682                | NODE_124_length_4137_cov_27.241480    | 1606..2287 |      | AP001918 |
| IncFII(pSE11)    | 95.45    | 264 / 264                | NODE_56_length_14019_cov_18.551752    | 1097..1026    | pSE11 | AP009242 |

---

The table lists virulence factors and their corresponding genes for Escherichia coli, including their identity, query/template length, contig information, position in contig, protein function, and accession number. Similarly, the table for Enterobacteriaceae plasmids provides information on plasmids, their identity, query/template length, contig, position in contig, note, and accession number.
| Gram Positive plasmids |  |
|------------------------|----------------|
| **Plasmid** | **Identity** | **Query / Template length** | **Contig** | **Position in contig** | **Note** | **Acc. No.** |
| No hit found |  |  |  |  |  |  |

| MLS - Macrolide, Lincosamide and Streptogramin B |  |
|-------------------------------------------------|----------------|
| **Resistance gene** | **Identity** | **Query/HSP** | **Contig** | **Position in contig** | **Acc. No.** |
| mdf(A) | 98.78 | 1233/1233 | NODE_5_length_336678_cov_27.747412 | 30218..31450 | Y0874 3 |

Green color indicates matching of O55 sequence with database target (identity), light green indicates partial matching, and magenta indicates no matching. The search was performed by the use of ResFinder-3.1 Server, FimTyper-1.0 Server, PlasmidFinder-2.0 Server, and SerotypeFinder-2.0 Server all available on https://cge.cbs.dtu.dk, and by the use of https://bitbucket.org/genomicepidemiology/fimtyper.
Supplementary Figure 1. Detection of IgA within duodenal vacuolated enterocytes.

Frozen tissue section of duodenum from piglet infected with *E. coli* O55 preincubated with SlgA and from germ-free piglet. Tissue sections were stained with human IgA-specific FITC-labeled mAb. Nuclei were stained with DAPI. Left section from piglet infected with *E. coli* O55 incubated with SlgA, right section from germ-free piglet not exposed to human SlgA.
Supplementary Figure 2. Human IgA in the blood and intestine in piglets infected with *E. coli* O55-incubated with SlgA and dg-SlgA.

A) The concentration of IgA1 and IgA2 in the serum of three randomly selected piglets per group. The level of IgA1 was increased in piglets infected with *E. coli* O55 preincubated with both SlgA and dg-SlgA. The level of IgA2 was slightly increased in group infected with *E. coli* O55 preincubated with dg-SlgA. B) The concentration of IgA1 and IgA2 in the lumen of jejunum, ileum, and the colon. IgA2 was increased in jejunum, ileum, and colon in the group of piglets infected with *E. coli* O55 preincubated with SlgA and dg-SlgA. IgA1 was slightly increased (ns) only in jejunum of piglets infected with *E. coli* O55 preincubated with SlgA and dg-SlgA. There is no significant difference between the amounts of IgA2 in jejunum of piglets infected with *E. coli* O55 preincubated with SlgA and dg-SlgA. Data are means ± SD. P values were calculated using Kruskal-Wallis test with Dunn-Bonferroni post-hoc test. *p < 0.05, **p < 0.01.
Supplementary Figure 3. Determination IgA and bacteria content in the stool during the course of experiment.

A) Bacterial load was determined from stool samples taken at indicated time points. The numbers of stool samples analyzed varies (n = 1 - 4) and for individual time point it corresponds to numbers of group-specific colored squares. The bacterial load is
expressed as means bacterial colony forming unite count calculated on agar plates (CFU/mg of intestine content). B) IgA1 and IgA2 were determined in stool samples of piglets infected with *E. coli* O55 + SlgA or with *E. coli* O55 + dg-SlgA taken before, 9, 24 hours after infection, and at the termination of the experiment (35 h after infection). Analyzed were samples from 1 to 4 piglets per group. IgA levels were determined by ELISA assay using monoclonal antibodies against IgA1 and IgA2 and expressed as means. Because the number of stool samples differs among groups and time points, we did not use statistical analysis here. At the termination of experiment the concentration of individual IgAs was below 13 μg/mg of stool. The curves represent polynomial approximation of time course of individual IgA sequestration.
A) *E. coli* O55 was grown in LB agar at 37°C for 16 h and thereafter culture was placed in 4°C overnight. Bacteria cells were harvested by mild centrifugation, washed, and fixed and mounted for observed by transmission electron microscopy. B) Detail of macroscopic agglutination assay performed with SlgA preparations at 2 mg/ml incubated with 2x10⁹/ml *E. coli* O55 at 37°C followed by 4°C incubation overnight. Sedimented non-agglutinated bacteria pellets are visible.
Supplementary Figure 5. Binding of SlgA preparations to \textit{E. coli} O55.

O55

Experiment no. 1

Experiment no. 2

Experiment no. 3

O55 + SlgA

O55 + dg SlgA

O55 + Fab

O55 + Fc-SC

O55 + dg Fab

11
E. coli O55 was incubated with individual SIgA preparations conjugated with DyLight 488, washed, and analyzed by flow cytometry. Individual plots are provided with median MFI.