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Abstract

Mycoplasma suis belongs to the hemotrophic mycoplasma group and causes infectious anemia in pigs. According to the present state of knowledge, this organism adheres to the surface of erythrocytes but does not invade them. We found a novel M. suis isolate that caused severe anemia in pigs with a fatal disease course. Interestingly, only marginal numbers of the bacteria were visible on and between the erythrocytes in acridine orange-stained blood smears for acutely diseased pigs, whereas very high loads of M. suis were detected in the same blood samples by quantitative PCR. These findings indicated that M. suis is capable of invading erythrocytes. By use of fluorescent labeling of M. suis and examination by confocal laser scanning microscopy, as well as scanning and transmission electron microscopy, we proved that the localization of M. suis was intracellular. This organism invades erythrocytes in an endocytosis-like process and is initially surrounded by two membranes, and it was also found floating freely in the cytoplasm. In conclusion, we were able to prove for the first time that a member of the hemotrophic mycoplasma group is able to invade the erythrocytes of its host. Such colonization should protect the bacterial cells from the host's immune response and hamper antibiotic treatment. In addition, an intracellular life cycle may explain the chronic nature of hemotrophic mycoplasma infections and should serve as the foundation for novel strategies in hemotrophic mycoplasma research (e.g., treatment or prophylaxis).
Mycoplasma suis Invases Porcine Erythrocytes

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Mycoplasma suis belongs to the hemotrophic mycoplasma group and causes infectious anemia in pigs. According to the present state of knowledge, this organism adheres to the surface of erythrocytes but does not invade them. We found a novel M. suis isolate that caused severe anemia in pigs with a fatal disease course. Interestingly, only marginal numbers of the bacteria were visible on and between the erythrocytes in acridine orange-stained blood smears for acutely diseased pigs, whereas very high loads of M. suis were detected in the same blood samples by quantitative PCR. These findings indicated that M. suis is capable of invading erythrocytes. By use of fluorescent labeling of M. suis and examination by confocal laser scanning microscopy, as well as scanning and transmission electron microscopy, we proved that the localization of M. suis was intracellular. This organism invades erythrocytes in an endocytosis-like process and is initially surrounded by two membranes, and it was also found floating freely in the cytoplasm. In conclusion, we were able to prove for the first time that a member of the hemotrophic mycoplasma group is able to invade the erythrocytes of its host. Such colonization should protect the bacterial cells from the host’s immune response and hamper antibiotic treatment. In addition, an intracellular life cycle may explain the chronic nature of hemotrophic mycoplasma infections and should serve as the foundation for novel strategies in hemotrophic mycoplasma research (e.g., treatment or prophylaxis).

Mycoplasma suis is a member of the family Mycoplasmataceae. This organism belongs to a group of uncultivable highly specialized bacteria which parasitize the surface of erythrocytes of a variety of mammals (34). These species represent a distinct new cluster in the genus Mycoplasma and have been given the trivial name hemotrophic mycoplasmas (HM). Infections with HM are identified clinically by overt life-threatening hemolytic anemia or by subtle chronic anemia characterized by infarctility, immune suppression, and greater susceptibility to infections (34). It is noteworthy that organisms that morphologically resemble HM have also been detected in the blood of humans (1, 8, 42, 50).

M. suis causes febrile acute icteroanemia in pigs (IAP), which is accompanied by high numbers of M. suis cells in the blood, as confirmed by microscopy as well as by PCR (18, 21, 34). Clinical symptoms are successfully cured by treatment with tetracycline. Nevertheless, once pigs are infected with M. suis, they remain lifelong carrier animals and therefore are epidemiologically important (19). Chronic M. suis infections result in reproductive disorders in sows, growth retardation in piglets, and increased susceptibility to respiratory and enteric infections in feeder pigs. M. suis occurs worldwide, and chronic IAP, in particular, is of major economic importance (19).

Contrary to the well-established clinical picture of IAP (i.e., high morbidity and low mortality), we recently observed an increased incidence of acute IAP in feeder pigs with a predominantly fatal outcome despite antibiotic therapy. And contrary to the expected high numbers of M. suis cells on and between the erythrocytes in acridine orange-stained blood smears (the established diagnostic feature of acute IAP), only marginal numbers of bacterial cells were observed. This microscopic finding conflicted with the results of quantitative real-time PCR, which detected high numbers of M. suis cells (10^9 to 10^10 cells per ml of blood) in the same blood samples. The striking difference between the microscopy and PCR results raises the issue of putative intracellular localization of a novel M. suis isolate within the erythrocytes.

