Genomic Characterization of *Haemophilus parasuis* SH0165, a Highly Virulent Strain of Serovar 5 Prevalent in China

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**Abstract**

*Haemophilus parasuis* can be either a commensal bacterium of the porcine respiratory tract or an opportunistic pathogen causing Glässer’s disease, a severe systemic disease that has led to significant economical losses in the pig industry worldwide. We determined the complete genomic sequence of *H. parasuis* SH0165, a highly virulent strain of serovar 5, which was isolated from a hog pen in North China. The single circular chromosome was 2,269,156 base pairs in length and contained 2,031 protein-coding genes. Together with the full spectrum of genes detected by the analysis of metabolic pathways, we confirmed that *H. parasuis* generates ATP via both fermentation and respiration and possesses an intact TCA cycle for anabolism. In addition to possessing the complete pathway essential for the biosynthesis of heme, this pathogen was also found to be well-equipped with different iron acquisition systems, such as the TonB system and ABC-type transport complexes, to overcome iron limitation during infection and persistence. We identified a number of genes encoding potential virulence factors, such as type IV fimbriae and surface polysaccharides. Analysis of the genome confirmed that *H. parasuis* is naturally competent, as genes related to DNA uptake are present. A nine-mer DNA uptake signal sequence (ACAAGCGGT), identical to that found in *Actinobacillus pleuropneumoniae* and *Mannheimia haemolytica*, followed by similar downstream motifs, was identified in the SH0165 genome. Genomic and phylogenetic comparisons with other *Pasteurellaceae* species further indicated that *H. parasuis* was closely related to another swine pathogenic bacteria *A. pleuropneumoniae*. The comprehensive genetic analysis presented here provides a foundation for future research on the metabolism, natural competence and virulence of *H. parasuis*.

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**Introduction**

The Gram-negative bacterium *Haemophilus parasuis* is a strictly swine pathogen. It is a non-motile, pleomorphic NAD-dependent coccobacillus belonging to the family *Pasteurellaceae* of the γ-proteobacteria [1]. As a commensal colonizing the upper respiratory tract of pigs, *H. parasuis* is also an opportunistic etiologic agent that can cause serious systemic disease, namely Glässer’s disease, which is characterized by fibrinous polyserositis, arthritis and meningoitis, or acute pneumoniae and acute septicemia [2]. In recent years, infections caused by this bacterial pathogen have led to great economic losses in the pig industry worldwide.

Based on the heat-stable antigens and the gel diffusion test, 15 serovars of *H. parasuis* have been classified, with apparent differences in virulence [3]. However, there are a large number of non-typeable *H. parasuis* strains exhibiting high heterogeneity at the molecular level [4], perhaps due to recombination or lateral gene transfer. *H. parasuis* serovar 4 predominates in North American herds, whereas serovar 5 and non-typeable strains are commonly isolated in many European countries [5,6]. In China, the most prevalent serovars are 4, 5 and 13 [7].

To date, many potential virulence-associated factors investigated in the family *Pasteurellaceae* have been identified in *H. parasuis*, including lipopolysaccharide (LPS), capsular polysaccharide, fimbriae, outer membrane proteins, neuraminidase and iron acquisition systems [6,8–10]. However, the molecular basis underlying these candidate virulence factors is yet to be fully elucidated.

Currently, the complete genome sequences of eight strains from four species within the genus *Haemophilus* are publicly available (up to June, 2010). These include *H. parasuis* SH0165 (GenBank accession no. CP001321), *H. ducreyi* 35000HP (AE017143), *H. influenzae* Rd KW20 (L42023), 86-028NP (CP000057), Pitt-EE (CP000671), PittGG (CP000672), *Histophilus somni* EE (CP000671), *H. ducreyi* 35000HP (AE017143), *H. influenzae* Rd and *H. somni* 129PT are both nonpathogenic strains of human and bovine origin, respectively, and *H. ducreyi* 35000HP causes chancroid, a human sexually transmitted disease [11,12]. In this study, we carried out a comprehensive genomic characterization...
of *H. parasuis* SH0165, a high virulent serovar 5 strain recovered from the lung of a diseased piglet in North China. Comparisons of the genomic components between *H. parasuis* SH0165 and other representative species within the family *Pasteurellaceae* were performed in detail. Our work focused on identifying the particular genes/pathways that play a role in carbon metabolism, natural competence, virulence and host colonization of *H. parasuis*.

**Methods**

**Bacterial strain and genome sequencing**

*H. parasuis* strain SH0165 was isolated in 2001 from the lung of a diseased piglet in North China and was identified as serovar 5. This bacterial strain was cultured on tryptic soy agar (TSA) plates supplemented with 5% bovine serum and 10 μg/ml nicotinamide adenine dinucleotide (NAD). Bacterial DNA was extracted and sequenced using a shotgun sequencing strategy, as described previously [13].

**Sequence analysis**

Glimmer3 [14], tRNASCAN-SE [15] and RNAmer [16] were used to predict protein coding sequences (CDSs), tRNAs and rRNAs, respectively. BLASTP [17] was used for automated annotation based on sequence similarity in conjunction with searching the CDS set against the cluster of orthologous groups (COG) database, Kyoto Encyclopedia of Genes and Genomes (KEGG) database, Pfam protein families database and NCBI non-redundant protein database. A genome comparative circular map was implemented using the program CGview [18]. The sequenced draft genome of *H. parasuis* serovar 5 strain 29755 (NZ_ABKM00000000) was aligned to the complete genome of *H. parasuis* strain SH0165 (CP001321) using BLASTN (minimum sequence identity of 95% and expected threshold of 1e⁻⁵). Short DNA repeats in the genomes were searched with a Perl script repeat_finder (http://folk.uio.no/stephanf/repeat_finder.html) and graphical representations of the patterns in sequence conservation were created using WebLogo [19]. Protein transmembrane helices were predicted by the package TMHMM 2.0 [20].

