AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

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1. SUBJECT CLASSIFICATION

A. PRIMARY: Agriculture

B. SECONDARY: Veterinary Medicine

2. TITLE AND SUBTITLE

Infectivity and Immunogenicity of Irradiated Babesia rodhaini

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4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1974	3p.	ARC 636.089072-B622

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Texas A&M University
Institute of Tropical Veterinary Medicine
College Station, Texas 77843

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)

Published in J. Protozool. vol. 21, no.5

9. ABSTRACT

Babesia rodhaini-parasitized mouse blood exposed to varied doses of y radiation up to 30 kRad was inoculated into mice. Mice inoculated with nonirradiated B. rodhaini developed progressive infections and died 7-11 days postinoculation. Mice infected with B. rodhaini-parasitized blood exposed to doses up to and including 22 kRad developed progressive parasitemias which were delayed in comparison to mice inoculated with non-irradiated B. rodhaini. Some mice receiving parasitized blood irradiated at 26 kRad did not develop progressive parasitemias. Progressive infections were prevented by exposure to irradiation at 30 kRad.

The results of 2 separate experiments revealed that one inoculation of parasitized blood exposed to 30 kRad or higher apparently stimulated a resistance to a challenge infection with nonirradiated parasitized blood. While 20 of 20 control mice died as a result of challenging infections, 9 of 28 mice previously exposed to irradiated parasitized blood survived.

The injection of irradiated nonparasitized blood did not produce a discernible acquired resistance to B. rodhaini. Presumably the irradiated blood was responsible for the development of acquired resistance to B. rodhaini.

10. CONTROL NUMBER	11. PRICE OF DOCUMENT
PN-AAB- 113	

12. DESCRIPTORS

Babesiosis, Irradiation, Attenuation, Immunity, Inoculation, Parasite

13. PROJECT NUMBER: 931-17-130-475

14. CONTRACT NUMBER: AID/CSD-1947

15. TYPE OF DOCUMENT: Research Pamphlet

J. PROTOZOOL. 21(5), 758-760 (1974).

Infectivity and Immunogenicity of Irradiated *Babesia rodhaini**

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SYNOPSIS. *Babesia rodhaini*-parasitized mouse blood exposed to varied doses of γ radiation up to 30 kRad was inoculated into mice. Mice inoculated with nonirradiated *B. rodhaini* developed progressive infections and died 7-11 days postinoculation. Mice infected with *B. rodhaini*-parasitized blood exposed to doses up to and including 22 kRad developed progressive parasitemias which were delayed in comparison to mice inoculated with non-irradiated *B. rodhaini*. Some mice receiving parasitized blood irradiated at 26 kRad did not develop progressive parasitemias. Progressive infections were prevented by exposure to irradiation at 30 kRad.

The results of 2 separate experiments revealed that one inoculation of parasitized blood exposed to 30 kRad or higher apparently stimulated a resistance to a challenge infection with nonirradiated parasitized blood. While 20 of 20 control mice died as a result of challenging infections, 9 of 28 mice previously exposed to irradiated parasitized blood survived.

The injection of irradiated nonparasitized blood did not produce a discernible acquired resistance to *B. rodhaini*. Presumably the irradiated parasitized blood was responsible for the development of acquired resistance to *B. rodhaini*.

Index Key Words: *Babesia rodhaini*; babesiosis; irradiation; attenuation; immunity.

BABESIA RODHAINI is a protozoan parasite of the red blood corpuscles of rodents first isolated from *Thamnomys surdaster surdaster* in the Congo in 1950 (21). *Babesia rodhaini* was easily transmissible to laboratory mice (21) and became increasingly virulent with repeated serial transfers in mice (6). Colas-Belcour & Vervent (6) found that the majority of mice died from the infection but those that recovered were resistant to challenge.

Babesia rodhaini in rodents has been used widely as a model for the study of *Babesia* infections of cattle, especially in the field of chemotherapy. The immunology of *B. rodhaini* in rats and mice has recently received attention (9, 11, 12, 17, 18, 20). Attempts to induce protective immunity in rats and mice by use of irradiated *Plasmodium berghei* have been relatively successful (1, 7, 8, 13-15, 22-25). Recent studies (19) indicated that essentially similar results could be achieved in mice and rats by irradiation of the erythrocytic forms of *B. rodhaini*.