To date, several Mycoplasma species are known to invade cells (2, 28, 33, 46); for example, M. penetrans invades the epithelial cells of the human urogenital tract (28), and M. genitalium infects human lung fibroblasts (2, 33). M. gallisepticum can invade nonphagocytic cell lines, such as HeLa cells and chicken embryo fibroblasts (49), and is the only known Mycoplasma species that is able to invade erythrocytes (47). The general advantages associated with invasion of eukaryotic cells by bacterial pathogens include protection from the immune system, reduction in the efficacy of antibiotics during treatment, and nutritional benefits. The intraerythrocytic localization of M. suis might provide the organism with a supply of iron, large amounts of which can be found inside the red blood cells (RBCs) in the form of hemin, or other trace metals (47). It is known that hemin can support the growth of invasive bacteria, such as Bartonella quintana (43). Moreover, the persistence of some Bartonella species is directly linked to nonhemolytic erythrocyte parasitism (43). However, no influence of hemin on the growth of mycoplasmas has been demonstrated so far.

It is interesting that hemotrophic mycoplasmas have a tendency to establish chronic infections that often are not apparent clinically (34). The long persistence of M. suis (and other HM) might be linked to their ability to invade the cytoplasm of host cells. Dallo and Basemann (6) assumed that the chronic nature of mycoplasma infections (M. pneumoniae, M. penetrans, M. genitalium) can be explained by a subpopulation of mycoplasmas that enter mammalian cells, where they can per-
sis in a latent or nongrowth maintenance form. Such biological latency would circumvent the killing action of antimicrobials. The usual establishment of chronic disease in *M. gallisepticum*-infected birds and the limited effects of antibiotic treatments (44) have also been linked to the ability of this agent to enter nonphagocytosing host cells (35, 49).

In order to confirm our hypothesis that *M. suis* invades blood cells, splenectomized pigs were experimentally infected with a novel *M. suis* isolate. The clinical course was monitored, and blood samples were taken during the experiments. Furthermore, we used fluorescent labeling of *M. suis* coupled to confocal laser scanning microscopy (CLSM), as well as scanning electron microscopy (SEM) and transmission electron microscopy (TEM), in order to investigate the intracellulal life cycle of *M. suis*.

**MATERIALS AND METHODS**

**Bacterial isolate and experimental infection.** *M. suis* isolate **08/07** originated from a representative pig in a stock of feeder animals that had severe IAP infections with high mortality. The pig used suffered from acute IAP/08/07-induced hypoglycemia. The values obtained were compared to data obtained from Metabion (Martinsried, Germany). Real-time PCR was performed using a Lightcycler Fast Start DNA MasterPLUS hybridization probe kit (Roche Diagnostics, Mannheim, Germany). The clinical course was monitored, and clinical attacks. Clinical diagnosis of acute IAP was confirmed by quantitative real-time PCR was performed using anticoagulated blood were fixed in ethanol for 1 min and air dried. Staining was done by using an acridine orange solution (0.25 µg/ml) for 1 h in the dark. The slides were rinsed with water and air dried. *M. suis* cells on and between the erythrocytes were detected using UV light excitation with a fluorescence microscope.

When there were acute clinical attacks, pigs were treated with tetracycline (40 mg/kg of body weight) and glucose (35 g glucose/liter of drinking water) to prevent death caused by *M. suis* 08/07-induced hypoglycemia.

**Hematological investigations.** The erythrocyte counts, hemoglobin concentrations, and hematocrit levels of acutely diseased pigs infected with *M. suis* 08/07 were determined using blood that was anticoagulated by EDTA and an automatic blood cell counter (Cellek; Bayer Diagnostics, Munich, Germany). Serum was obtained by centrifugation of a serum monovette (Hettich Rotap/A; Hettich, Tuttinglen, Germany). Glucose and bilirubin levels were determined in the laboratory of the farm animal clinic of Ludwig Maximilians University of Munich by using a selective, discrete, multiple-analysis system (Hitachi 911; Roche, Mannheim, Germany). The values obtained were compared to data obtained from classical *M. suis* infections (17). Reference values (erythrocyte counts, hematocrit levels, hemoglobin concentrations) for healthy pigs were obtained from previously published data of Kraft and coworkers (25). Reference ranges for bilirubin and blood glucose levels were obtained from the data of Kasmoller et al. (24).

