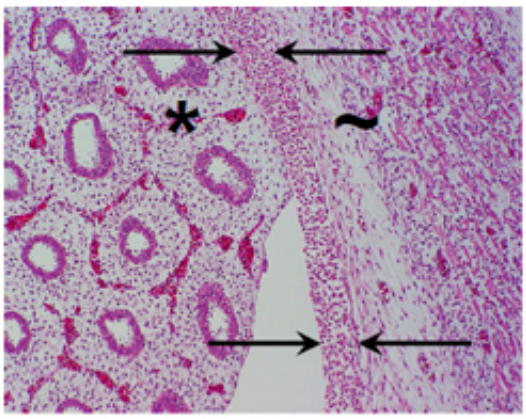
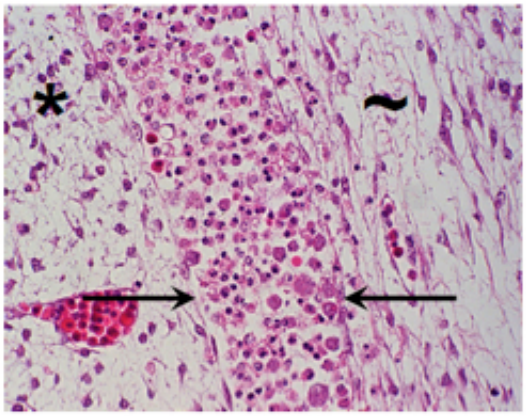
Pathogenicity of Brucella microti in chicken embryos

Brucellosis is one of the major bacterial zoonosis spread worldwide. The disease has different names: Infectious abortion, contagious abortion, enzootic abortion in animals; and Crimean fever, Mediterranean fever, Rock fever, Undulant fever or Malta fever in humans. According to World Health Organization, brucellosis is classified among the top seven world neglected zoonotic diseases. The true incidence of human brucellosis is not easy to estimate globally but an estimated number of more than 500,000 persons get infected newly every year. Genus *Brucella* (B.) is a Gram negative, intracellular pathogens encompassed 11 accepted nomo-species. *B. microti* is one of a recently recognized species and characterized by fast growth on standard media. It was isolated from common voles, red foxes and soil in Austria and Czech Republic and from a wild boar in Hungary. This wide diversity in the reservoir species may plays a significant role in its epizootic spread. Its pathogenicity for human and livestock has not been confirmed yet.



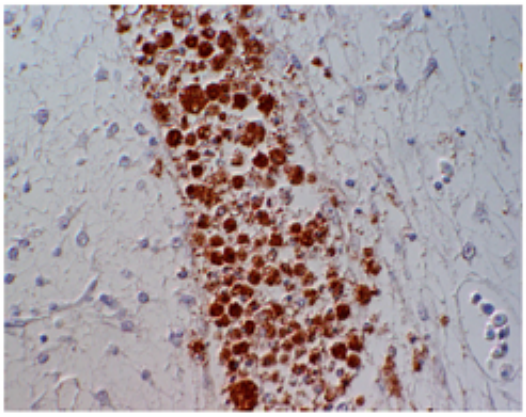
inflammatory cell infiltration comprising mainly macrophages in the gap (arrows indicating borders) between lung (asterisk) and thoracic wall (tilde)

BODY CAVITY



higher magnification of the photomicrograph shown above (using the same captions)

BODY CAVITY



immunohistochemical detection of *Brucella* sp. within inflammatory cells in the gap between lung and thoracic wall as well as scattered in the adjacent tissue

BODY CAVITY

To investigate the pathogenicity of *B. microti* murine strain for a non-mammalian host, a specific pathogenic free chicken embryos (CE) were inoculated at day 11 of age with two different doses of *B. microti* (1.6×10^3 and 1.6×10^5) and by two different routes (yolk sac and allantoic sac routes). 100% mortalities were recorded in all inoculated embryos four days post inoculation. Pure cultures of *B. microti* were isolated from all infected chicken embryos. The numbers of the re-isolated bacteria is nine million time higher than inoculated doses which indicated the rapid multiplication of bacteria in the CE. The bacteria provoked marked gross lesions. Signs of generalized infection in

the form of mild to severe congestion all over the abdomen with prominent hemorrhages streaks in the dorsum and the cranium were prominent. Chorioallantoic membrane showed prominent congestion of blood vessels. The predominant histopathological lesion was necrosis in liver, kidneys, lungs, spleen, gastrointestinal tract, spinal meninges, yolk sac and chorioallantoic membrane. An inflammatory cell infiltration was observed in the gap between the lung and the thoracic wall. Besides the morphological signs of parenchymatous cell death, moderate to marked hemorrhages were found in kidneys, lungs and livers. Colonies of bacteria were observed not only in parenchyma and the interstitium of the organs, but also be recognized within the lumina of blood vessels, in the chorioallantoic membrane and within the yolk sac. By immunohistochemically, *Brucella* antigen could often be detected in all organs.

Inoculation of chicken embryo is an approved tool for the isolation of intracellular infectious agents. The present study showed that *B. microti* can replicates in chicken embryos effectively. As an alternative model of infection, CE provides sterile conditions, easy to handle and offer different routes of inoculation. Moreover, it is cheaper and do not require ethical approval. Our results suggest that, even though chicken are no mammals, they are useful experimental animals to study the pathogenesis of *B. microti*.

Publication

Experimental infection of chicken embryos with recently described Brucella microti: Pathogenicity and pathological findings.
Wareth G, Böttcher D, Melzer F, Shehata AA, Roesler U, Neubauer H, Schoon HA
Comp Immunol Microbiol Infect Dis. 2015 Aug;