

Effect of ultraviolet radiation on the hatchability and survival of eggs and larvae of sheep nematode

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The hatchability of sheep gastrointestinal nematode eggs exposed to ultraviolet (UV) radiation and the activity of the hatched larvae were examined. Hatchability decreased with increasing exposure to radiation. The difference in hatchability of eggs irradiated for 15, 30 and 60 minutes were highly significant ($p < 0.01$ $d\alpha = 3.07, 3.24$ and 3.75) compared with the hatchability of the non-irradiated eggs. The life span of irradiated larvae was shortened, only 20% of those exposed to UV radiation 60 minutes survive for 2 days as against 100% survival rate in the non-irradiated larvae. Batches of nematode larvae (L1) were irradiated with ultraviolet (UV) light for varying time interval to determine the influence of radiation on the transmission potential of the irradiated larvae. There was a decrease in the survival rate of the hatched free-swimming larvae that corresponded with the increasing radiation exposure time.

Key words : Ultraviolet radiation, hatchability, survival, nematode, sheep

Introduction

Several authors have reported on the influence of such factors as temperature, pH, light, salinity and redox potential on hatchability of nematode eggs. Radiation influence on a variety of living organisms has been observed to vary from partial to total interference with normal development process [17,14,3,15].

The Nairobi declaration on climatic change in 1990 confirmed a significant increase in trace gases which is responsible for a gradual erosion of the stratospheric ozone layer which is expected to lead to an increase of ultra violet (UV) radiation at the earth surface. An argumentation of incident biologically effective U.V.B radiation wavelength

band between 290 and 325 nm could be a serious risk factor in the future [11,9].

In view of an expected changes in radiation intensities associated with the mean annual global temperature increase, the present study therefore aimed at determining the possible influence of change in UV radiation intensities base on length of exposure on the hatching of nematode eggs as well as the survival rate of the larvae obtained from the irradiated eggs and irradiated larvae.

Materials and Methods

Nematode egg recovery technique

The technique used was that previously described by Hubert and Kerbouef (1992). Briefly, 10 to 15 gm of faeces were suspended in water and cleaned of organic debris by filtration through sieves (1 mm and 100 μ m) the eggs being collected on a 20 μ m sieve. The eggs were further cleaned from organic debris by centrifugation in magnesium sulphate (density 1.16) for five minutes at 1000 g. The supernatant was filtered through 100 μ m and 60 μ m sieves and the eggs were washed in water and collected on a 20 μ m sieve.

Egg suspension

The concentration of eggs was in five 50 μ l samples and adjusted to 1200 to 1300 egg/ml. Bacteria are necessary for the development of the nematode larvae and must be added to the medium. The egg suspension was diluted with filtrate from the first step of egg extraction, which had been centrifuged for five minutes at 1000 g to eliminate organic debris. To avoid the proliferation of fungi, 5 μ g of amphotericin B (fungizon ND; squibb) was added per ml of egg suspension.

Nutritive medium

The nutritive medium was as described by Hubert and Kerbouef (1984) and composed of Earle's balance salt solution plus yeast extract (Difco laboratories) diluted in saline solution (1 g of yeast extract/90 ml of saline solution)

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in the proportion 1:9 volume to volume.

Hatchability test

The test was carried out in a 90 mm diameter petri dish. 100 µl of egg suspension containing approximately 100 eggs was added to each of the six petri dishes used. Five of these were exposed to UV radiation from a lamp source emitting a wavelength of 2.54 mm from a distance of 560 mm from the base of the containers to the radiation source. Radiation exposures of the egg batches were for 0, 0.5, 5.0, 15, 30 and 60 minutes respectively. 20 µl of nutritive medium was later added to egg suspension and put in an incubator at 27°C. The first stage larvae were obtained two days later. At this time, the parasites were counted. The counting is done for another 6 days to determine the survival rate of the larvae. By this time the larvae has developed to the infective third stage larvae.

Larval development test

The test was carried out in a 90 mm diameter petri dish. 100 µl of the egg suspension containing approximately 100 eggs was added to each of the six petridishes and put in an incubator at 27°C for 48 hours. By then the parasites had developed to first stage larvae. The larvae were then exposed to radiation as described above and later returned to the incubator.

Results

Egg viability and hatchability

The nematode eggs identified were *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Oesophagostomum columbianum*, *Strongyloides papillosus*, and *Trichuris ovis*. Microscopic examination of irradiated nematode eggs did not reveal any drastic morphological changes. In the batches of eggs exposed for 60 minutes, an insignificant number of eggs were observed to have become darkened in colour.