**Quantitative real-time PCR.** *M. suis* DNA was quantified using the Light-Cycler 2.0 system (Roche Diagnostics, Rotkreuz, Switzerland), as described previously (20). Primers msgl-Fw (5′-ACAATCTGGACTGATCTCCAT-3′) and msgl-Rv (5′-GCTGTAGTGAGAATGTTAAGTTA3′), as well as probes 5′-TTCGCCCTTCACTGCGCAAGAC-fluorescein-3′ and 5′-LACCCGAAAGCCTAGTTTTCT TC-3′, were purchased from Metabion (Martinsried, Germany). Real-time PCR was performed using a LightCycler Fast Start DNA MasterPLUS hybridization probe kit (Roche Diagnostics) with 0.5 µM of each primer, as well as 0.2 µM of each probe. For quantification, *M. suis* genomic DNA was extracted from experimentally infected pigs and quantified as described previously (20).

**Antibody reagents.** Polyclonal monospecific antisera for the detection of *M. suis* immunostaining were obtained by immunization of rabbits with recombinant HspA1, as described elsewhere (21). HspA1 is a surface protein of *M. suis* (21).

**CLSM.** For staining of the erythrocyte surface and differential staining of intracellular and extracellular *M. suis*, a modified version of the double-immuno-fluorescence (DIF) method adapted for use with erythrocytes (47) was used, with the following modifications. Peripheral anticoagulated blood samples (40 µl) were diluted in 2.5 ml phosphate-buffered saline (PBS) containing 10 mM glucose and 0.1% bovine serum albumin (BSA). Cells were fixed in 4% PBS-buffered paraformaldehyde (Sigma-Aldrich, Steinheim, Germany) containing 0.01% glutaraldehyde (grade I; Sigma-Aldrich, Buchs, Switzerland) and seeded onto poly-l-lysine-coated glass slides (SuperFrost, Menzel, Braunschweig, Germany). Monoclonal antibody against mouse anti-pig CD235a (glycophorin A) monoclonal antibody (1:100; Pharmingen, Bding Biosciences, Europe) and rabbit monospecific anti-HspA1 serum (1:100) for 1 h, followed by tetramethyl rhodamine isocyanate (TRITC)-conjugated goat anti-rabbit IgG (Molecular Probes, Basel, Switzerland) for 1 h. The staining procedure was repeated in Alexa Fluor 405-labeled goat anti-rabbit IgG (Molecular Probes, Basel, Switzerland) for 1 h and incubated in blocking buffer for 30 min. Intracellular *M. suis* cells were detected after incubation with rabbit monospecific anti-HspA1 serum for 1 h and Alexa Fluor 405-labeled goat anti-rabbit IgG (Molecular Probes, Basel, Switzerland) for 1 h. The staining procedure resulted in Alexa Fluor 405-labeled intracellular bacteria (blue) and FITC- and Alexa Fluor 405-labeled extracellular bacteria (green and red). doi:10.1128/aiai.08775-09}
RESULTS

Clinical manifestations and bacteriology of *M. suis* novel isolate 08/07. For blood samples taken from experimental pigs on day 3 postinfection (p.i.), no *M. suis* cells were found in acridine orange-stained (A) blood smears, whereas in SEM single *M. suis* cells were visible (B). During acute clinical attack on day 7 p.i. numerous *M. suis* cells were identified on the erythrocytes by fluorescence microscopy (C) and SEM (D). Both methods revealed a significant reduction in the number of *M. suis* cells on the surface of RBCs on day 11 p.i. (E and F). Ms, *M. suis*. (A, C, and E) Magnification, ×1,000. (B, D, and F) Bar = 2 μm.

FIG. 1. Acridine orange-stained blood smears from pigs infected with *M. suis* 08/07 (A, C, and E) and corresponding SEM images (B, D, and F). On day 3 p.i. no mycoplasmas were detected in acridine orange-stained (A) blood smears, whereas in SEM single *M. suis* cells were visible (B). During acute clinical attack on day 7 p.i. numerous *M. suis* cells were identified on the erythrocytes by fluorescence microscopy (C) and SEM (D). Both methods revealed a significant reduction in the number of *M. suis* cells on the surface of RBCs on day 11 p.i. (E and F). Ms, *M. suis*. (A, C, and E) Magnification, ×1,000. (B, D, and F) Bar = 2 μm.

