

## **Evaluation of Bovine Neutrophil Activation by *Leptospira***

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Early studies with human innate immune cells (macrophages and polymorphonuclear neutrophils [PMNs]) showed that some pathogenic *Leptospira* are efficiently phagocytosed and killed. However, these studies are lacking in bovines, which can be chronically infected with host-adapted *Leptospira* strains and can become reservoirs of disease. To evaluate the response of bovine PMNs to the presence of pathogenic (*L interrogans* serovar Pomona strain RM211, *L interrogans* serovar Copenhageni strain Fiocruz L1-130), host-adapted (*L borgpetersenii* serovar Hardjo strain JB197 and strain 203), and saprophytic *Leptospira* (*L biflexa* strain Patoc I) strains, PMNs were isolated from bovine whole blood. After incubation of PMNs with *Leptospira*, various assays measuring neutrophil activation (NET formation, MPO accumulation, cytokine expression, and bacterial killing) were performed. Neutrophil extracellular traps, or NETs, are formed in response to microbial pathogens and have been shown to ensnare these pathogens and inactivate or kill them. Leptospiral species, including the heat-killed and nonpathogenic saprophytic strain, induced NET formation as measured both by visual examination of cells adhered to microscope slides and by assays for quantification of extracellular DNA and increased myeloperoxidase accumulation in cultured cells. Leptospiral antigen could be observed in association with NET-like formations; however, many intact *Leptospira* were also observed not in association with cells or NETs. Limiting dilution culture of PMN-incubated *Leptospira* showed no reduction in viable cell numbers, including the saprophytic strain. In contrast with earlier studies with human cells, bovine PMNs, while activated by the presence of *Leptospira*, are not effective in killing the *Leptospira*. Further studies on the difference in innate immunity between species will lead to better infection models, treatments, and preventive measures for leptospirosis.

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*All animal use was carried out in accordance with all applicable animal care and use laws, regulations, and guidelines and the Institutional Animal Care and Use Committee approved the studies.*