The cumulative percentage (%) hatchability after two days of incubation showed that there was a decrease in the hatching rate of eggs exposed to irradiation as compared to the result obtained in the control (Fig. 1). The difference in % hatchability compared with the control was not significant at 0.5 and 5.0 minutes irradiation level but highly

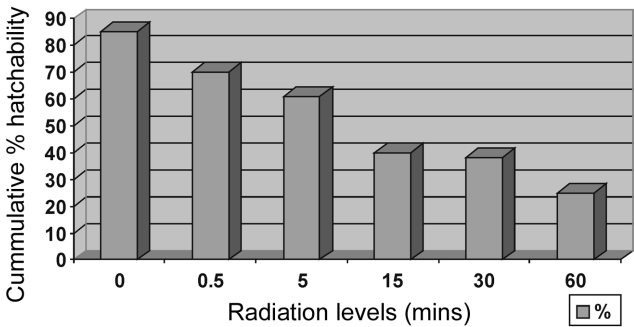


Fig. 1. Cumulative hatchability rate of irradiated nematode eggs.

significant at 15.0, 30.0 and 60.0 minutes ($p < 0.01$) $d\alpha = 3.07, 3.24$ and 3.75 respectively. After 2 days of incubation, no further hatching of eggs occurred in the irradiated eggs. An additional 2% of the eggs in the control batch hatched following a further 1-day incubation. As the number of eggs that hatched increased with the duration of hatching, the proportion of hatched eggs at a particular time interval varied with the radiation level (Fig. 1).

Larvae life span

The percentage survival rate (Table 1) showed that under the experimental condition the control larvae survived for 6days while only 20% of those exposed for 60 minutes survived for 2 days. The activity (the rate of movement) was also radiation level dependent.

Irradiated larvae life span

Direct exposure of nematode larvae to U.V had an inhibiting effect on the rate of activity and survival of the larvae. The findings are presented in Table 2. While only 20% of the 60 minutes irradiated larvae survived for 2 days, the control experiment showed that 100% of the larvae were alive during the same period post-irradiation.

Discussion

Radiation is expected to influence living organisms to varied degree [1,2,16]. The significance of such influence will be related to the level of inhibition-radiation can have on the ability of parasitic or pest organisms to maintain

Table 1. Life span of nematode L1 (larvae) hatched from UV radiated eggs

| Radiation level (mins) | Time of observation (days) | | | | | | |
|---------------------------|----------------------------|-----|----|----|----|----|----|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| 0.0 | 100 | 100 | 86 | 80 | 80 | 78 | 75 |
| 0.5 | 100 | 80 | 67 | 50 | 30 | 0 | 0 |
| 5.0 | 100 | 75 | 59 | 27 | 8 | 0 | 0 |
| 15.0 | 100 | 69 | 51 | 25 | 8 | 0 | 0 |
| 30.0 | 100 | 69 | 44 | 19 | 8 | 0 | 0 |
| 60.0 | 100 | 50 | 20 | 0 | 0 | 0 | 0 |

Table 2. The survival rate of ultraviolet radiated nematode larvae

| Radiation dose (mins) | Time of observation (days) | | | | | |
|--------------------------|----------------------------|-----|----|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 0.0 | 100 | 100 | 80 | 80 | 75 | 70 |
| 0.5 | 100 | 100 | 65 | 30 | 25 | 0 |
| 5.0 | 100 | 97 | 50 | 23 | 0 | 0 |
| 15.0 | 93 | 64 | 29 | 0 | 0 | 0 |
| 30.0 | 79 | 47 | 5 | 0 | 0 | 0 |
| 60.0 | 47 | 20 | 0 | 0 | 0 | 0 |

continuity of life.

The present study confirms the previous findings. In spite of unobserved drastic changes in the egg morphology or viability of the irradiated eggs, there was a significant interference with the hatching rates of eggs. The decreasing hatching rate with an increasing UV irradiation exposure is in line with the findings of Samuelson *et al.*, (1984). The decrease hatchability in eggs expose to high level of (60 minutes) of radiation might have been as a result of radiation damage to the larvae and hence interference with larval ability to induce hatching.

UV radiation cannot explain any inhibition in the built-up of internal osmotic pressure needed for hatching as reported by Kusel (1970) nor can UV support the proposed hatching mechanism by Bair and Etges (1973) that hatching may be induced by enzymatic degradation of the egg shell. Higgins-Optiz and Evers (1983) observed that hatching of eggs occurred as a result of the shell rupturing on one of the two lateral sides of the egg surface. The observed inhibiting effect UV light had on hatchability in this study could have explained such distinctive longitudinal orifice but for localised damage or focal action by enzymes [12] on the rupture line assuming they were present. Radiation could not have selectively affected particular location on the eggshell. On this line of thought, the decreased activity of larvae exposure to high exposure level, (60 minutes) might have been as a result of radiation damage of the larvae and hence the reduce hatchability rate with increase radiation exposure.

However the most tenable assumption like Prah and James (1977) suggested appears to be that the influence of UV radiation on metabolic process in the larvae may directly affect activity and hence survival. UV radiation also had significant influence on larvae from hatched nematode eggs. Investigation, reveal a reduction in the survival ability of the irradiated larvae, suggesting a possible reduction in transmission with respect to population dynamics of the nematode development. It is however pertinent to mention that this observation shows a break-point in the lifecycle of irradiated nematode larvae for the purpose of reduction in parasite number and transmission, particularly as there is a level below which no transmission can successfully take

place [7].

This study did not establish the direct effect of natural UV radiation from sunlight on the larvae which is positively phototropic, it however confirms in the future, the increase UV reaching the earth, if not controlled have a significant effect on organisms activity positively or negatively. A reduction in the transmission level of nematode could be forced.

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