Polymorphonuclear leukocytes (PMNs) are an essential component of the human innate immune system. Neutrophils are rapidly recruited from circulation and bone marrow reserves to sites of bacterial infection in response to host- and pathogen-derived chemotactic factors (37). PMNs bind and ingest bacteria through a process known as phagocytosis, which promotes the production of reactive oxygen species (ROS) and the fusion of cytoplasmic granules with pathogen-containing vacuoles. Phagocytosis of bacteria by PMNs is initiated most efficiently by receptor recognition of host antibody and complement molecules deposited on the invading microbe (37). During phagocytosis, ligation of these receptors leads to initiation of diverse neutrophil signal transduction pathways, including assembly of the NADPH-oxidase complex (35). Subsequent activation of the NADPH-oxidase results in the production of several types of ROS, including superoxide, hydrogen peroxide, and hypochlorous acid (36). The cumulative effect of neutrophil ROS and granule components is sufficient to kill most invading bacterial pathogens. Notwithstanding, some human pathogens have evolved sophisticated methods to evade the innate immune response, including inhibition of phagocytosis and ROS production and resistance to oxidative stress and microbicidal granule constituents (2). Importantly, subversion of the innate immune system is linked to increased bacterial pathogenicity (23).

The genus Yersinia contains three pathogens of human importance: Yersinia enterocolitica, Yersinia pseudotuberculosis, and Yersinia pestis. The pathogenic Yersinia share several common virulence determinants, such as variable lipopolysaccharide (41), a high-pathogenicity island (39), and, perhaps most notably, the virulence plasmid encoding the type III secretion system (TTSS) (11). The TTSS is assembled during growth at 37°C but remains blocked until physical contact with a host cell is achieved or the calcium concentration is depleted (10). The six known TTSS effector Yops are injected into mammalian host cells and together are indispensable for virulence (11). Recently, Y. pestis Yop proteins were shown to selectively target host immune cells in vivo, with a demonstrated preference for dendritic cells, macrophages, and neutrophils (30). Extensive studies performed on Yop function in Yersinia spp. have collectively shown that the primary mechanism of action for these effector proteins is interference with host cell signal transduction pathways (10). YopJ/P inhibits both the mitogen-activated protein kinase and NF-κB signaling pathways and influences both inflammatory capacity and apoptosis of host immune cells (33, 38). In addition, YopE, YopH, YopO/YpkA, and YopT interfere with signaling pathways involved in maintenance of the host cytoskeleton (11). The cumulative effects of these four Yops from Y. enterocolitica and Y. pseudotuberculosis result in inhibition of ROS production and phagocytosis by macrophages and neutrophils (10).

Several important differences exist between pathogenic Yersinia spp. Infections with Y. enterocolitica and Y. pseudotuberculosis result in a primarily self-limiting enterocolitis, whereas Y. pestis causes severe, life-threatening systemic disease (7). The increased pathogenicity and diverse life cycle of Y. pestis are due, in part, to the presence of additional genetic features that enable capsule production (13), pulmonary dissemination (25), biofilm formation (18), and survival in the flea (16). Further differences exist between the pathogenic Yersinia spp. and are defined by the evolutionary loss of genetic elements (1). For example, Y. pestis lacks two functional adhesins, invasin and virulence plasmid-encoded YadA, which are important
for \textit{Y. enterocolitica} and \textit{Y. pseudotuberculosis} attachment to host cells (9, 43, 48). Although significant species-specific differences exist between the pathogenic yersiniae, it is unclear if these changes affect interactions with the host innate immune system.

\textit{Y. pestis} infection in humans results in elevated leukocyte counts, with a predominance of immature and mature neutrophils (7). In addition, PMNs are recruited to infection sites resulting from fliA transmission of bubonic plague (44). However, the persistence of extracellular bacteria during infection suggests that \textit{Y. pestis} resists PMN phagocytosis and killing (7). The interactions between human PMNs and \textit{Y. pestis} have not been investigated, and thus the role of the neutrophil in the pathogenesis of plague is unclear. Limited inferences on \textit{Y. pestis} interactions with human PMNs can be deduced from experiments with \textit{Y. enterocolitica} (15, 43, 47) or cells of rodent origin (6, 8). However, significant differences exist between human and murine neutrophils, including the apparent lack of inducible nitric oxide synthase in human PMNs (51) and variations in Toll-like receptor recognition of lipopolysaccharide (34). In this study, we examine directly the effects of \textit{Y. pestis} TTSS expression on human neutrophil function. We now show that the \textit{Y. pestis} strain KIM5, containing the pCD1 virulence plasmid, inhibits phagocytosis and ultimately survives following interactions with human PMNs. Although \textit{Y. pestis} KIM5 completely inhibits PMN ROS production, we demonstrate that the majority of ingested bacteria are killed and that residual intracellular survival occurs by a TTSS-independent mechanism.