MATERIALS AND METHODS

The strain of *B. rodhaini* used throughout this study was obtained from Dr. C. G. Ludford, Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan, and was maintained by serial passage in albino mice in the Institute of Tropical Veterinary Medicine, Texas A&M University, College Station, Texas.

Male inbred albino mice each weighing 30-40 g were used as experimental hosts. The organisms used for immunization and infection were prepared as follows: blood containing 60-70% parasitized erythrocytes was harvested from infected mice 7 days after a 0.5 ml intraperitoneal (ip) inoculation of a 1:1000 dilution of *B. rodhaini* parasitized mouse blood. The number of parasitized erythrocytes/ml was determined by red blood cell counts and thin blood smears stained with Giemsa stain.

The inoculum was exposed to the desired amount of irradiation using an 800 curie (c) source of cobalt-60. The 800 c source delivered a dose rate of ~1550 Rad/min. The inoculum was kept in an ice bath until it was irradiated and afterwards until the mice were injected. Other inocula were maintained under similar conditions for the duration of irradiation and were used as nonirradiated controls. All inoculations and challenges were given ip.

The first series of experiments was undertaken to determine the effect of γ radiation on the infectivity of *B. rodhaini* in mice. Seventy mice were separated into 7 groups. The mice of the 1st group received 7×10^5 nonirradiated *B. rodhaini* parasitized erythrocytes. The other 6 groups received approximately the same number of parasitized erythrocytes irradiated respectively with 10, 14, 18, 22, 26, and 30 kRad. All mice were examined daily for parasitemia by the use of Giemsa stain on thin blood smears.

In a 2nd experiment the effect of γ radiation on the immunogenicity of *B. rodhaini* was studied. Eight mice were inoculated with 7×10^5 parasitized erythrocytes irradiated at 30 kRad and 10 served as controls. All mice were examined daily for parasitemia by the use of Giemsa stain on thin blood smears. All mice were challenged 3 weeks later with nonirradiated, parasitized blood. Before challenge, blood from mice which had received irradiated, parasitized blood was subinoculated into susceptible mice to determine whether a subpatent parasitemia might have resulted. Subinoculations were accomplished by diluting 3 drops of tail blood from each mouse with 0.5 ml of saline and injecting ~0.25 ml ip into susceptible mice.

A 3rd experiment was undertaken to evaluate the immunogenicity of parasitized and nonparasitized irradiated erythrocytes. Thirty mice were divided into 7 groups, with 1 group receiving 7×10^5 parasitized erythrocytes exposed to 36 kRad and the other group receiving approximately the same number of nonparasitized erythrocytes exposed to 36 kRad. All mice were challenged 3 weeks later with nonirradiated, parasitized blood and then examined daily for parasitemia by the use of Giemsa stain on thin blood smears.

RESULTS

The 1st series of experiments was undertaken to determine the effect of γ radiation on the infectivity of *Babesia rodhaini* in mice. The results summarized in Table 1 show that all mice inoculated with nonirradiated *B. rodhaini* developed progressive infections and died 7-11 days postinoculation. Mice inoculated with *B. rodhaini*-parasitized erythrocytes exposed to doses up to and including 22 kRad developed progressive parasitemias which were delayed in comparison to mice inoculated with nonirradiated *B. rodhaini*. A direct and significant ($p < 0.01$) correlation between the irradiation dose and the time of death

* This study was assisted by the United States Agency for International Development and the Rockefeller Foundation.

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TABLE 1. *Infectivity of irradiated* Babesia rodhaini *parasitized mouse blood*

Radiation dosage in kRad*	No. with parasitemia	No. survivors	Day of Death	
	No. inoculated*	No. inoculated	Mean	Range
0	10/10	0/10	7.8	7-11
10	10/10	4/10	10.0	10-10
14	10/10	2/10	10.9	10-14
18	10/10	0/10	12.3	11-15
22	10/10	2/10	14.3	13-18
26	8/10	7/10	14.0	13-15
30	0/10	10/10	-	-

* Size of inoculum: 7×10^3 parasitized red blood cells.
† Detected by either thin blood films or subinoculations.

was noted. Some mice receiving parasitized erythrocytes irradiated at 26 kRad did not develop progressive infections. Infections were not detected in mice receiving parasitized erythrocytes irradiated at 30 kRad.

