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Brucella melitensis Differs from *B. suis* in Growth and Urease Activity *In-Vitro*, and Infectivity in Fisher-344 Rats *In-Vivo*

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ABSTRACT

Importance of urease activity on pathogenic differences among *Brucella* species was evaluated. In cell-free extracts, the *B. suis* urease showed 12 times greater specific activity than the *B. melitensis* urease. When Fisher-344 rats were inoculated intraperitoneally (IP), at 1 week post-inoculation (PI), *B. melitensis* wild type 16 M was recovered from spleens and livers in greater numbers than *B. suis* wild type 1330. At 8 weeks PI, spleens were clear of *B. melitensis*, whereas *B. suis* remained. The wild type and the urease deficient strains of *B. suis* did not differ from each other in terms of recovery from spleen or liver. Our observations suggest that *B. melitensis* induces greater acute infectivity in Fisher-344 rats, whereas *B. suis* causes chronic infectivity; and urease activity has no influence on *Brucella* infection using an IP route.

Keywords: Brucella; Urease Activity; Splenomegaly; Infectivity; Pathogenicity

1. Introduction

Brucellosis is a disease in humans and animals, resulting from infection with bacteria belonging to the genus *Brucella* [1]. The genus *Brucella* consists of 10 known species designated on the basis of host preference, and antigenic and biochemical characteristics. These include *B. abortus*, *B. canis*, *B. melitensis*, *B. neotomae*, *B. ovis*, *B. suis*, *B. ceti*, *B. pinnipedialis*, *B. microti*, and *B. inopinata* [2-4]. Abortion and sterility are the major manifestations of brucellosis among livestock. Fever, sweats, malaise, weight loss, arthralgia, splenomegaly, and heaptomegaly are common clinical presentations in humans [2,3]. Relatively little is known about the genetic elements regulating pathogenicity or host-preference among *Brucella* species.

Microbial ureases are multi-subunit metalloenzymes that hydrolyze urea to form carbon dioxide and two molecules of ammonia that protonate to form ammonium causing the pH to increase. Thus, the hydrolysis of urea provides ammonium for incorporation into intracellular metabolites and facilitates survival in acidic environments. Among several functional differences among *Brucella* species, the difference in urease enzyme activity is prominent [5].

In this study, we sought to determine whether the pathogenicity between two major *Brucella* species differs as a function of urease enzyme activity. We compared *B. melitensis* and *B. suis* in terms of their *in vitro* growth, urease activity and infectivity in rats.

2. Materials and Methods

B. melitensis wild type strain 16M, *B. suis* wild type 1330, and the urease-deficient *B. suis* mutant 1330Δ*ure*1*K* [6] were obtained from our bacterial culture collection. The attenuated *ctpA* mutant of *B. suis* (1330Δ*ctpA*) [7] was used as a control. *Brucella* was grown in trypticase soy broth (TSB) or on trypticase soy agar (TSA) (Difco) at 37˚C in the presence of 5% CO_2 as previously described [8]. The cultures were grown in 25 mL TSB at 37˚C with shaking at 180 rpm, and Klett units were recorded every three hours using a Klett-Summerson colorimeter. The specific activity of urease was determined using the extracts prepared from the strains grown in TSB and harvested during logarithmic growth, as described elsewhere [6,9].

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All experiments with animals were approved by the Virginia Tech Institutional Animal Care and Use Committee. Five to six week old female Fisher-344 rats (Charles River Laboratory) were injected intraperitoneally (IP) with 4.3×10^4 to 4.6×10^4 colony forming units (cfu)/animal of *Brucella* strains. Groups of five rats inoculated with each *Brucella* strain were humanly sacrificed by exposing to excess CO_2 at 1, 4, and 8 weeks post-inoculation (PI). Parts of liver and spleen were used to determine the *Brucella* cfu as described previously [8].