\textbf{MATERIALS AND METHODS}

\textbf{Isolation of human PMNs and cell viability.} Neutrophils were obtained from heparinized venous blood of healthy individuals and isolated by dextran sedimentation followed by Ficoll-Paque Plus (Amersham Biosciences, Piscataway, NJ) gradient separation as described previously (21). All studies with human PMNs were performed in accordance with a protocol approved by the Institutional Review Board for Human Subjects, University of Idaho. The purity of PMN preparations and cell viability were routinely assessed by flow cytometry (FACSCalibur; BD Biosciences, San Jose, CA), and preparations contained \approx 98\% neutrophils. PMN apoptosis following phagocytosis was measured using annexin V-fluorescein isothiocyanate (annexin V-FITC apoptosis detection kit II; BD Biosciences) as described previously (21).

\textbf{Bacterial strains and culture conditions.} Isolated colonies were obtained from overnight cultures of viable frozen stocks of \textit{Y. pestis} strains KIM5 (pCD1 positive and pgm negative) and KIM6 (pCD1 negative and pgm negative). The presence of the pCD1 virulence plasmid in \textit{Y. pestis} strain KIM5 was routinely verified by selective growth on Congo red-magnesium oxide plates (42) and by PCR. A \textit{Y. pestis} KIM5 isogenic pCD1-negative control strain (KIM5(-)) was obtained by selection for plasmid loss on Congo red-magnesium oxide plates and was verified by PCR. Overnight cultures of \textit{Y. pestis} strains were obtained by growth in BBL brain heart infusion medium (Becton Dickinson and Co., Sparks, MD) at 22°C. Prior to each assay, overnight cultures were diluted 20-fold in brain heart infusion medium supplemented with 2.5 mM CaCl$_2$, grown at 28 or 37°C with shaking (225 rpm), and harvested at mid-exponential growth phase (optical density at 600 nm, \approx 0.6). The final concentrations of \textit{Y. pestis} cultures were determined by enumeration in a Petroff-Hauser counting chamber by light microscopy.

\textbf{Phagocytosis experiments.} Phagocytosis of \textit{Y. pestis} by human PMNs was determined by fluorescence microscopy as described previously (20), with the following modifications. Bacteria were grown to exponential phase, opsonized with 20% autologous normal human serum for 30 min at 37°C, and washed in Dulbecco's phosphate-buffered saline (Invitrogen, Rockville, MD). \textit{Y. pestis} strains KIM5 and KIM6 were transformed with plasmid pFPV25.1 (24), encoding constitutively expressed green fluorescent protein (GFP). PMNs (\times 10^6) suspended in RPMI 1640 medium (Invitrogen) were added to glass coverslips coated with 5 \(\mu\)g/cm$^2$ human fibronecin (BD Biosciences) in 24-well tissue culture dishes and allowed to adhere at room temperature for 15 min. PMNs were chilled on ice for 10 min. GFP-expressing bacteria were added (10^3 bacteria-to-PMN ratio), and plates were centrifuged at 400 \(\times\) g for 8 min at 4°C to synchronize phagocytosis. Following centrifugation, samples were processed before incubation at 37°C (0 min) or plates were incubated at 37°C in a CO$_2$ incubator for the remainder of the assay. Medium was removed from the wells by aspiration, and cells were fixed on ice for 30 min with 4\% paraformaldehyde. Fixation was removed by aspiration, and unengested bacteria were counterstained with Alexa Fluor 594-conjugated antibody specific for human complement component C3 (MP Biomedical, Irvine, CA) for 15 min at room temperature. Samples were visualized with a Nikon Eclipse 80i (Nikon Instruments Inc., Melville, NY) fluorescence microscope with phase contrast. Percent phagocytosis was calculated by subtracting the number of extracellularly associated bacteria from the total number of cell-associated bacteria, dividing the result by the total number of associated bacteria, and multiplying by 100 for at least 100 PMNs from random fields for each experiment.

