The Irradiation of Babesia bovis

1. The Difference in Pathogenicity Between Irradiated and Non-Irradiated Populations

I.G. Wright, B.V. Goodger, and D.F. Mahoney

CSIRO, Division of Animal Health, Long Pocket Laboratories, Private Bag No. 3, P.O., Indooroopilly, Queensland, Australia 4068

Abstract. Babesia bovis parasites attenuated by 35 krads γ irradiation and parasites not exposed to irradiation, were injected into intact 2-year-old Hereford steers. All five animals receiving non-irradiated blood died but the five animals which received irradiated blood were only mildly affected. Highly significant differences were observed in changes to plasma fibrinogen, serum fibrinogen-like proteins, packed cell volume, partial thromboplastin time, prothrombin time, blood kinins, and plasma kininogen levels in the control animals but non-significant changes in these parameters occurred in the group receiving irradiated blood. Significant changes in the antiplasmin, α2M, and the antithrombin levels occurred in control cattle but not in the group receiving irradiated blood. Parasite multiplication rates and maximum parasitaemias were similar in both groups. Irradiation reduced the dose of living parasites from $1 \times 10^8$ to $2.5 \times 10^3$, but this was not the reason for the mild reactions. It was concluded that irradiation had selected an avirulent parasite population.

Key words: Babesia – Irradiation – Virulence – Pathogenicity.

Introduction

In a previous paper Mahoney et al. (1973) reported that Babesia bovis parasites irradiated with doses between 20 and 50 krads produced only a mild disease that left the recipient animals strongly immune to reinfection. The multiplication rate of the organisms was reduced and these data suggested that parasites after exposure between 30 and 35 krad became attenuated. It has also been demonstrated that severe hypotension occurs during primary acute B. bovis infections and that much of the vascular disturbance is linked to the activation of the plasma kallikrein and coagulation systems (Wright and Mahoney, 1974; Wright and Kerr, 1977; Goodger and Wright, 1977). The object of this study
was to determine whether the mild reactions produced by irradiated parasites in non-immune hosts were attributed to a reduction in the involvement of these host-systems.

**Materials and Methods**

*Experimental Animals*

Calves and cattle of mixed breeds (*Bos taurus*) and sexes obtained from a *Babesia*-free area were used in these experiments. Calves were splenectomised prior to use and all animals were determined to be serologically negative to *Babesia* using the passive haemagglutination test (PHA) (Goodger and Mahoney, 1974).

*The Parasites*

The Lismore (L) strain of *B. bovis*, described in the previous experiments (Mahoney et al., 1973) was used for infection. All parasite populations were stabilates stored in nitrogen vapour, and were then passaged in a donor calf from which the infective dose was then obtained.

*Irradiation of Blood*

The methods described by Mahoney et al. (1973) were used.

*Infection of Animals*

Steers were generally infected intravenously with a standard dose of $1 \times 10^8$ irradiated or non-irradiated parasites in whole blood. Jugular blood was taken daily for packed cell volume (PCV) estimation and thick blood films (Mahoney and Saal, 1961) which were examined to estimate the number of parasites/mm³ blood. Parasite doubling time was estimated according to the method described by Mahoney et al. (1973).

*Experiment 1*

Four groups of splenectomised, 3-month-old calves each composed of four animals were infected with $1 \times 10^8$ *B. bovis* “L” strain parasites exposed to either 20, 30, 40, or 50 krads, and the fifth group received $1 \times 10^8$ non-irradiated parasites of the parent strain.

*Experiment 2*

Ten two-year old *Bos taurus* steers were randomly divided into two equal groups. Blood containing approximately $2 \times 10^7$ virulent *B. bovis* (L strain) parasites/ml was taken from a splenectomised donor calf and samples were irradiated with 35 krad as described previously (Mahoney et al., 1973). Experimental animals received $1 \times 10^8$ irradiated parasites and the control animals $1 \times 10^8$ non-irradiated parasites of the parent strain.

*Blood Collection*

Nine millilitres of jugular blood was collected into 1 ml of sterile 3.13% tri-sodium citrate from each animal just prior to infection and further samples were collected every second day of infection.