Due to the life-threatening effect of the IAP attack, tetracycline therapy and glucose infusion over a 5-day period were inevitable. The animals, however, did not respond to the therapy, and the severe clinical IAP continued. To examine the cause of this therapy failure, we performed a microarray-based detection assay used to screen for antibiotic resistance genes in gram-positive bacteria. However, hybridization analysis of *M. suis* 08/07 genomic DNA with the microarray revealed no common tetracycline resistance genes.

Especially remarkable on day 11 p.i. were the different sizes (anisocytosis) of the erythrocytes and the formation of echinocytes, as observed by fluorescent examination. Compared to...
blood samples taken on day 7 p.i., blood samples taken from diseased animals on day 11 p.i. showed a clear reduction in the number of visible *M. suis* cells in acridine orange-stained smears, as well as in SEM (Fig. 1E and F). As determined by real-time PCR, the high bacterial loads (10¹⁰ to 10¹¹ *M. suis* cells per ml of blood) persisted. The striking discrepancy between the microscopy results on the one hand and the PCR results on the other hand suggests that *M. suis* 08/07 might enter the erythrocytes after day 7 p.i., thus escaping detection by surface scanning microscopy.

**Localization of *M. suis* within porcine erythrocytes.** In order to investigate whether *M. suis* 08/07 is able to invade porcine erythrocytes, blood samples from experimental pigs were analyzed in detail by using SEM, TEM, and CLSM.

The interaction of *M. suis* and erythrocytes is marked by the formation of invaginations of variable depth in the erythrocyte membrane (Fig. 2A to D and 3A). In addition to superficially attached *M. suis* cells, the agents were also found in shallow pits. It is noteworthy that deep invaginations in which *M. suis* was surrounded almost completely by the erythrocyte membrane (Fig. 2C and D and 3A) occurred sporadically. In addition, in the *M. suis*-containing sections with deep invaginations, as shown by TEM, there were vacuoles in which the organism was entirely enclosed and therefore located in the RBC cytoplasm (Fig. 3A and D). *M. suis* cells in deep invaginations were covered by membrane material (Fig. 2B and 3B and C), leading to the formation of invasion scars (Fig. 2B).

We also detected intracellular mycoplasma cells which were not surrounded by the vacuole membrane (Fig. 3E), indicating that these bacterial cells were free in the cytoplasm. These free intracellular *M. suis* cells were approximately 300 to 500 nm in diameter, like extracellular attached *M. suis* cells (Fig. 3F), and they had a similar morphology.

The intracellular localization of *M. suis* was confirmed by a DIF technique (Fig. 4). Using this method, extra- and intracellular mycoplasmas were distinguished by consecutive incubation of infected erythrocytes with *M. suis*-specific antisera and different fluorescently labeled secondary antibodies before and after chemical permeabilization of the erythrocyte membrane. Bacteria with both green and blue marks were extracellular bacteria, whereas bacteria that stained only blue were intracellular (Fig. 4). In superimposed images (Fig. 4D) green and blue fluorescence signals appeared to be light blue. By using this method we clearly showed that *M. suis* invaded the porcine erythrocytes. Uninfected porcine erythrocytes that were stained in parallel and served as negative controls did not exhibit any fluorescence signals (data not shown). Figure 5 shows erythrocytes that had a green label (FITC) due to glycophorin A. We used the Imaris Colocalization software to analyze whole stacks of confocal sections of erythrocytes that were double labeled for extra- and intracellular *M. suis* cells. When this method was used, extracellular *M. suis* cells stained red and blue with TRITC and Alexa Fluor 405 appeared to be white, while intracellular *M. suis* cells were blue. This method allowed definite discrimination of double-stained (extracellular) and single-stained mycoplasmas, confirming the intraerythrocytic localization of *M. suis*.

