DOI: 10.1017/S1431927603447053
Microsc Microanal 9(Suppl 2), 2003


# Differential Antibody Response of Cattle Immunized with *Anaplasma marginale* Derived from Bovine Erythrocytes or Cultured Tick Cells

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Anaplasmosis is a hemolytic disease of cattle caused by the rickettsial tick-borne pathogen *Anaplasma marginale* (Rickettsiales: Anaplasmataceae). Killed vaccines have been used for the control of anaplasmosis in the United States. These previous vaccines used *A. marginale* antigen harvested from infected bovine erythrocytes and bore the risk of being contaminated with bovine cells and other pathogens. Recently, a cell culture system was developed for the propagation of *A. marginale* using a continuous cell line (IDE8) that was originally derived from embryos of *Ixodes scapularis.* The six major surface proteins (MSPs) described on the erythrocytic stage of *A. marginale* were found to be conserved on cell culture-derived organisms. *A. marginale* antigen derived from cell culture was tested as an immunogen for cattle and the cell culture-derived vaccine prevented clinical anaplasmosis in vaccinated cattle. In this research we report the differential antibody response of cattle to erythrocyte- and cell culture-derived antigen that was demonstrated in three independent vaccine trials. Cattle immunized with *A. marginale* from tick cells or bovine erythrocytes produced antibodies against the *A. marginale* MSP5 but a differential antibody response to MSP1a and MSP1b was observed. Cattle immunized with erythrocyte-derived *A. marginale* elicited an antibody response mainly against MSP1a, while animals immunized with cell culture-derived antigen produced predominantly antibodies to MSP1b. The molecular basis of this differential antibody response was then studied by comparing the amounts of MSP1a, MSP1b and MSP5 on *A. marginale* harvested from the two host cells. The amount of MSP1b and MSP5 was similar on *A. marginale* from both host cells, but the amount of MSP1a was higher in the erythrocyte-derived *A. marginale*. These results were further demonstrated using confocal microscopy (Fig. 1). These differences could be regulated at transcriptional, translational or post-translational levels. Since MSP1a has been shown to be an *A. marginale* adhesin for tick cells, biological transmission of the pathogen could be enhanced by increased levels of this surface protein. Differences in the level of surface exposed molecules also may contribute to phenotypic and antigenic variation in the pathogen.

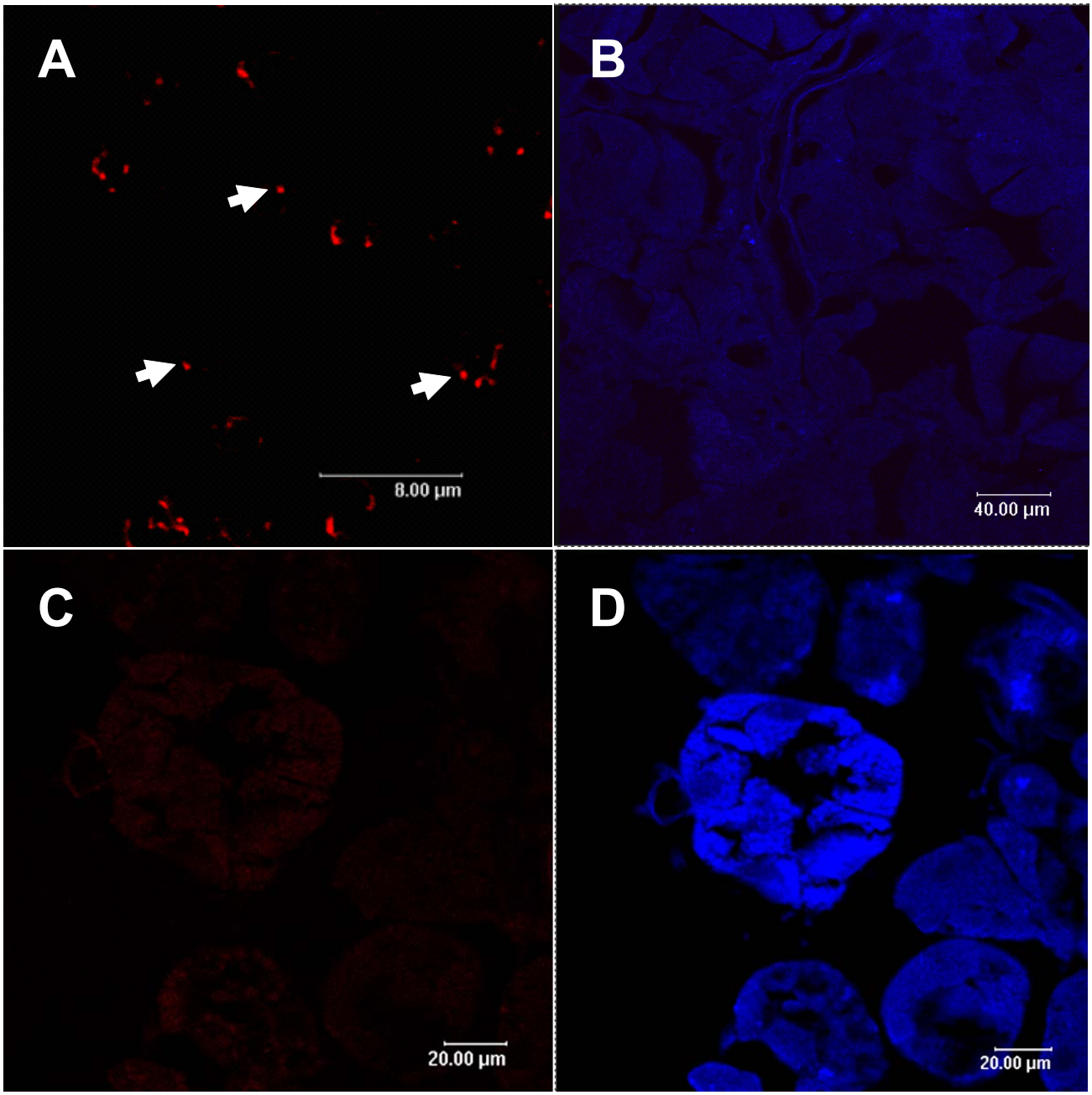


Figure 1. Confocal microscopy of (A) a smear of *A. marginale* infected bovine erythrocytes, (B) a cross-section of uninfected *D. variabilis* salivary glands and (C,D) a cross-section of *A. marginale* infected *D. variabilis* salivary glands, probed with (A,C) MAb ANA15D2 (anti-MSP1a) or (B, D) MAb ANAF16C1 (anti-MSP5), labeled with Alexa Fluor 546 and Alexa Fluor 633, respectively. Arrows indicate expression of MSP1a in the *A. marginale* inclusion bodies in bovine erythrocytes. Figures C and D correspond to a cross-section of infected salivary gland that was simultaneously incubated with labeled anti-MSP1a and anti-MSP5 MAbs and examined for the presence of both labels.

https://doi.org/10.1017/S1431927603447053 Published online by Cambridge University Press