3. Results

After 4 days of growth on TSA, colonies of *B. suis* strains 1330 and 1330Δ*ure*1*K* appeared approximately twice the size of colonies of *B. melitensis* 16 M (data is not shown). In TSB media during the logarithmic phase, the doubling time of the strain 1330 (11.5 h) and 1330Δ*ure*1*K* (11.3 h) were almost twice as long as that of the strain 16 M (6.5 h). As expected, the urease mutant 1330Δ*ure*1*K* displayed no measurable enzyme activity. The strain 1330 displayed twelve times greater urease specific activity than the strain 16M (9.12 and 0.73 μmoles/min/mg of protein, respectively).

The rats inoculated with all three test strains displayed substantial splenomegaly compared to those inoculated with the control 1330Δ*ctpA* (**Figure 1**). At 1 week PI, rats injected with the strain 16 M had nearly two-fold larger spleens than those injected with the strain 1330 or 1330Δ*ure*1*K*. At 4 and 8 weeks PI, rats in all test groups displayed moderate spleen weights.

At 1 week PI, the strain 16 M was recovered in greater numbers than strains 1330 and 1330Δ*ure*1*K* from spleens of rats (**Figure 2**). At 4 weeks PI, all three test strains

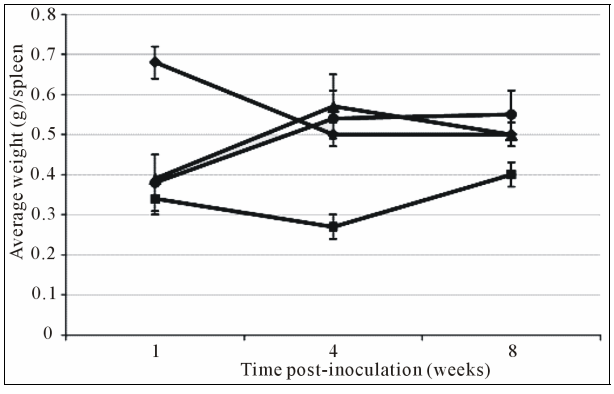

Figure 1. Splenomegaly in Fisher-344 rats. Rats were inoculated IP with *B. melitensis*wild type 16M (_____♦_____), *B. suis* wild type 1330 (_____●_____), *B. suis* urease-deficient mutant 1330Δ*ure*1*K* (_____▲_____), or *B. suis* attenuated mutant 1330Δ*ctpA* (control) (_____■_____). Groups of rats were euthanized at 1, 4, and 8 weeks PI, and the average weight of spleens (g) was determined. *P* values for the difference among mean values were: <0.005 at 1 week <0.005 at 4 weeks, and <0.025 at 8 weeks.

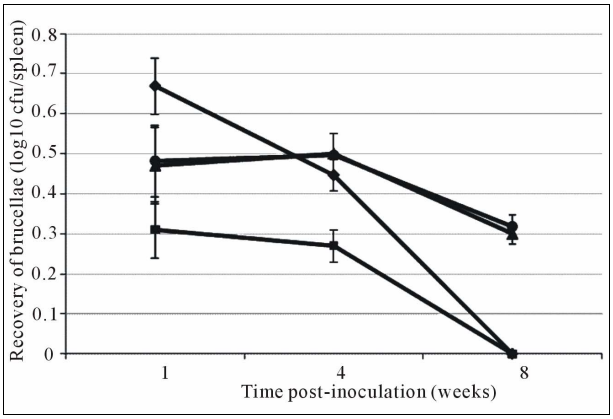

Figure 2. Recovery of Brucella from spleens of Fisher-344 rats. Rats were inoculated IP with B. melitensiswild type 16M (_____♦_____), *B. suis* wild type 1330 (_____●_____), *B. suis* urease-deficient mutant 1330Δure1K (_____▲_____), or *B. suis* attenuated mutant 1330ΔctpA (control) (_____■_____). Groups of rats were euthanized at 1, 4, and 8 weeks PI, and the recovery of Brucella from spleens (log10cfu/spleen) was determined. P values for the difference among mean values were: <0.01 at 1 week, <0.005 at 4 weeks, and <0.005 at 8 weeks.