\textbf{Assay for ROS production.} Neutrophil ROS production was measured using a published fluorometric method (20), but with modifications. PMNs were incubated with 25 \muM 2',7'-dichlorodihydrofluorescein diacetate (Invitrogen) for 30 min at room temperature in RPMI 1640. Subsequently, PMNs (\times 10^6) and bacteria (\times 10^7 \textit{Y. pestis} organisms) opsonized with 20\% autologous normal human serum were combined in serum-coated wells of a 96-well microtiter plate at 4°C. The plates were centrifuged for 5 min at 400 \(\times\) g and immediately transferred to a microplate fluorometer (SpectraMax M2; Molecular Devices, Sunnyvale, CA). ROS production was measured for up to 180 min at 37°C, with excitation and emission wavelengths of 485 and 538 nm, respectively. V$_{max}$ was calculated as the highest rate of ROS production within a 10-min period, using SoftMax Pro, version 5 (Molecular Devices).

\textbf{PMN bactericidal activity.} Killing of bacteria by human PMNs was determined as described previously (20), with the following modifications. Briefly, PMNs (\times 10^6) were combined with opsonized bacteria (\times 10^7) in 96-well plates (10:1 bacterium-to-PMN ratio), centrifuged at 400 \(\times\) g for 5 min, and incubated at 37°C for times of up to 300 min. Alternatively, PMNs were treated with 250 \mug/ml gentamicin (Sigma) 15 min following the addition of \textit{Y. pestis} to remove any remaining extracellular bacteria. Cells were incubated with gentamicin for an additional 30 min at 37°C prior to commencement of the assay (time zero) and further incubation for times of up to 240 min. Gentamicin was removed by aspiration, and cells were gently washed by the addition of fresh RPMI 1640. Viable bacteria were recovered by use of a previously published method (40). Briefly, PMNs were lysed with 0.1\% Triton X-100 (EMD Chemicals, Gibbstown, NJ) in Dulbecco's phosphate-buffered saline for 10 min on ice, followed by sonication (3 times for 1 s each) (150\% microtip; Branson, Danbury, CT), and bacteria were plated on LB agar. Colonies were enumerated following incubation for 2 to 3 days, and the percentage of bacteria killed was calculated by using the equation (CFU$_{PMN}$/CFU$_{tot}$) \times 100. The assay measures the total number of viable ingested and unengusted bacteria or ingested bacteria only (gentamicin treatment).

\textbf{Statistics.} Statistics were performed using repeated-measures analysis of variance with Tukey's correction for all multiple pairwise comparisons or with Student's t-test, using Prism 4 (GraphPad, San Diego, CA).

\textbf{RESULTS}

\textbf{Phagocytosis of \textit{Y. pestis} by human neutrophils.} Pathogenic yersiniae possess a common TTSS that is involved in the inhibition of phagocytosis by both neutrophils and macrophages. Although the ability of \textit{Y. enterocolitica} to limit ingestion by human PMNs is well documented, a similar role for \textit{Y. pestis} has simply not been tested. Therefore, we used differential fluorescence microscopy to assess PMN phagocytosis of \textit{Y. pestis} isogenic strains either with or without the presence of the TTSS-encoding plasmid pCD1 (Fig. 1). Phagocytosis of both strains of serum-opsonized \textit{Y. pestis} occurred rapidly and approached 70\% within 15 min. PMN phagocytosis of \textit{Y. pestis} strain KIM5 increased to approximately 90\% at 45 min, whereas ingestion of KIM5 remained relatively unchanged. Phagocytosis of either strain of \textit{Y. pestis} reached a maximum by 45 min, with little evidence of increased ingestion at later time points. It is possible that following phagocytosis, ingested GFP-
expressing *Y. pestis* cells were lysed, thus confounding enumeration. However, similar results were obtained with *Y. pestis* directly labeled with Alexa Fluor 488 (data not shown). In addition, PMN phagocytosis of both KIM5 and KIM6 grown at 28°C was nearly identical to ingestion of KIM6 grown at 37°C (data not shown). The results of the phagocytosis assays suggest that human PMN ingestion of *Y. pestis* is inhibited by the TTSS, which is in direct agreement with findings reported for *Y. enterocolitica* (15, 43, 47).