On the basis of the above results, a 2nd experiment was undertaken to determine whether an inoculation with parasitized blood exposed to 30 kRad or higher stimulated a demonstrable resistance to a challenge infection with nonirradiated parasites. Before challenge, blood from the mice which had received irradiated, parasitized blood was subinoculated into susceptible mice to determine whether a subpatent parasitemia might have resulted. No evidence of infection was discernible in these mice. After challenge with nonirradiated infected erythrocytes, the % survival was compared in the 2 groups of mice (Table 2). The animals that were previously inoculated with irradiated parasitized blood all developed parasitemias and had a 50% survival after the challenging infection. Although the mean survival time for the "immunized" mice was slightly longer than their controls, the difference between them was not significant.

Since previous experiments (12) had indicated that resistance to *B. rodhaini* could be induced nonspecifically by substances other than those of parasitic origin, a 3rd experiment was designed to determine whether the irradiated parasites were responsible for stimulating the observed acquired resistance. As indicated in Table 3, all the animals previously inoculated with irradiated, parasitized blood developed parasitemias and had a 25% survival after the challenging infection, whereas the animals previously inoculated with irradiated, nonparasitized blood had a 0% survival following the challenging infection. No significant difference was observed in the mean survival time of the animals in the 2 groups.

DISCUSSION

The results from studies designed to determine the effect of various radiation dosages on the infectivity of *Babesia rodhaini* indicate that mice infected with *B. rodhaini*-parasitized

TABLE 2. *Immune response of mice inoculated once with irradiated* Babesia rodhaini

Treatment	No. survivors / No. challenged*
7×10^6 parasitized RBC's exposed to 30 kRad	5/9
None	0/10

* Challenge dose: 7×10^3 parasitized red blood cells.

TABLE 3. *Immune response of mice inoculated once with irradiated* Babesia rodhaini

Treatment	No. survivors / No. challenged*
7×10^6 parasitized RBC's exposed to 36 kRad	5/20
7×10^6 normal RBC's exposed to 36 kRad	0/10

* Challenge dose: 7×10^3 parasitized red blood cells.

erythrocytes exposed to 26 kRad developed progressive infections which were prevented by exposure to irradiation at 30 and 36 kRad. These results confirm and extend those reported previously from mice inoculated with irradiated *B. rodhaini* (19, 20). These studies indicated that mice infected with *B. rodhaini*-parasitized erythrocytes exposed to 25 kRad developed progressive parasitemias whereas progressive infections were prevented by exposure to irradiation at 40 and 60 kRad.

An analysis of the foregoing experiments seems to indicate the development of acquired resistance to *B. rodhaini* in mice inoculated with irradiated *B. rodhaini*. The results of 2 separate experiments revealed that 25-50% of mice inoculated once with irradiated *B. rodhaini* survived otherwise lethal infections. These results compare favorably with the 20-30% recovery rate among mice inoculated with irradiated blood forms of *B. rodhaini* reported by Phillips (20). The results obtained in our study are in agreement with those reported from rats immunized with irradiated *B. rodhaini* (19) and from cattle immunized with irradiated *B. bigemina* (1). These authors report that the resistance developed was sufficient to suppress multiplication of the *Babesia* and to permit rats and cattle to survive otherwise severe clinical infections due to challenge with nonirradiated parasites.

The injection of irradiated nonparasitized blood in the 3rd experiment did not produce a discernible acquired resistance to *B. rodhaini*. Presumably the irradiated parasitized blood was responsible for the development of acquired resistance to *B. rodhaini*.

The observation that inoculation of mice with irradiated *B. rodhaini* may induce protective immunity suggests that the presence of replicating *Babesia* in the host may not be necessary for the development of acquired resistance. This is in agreement with the concept of "sterile immunity" described for *B. argentina* (4), *B. bigemina* (2, 3), *B. divergens* (10), *B. microti* (9) and *B. rodhaini* (9, 16).

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