

## SOME EFFECTS OF <sup>60</sup>CO IRRADIATION ON *COWDRIA RUMINANTIIUM* IN ITS TICK HOST *AMBLIOMMA HEBRAEUM* KOCH (ACARINA: IXODIDAE)

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### ABSTRACT

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An attempt was made to attenuate *Cowdria ruminantium* by irradiation of the nymphal stage of its tick host, *Amblyomma hebraeum*. The irradiated nymphae were homogenized and serial dilutions of the resultant suspension were injected intravenously into heartwater-susceptible sheep. No attenuation could be demonstrated but the results indicate that progressively more micro-organisms will be destroyed the higher the irradiation dosage applied, and that dosages between 20 and 30 kilorad apparently prove fatal to all the pathogenic organisms.

### Résumé

CERTAINS EFFETS D'IRRADIATION <sup>60</sup>CO SUR LE *COWDRIA RUMINANTIIUM* DANS SON TIQUE HÔTE, *AMBLIOMMA HEBRAEUM* KOCH (ACARINA: IXODIDAE)

Une tentative a été faite pour atténuer le *Cowdria ruminantium* par irradiation de son tique hôte, *Amblyomma hebraeum*, au stade de nymphe. Les nymphes irradiées furent broyées et des dilutions en série de la suspension résultante furent injectées par voie intra-veineuse aux moutons susceptible de la cowdrose. Aucune atténuation ne put être démontrée mais les résultats indiquent que progressivement plus de microbes sont détruits avec un dosage d'irradiation plus élevé et que les dosages situés entre 20 et 30 kilorad s'avèrent apparemment fatals pour tous les organismes pathogènes.

### INTRODUCTION

Abramov & Duranov (1965) succeeded in producing a mild form of babesiosis by irradiating (2 kilorad) *Rhipicephalus bursa* infected with *Babesia ovis* and subsequently feeding the ticks on sheep. Chakhmatov (1969) irradiated *Hyalomma anatolicum* infected with *Theileria annulata* to 2,4 kilorad. Subsequent feeding on bovines caused only a mild theileriosis. Cunningham, Brown, Purnell, Musoke & Burridge (1971) suggested that a concentrated suspension of infective particles of *Theileria parva* may be attenuated by irradiation. More recently Purnell, Lewis, Brocklesby & Taylor (1980) vaccinated calves infected with a strain of *Babesia divergens* that had apparently been successfully attenuated by irradiation at 25 and 30 kilorad. When the calves were subsequently subjected to field challenge they were found to be resistant to this parasite.

Recently a series of experiments was designed to find methods of immunizing animals against heartwater other than by the use of the existing blood vaccine issued by the Institute. These experiments include the present attempt to attenuate the causative organism, *Cowdria ruminantium*, by irradiation.

### MATERIALS AND METHODS

*A. hebraeum* larvae were fed on sheep that had been infected with *Cowdria ruminantium* by intravenous blood inoculation. Engorged larvae were collected at the peak of the febrile reaction and allowed to moult in an incubator (26 °C ± 2°, 80% RH). The resultant, transstadially infected nymphae were irradiated by the Atomic Energy Board at the Pelindaba Research Institute, using a <sup>60</sup>Co source.

#### Unfed nymphae

Three groups, each containing 200 unfed, infected nymphae, were irradiated at 96 kilorad (kR) per hour for set times to attain dosages of 3; 6 and 8 kR respectively. The nymphae were subsequently fed on susceptible sheep to determine (a) the effect of irradiation on the pathogen in the early stages of nymphal

development as shown by host reaction and (b) whether these nymphae would engorge, moult and thus promote possible multiplication of the micro-organisms.

#### Engorged nymphae

In the 2nd series of experiments infected nymphae were allowed to engorge on sheep and subsequently 7 groups, each containing 100 of these engorged nymphae, were irradiated at 96 kR per hour for set times to attain dosages of 3; 6; 8; 10; 20; 30 and 40 kR.

Each group of engorged irradiated nymphae was separately homogenized and the resulting suspension was made up to 100 ml with buffered lactose phosphate. Each suspension was then diluted serially so that 1 nymph represented 1 ml of a 1/1 dilution. One ml of each dilution (Table 1) was injected intravenously into a susceptible sheep. Control dilutions from unirradiated infected nymphae were simultaneously inoculated intravenously into susceptible sheep.

The temperatures of the sheep were taken daily to determine when a primary reaction to heartwater infection took place. Surviving sheep were subsequently challenged with 5 ml of heartwater blood vaccine inoculated intravenously to determine if immunity had been induced. Hippocampal smears were made from fatal cases and stained with Giemsa to confirm that deaths were due to heartwater.

### RESULTS AND DISCUSSION

#### Unfed nymphae

Unfed nymphae, irradiated to 3; 6 and 8 kR and subsequently placed on susceptible hosts engorged to different stages of repletion. They transmitted parasites that caused typical heartwater temperature reactions and, at the 6 and 8 kR levels, the deaths of their hosts. The host of the 3 kR group of nymphae survived the primary reaction and proved immune upon challenge with heartwater blood vaccine. Irradiation at these levels affected the ticks in that no fully engorged nymphae were found and those that were collected did not moult to adults.