**DISCUSSION**

Our clinical data for experimentally infected splenectomized pigs revealed that *M. suis* 08/07 is more virulent than “classical” *M. suis* isolates. All infected pigs developed severe life-threatening hemolytic anemia (significant lower erythrocyte counts and hemoglobin and hematocrit levels), which was not affected by the antibiotic therapy. Despite the fact that as determined
by SEM the number of extraerythrocytic attached M. suis cells on day 11 was less than the number on day 7, the bacterial load (10^{10} to 10^{11} M. suis cells per ml of blood) was the same. These findings strongly indicated that there was intraerythrocyte localization of M. suis. The HM have previously been described as exclusively epicellular organisms (18, 40, 42), which was the basis for their initial designation as Eperythrozoon suis before they were reclassified in the genus Mycoplasma (37, 38, 43).

In order to define the localization of bacteria inside eukaryotic cells, the two approaches generally used are (i) the gentamicin invasion assay (9, 23, 49), which is a semiquantitative method for comparison of the invasion frequencies of different mycoplasma populations, and (ii) the DIF labeling technique (47, 49) coupled to CLSM, which provides simple and accurate differentiation between intracellular and extracellular mycoplasmas. As the gentamicin invasion assay cannot be used for examination of uncultivable bacteria, such as M. suis, for verification of an intracellular life cycle we had to rely on the DIF assay combined with CLSM, as well as on SEM and TEM. By using these methods we showed for the first time that a member of the HM group is able to invade erythrocytes. The reproducibility of the DIF assay was proved by the results of several repeated experiments performed with the blood of M. suis 08/07-infected pigs, and the data left no doubt concerning the intracellular localization of this bacterium.

Given the fact that acridine orange is a cell-permeable nucleic acid stain, one would expect intraerythrocytic M. suis to be detected by this technique. However, against the intense autofluorescence background of erythrocytes after chemical fixation with ethanol and staining with acridine orange, it is virtually impossible to differentiate the restrained light emission of intracellular M. suis.

Erythrocytes are markedly nonendocytic cells (15) and therefore are suitable hosts for studying intracellular parasitism and potential alternative pathways. In our case, entry of M. suis into the RBCs seemed to begin with invagination of the erythrocyte membrane. As invasion progressed, the depression in the erythrocyte deepened and conformed to the shape of the pathogen. Upon entry, the orifice of the invaginated erythrocyte membrane apparently fused. As a result, M. suis was found in intracellular vacuoles within the erythrocytes.

A similar endocytosis-like mechanism of erythrocyte invasion has been described for Plasmodium falciparum in malaria (14) and for Bartonella bacilliformis, which is responsible for human Oroya fever (3). The finding that M. suis was located within vacuole-like structures indicates that the bacteria must initiate and mediate the process leading to their uptake by the RBCs. The formation of deep invaginations during entry is therefore aided either by mechanical forces generated by the adhering bacteria or by cellular factors secreted by M. suis. In B. bacilliformis a small, hydrophobic molecule named deformin was identified, which causes deformation and invagination of RBC membranes, leading to the formation of intraerythrocytic vacuoles formed by a process similar to endocytosis (32).

In a study carried out by Murphy and coworkers, the chemical composition of drug-induced endovesicle membranes of erythrocytes was compared with that of the host-derived parasitophorous vacuolar membrane in malaria-infected RBCs (36). The results clearly showed that in pathogen-induced en-
dovacuolation specific raft and cytoskeletal proteins, as well as lipids, are actively excluded from the vacuole, e.g., PIP$_2$, which displays a major phosphoinositide in erythrocyte membranes influencing membrane junctional complex stability. Interestingly, PIP$_2$ is present in the drug-induced endovesicle whereas it cannot be found in the parasitophorous membrane, suggesting an unknown erythrocyte phospholipid remodeling in the malarial vacuole during invasion of *P. falciparum*. Further studies are necessary to examine whether the *M. suis*-induced endovacuole has a composition similar to that of the parasitophorous vacuolar membrane in malaria.

Sometimes we found that *M. suis* cells were located free in the RBC cytoplasm. These bacterial cells might have corresponded to *M. suis* cells that either used a different mechanism for erythrocyte invasion or escaped from the initial vacuole. Further investigation (e.g., staining of infected erythrocytes with FM4-64 coupled to CLSM) will be done to clearly distinguish *M. suis* located within RBC-derived vacuoles from free forms located in the RBC cytoplasm. FM4-64 is an amphiphilic plasma membrane stain used to investigate endocytosis and vesicle trafficking in living eukaryotic cells.