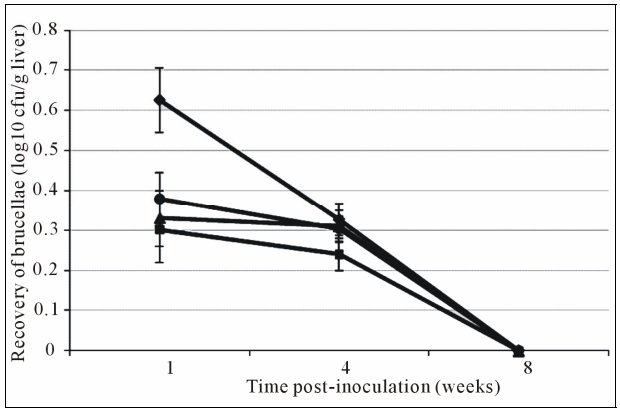

Figure 3. Recovery of Brucella from livers of Fisher-344 rats. Rats were inoculated IP with B. melitensiswild type 16M (_____♦_____), *B. suis* wild type 1330 (_____●_____), *B. suis* urease-deficient mutant 1330Δ*ure*1*K* (_____▲_____), or *B. suis* attenuated mutant 1330ΔctpA (control) (_____■_____). Groups of rats were euthanized at 1, 4, and 8 weeks PI, and the recovery of Brucella in livers was determined. P values for the difference among mean values were: <0.01 at 1 week, and <0.90 at 4 weeks for cfu/gram liver.

were recovered in similar numbers from spleens. However, at 8 weeks PI, the strain 16M completely cleared from spleens, but strains 1330 and 1330Δ*ure*1*K* were. recovered in substantial numbers. The attenuated *B. suis* mutant 1330Δ*ctpA* was recovered in significantly smaller numbers than other strains at 1 and 4 weeks PI, and was completely cleared by 8 weeks PI.

At 1 week PI, the strain 16M was recovered from the livers of infected rats in significantly greater number than

the strains 1330 and 1330Δ*ure*1*K* (**Figure 3**). Nevertheless, all three strains were recovered in similar numbers at 4 weeks PI, and completely cleared from livers by 8 weeks PI.

4. Discussion

The aim of our study was to determine the importance of urease enzyme activity to the species-specific pathogenicity among *Brucella* species. We compared two of the most pathogenic species of *Brucella* in terms of their *in vitro* growth and urease activity, and *in vivo* infectivity. We report that in spite of its relatively very low urease activity, *B. melitensis* wild type induced greater splenomegaly and was recovered from liver and spleen in greater numbers during the early phase of infection in Fisher-344 rats. These observations suggest that infectivity of this species is not related to its low urease activity. Nevertheless, *B. melitensis* was cleared from spleens and livers of rats in less than 8 weeks. Young *et al.*, [10] reported that in C3H female mice, *B. melitensis* strain EP cleared from spleens and liver 30 days after IP inoculation, whereas nearly 5.0 $\log_{10}$ cfu per organ (spleen or liver) of *B. abortus* strain 2308 was still present. Thus, our observations support those of Young *et al.*, [10] in that *B. melitensis* is less persistent albeit in a different rodent *i.e.* Fisher-344 rats and with respect to *B. suis.*

B. suis wild type exhibited relatively less splenomegaly and recovered from spleens and livers in smaller numbers at an early phase of infection, but managed to persist longer in spleens (past 8 weeks PI). Based on these observations, one may speculate that the relatively greater urease enzyme activity of this strain enables its longer persistence in spleens. Nevertheless, the *B. suis* mutant 1330Δ*ure*1*K* that displayed zero urease enzyme activity also persisted in spleens for longer time periods exactly as the wild type *B. suis* did. These observations suggest that the infectivity of *B. suis* is not related to its greater urease activity.

5. Conclusion

B. melitensis differs from *B. suis* in growth and urease activity *in vitro* and persistence *in vivo*. *B. melitensis* induces greater acute infectivity in rats, whereas *B. suis* causes chronic infectivity. The urease enzyme activity does not have an influence on *Brucella* infectivity in Fisher-344 rats when inoculated IP. The findings on differences between *B. melitensis* and *B. suis* in urease enzyme activity and pathogenicity will be useful in development of measures to prevent and control brucellosis.

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