**Ortholog identification and phylogenetic reconstruction**

All protein sequences from the complete genomes of *H. somni* 129PT (CP000436), *H. influenzae* Rd (L24025), *H. ducreyi* 35000HP (AE017143), *Actinobacillus pleuropneumoniae* JL03 (CP000687), *A. succinogenes* 130Z (CP000746), *Aggregatibacter aphrophilus* NJ8700 (CP001607), *A. actinomycetemcomitans* D11S-1 (CP001733), *Pasteurella multocida* Pm70 (AE004439) and *Mannheimia haemolytica* MBEL55E (AE016827), were retrieved from the GenBank database. Protein sequences from the draft genome assemblies of *H. parasuis* strain 29755 were also retrieved. In this study, orthologous pairs of proteins between the CDS sets of two genomes were defined if protein identity was above 45%, alignment coverage above 70% and E-value below 1e⁻³⁰. Orthologs present in the complete genomes of ten *Pasteurellaceae* species were retrieved. These protein sequences were aligned using the program MUSCLE 3.6 [21] and 727 alignments were then concatenated into a large alignment of 245,554 amino acids. A neighbor joining tree was reconstructed in the software MEGA 4 [22] via the bootstrap test of 1,000 replicates.

**Results and Discussion**

**Genome structure and general features**

*H. parasuis* SH0165 contains a single, circular chromosome that is 2,269,156 base pairs (bp) in length (Figure 1). The chromosome encodes 2,031 protein-coding genes (192 pseudogenes not included herein), 36 tRNA genes and 20 rRNA genes on six ribosomal rRNA operons. Global characterizations of the *H. parasuis* SH0165 genome were summarized and compared to those of strains 129PT of *H. somni*, Rd of *H. influenzae*, 35000HP of *H. ducreyi*, JL03 of *A. pleuropneumoniae* and Pm70 of *P. multocida* (Table 1). In comparison with the other *Haemophilus* spp., *H. parasuis* has a considerably larger chromosome, which is almost equal in size to those of *P. multocida* and *A. pleuropneumoniae*. The overall G+C content of the *H. parasuis* SH0165 genome was 40.0%, a bit higher than those of the other *Haemophilus* genomes. Pairwise nucleotide sequence alignments between the complete genome of *H. parasuis* SH0165 and the contigs of *H. parasuis* serovar 5 strain 29755 indicated that approximately 2,003 kb of sequence (above 95% identity) was shared by both strains, accounting for 88% of the total length of the SH0165 genome. There were 1,714 orthologous pairs of protein-coding genes between both *H. parasuis* strains of serovar 5, and *H. parasuis* strain SH0165 has 317 genes absent in strain 29755. It is worth noting that one third (104 genes) of these unique genes were phage-related. The *H. parasuis* SH0165 genome had about 198 CDSs encoding proteins involved in phage functions or phage islands, accounting for approximately 10% of the total CDSs. The majority of phage-related genes were mainly located in three phage islands designated HP-P1 (68.4 kb ranging from HAPS0536 to HAPS0638), HP-P2 (38.8 kb, HAPS0859–0910) and HP-P3 (9 kb, HAPS1147–1160). It has been suggested that the integration of phage elements, as a strategy of horizontal gene transfer, play a potentially important role in genetic diversity and virulence variations in many bacteria [23]. The phage-related genes found in the *H. parasuis* SH0165 genome may be also a putative contributor to virulence and inheritance differences.

Genome-wide comparisons of orthologous gene pairs between *H. parasuis* and other organisms within the family *Pasteurellaceae* showed that *H. parasuis* is more closely related to *A. pleuropneumoniae* (1,341 pairs of orthologs), which also colonizes the upper respiratory tract of pigs [24], but is more distantly related to the other three *Haemophilus* species, with only 1,095 orthologs found in *H. influenzae* (Table 2). Phylogenetic tree reconstruction based upon 727 conserved coding genes demonstrated that *H. parasuis*, *A. pleuropneumoniae*, and *H. ducreyi* were grouped into a subclade (Figure 2). However, the evolutionary relationship between *H. parasuis* and the strict human pathogen *H. ducreyi* is less obvious [25], as only 1,096 orthologs were identified in the genomes of both species. This may be due to adaptation of the two pathogens to different host species and tissues. These findings may indicate that, phylogenetically, the swine pathogens *H. parasuis* and *A. pleuropneumoniae* probably derive from a recent common ancestor and their adaptations reflect uniform host niches.

**Carbon source utilization**

*H. parasuis* is a facultative anaerobe which possesses metabolic pathways of both fermentation and respiration for energy generation [26]. Two kinds of sugar transport systems were identified in the *H. parasuis* SH0165 genome. We identified a predicted set of genes encoding ATP-binding cassette (ABC) transport complexes involved in the utilization of distinct sugars, including ribose (*rbsDACB*, HAPS1629–1632; *rbsACB*, HAPS1727–1725), galactose (*mgBGC*, HAPS0442–0444) and maltose (*maIEFGK*, HAPS0236–0234, HAPS0237) (Figure 3). *H. parasuis* SH0165 also possesses genes encoding phosphotransferase systems (PTS), which can be devoted to the uptake of other sugars, including glucose (*ptsHI-err*, HAPS0960–0958; *ptsG*, HAPS2092), fructose (*ptsEII*, HAPS0193–0189), sucrose (*ptsB*,...
Table 1. General features of whole genomes of *H. parasuis* (SH0165), *H. somni* (129Pt), *H. influenzae* (Rd), *H. ducreyi* (35000HP), *A. pleuropneumoniae* (JL03) and *P. multocida* (Pm70).

| GenBank accession No. | CP001321  | CP000436  | L42023  | AE017143  | CP000687  | AE017143  |
|-----------------------|-----------|-----------|---------|-----------|-----------|-----------|
| Strain                | SH0165    | 129Pt     | Rd      | 35000HP   | JL03      | Pm70      |
| Total length (bp)     | 2,269,156 | 2,007,700 | 1,830,138 | 1,698,955 | 2,242,062 | 2,257,487 |
| Number of CDSs        | 2,031     | 1,792     | 1,709   | 1,717     | 2,036     | 2,014     |
| Average length of CDS (bp) | 907    | 989       | 918     | 842       | 944       | 997       |
| CDS genome coverage   | 81%       | 88%       | 86%     | 85%       | 86%       | 89%       |
| GC content            | 40.0%     | 37.2%     | 38.2%   | 38.2%     | 41.2%     | 40.4%     |
| Ribosyme RNA          | 16S rRNA  | 6         | 5       | 6         | 6         | 6         |
| 23S rRNA              | 6         | 6         | 6       | 6         | 6         | 6         |
| 5S rRNA               | 8         | 5         | 6       | 7         | 7         | 6         |
| Number of tRNA        | 56        | 50        | 54      | 45        | 63        | 57        |

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HAPS1008) and mannose (manXYZ, HAPS1732–1730). These confirmed genotypes support the previously observed biochemical patterns of sugar fermentation in *H. parasuis* [26].