**Inhibition of human neutrophil ROS production by *Y. pestis* is dependent on TTSS expression.** Human PMNs produce a rapid oxidative burst following ingestion of bacterial pathogens (20). However, several bacterial pathogens, such as *Helicobacter pylori* (3), *Franciscella tularensis* (31), and *Y. enterocolitica* (27), have been shown to inhibit neutrophil ROS production. *Y. enterocolitica* inhibition of the PMN oxidative burst is due, at least in part, to the concerted activity of secreted Yop proteins on neutrophil signal transduction (4, 49). To test the hypothesis that *Y. pestis* inhibition of PMN ROS production is TTSS dependent, the neutrophil oxidative burst was measured following phagocytosis of *Y. pestis* strains KIM5 and KIM6 grown at either 28 or 37°C. Phagocytosis of either *Y. pestis* strain grown at 28°C, a condition not conducive to Yop expression, resulted in robust PMN ROS production (Fig. 2A). In contrast, *Y. pestis* strain KIM5 grown at 37°C completely inhibited the neutrophil oxidative burst, whereas identically grown KIM6 and pCD1-cured KIM5(−) elicited pronounced ROS production (Fig. 2B). To verify that the *Y. pestis* inhibition of PMN ROS production was not due to strain-specific differences in phagocytosis (Fig. 1), PMNs were pretreated with 100 μM cytochalasin D to prevent ingestion of all bacteria (19). Cytochalasin D-treated PMNs produced a robust oxidative burst following interactions with KIM6, and ROS production remained inhibited by KIM5 (data not shown). Together, these data indicate that *Y. pestis* inhibits ROS production in human PMNs by a TTSS-dependent mechanism that is independent of changes in phagocytosis.

**Persistence of TTSS-expressing *Y. pestis* following interaction with human PMNs.** The primary role of the neutrophil in host defense is to kill invading pathogens (36). Similarly, the ability of bacterial pathogens to evade PMN killing mechanisms is linked to increased pathogenesis (2). To determine if *Y. pestis* survives following interactions with human neutrophils, PMN bactericidal activity was measured following synchronized phagocytosis assays of *Y. pestis* strains KIM5 and KIM6. Both *Y. pestis* strain KIM6 grown at 37°C and strain KIM5 grown under conditions not conducive to TTSS expression (28°C) were rapidly killed (~20% survival) within 45 min and remained relatively constant at later time points (Fig. 3). In comparison, PMN killing of TTSS-expressing KIM5 (37°C) was somewhat lower (~30% survival) at 45 min and dramatically decreased over time (Fig. 3) (~65% at 285 min). In addition, the viability of *Y. pestis* was not affected by treatment with 0.1% Triton X-100 and sonication in control experiments (data not shown), which is consistent with previous reports (40). These data indicate that *Y. pestis* survives interactions with human PMNs by a TTSS-dependent mechanism.

**Despite TTSS-dependent inhibition of PMN ROS production, intracellular *Y. pestis* is effectively killed by human neutrophils.** The microbicidal contribution of PMN oxygen radicals is evidenced by the increased susceptibility to bacterial infection of chronic granulomatous disease patients who are deficient in NADPH-oxidase activity (26). Likewise, the ability of bacterial pathogens to inhibit PMN ROS production may contribute to intracellular survival and facilitate the progression of disease. Inhibition of PMN ROS production correlates

---

**FIG. 1.** Influence of the *Y. pestis* TTSS on phagocytosis by human neutrophils. *Y. pestis* isogenic strains with the presence (KIM5) or absence (KIM6) of the pCD1 virulence plasmid were grown at 37°C to induce expression of the TTSS. Phagocytosis was assessed by immunofluorescence microscopy, and the results are expressed as means ± standard deviations (SD) for three experiments. *, significant difference between strains (*P* < 0.01) at indicated time points.

