Infection Exclusion of the Rickettsial Pathogen Anaplasma marginale in the Tick Vector Dermacentor variabilis

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Anaplasma marginale is a tick-borne, rickettsial cattle pathogen that is endemic in several areas of the United States. Recent studies (J. de la Fuente, J. C. Garcia-Garcia, E. F. Blouin, J. T. Saliki, and K. M. Kocan, Clin. Diagn. Lab. Immunol. 9:658-668, 2002) demonstrated that infection of cultured tick cells and bovine erythrocytes with one genotype of A. marginale excluded infection with other genotypes, a phenomenon referred to as infection exclusion. The present study was undertaken to confirm the phenomenon of infection exclusion of A. marginale genotypes in a tick vector, Dermacentor variabilis. Only one genotype of A. marginale (Virginia isolate) was detected by PCR in ticks that fed first on a calf infected with a Virginia isolate and second on a calf infected with an Oklahoma isolate. These studies demonstrate that infection exclusion of A. marginale genotypes also occurs in naturally infected ticks, as well as in cattle and cultured tick cells, and results in establishment of only one genotype per tick.

Anaplasmosis, a tick-borne cattle disease caused by the obligate intraerythrocytic bacterium Anaplasma marginale (Rickettsiales: Anaplasmataceae), is endemic in many tropical and subtropical regions, including several areas of the United States. Several geographic isolates of A. marginale, which differ from each other in biology, morphology, and sequence and antigenic characteristics, have been identified in the United States (reviewed in reference 3). Feeding ticks effect biological transmission of A. marginale, while mechanical transmission occurs when infected blood is transferred to susceptible animals by biting flies or by blood-contaminated fomites. Cattle that recover from acute infection remain persistently infected and serve as reservoirs for mechanical transmission and infection of ticks (reviewed in reference 9). A. marginale multiplies only within membrane-bound inclusions in the cytoplasm of host cells. The only known site of A. marginale development in cattle is within erythrocytes (14), while A. marginale develops in several tissues in ticks, including the salivary glands from which A. marginale is transmitted to susceptible cattle (10-12).

Major surface protein 1a (MSP1a) is one of six MSPs that have been described for A. marginale from bovine erythrocytes. MSP1a is encoded by a single gene, msp1a, which is conserved during the multiplication of the parasite in cattle and ticks, therefore resulting in a stable genetic marker of A. marginale geographic isolates (2). The molecular weight of MSP1a is variable among geographic isolates because of a variable number of tandem 28- or 29-amino-acid repeats (1, 3, 4, 7). Recent reports by Palmer et al. (13) and J. de la Fuente, R. A. Van Den Bussche, T. Prado, and K. M. Kocan (submitted for publication) documented genetic heterogeneity in the structure of msp1a sequences that have been recovered from infected animals in endemic areas in Oregon and Oklahoma, respectively.

However, only one msp1a genotype was identified in individual cattle that were naturally or experimentally infected and sampled at different stages of infection (2, 13). These findings were recently explained by the demonstration of infection exclusion of other A. marginale genotypes in infected cattle and cultured tick cells (8).

The present study was undertaken to determine whether the phenomenon of infection exclusion of A. marginale also occurs in Dermacentor variabilis, a tick vector of A. marginale in the United States. Two tick-transmissible isolates of A. marginale were used in this study (8), a Virginia isolate obtained originally in 1978 from the USDA Animal Disease Research Laboratory, Beltsville, Md., and an Oklahoma isolate obtained from a naturally infected bovine from Wetumka, Okla., in 1997. Both isolates have been used for tick transmission and cell culture studies in our laboratory (2, 5, 8). Splenectomized calves were inoculated with each isolate, and blood collected at the infection peak was prepared as stablates and stored in liquid nitrogen until used for infection of the experimental calves.