Complete sets of genes coding for enzymes involved in glycolysis and gluconeogenesis, the tricarboxylic acid (TCA) cycle, as well as the pentose phosphate pathway, were identified in the SH0165 genome (Figure 3). The Entner–Doudoroff pathway, an alternative bypass of classical glycolysis, was not encoded in the *H. parasuis* SH0165 genome, as genes *edd* and *eda* encoding 6-phosphogluconate dehydratase and 2-keto-3-deoxygluconate 6-phosphate aldolase, respectively, are absent. By contrast, *H. somni* 129PT and *H. influenzae* Rd both have an active Entner–Doudoroff pathway responsible for the conversion of glucanate-6-phosphate to pyruvate [11]. In addition, *H. parasuis* SH0165 possesses an enzyme (307 amino acids, aa), encoded by the *rbsK2* (HAPS1688) gene, that was homologous to the fructokinase CscK (307 aa) of *Escherichia coli* EC3132, which can catalyze the phosphorylation of fructose to fructose-6-phosphate [27]. RbsK2 is capable of metabolizing fructose as an alternative start point to glycolysis. However, *H. influenzae* Rd, *H. somni* 129PT and *H. ducreyi* 35000HP all lack this gene. Five *H. parasuis* genes coding for enzymes involved in the conversion of pyruvate were identified, which are necessary for ATP production through lactic acid fermentation (*ldhA*, HAPS2117; *dld*, HAPS1875), and for the generation of formate (*pflB*, HAPS0155) and acetate (*pta*, *ackA*, HAPS0392). Besides fermentation, *H. parasuis* may conduct the entire oxidation of glucose derivatives aerobically to release energy through an intact TCA system (Figure 3). In contrast, several other sequenced species in the genus *Haemophilus*, e.g., *H. influenzae*, *H. ducreyi* and *H. somni*, have been found to harbor deficiencies in the TCA cycle, as they are missing one or three genes coding for citrate synthase, aconitase and isocitrate dehydrogenase [11]. In addition, genes *aceA* and *aceB* encoding isocitrate lyase and malate synthase, respectively, which code for key enzymes involved in the glyoxylate shunt, are absent in *H. parasuis* SH0165. Moreover, all known sequenced genomes of *Haemophilus* spp. are lacking the entire glyoxylate bypass.

*H. parasuis* may perform not only aerobic respiration but also anaerobic respiration, as approximately 61 genes involved in the branched electron respiration transport chains were identified in the SH0165 genome (Table S1). Besides cytochrome D ubiquinol oxidase encoded by *cydAB* (HAPS0067, 0068), which is used for aerobic respiration in the presence of oxygen, *H. parasuis* SH0165 possesses an intact *nagF/DAGHBC* (HAPS1796–1790) operon encoding a putative periplasmic nitrate reductase responsible for the reduction of terminal electron acceptor nitrates in anaerobic environments [28,29].

To effectively control gene expression in response to environmental stimuli, *H. parasuis* SH0165 encodes about 94 genes related to regulatory functions (Table S2). Genes encoding conserved global

### Table 2. Orthologs of predicted CDSs of *H. parasuis* SH0165 compared with complete genomes of related organisms.

| Homologous to                                | number of orthologs | % of CDSs in *H. parasuis* |
|---------------------------------------------|---------------------|-----------------------------|
| *A. pleuropneumoniae* JL03                   | 1,341               | 66.0%                       |
| *P. multocida* Pm70                         | 1,213               | 59.7%                       |
| *M. succiniciproducens* MBEL55E              | 1,184               | 58.3%                       |
| *A. aphrophilus* NJ8700                      | 1,149               | 56.6%                       |
| *M. succinogenes* 130Z                       | 1,145               | 56.4%                       |
| *A. actinomycetemcomitans* D115-1            | 1,111               | 54.7%                       |
| *H. somni* 129PT                             | 1,103               | 54.3%                       |
| *H. ducreyi* 35000HP                         | 1,096               | 54.0%                       |
| *H. influenzae* Rd                           | 1,095               | 53.9%                       |

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**Figure 2. Neighbor-joining phylogeny.** Tree derived from 727 concatenated, conserved protein sequences in the complete genomes of 10 *Pasteurellaceae* species. The scale indicates the number of substitutions per residue. Node support after 1,000 bootstrap replicates is shown. doi:10.1371/journal.pone.0019631.g002
regulatory proteins, such as Crp (HAPS2043), CyaA (HAPS0993), Fur (HAPS0167), ScrR (HAPS1203), Mlc (HAPS0390) and CsrA (HAPS0464), are present and intact in the SH0165 genome. These regulators are able to play a role in activating or repressing the transcription of genes involved in carbon metabolism or other central metabolisms [30,31]. In addition, three predicted two-component signal transduction systems, encoded by cpxAR, arcAB, and qseBC, were also identified in the genome of H. parasuis SH0165. Two-component systems are known to mediate adaptive responses to various environmental signals [32]. For instance, under anaerobic conditions, the ArcA-ArcB system may upregulate genes for anaerobic respiration while downregulate genes for aerobic respiration and fermentation [33,34].

NAD biosynthesis

H. parasuis requires supplementation of the culture media with NAD (V factor) for growth, but NAD is not necessary for the in vitro growth of H. somni or H. ducreyi [11]. Corresponding to these phenotypic differences, gene nadV, which encodes the nicotinamide phosphoribosyltransferase responsible for NAD salvage, is present in the genomes of H. somni and H. ducreyi (HS0002, HD1447 and HD1455) [11,35], but is absent from the SH0165 genome. This is the first time that the NAD-dependent growth of H. parasuis can be explained genetically [1].

