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Concomitant infection of cattle with the vaccine strain *Anaplasma marginale* ss *centrale* and field strains of *A. marginale*

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Abstract

Bovine anaplasmosis, caused by *Anaplasma marginale*, the intraerythrocytic rickettsia, is controlled by vaccination with live *Anaplasma marginale* ss *centrale* (*A. centrale*), a subspecies of relatively low pathogenicity. We have experimentally demonstrated that an animal primarily infected with *A. marginale*, or with the related vaccine subspecies *A. centrale* can be infected with the heterologous subspecies, and carries both bacteria. The co-infection was detected in experimentally cross-infected calves for up to 3 months after the last inoculation with the heterologous subspecies. The occurrence of characteristic cyclic rickettsemia of *A. centrale* and *A. marginale* was observed by examination of Giemsa-stained blood smears, or by the presence of specific rickettsial DNA confirmed in PCR assays based on specific *msp1a* and *msp4* for *A. marginale*, and on specifically designed *msp3* and *msp4* primers for *A. centrale*. Sequence analysis of *msp4*-specific fragments for each subspecies revealed the presence of dual infection in both calves on days 30 and 60 after cross-inoculation with the heterologous *Anaplasma* subspecies. The experimental cross-infection of calves clearly demonstrated that the concept of ''infection exclusion'' does not apply to *Anaplasma* infection in cattle; as there was no infection exclusion of *A. marginale* in *A. centrale*-infected cattle, and *vice versa*. The present results confirmed our previous findings that cattle grazing in an anaplasmosis-endemic field were subject to concomitant infection with both the vaccine *A. centrale* and the field *A. marginale* strains.

Keywords: *Anaplasma centrale*; *A. marginale*; Infection exclusion; Cattle

1. Introduction

Bovine anaplasmosis, a disease caused by the intraerythrocytic rickettsial pathogen *Anaplasma marginale*, is characterized by progressive hemolytic anemia, abortions, loss of condition, low milk production, and death (Theiler, 1911; Palmer,

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1989). *A. marginale* ss *centrale* (*A. centrale*), a closely related subspecies, exhibits moderate pathogenicity compared with *A. marginale*. It has been demonstrated that established infection with *A. centrale* did not prevent *A. marginale* infection, but resulted in reduced severity of the clinical disease (Theiler, 1912). To reduce the impairment of animal health and the heavy losses of production, a live strain of *A. centrale* is routinely used for vaccination in South Africa, Australia, Israel, and several South American countries (Potgeiter, 1979; Callow and Dalgliesh, 1980; Guiglielmone et al., 1988; Krigel et al., 1992). Membrane surface proteins MSP1–MSP5 were described in all examined *A. marginale* strains from various geographical regions (Palmer et al., 1999; de la Fuente et al., 2005). Orthologs of *A. marginale* MSP2, encoded by the operon-associated gene, and highly conserved non-operon-linked proteins MSP4 and MSP5 were described in both rickettsial species (Lohr et al., 2002; Shkap et al., 2002a; Molad et al., 2004). The MSP1 heterodimer (MSP1a and MSP1b) was detected in all *A. marginale* isolates, but the protein and the encoding gene have not been found in *A. centrale*. The *msp1a*, encoding for the MSP1a, is used to differentiate *A. marginale* strains (Allred et al., 1990). On the basis of the number and sequence of *msp1a* tandem repeats, examination of naturally or experimentally infected cattle grazing in an anaplasmosis-endemic area revealed distinct genotypes of *A. marginale*, but only a single genotype was detected in any individual animal over a period of 2 years (Palmer et al., 2001). These findings led to a suggestion that in cattle persistently infected with a specific genotype, there is exclusion of other *A. marginale* genotypes. The phenomenon of infection exclusion of *A. marginale* infection has been reported by the detection of only one genotype in bovine erythrocytes and infected tick (de la Fuente et al., 2002), although in cattle, the ability to establish a new infection in already infected animals was found as a rare event associated with presence of strains with distinct *msp2* pseudogene repertoires (Rodriguez et al., 2005).

In the present study, we show the presence of concurrent infection with *A. marginale* and *A. centrale*, and provide new experimental data demonstrating that the concept of ‘‘infection exclusion’’ does not apply to *Anaplasma* infection in cattle.

2. Materials and methods

2.1. Anaplasma organisms

The *A. centrale* vaccine strain was obtained from South Africa in 1952, and has been used since then as the standard vaccine in Israel (Tsur, 1953). The Virginia isolate of *A. marginale* was obtained from K. Kocan, Oklahoma State University, USA. Both, *A. marginale* and *A. centrale* were inoculated into splenectomized calves, and frozen blood stabilates were cryopreserved in 10% dimethyl sulfoxide (DMSO) in liquid nitrogen pending use (Love, 1972).

2.2. Infection of calves and monitoring of infection

The Kimron Veterinary Institutional Animal Welfare Committee approved all the experiments. Two splenectomized Friesian–Holstein calves (nos. 592 and 603) were infected with *A. centrale* and *A. marginale*, respectively. The calves were found to be seronegative for *Anaplasma* in the IFA test (Shkap et al., 1990). On day 29 after infection, at 23% rickettsemia, calf 603 was inoculated i.v. with 1×10^5 *A. centrale*-infected erythrocytes drawn from calf 592. Similarly, the same number of *A. marginale*-infected cells from calf 603 was administered into calf 592 on day 31, at rickettsemia of 30%. The calves were monitored daily for more than 3 months by counting rickettsemia in Giemsa-stained thin blood smears, recording fever, and determining packed cell volume (PCV).

2.3. Blood samples

For PCR assays, blood samples from the experimentally infected calves were collected weekly from the day of the initial infection up to 3 months after the cross-infection. The blood was washed three times with phosphate-buffered saline (PBS), pH 7.4 by centrifugation at $1200 \times g$, the buffy coat was discarded and the blood kept frozen at $-70\,^{\circ}C$ pending use.

Blood samples ($n = 27$) were collected from a herd grazing in the anaplasmosis-endemic northern part of Israel. The herd had been vaccinated with *A. centrale* a year earlier, at the weaning age of 6–8 months.