The central considerations arising from our observations are unquestionable. Does the intraerythrocytic lifestyle of *M. suis* provide any benefit to the organism, and are there any hints that it has an impact on the life cycle of this organism? Obviously, the intracellular localization of *M. suis* can prevent damage of the bacteria by antibiotics. This conclusion is supported by the fact that it was impossible to cure the high level of bacteremia with tetracycline, the antibiotic which is generally used to treat pigs with acute IAP. Tetracycline is, however, the drug of choice for elimination of obligate intracellular bacteria (22, 29). Thus, it is more probable that *M. suis* developed tetracycline resistance mechanisms. In a study of *M. synoviae*, an increase in resistance to enrofloxacin could be demonstrated after several in vivo treatments of experimentally infected hens (27). However, when we used a microarray-based detection assay used to screen for antibiotic resistance genes in gram-positive

![Image](https://via.placeholder.com/150.png?text=FIG. 3. Invasion of porcine erythrocytes by *M. suis* 08/07 shown by SEM (B) and TEM (A and C to F). During invasion *M. suis* 08/07 is located in deep invaginations of the RBC membrane (A). As invasion progresses, the erythrocyte membrane conforms to the shape of the bacterial cells (A and C), and newly formed membrane material covers the surface of the bacteria (B and C). As a result, invading mycoplasmas are located within an intraerythrocytic vacuole (D). Intracellular forms that are free in the RBC cytoplasm (E) have a shape and size similar to the shape and size of extracellular attached *M. suis* cells (F). exMs, extracellular *M. suis*; inMs, intracellular *M. suis*; IS, invasion scar; Ms, *M. suis*; MsV, *M. suis* vacuole; RBCC, RBC cytoplasm.)
bacteria (39), we did not find any common tetracycline resistance genes (tetP, tetS, tetT, tetU, tetW, tetZ, tetK, tetL, and tetM) in M. suis. Therefore, M. suis 08/07 might have unknown or uncommon resistance mechanisms. The presence of M. suis within porcine erythrocytes nonetheless has significant clinical implications. It is possible that the internalized bacteria are able to exploit host cell functions and elude host defense mechanisms, such as complement-mediated lysis and circulating phagocytic cells and antibodies (5, 6, 10, 13). Moreover, an intraerythrocytic localization of M. suis aggravates the natural turnover of infected RBCs in the spleen (30). The RBCs carrying only cytoplasmic M. suis cells do not have modified surfaces and cannot be recognized by spleen macrophages. This conclusion was supported by the findings obtained with experimentally infected pigs that obviously had constant numbers of RBCs that were invaded by M. suis in the peripheral blood and the duration of the severe clinical symptoms. Nevertheless, further verification is needed.

The bacterial invasion of erythrocytes is a special feature that has been described previously only for members of the genera Bartonella (3, 7, 31, 43) and Anaplasma (12) and for M. gallisepticum (47). In contrast to the establishment of a persistent stage of Bartonella, erythrocyte invasion by M. suis 08/07 leads to severe hemolysis and to greater virulence. Furthermore, in contrast to previously described infections with common M. suis isolates, a chronic infection is not established upon infection with M. suis 08/07 due to the fatal course of the disease. To further define whether internalization of M. suis occurs in naturally chronically infected pigs and the potential impact of this phenomenon in these animals, TEM studies, as well as CSLM, will have to be performed with samples collected from such pigs. Detection of intracellular M. suis bacteria in asymptomatic persistently infected pigs would be a milestone in research on HM, as it would help explain the long-term survival of the organisms and the associated persistence.

We found many erythrocytic precursor cells in the circulating blood using TEM. It is well known from former studies that RBC precursors can be found in the circulation of patients with chronic and acute anemia (48) and in animals infected with HM (11). However, the presence of M. suis-infected reticulocytes and normoblasts in the bloodstream is a novel finding for the pathogenesis of HM (data not shown). Further investigations of the bone marrow of infected pigs should reveal whether M. suis 08/07 can propagate within the blood-building tissue.

In conclusion, this is the first report to show that a member of the HM group is able to invade the erythrocytes of its host. Our studies clearly showed that the intracellular life cycle of M.
suis dramatically increased the virulence of the organism. Further studies are required to understand the cellular interactions and factors that contribute to host cell invasion by M. suis 08/07 and to investigate the ability of common M. suis isolates to invade erythrocytes.

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