Heme biosynthesis

Unlike H. influenzae and H. ducreyi, H. parasuis can grow well in vitro without requiring additional supplementation of iron porphyrin (X factor) [1]. This is because the heme biosynthetic pathway is present and intact in H. parasuis SH0165. An entire set of genes encoding enzymes that are responsible for the conversion of L-glutamate to protoheme were identified in our study (Table S3). Notably, H. influenzae Rd and H. ducreyi 35000HP were missing the main enzymes necessary for heme biosynthesis, but the animal-originated members within the Pasteurellaceae family, e.g. H. parasuis, H. somni, A. pleuropneumoniae and P. multocida, all had a complete biosynthetic pathway for the production of the porphyrin ring. Two hemN genes (HAPS0238 and 0482) encoding coproporphyrinogen III oxidases (EC: 1.3.99.22) were identified in H. parasuis SH0165. Despite the identity between HemN1 and HemN2 only being 23%, they both belong to the HemN_C (PF06969) family whose members catalyze the conversion of coproporphyrinogen-III to protoporphyrinogen-IX [36]. Otherwise, the SH0165 genome encodes almost all indispensable enzymes involved in the biosynthesis of amino acids, nucleotides, fatty acids and cofactors. The catalase encoded by hktE (HAPS2238) may support a catalase-positive phenotype [2]. It is worth noting that genes arcAB encoding urease are all absent from the H. parasuis SH0165 genome, explaining the urease-negative characteristic of this species [2].

Natural competence

Many bacteria are competent, indicating that they have the ability to actively acquire extracellular DNA from the environment [37]. In comparison with the competence genes in H. influenzae and Mannheimia haemolytica [38,39], the H. parasuis SH0165 genome also
contains homologous genes encoding proteins involved in the assembly of the DNA uptake machinery. Outer membrane proteins ComE (HAPS2289) and PilA (HAPS2013) play a potential role in binding liberated DNA segments at the cell surface [38]. Orthologs of proteins that function in the transport of DNA across the cell membrane, periplasmic space, then into the cytoplasm, include lipoprotein ComL (HAPS9026), periplasmic protein ComF (HAPS1497) and cytoplasmic membrane protein ComEA (HAPS0844) [38]. Additionally, several cytoplasmic competence proteins coding genes were identified in the H. parasuis SH0165 genome, including comA (HAPS2285), dprA (HAPS1573) and comM (HAPS1740) [39]. These genetic components may contribute to the natural competence of H. parasuis [40].

It has been shown that Pasteurellaceae species preferentially take up short DNA sequences, called uptake signal sequences (USSs), that are overrepresented in their respective genomes [41]. In H. influenzae, the highly redundant USS has the sequence AAGTGCGGT, and this is also the most common uptake core USS in the genomes of H. somni, A. actinomycetemcomitans, P. multocida and M. succiniciproducens [41]. However, in the genome sequence of H. parasuis SH0165, the most frequent nine-mer repeat is ACAAGCGGT, being present in 523 copies, whereas only 109 copies of the H. influenzae core USS were identified in H. parasuis. Moreover, the core USS of H. parasuis is identical to the most common nine-mer repeats previously identified in A. pleuropneumoniae and M. haemolytica [41]. Figure 4 shows the 50-bp consensus sequences flanking the core USSs in the H. parasuis SH0165 genome. Two AT-rich motifs were observed downstream of the H. parasuis USSs and similar patterns of sequence conservation have been found in M. haemolytica and A. pleuropneumoniae, further confirming that these three microorganisms belong to the same evolutionary subclade [41,42].

**Adherence and secretion**

Pili (namely pili) are a common virulence factor that mediate bacterial adherence to mucosal epithelia [43]. Fimbria-like structures have been observed on the surface of H. parasuis after in vivo passage [44]. As previously reported, a gene cluster pilABCD coding for type IV fimbriae has been identified in a number of Gram-negative pathogens in the genera of Haemophilus, Actinobacillus, Pseudomonas, Vibrio and others [45,46]. H. parasuis SH0165 also possesses four type IV fimbrial genes encoding the major structural unit PilA (HAPS2013) and three biogenesis proteins PilB (HAPS2011–2009) for mediating bacterial adherence. The proteins encoded by these CDSs share 68%, 68%, 50% and 44% identity with the ApABCD proteins of A. pleuropneumoniae HK361, respectively [46]. In addition to PilABCD, H. parasuis SH0165 also encodes a protein (HAPS2143, 22.3 kDa) that shares 54% similarity with Pseudomonas aeruginosa PilF (22.4 kDa), which is involved in type IV fimbrial biogenesis and twitching motility [47].

Genes coding for proteins that constitute the classical Sec and Tat secretion systems were identified in the genome of H. parasuis SH0165 (Table 3), which are required for protein secretion and trafficking. Ten paralog genes vtaA coding for virulence-associated trimeric autotransporters of the type V secretion system were also found in SH0165, the products of which are generally exported through the cytoplasmic membrane via the Sec system [48]. Recent studies have indicated that the H. parasuis vtaA genes reveal diversity and the expressed products are good candidate immunogens [49,50]. Furthermore, three potential autotransporters related to the type V protein secretion pathway were encoded in the SH0165 genome, including two EspP proteins (HAPS1370, 771 aa; HAPS1381, 780 aa) and AaiA (HAPS0733, 858 aa), all of which have a C-terminal translocator domain (PF03797) responsible for the transport of the N-terminal passenger domain across the outer membrane [51]. The amino acid sequence identity between both EspP proteins was high (73%), indicating that they may be encoded by duplicate copies of the same gene.

**Biosynthesis of surface polysaccharide**

LPSs are the primary structural and functional components of the Gram-negative bacterial outer membrane, which can be recognized and targeted by the mammalian immune system [52]. Typical LPS molecules consist of three covalently linked biochemical moieties: the lipid A, the core oligosaccharide and the major surface-exposed O-specific antigen (known as the O polysaccharide). The O polysaccharide is composed of many repeats of an oligosaccharide unit (O unit) and plays a vital role in bacterial adherence, invasion and immune evasion [53]. The LPSs of H. parasuis, also termed lipo-oligosaccharides (LOSs) in this bacterium, are considered a virulence factor associated with disseminated intravascular coagulation and thrombosis [5,54].