TABLE 1 Serial dilutions made from homogenized, engorged, infected nymphae and inoculated intravenously into sheep

| kR Group | Dilutions |      |      |      |      |       |       |       |       |
|----------|-----------|------|------|------|------|-------|-------|-------|-------|
|          | 1/1       | 1/10 | 1/20 | 1/40 | 1/80 | 1/100 | 1/160 | 1/200 | 1/320 |
| 3.....   | x         | x    | x    | x    | x    |       | x     |       | x     |
| 6.....   | x         | x    | x    | x    | x    |       | x     |       | x     |
| 8.....   | x         | x    | x    | x    | x    |       | x     |       | x     |
| 10.....  |           | x    |      |      |      |       |       |       |       |
| 20.....  |           | x    |      |      |      | x     |       | x     |       |
| 30.....  |           | x    |      |      |      | x     |       | x     |       |
| 40.....  |           | x    |      |      |      | x     |       | x     |       |

The results show that no change in the degree of infectivity of *C. ruminantium* to sheep took place when low levels of irradiation (3; 6 and 8 kR) were applied in the early stages of nymphal development. Since the nymphae were unable to engorge fully and moult to the adult stage, it could not be determined if induced changes would be exhibited at a later stage of development of the pathogen within the tick host.

#### Engorged nymphae

Dilutions of the 7 irradiation groups which did not cause primary temperature reactions are given in Table 2. All other dilutions of the irradiation groups and of the controls caused primary temperature reactions in the sheep, which either died or were immune upon challenge with heartwater blood vaccine. Hippocampal smears from fatal cases all showed organisms indistinguishable from *C. ruminantium*.

TABLE 2 Dilution rates not causing primary temperature reactions in sheep and their immune status when challenged

| Irradiation group (kR) | Dilution | Immune status |
|------------------------|----------|---------------|
| 3.....                 | 1/320    | I             |
| 6.....                 | 1/320    | I             |
| 8.....                 | 1/320    | S             |
| 10.....                | 1/100    | S             |
|                        | 1/200    | S             |
| 20.....                | 1/200    | S             |
| 30+40.....             | 1/10     | S             |
|                        | 1/100    | S             |
|                        | 1/200    | S             |

I=immune, S=susceptible

No attenuating effect on *C. ruminantium* due to irradiation was demonstrated at dilution levels of 1/1 to 1/160. It is interesting that the sheep in both the 3 and 6 kR groups, at the 1/320 dilution level, showed no primary reactions, but were immune when challenged (Table 2). Alexander (1931), however, states that animals exhibiting immunity without undergoing a primary reaction possess natural resistance and that less than 2% of sheep fail to show such a reaction. This finding therefore requires further investigation on a larger scale than in the present experiments. The

8 kR group sheep proved to be susceptible when challenged, which suggests that the pathogenic organisms had been destroyed to the extent that a dilution of 1/320 produced no effect at all on the sheep. This is borne out by the fact that both 10 and 20 kR group sheep also showed no effect at a 1/200 dilution. At the 30 and 40 kR levels all the organisms seem to have been destroyed, as no effect was shown at any of the 3 dilution levels employed (Table 2).

In all the sheep that showed primary reactions, these reactions were severe, which also suggests that no attenuation of the pathogen had taken place. It appears from the data obtained that progressively more organisms are killed the higher the irradiation dosage applied, and that dosages between 20 and 30 kR are sufficient to kill all the organisms. This is similar to the results of Purnell (1978), who found that irradiation doses of 36 and 40 kR were apparently fatal to *Babesia divergens*, and those of Cunningham, Brown, Burrige, Musoke, Purnell & Dargie (1973), who found that increasing doses of irradiation destroy increasing numbers of *Theileria parva* infective particles.

#### REFERENCES

- ABRAMOV, I. V. & DURANOV, V. S., 1965. Effect of ionizing radiation on *Babesia ovis*—the etiological agent of sheep babesiosis. Abstracted paper. *International Conference of Protozoology, London, July 29–August 5, 1965*, 178–179.
- ALEXANDER, R. A., 1931. Heartwater. The present state of our knowledge of the disease. *17th Report of the Director of Veterinary Services and Animal Industry, Onderstepoort, Pretoria*. Part 1, 89–150.
- CHAKHMATOV, G. N., 1969. Étude de l'action des rayons ionisants sur les agents de la nuttalliose des souris et de la theileriose des bovidés. In: STRELKOV, A. A., SUKHANOVA, K. M., & RAIKOV, I. B. *Progress in Protozoology*, p. 254.
- CUNNINGHAM, M. P., BROWN, C. G. D., BURRIDGE, M. J., MUSOKE, A. J., PURNELL, R. E. & DARGIE, J. D., 1973. East Coast fever of cattle: <sup>60</sup>Co irradiation of infective particles of *Theileria parva*. *Journal of Protozoology*, 20, 298–300.
- CUNNINGHAM, M. P., BROWN, C. G. D., PURNELL, R. E., MUSOKE, A. J. & BURRIDGE, M. J., 1971. Some effects of irradiation on the infective stage of *Theileria parva* harvested from infected ticks. *Isotopes and Radiation in Parasitology*, 3, 145–154.
- PURNELL, R. E., 1978. Redwater in British cattle: Steps towards an irradiated vaccine. *A.R.C. Research Review*, 4, 43–45.
- PURNELL, R. E., LEWIS, D., BROCKLESBY, D. W. & TAYLOR, S. M., 1980. Bovine babesiosis: Steps towards an irradiated vaccine. *Journal of the South African Veterinary Association*, 50, 339–344.