**A**

![Phagocytosis Graph](image)

**B**

![Phagocytosis Graph](image)
with an increased intracellular survival of both *F. tularensis* and *Salmonella enterica* (14). To test this hypothesis with *Y. pestis*, we measured intracellular survival in human neutrophils. Following PMN phagocytosis of *Y. pestis* strains KIM5 and KIM6 grown at 37°C, extracellular *Y. pestis* (Fig. 1) was removed by treatment with gentamicin. Regardless of the strain, *Y. pestis* cells were equivalently killed by human PMNs over time (Fig. 4A). Survival of both *Y. pestis* strains remained constant and persisted (~30%) at time points beyond 240 min (data not shown). It is important that there were no appreciable differences in induction of early PMN apoptosis between *Y. pestis* strains KIM5 and KIM6, as evidenced by annexin V staining (Fig. 4B). In addition, there was only a slight increase in late PMN apoptosis/necrosis detected by annexin V and propidium iodide staining (Fig. 4C). Previous reports have shown that *Y. enterocolitica* and *Y. pseudotuberculosis* induce increased macrophage apoptosis and cell death via the TTSS effector YopP/J (32, 33). In vivo data indicate that *Y. pestis* is also capable of inducing apoptosis of macrophages and neutrophils (29), although the translocation of YopJ is markedly less efficient (>10-fold) than that for either of the enteropathogenic species (52). Similarly, our data indicate that a multiplicity of infection of 10 does not impact PMN viability by a TTSS-dependent mechanism, whereas ROS production is inhibited completely. Furthermore, the ability of *Y. pestis* KIM5 to inhibit the neutrophil oxidative burst was not altered by the addition of gentamicin following phagocytosis (Fig. 4D). Taken together, these findings suggest that *Y. pestis* intracellular survival in human PMNs is independent of the TTSS.

### DISCUSSION

Neutrophils are an essential first-line defense against invading bacteria. Human deficiencies in neutrophil function routinely lead to increased susceptibility to life-threatening bacterial infections. Similarly, several bacterial pathogens are able to circumvent neutrophil killing mechanisms to cause disease. We show here that *Y. pestis* inhibition of neutrophil phagocytosis and ROS production is TTSS dependent and that bacteria survive following interaction with human PMNs. These findings are in general agreement with previous studies demonstrating that the TTSS of *Y. enterocolitica* is involved in the inhibition of phagocytosis, ROS production, and killing by human PMNs (15, 43, 47). In addition, we show that PMN bactericidal activity against *Y. pestis* is independent of inhibition of the oxidative burst.

During the course of infection, *Y. pestis* is capable of achieving high levels of bacteria in the bloodstream and in tissue (45). The finding that *Y. pestis* often exists extracellularly during plague suggests that *Y. pestis* is capable of evading phagocytosis (7). *Y. pestis* grown at temperatures lower than 37°C is readily phagocytosed by both human (18, 28) and murine (6, 8) PMNs. However, expression of both F1 capsular antigen and TTSS during growth at 37°C results in a high level of resistance to phagocytosis in rodent neutrophils (12, 17). Our data suggest that *Y. pestis* inhibition of phagocytosis by human PMNs is TTSS dependent. *Y. pestis* strain KIM5 showed ~20% less PMN phagocytosis than that of strain KIM6 lacking the pCD1 virulence plasmid (Fig. 1). Both *Y. pestis* strains were grown at 37°C for >5 h under conditions conducive to capsule formation, and although PMN phagocytosis of KIM6 was relatively complete by 45 min (~90%), we did not test for the contribution of the F1 capsule. The maximum level of PMN phagocytosis of *Y. pestis* (~70%) is somewhat higher than that reported for *Y. enterocolitica* (~12 to 37%) (15, 43, 47) but may be accounted for by the inactivation of YadA in *Y. pestis* (9, 43). Notwithstanding, wild-type *Y. pestis* was consistently intracellular within neutrophils 1 to 2 days following infection in a murine model of plague (29). Regardless of the degree, *Y. pestis* inhibition of human PMN phagocytosis likely contributes to increased bacterial survival in the host and thus facilitates disease.