All genes coding for enzymes involved in the biosynthesis of LOS were identified in the SH0165 genome (Table S4). Unsurprisingly, according to sequence comparisons and function assignment, genes essential for the synthesis of lipid A (lpxC, kdsA, lpxB, kdtB, lpxH, lpxK, lpxD, lpxA, kdtA, lpxM, kdtC and lpxL) and core oligosaccharide (gufA, gufB, gufD, gufE and gmkA) are present and highly conserved among the genus Haemophilus. Furthermore, a 13,964 bp genomic region that is likely associated with O-antigen biosynthesis was predicted in the H. parasuis SH0165 genome. Twelve putative CDSs were identified within this potential O-antigen region located between HAPS0039 and HAPS0052, and all of these share the same transcriptional orientation (Figure 5). Like many other Gram-negative bacteria, the H. parasuis O-antigen gene cluster had a G+C content (32.8%) lower than that of the SH0165 genome (40.0%). Nine of these
CDSs could be divided into three categories according to their functional roles in the biogenesis of O-antigen [55]: sugar synthesis-related genes (neuA1 and wbgX); glycosyltransferase genes (lbgB, wzaK, wzfQ, and wbgY); and O unit processing genes wcx, wzy and wzz involved in the assembly of O-polysaccharide.

In general, O-unit-processing enzymes (Wzx, Wzy and Wzz) in diverse Gram-negative bacteria show considerable sequence variation but share conserved topologies in membrane spanning regions [53]. The wcx (HAPS0041) gene of H. parasuis encodes an O-antigen flippase (390 aa) which has 12 predicted transmembrane helices and shares 48% identity and 69% similarity with a Wzx homologue (404 aa) from the Shigella boydii O-antigen gene cluster [56]. Although BLASTP searches did not detect any significant homologues of the product of HAPS0043, it was predicted to be an inner-membrane protein with 10 transmembrane helices that contains a large periplasmic loop of 44 amino acid residues (Figure 6), a topology typical of all known O-antigen polymersases [57,58]. Thus, we propose that HAPS0043 of H. parasuis is the O-antigen polymerase gene, wcx. More experimental works are still required to verify the function of H. parasuis Wzy in the O-antigen-processing process.

As is well known, sialylated oligosaccharide units in bacterial polysaccharides play a role in evading the host immune response by mimicking the glycolipid components of mammalian tissues [59]. A CMP-NeuNac synthetase (N-acetylmuraminic acid cytidylylsynthe-

Table 3. Genes encoding proteins with a putative role in adherence and secretion of strain SH0165.

| CDS no. | Name       | Function                              |
|--------|------------|---------------------------------------|
| HAPS003 | vtaA1      | virulence-associated trimeric autotransporter |
| HAPS012 | lsgB       | signal peptidase I                    |
| HAPS020 | vtaA2      | virulence-associated trimeric autotransporter |
| HAPS026 | secA       | preprotein translocase subunit SecA  |
| HAPS029 | wzy        | lipoprotein copper homeostasis and adhesion, NlPE |
| HAPS025 | lolA       | outer-membrane lipoprotein carrier protein precursor |
| HAPS038 | vtaA3      | virulence-associated trimeric autotransporters |
| HAPS042 | secG       | preprotein translocase subunit SecG  |
| HAPS052 | vtaA4      | virulence-associated trimeric autotransporters |
| HAPS054 | vtaA5      | virulence-associated trimeric autotransporters |
| HAPS059 | vtaA6      | virulence-associated trimeric autotransporters |
| HAPS067 | vtaA7      | virulence-associated trimeric autotransporters |
| HAPS073 | aidA       | Type V secretory pathway, adhesin AidA |
| HAPS076 | fimB       | fimbrial assembly chaperone           |
| HAPS095 | vtaA8      | virulence-associated trimeric autotransporters |
| HAPS096 | secF       | preprotein translocase subunit SecF  |
| HAPS097 | secD       | preprotein translocase subunit SecD  |
| HAPS098 | yycC       | preprotein translocase subunit YycC  |
| HAPS130 | ispA       | lipoprotein signal peptidase         |
| HAPS137 | espP1      | extracellular serine protease (autotransporter) |
| HAPS138 | espP2      | extracellular serine protease (autotransporter) |
| HAPS139 | tatC       | Sec-independent protein translocase protein TatC |
| HAPS139 | tatB       | Sec-independent protein translocase protein TatB |
| HAPS139 | tatA       | Sec-independent protein translocase protein TatA |
| HAPS139 | yidC       | putative inner membrane protein translocase component |
| HAPS143 | secY       | preprotein translocase subunit SecY  |
| HAPS147 | secB       | preprotein translocase subunit SecB  |
| HAPS150 | ffh         | signal recognition particle GTase    |
| HAPS151 | vtaA9      | virulence-associated trimeric autotransporters |
| HAPS181 | secE       | preprotein translocase subunit SecE  |
| HAPS185 | fbsY       | cell division protein, signal recognition particle GTase |
| HAPS200 | pilD       | Tfp pilus assembly pathway, fimbrial leader peptidase PIID |
| HAPS201 | pilC       | Tfp pilus assembly pathway, component PIIC |
| HAPS201 | pilB       | Tfp pilus assembly pathway, ATPase PIIB |
| HAPS203 | pilA       | Tfp pilus assembly protein, major pilin PIIA |
| HAPS207 | lolA       | outer membrane lipoprotein LolA      |
| HAPS206 | vtaA10     | virulence-associated trimeric autotransporters |
| HAPS214 | pilF       | fimbrial biogenesis and twitching motility protein |
| HAPS224 | pilG       | Type II secretory pathway, pseudopilin PuLG  |

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Haemophilus (Table S4). The products encoded by these CDSs belong to the glycosyltransferase family 1 (HAPS1021, 1022), 9 (HAPS1017, 1019) and 25 (HAPS1020, 1023), respectively.

Interestingly, nine genes were identified in the *H. parasuis* genome that encode enzymes homologous to those encoded by the *wec* genes associated with the synthesis of LPS O-antigen.

Figure 5. The genetic organization of the LPS O-antigen biosynthetic region in *H. parasuis* SH0165. The coding sequences (CDSs) are drawn to scale, with 1 kb increments indicated.

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Figure 6. Schematic comparisons of the topological models of transmembrane proteins and functional assignment of genes encoding enzymes with a putative role in the biogenesis of O-antigen. A. HAPS0041 (APJL1491, wzx), encoding an O-antigen flippase; B. HAPS0043 (APJL1490, wzy), encoding a putative O-antigen polymerase; C. HAPS0051 (APJL1485, wzz), encoding an O-antigen chain length determining protein. All the amino acid sequences were retrieved from the genomes of *H. parasuis* SH0165 and *A. pleuropneumoniae* JL03. The posterior probability of the transmembrane helix, intracellular side and extracellular side are indicated in green, red and blue, respectively.