Two mixed-breed calves (4 to 6 months old), determined to be free of infection by an A. marginale-specific enzyme-linked immunosorbent assay (15), were used for the study. The calves were housed in the Anaplasmosis Research Barn and cared for by the Oklahoma State University (OSU) Laboratory Animal Research Unit with the approval of the OSU Institutional Animal Care and Use Committee. The calves were splenectomized to cause higher levels of A. marginale infection, and each calf was then experimentally infected with one of the two A. marginale isolates. The calves were monitored three times a week by examination of stained blood smears and determination of the packed cell volume. Thin smears were made on glass slides from blood collected from the calves by venipuncture in EDTA-treated Vacutainer tubes and stained with Protocol Hema 3 stain (Biochemical Sciences, Inc., Swedesburg, N.J.). The percentage of parasitized erythrocytes (PPE) out of 500 was determined. Once infection was detected in blood...
smears, the calves were monitored daily. Calf PA482 was inoculated intravenously with 5 ml of the Oklahoma isolate stabilate made from blood collected from calf PA407 at a 16.5% PPE, and calf PA481 was inoculated intravenously with 8 ml of the Virginia isolate stabilate made from blood collected from calf PA433 with a 12.2% PPE. Both calves were infused with approximately 300 D. variabilis male ticks in two orthopedic stockinettes (150 ticks each) glued to the side of the calf when the parasitemia was 3 to 10%. The ticks were allowed to feed for 7 days, after which they were held in a humidity chamber. The ticks were then allowed to feed for 7 days on a sheep to cause development of A. marginale infection in the ticks’ salivary glands. Group 1 ticks fed only on calf PA481, which was infected with the Virginia isolate of A. marginale. Group 3 ticks fed only on calf PA482, which was infected with the Oklahoma isolate of A. marginale. Group 2 ticks were allowed to feed first on calf PA481 and then on calf PA482, before being allowed to feed on the sheep (Fig. 1). After feeding on the sheep, all the ticks were removed; the salivary glands from groups of 30 ticks were dissected and placed in 500 μl of a commercial solution for preserving biological samples for RNA analysis (RNALater; Ambion, Austin, Tex.) to be used for PCR studies. Two groups of tick salivary glands were analyzed, with similar results.

A. marginale from tick salivary glands was characterized by PvuII restriction analysis of the msp1α gene amplified by PCR as previously reported (8). The sequence of the msp1α gene varies among different geographic isolates of A. marginale (1, 3, 4, 7). The msp1α gene of the Virginia isolate has two tandem repeats, while the Oklahoma isolate gene has three tandem repeats (4, 8). The numbers of PvuII sites in the msp1α gene are different for the two isolates: the Oklahoma isolate gene has two sites, at positions 31 and 879 with respect to the translation initiation codon, while the Virginia isolate gene has only one, at position 712 (4, 8). The PvuII digestion products of the msp1α PCR therefore result in distinguishable patterns for the two isolates (8). The PCR product of the PvuII restriction analysis from group 2. DNA was extracted from tick salivary glands after tick transmission feeding on a sheep, and the msp1α genotype was analyzed after PvuII digestion of the PCR products. The samples were analyzed on a 1% ethidium bromide-stained agarose gel. Lane MW, 1-kb Plus DNA ladder (Gibco BRL); lane C-, control sample with no DNA added before PCR. Two groups of tick salivary glands were analyzed, with similar results.

![FIG. 1. Infection exclusion of A. marginale in ticks. Groups of D. variabilis male ticks were allowed to acquisition feed on cows infected with a Virginia isolate of A. marginale (VaAM) (PA481; group 1) and an Oklahoma isolate (OkAM) (PA482; group 3). One group of ticks was allowed to feed first on calf PA481 and subsequently on calf PA482 (group 2). The levels of infection in cattle during tick feeding are indicated as PPE. Only the Virginia isolate was present in the ticks from group 2. DNA was extracted from tick salivary glands after tick transmission feeding on a sheep, and the msp1α genotype was analyzed after PvuII digestion of the PCR products. The samples were analyzed on a 1% ethidium bromide-stained agarose gel. Lane MW, 1-kb Plus DNA ladder (Gibco BRL); lane C-, control sample with no DNA added before PCR. Two groups of tick salivary glands were analyzed, with similar results.](http://cvi.asm.org/)
opportunity to be exposed to more than one _A. marginale_ genotype. However, the results of this study suggest that the males will become infected with only one genotype. The demonstration of infection exclusion of _A. marginale_ in ticks further confirms that different _A. marginale_ genotypes survive in nature by individual transmission events and explains the existence of several _A. marginale_ genotypes in infected cattle in endemic regions (13; de la Fuente et al., submitted). Genotypes introduced into a herd in an endemic area via cattle shipment would most likely be maintained and become endemic if they were transmitted to susceptible cattle. Both persistently infected cattle and ticks could therefore serve as reservoirs of the introduced genotype. These results have important implications for the epidemiology and control of anaplasmosis.

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