The production of NADPH-derived oxidants contributes to the overall microbicidal capacity of the neutrophil. Inhibition of ROS production by *F. tularensis* (3), *H. pylori* (31), and *Y. enterocolitica* (27) in PMNs and by pathogenic yersiniae (40, 50) and *S. enterica* (46) in macrophages correlates with increased intracellular survival. However, in addition to TTSS-dependent repression of the oxidative burst, pathogenic yersiniae also inhibit phagocytosis in both PMNs and macrophages. Our results indicate that *Y. pestis* survival following interactions with human PMNs is TTSS dependent (Fig. 3), similar to the results of previous studies using *Y. enterocolitica* (9, 43, 47). The observation that *yersinia* spp. survive following interactions with human PMNs is expected given that TTSS-expressing bacterial cells are somewhat resistant to phagocytosis and likely replicate extracellularly. However, neither our experiments nor those performed with *Y. enterocolitica* addressed specifically the intracellular fate of bacteria ingested by PMNs. Inasmuch as a TTSS-expressing strain of *Y. pestis* com-
pletely inhibited PMN ROS production (Fig. 2), we used gentamicin to test directly the fate of phagocytosed (intracellular) Y. pestis (Fig. 4). Our data indicate that intracellular survival of Y. pestis in human PMNs is independent of TTSS expression. The observation that the majority of ingested Y. pestis cells are killed by human PMNs is consistent with previous studies demonstrating that Y. pestis organisms residing in PMNs from infected mice are nonviable (6, 29). In addition, our data indicate that a persistent level (~30%) of Y. pestis cells survive intracellularly for at least 4 h (Fig. 4A). Although Y. pestis is generally considered an intracellular pathogen of macrophages, Janssen and Surgalla (17) showed that a small percentage of virulent or avirulent Y. pestis cells survive in neutrophils obtained from the peritoneal cavity of guinea pigs. Notably, these experiments were performed at 23°C in the absence of CO₂ and used bacterial growth medium, which is at variance with physiological conditions that support optimal PMN function. Nonetheless, it was noted that the number of PMNs containing viable Y. pestis was always lower than the total number containing ingested organisms. These results are consistent with our finding that isolated human PMNs are able to dramatically decrease intracellular Y. pestis viability but (regardless of TTSS expression) do not completely eliminate bacteria (Fig. 4A). Our finding that inhibition of ROS has little apparent impact on PMN killing of Y. pestis suggests that ingested bacteria are susceptible to the microbicidal activity of neutrophil granules. Although Y. pestis is more resistant to cationic peptides than Y. enterocolitica is (5), murine neutrophil extracts are somewhat bactericidal to Y. pestis (8). In addition, Y. pestis is more susceptible to the activity of bactericidal cationic peptides when grown at 37°C than when grown at 21°C, due in part to temperature-dependent variations in lipopolysaccharide structure.

FIG. 4. Y. pestis intracellular survival following phagocytosis by human PMNs. PMNs were incubated with Y. pestis strains KIM5 and KIM6 grown at 37°C. (A) Gentamicin was added to PMNs 15 min following phagocytosis to eliminate noningested (extracellular) Y. pestis. At each time point, PMNs were washed to remove gentamicin, lysed, and plated on growth agar. PMN bactericidal activity was calculated as described in Materials and Methods. Results are expressed as means ± SD for three experiments. No differences (P > 0.05) were detected between KIM5 and KIM6 at any time point. (B) Flow cytometric analysis of early PMN apoptosis following phagocytosis of Y. pestis. PMNs with exposed phosphatidylserine (early apoptosis) bound annexin V-FITC. (C) Late apoptotic/necrotic PMNs dually stained with annexin V-FITC and propidium iodide (PI). Results are expressed as means ± SD for at least 12 experiments. No significant differences (P > 0.05) were detected between KIM5 and KIM6 at any time point. (D) Neutrophil ROS production in the presence of gentamicin. 2',7'-Dichlorodihydrofluorescein diacetate-treated PMNs were incubated alone or with Y. pestis strains KIM5 and KIM6 (37°C) for 15 min, followed by the addition of gentamicin. The rates of ROS production are means for three experiments. ∆FL, change in fluorescence. PMNs not treated with gentamicin were included as a reference control (none).
(41). Future studies that directly address the susceptibility of *Y. pestis* to specific neutrophil granule components will likely further our understanding of neutrophil bactericidal activity against the plague bacillus.

In summary, our data demonstrate that the *Y. pestis* TTSS contributes to extracellular survival following interactions with human PMNs and that the intracellular fate is independent of TTSS inhibition of neutrophil ROS production. Although *Y. pestis* inhibition of the neutrophil oxidative burst does not directly impact the fate of intracellular bacteria, it is possible that decreased levels of ROS may result in the alteration of directly impact the fate of intracellular bacteria, it is possible that decreased levels of ROS may result in the alteration of *Y. pestis* mission of the plague bacillus. Science 296:733–735.


Lillard, J. W., Jr., S. W. Bearden, J. D. Fetherston, and R. D. Perry, 1999. The haemin storage (Hms +) phenotype of *Yersinia pestis* is not essential for the pathogenesis of bubonic plague in mammals. Microbiology 145:197–209.