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Enterobacterial Common Antigen (ECA), a complex cell surface glycolipid found in all Gram-negative enteric bacteria [68]. Unlike the wee gene cluster in the E. coli K12 chromosome [69], the H. parasuis ECA-like related genes, weeA, weeB, weeC, mlb, weeD, weeE, weeF, weeG and weeO, are dispersed throughout the SH0165 chromosome (Table S4). The identification of these genes provides genetic evidence that H. parasuis may produce a putative ECA-like glycoconjugate. It is worth mentioning that the wee homologues are also present and linked in a cluster in H. ducreyi 35000HP [70].

Virulence-related genes

A number of enzymes, including neuraminidase and proteases, may be important for the virulence of H. parasuis. The gene nanH (HAPS1616) coding for a potential virulence factor, neuraminidase (sialidase), was identified in the SH0165 genome and its product may function in H. parasuis colonization [71]. The H. parasuis SH0165 genome contains approximately 37 genes encoding different proteases that may contribute to virulence (Table S5). The majority of protease-coding genes are conserved among members of the Pasteurellaceae, perhaps due to their role in proteolysis for protein quality control, but several other proteases (encoded by HAPS0648, 1928 and 2032) may be unique to H. parasuis. A clpPX (HAPS0206, 2007) operon encodes the structural subunits of the ATP-dependent Clp protease which proteinasefunction has been reported to be pivotal to the secretion processes of Gram-negative pathogens [72]. The gene lon (HAPS1011) encodes a cytoplasmic serine protease (800 aa) that shares 70% identity with Salmonella enterica serovar Typhimurium Lon protease (784 aa), a potential target of antimicrobial therapy [73].

Iron acquisition and utilization

Iron plays a crucial role in the basic physiological functions of mammalian pathogens, e.g., ATP synthesis, formation of heme, bacterial survival and persistent infection. To overcome iron restriction in host environments, many pathogenic bacteria have evolved different approaches for iron uptake, including synthesizing certain iron-chelating compounds, called siderophores, or scavenging host iron-binding complexes [74]. Unlike other bacterial pathogens that can produce high-affinity siderophores, such as enterobactin, alcaligin and pyochelin, it seems that H. parasuis may be deficient in production of these molecules, as reflected by the lack of related biosynthetic genes [74]. However, H. parasuis SH0165 was found to encode at least 37 genes involved in the capture and utilization of iron from porcine transferrin, heme and heme–hemopexin (Table S6).

The TonB system is used for iron acquisition by many Gram-negative bacteria [75]. Two clusters of consecutive genes exbB1–exbD1–tonB1 (257 aa) (HAPS1366–1364) and tonB2 (267 aa)–exbB2–exbD2 (HAPS2220–2222) were identified in the SH0165 genome, which are responsible for the formation of the TonB1 and TonB2 systems, respectively. Gene components of the TonB2 system have been described previously in H. parasuis [9]. Although the periplasmic proteins TonB1 and TonB2 share low sequence identity (24%), they contain an identical domain (PF03544) involved in the transfer of energy to transferrin binding proteins (TbpB and TbpA), both homologues of which are responsible for transport of iron across the outer membrane of A. pleuropneumoniae [76]. H. parasuis TbpA and TbpB (HAPS2224, 2223) have low sequence identity (28% and 42%) with H. influenzae Tbp1 and Tbp2 (H10094, 0995), respectively, but are highly homologous to the A. pleuropneumoniae TbpA and TbpB (APL1397, 1396) proteins, sharing 93% and 87% identity, respectively [77,78]. Consequently, the divergent residues inferred from the sequence alignments may help determine why H. parasuis and A. pleuropneumoniae preferentially bind porcine transferrin but not human transferrin [79].

Besides the TonB-dependent systems, H. parasuis seems to have various ABC transport systems involved in the uptake of extracellular iron, hemin and hemoprotein. A hemin transport system encoded by the hmuTUV operon (HAPS1572–1570) was identified in the SH0165 genome and was found to be homologous to a family of ABC transporters (HemTUV, ShuTUV) that function in transporting free heme into the cytoplasm [80]. Orthologs of yfeABC (HAPS1097–1100) and fhpABC (HAPS1129–1127) were found in A. actinomycetemcomitans and M. haemolytica, respectively, and their protein products make up periplasmic ABC transport complexes involved in iron acquisition [81,82]. In H. parasuis, the periplasmic iron transport system FhpABC together with the transferrin outer membrane receptor complex TbpAB may form another iron uptake pathway, which is used by Neisseria species to transport ferric iron into the cell [83].

Although the intact fhuCDBA operon involved in ferric hydroxamate uptake has been reported in strain Nagasaki of H. parasuis serovar 5 [84], it was found to be truncated in the SH0165 genome, only consisting of the 5′ portion of fhuC (HAPS0095) and 3′ portion of fhuA′ (HAPS0096), and lacking fhuDB. Genes hhdB′ and hhdA′ (HAPS1445, 1446), encoding two components of a putative hemolysin export system, and ciaA′ (HAPS0477), encoding an iron transporter, are also likely to be non-functional due to frameshift mutations [85].

In this study, we reported the complete genome sequence of H. parasuis strain SH0165, a highly virulent strain isolated in China, and further performed a comparative genomic analysis. Genomic and phylogenetic comparisons indicated that H. parasuis and A. pleuropneumoniae may derive from a recent common ancestor. Together with a full set of the relevant metabolic genes, we confirmed that H. parasuis generates ATP via both fermentation and respiration with an intact TCA cycle for anabolism. Furthermore, genes and operons related to bacterial virulence factors, such as surface polysaccharides, fimbriae and iron acquisition systems, were identified at the genomic level and compared with known virulence genes from other pathogens. A putative Wzy/Wzx-dependent biosynthetic pathway for O-chain polysaccharide was proposed and requires further experimental confirmation in H. parasuis. Our findings should provide a genetic foundation for future research into the mechanisms of pathogenesis of H. parasuis and will accelerate the development of safe and effective vaccines to prevent and control this severe swine disease.

Supporting Information

Table S1 Genes encoding enzymes involved in central metabolism of H. parasuis SH0165 and orthologs present in genomes of five representative species within Pasteurellaceae. (XLS)

Table S2 Genes coding for proteins related to regulatory functions of strain SH0165. (XLS)

Table S3 Heme biosynthetic enzymes encoded in the genome of H. parasuis SH0165 and orthologs from five representative Pasteurellaceae genomes. (DOC)

Table S4 Genes encoding enzymes with a role in surface polysaccharides of strain SH0165 and orthologs present in genomes of three representative Haemophilus spp. (DOC)
Table S5  Putative protease-encoding genes in the H. parasuis SH0165 genome. (XLS)

Table S6  Genes encoding proteins involved in iron metabolism of H. parasuis SH0165 and orthologous proteins from five representative genomes within Pasteurellaceae. (DOC)

References
1. Biberstein EL, White DC (1969) A proposal for the establishment of a new Haemophilus species. J Med Microbiol 2: 75–78.
2. Neddleman S, Sangermano C, Grosberg D, Gluszak C (2006) Haemophilus parasuis and Glässer’s disease in pigs: a review. Veterinarni Medicina 51: 169–179.
3. Kielstein P, Rapp-Gabrielson VJ (1992) Designation of 15 serovars of Haemophilus parasuis on the basis of immunodiffusion using heat-stable antigen extracts. J Clin Microbiol 30: 862–865.
4. Oliveira A, Caneva M, Arapaz V (2006) Genotypic diversity of Haemophilus parasuis field strains. Appl Environ Microbiol 72: 3984–3992.
5. Oliveira S, Blackall PJ, Pijuan C (2003) Characterization of the diversity of Haemophilus parasuis field isolates by use of serotyping and genotyping. Ann J Vet Res 64: 435–442.
6. Oliveira S, Pijuan C (2004) Haemophilus parasuis: new trends on diagnosis, epidemiology and control. Vet Microbiol 99: 1–12.
7. Cai X, Chen H, Blackall PJ, Yin Z, Wang L, et al. (2003) Serological characterization of Haemophilus parasuis isolates from China. Vet Microbiol 111: 231–236.
8. Amaro H, Shihata M, Takahashi K, Sasaki Y (1997) Effects on endothron pathogenicity in pigs with acute septicemia of Haemophilus parasuis infection. J Vet Med Sci 59: 431–435.
9. del Rio ML, Gutiérrez-Martin CB, Rodríguez-Barbosa JO, Navas J, Rodríguez-Ferri EF (2005) Identification and characterization of the TonB region in and role in transferrin–iron acquisition in Haemophilus parasuis. FEMS Immunol Med Microbiol 45: 75–86.
10. Ruiz A, Oliveira S, Torremorell M, Pijuan C (2001) Outer membrane proteins and DNA profiles in strains of Haemophilus parasuis recovered from systemic and respiratory sites. Clin Microbiol 39: 1577–1582.
11. Challacombe JF, Duncan AJ, Brettin TS, Bruce D, Cherterk O, et al. (2007) Complete genome sequence of Haemophilus somnus (Histophilus somnus) strain 129P4 and comparison to Haemophilus ducreyi strain 35000HP and Haemophilus influenzae Rd. J Bacteriol 189: 1090–1098.
12. Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, et al. (1995) Whole-genome random sequencing and assembly of Haemophilus influenzae Rd. Science 269: 496–512.
13. Yue M, Yang F, Yang J, Wei W, Cai X, et al. (2009) Complete genome sequence of Haemophilus parasuis SH0165. J Bacteriol 191: 1359–1360.
14. Salzberg SL, Delcher AL, Kasif S, White O (1998) Microbial gene identification using interpoated Markov models. Nucleic Acids Res 26: 541–548.
15. Lowe TM, Eddy SR (1997) RNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25: 955–964.
16. Lagergaard T (1995)
17. Lagensed K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, et al. (2007) RNAmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35: 3100–3103.
18. Abelsch SF, Gush W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215: 403–410.
19. Sneath P, Wistart DS (2005) Circular genome visualization and exploration using CGView. Bioinformatics 21: 537–539.
20. Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo: a sequence logo generator. Genome Res 14: 1188–1190.
21. Krogh A, Larsson B, von Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305: 567–580.
22. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32: 1797–1800.
23. Kumar S, Nei M, Dudley J, Tamura K (2008) MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform 9: 299–306.
24. Canchaya C, Fournier G, Chilani-Chennoufi S, Dillmann ML, Brusnow H (2003) The origin of monen as lateral gene transfer. Curr Opin Microbiol 6: 417–418.
25. Siddibe M, Messier S, Larrivée G, Gottschalk M, Mittal KR (1993) Detection of Actinobacillus pleuropneumoniae in the porcine upper respiratory tract as a complement to serological tests. Can J Vet Res 57: 204–208.
26. Laguzet T (1995) Haemophilus ducreyi: pathogenesis and protective immunity. Trends Microbiol 3: 87–92.
27. Fink DL, Genez JW (2006) The Genus Haemophilus. 1034–1061. In MDworkin, SFalkow, ERosenberg, K-HSchleifer, EBstaklebranch, eds. The prokaryotes, 3rd ed., vol. 2, Springer, New York, NY.
28. Jalbert K, Blumer B, Lobbink M, Hans S, Meyer A, et al. (2002) Adaptation of sucrose metabolism in the Escherichia coli wild-type strain EC3192. J Bacteriol 184: 5307–5316.

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Conceived and designed the experiments: QJ WB HC. Performed the experiments: ZX MY YF. Analyzed the data: ZX RZ. Contributed reagents/materials/analysis tools: ZX MY YF WB. Wrote the paper: ZX WB.
54. Reeves PR, Hobbs M, Valvano MA, Skurnik M, Whitfield C, et al. (1996) Bacterial polysaccharide synthesis and gene nomenclature. Trends Microbiol 4: 495–503.

55. Liu B, Knirel YA, Feng L, Perepelov AV, Senchenkova SN, et al. (2008) Structure and genetics of Shigella O antigens. FEMS Microbiol Rev 32: 627–653.

56. Liu B, Knirel YA, Feng L, Perepelov AV, Senchenkova SN, et al. (2008) O-antigen polymerase (Rfc/Wzy). Mol Microbiol 69: 1211–1222.

57. Wang L, Briggs CE, Rothemund D, Fratamico P, Luchansky JB, et al. (2001) Identification of putative O-antigen gene clusters and identification of O104 specific gene. J Bacteriol 183: 251–256.

58. Moran AP, Prendergast MM, Appelmelk BJ (1996) Molecular mimicry of host structures by bacterial lipopolysaccharides and its contribution to disease. FEMS Immunol Med Microbiol 16: 105–115.

59. Tullius MV, Munson RS, Jr., Wang J, Gibson BW (1996) Purification, cloning, and expression of a cytidine 5′-monophosphate N-acetylmuraminic acid synthetase from Haemophilus ducreyi. J Biol Chem 271: 15373–15380.

60. Gilbert M, Watson DC, Cunningham AM, Jennings MP, Young NM, et al. (1996) Cloning of the lipopolysaccharide alpha-2,3-sialyltransferase from the bacterial pathogens Neisseria meningitidis and Neisseria gonorrhoeae. J Biol Chem 271: 28271–28276.

61. Bozue JA, Tullius MV, Wang J, Gibson BW, Munson RS, Jr. (1999) Haemophilus ducreyi produces a novel sialytransferase. J Biol Chem 274: 4106–4114.

62. Shepherd JG, Wang L, Reeves PR (2000) Comparison of O-antigen gene clusters of Escherichia coli (Shigella) sonnei and Plesiomonas shigelloides O17: sonnei gained its current plasmid-borne O-antigen genes from P. shigelloides in a recent event. Infect Immun 68: 6056–6061.

63. Coyne MJ, Tzianabos AO, Mallory BC, Carey VJ, Kasper DL, et al. (2001) Polysaccharide biosynthesis locus required for virulence of Bacteroides fragilis. Infect Immun 69: 4342–4350.

64. Bentley SD, Aaensten DM, Mayrovitz A, Saunders D, Rabbinowitsch E, et al. (2006) Genetic analysis of the capsular biosynthetic locus from all 90 pneumococcal serotypes. PLoS Genet 2: e31.

65. Chatzidaki-Livanis M, Jones MK, Wright AC (2006) Genetic variation in the Vibrio vulniﬁcus group 1 capsular polysaccharide operon. J Bacteriol 188: 1987–1998.

66. Zhou H, Yang B, Xu F, Chen X, Wang J, et al. (2010) Identification of putative virulence-associated genes of Haemophilus parasuis through suppression subtractive hybridization. Vet Microbiol 144: 377–383.

67. Erlbruch P, Barr K, Gao N, Gerwig GJ, Rick PD, et al. (2005) Identification and biosynthesis of cyclic enterobacterial common antigen in Escherichia coli. J Bacteriol 187: 1905–2004.

68. Plattner FR, Plunkett G, 3rd, Bloch CA, Perna NT, Burland V, et al. (1997) The complete genome sequence of Escherichia coli K-12. Science 277: 1453–1462.

69. Banks KE, Fourney KR, Baker B, Billings SD, Katz BP, et al. (2008) The enterobacterial common antigen-like gene cluster of Haemophilus ducreyi contributes to virulence in humans. J Infect Dis 197: 1531–1536.

70. Lichtensteiger CA, Vinner ER (2003) Purification and renaturation of membrane neuramidase from Haemophilus parasuis. Vet Microbiol 93: 79–87.

71. Ingmer H, Brennerd L (2009) Proteases in bacterial pathogenesis. Res Microbiol 160: 704–710.

72. Frase H, Lee I (2007) Peptidyl boronates inhibit Salmonella enterica serovar Typhimurium Lon protease by a competitive ATP-dependent mechanism. Biochemistry 46: 6647–6657.

73. Cross JH, Walsh CT (2002) Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. Microbiol Mol Biol Rev 66: 223–239.

74. Poole K, Kadner RJ (2003) Touch and go: tying TonB to transport. Mol Microbiol 49: 869–882.

75. Bosse J, Janson H, Sheehan BJ, Beddek AJ, Rycroft AN, et al. (2002) Actinobacillus pleuropneumoniae: pathobiology and pathogenesis of infection. Microbes Infect 4: 225–235.

76. Gray-Owen SD, Loosmore S, Schryvers AB (1995) Identification and characterization of genes encoding the human transferrin-binding proteins from Haemophilus influenzae. Infect Immun 63: 1201–1210.

77. Xu Z, Zhou Y, Li L, Zhou R, Xiao S, et al. (2008) Genome biology of Actinobacillus pleuropneumoniae JL03, an isolate of serotype 3 prevalent in China. PLoS One 3: e1450.

78. Charlard N, D’Silva CG, Dumont RA, Niven DF (1995) Contact-dependent acquisition of transferrin-bound iron by two strains of Haemophilus parasuis. Can J Microbiol 41: 76–79.

79. Thompson JM, Jones HA, Perry RD (1999) Molecular characterization of the hmu locus of Escherichia coli: isolation of hmu mutants for hemin and hemoprotein utilization. Infect Immun 67: 3879–3892.

80. Rhodes ER, Tomaras AP, McGillivary C, Comerly PL, Actis LA (2005) Genetic and functional analyses of the Actinobacillus actinomycetemcomitans AfeACBD siderophore-independent iron acquisition system. Infect Immun 73: 3758–3763.

81. Rocheir SC, Tran HQ, Sperh V, Gunkel N, Selzer PM, et al. (2007) The response of Mannheimia haemolytica to iron limitation: implications for the acquisition of iron in the bovine lung. Vet Microbiol 121: 316–329.

82. Faraldo-Gómez JD, Sanson MS (2003) Acquisition of siderophores in gram-negative bacteria. Nat Rev Mol Cell Biol 4: 105–116.

83. del Río ML, Navas J, Martín AJ, Gutiérrez CB, Rodríguez-Barbosa JL, et al. (2006) Molecular characterization of Haemophilus parasuis ferric hydroxamate uptake (fhu) genes and constitutive expression of the FhuA receptor. Vet Res 37: 49–59.

84. Sack M, Baltes N (2009) Identification of novel potential virulence-associated factors in Haemophilus parasuis. Vet Microbiol 136: 302–306.