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DECONTAMINATION OF SHEEP FAECES INFESTED WITH GASTRO-INTESTINAL NEMATODES BY GAMMA IRRADIATION

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THE killing effects of ionizing radiation on *Rhabditidae takai* eggs and *Ascaris* eggs in the sewage have been reported earlier[1, 2]. When exposed to ionizing radiation, a 3 cm deep layer of pig manure was decontaminated from *Ascaris*, *Trichuris* and *Oesophagostomum* spp. eggs[3]. However, no information is available on the effect of γ-radiation as a decontaminant of sheep faeces infested with gastro-intestinal (GI) nematode eggs. The present investigation was aimed at finding out the optimum dose of γ-radiation suitable for decontaminating the sheep faeces infested with nematode ova.

The necropsied material was obtained from the intestines of sheep slaughtered in abbatoirs at Kashmir. The worm counts, the type of nematode infection and the respective eggs per gram (EPG) counts in each intestine were estimated and recorded using standard technique[4, 5] before a specified quantity of faeces was subjected to γ-radiation (Gamma chamber–900, BARC, Bombay) or to oven sterilization procedures. Experiment 1, was designed to determine the optimum dose of γ-irradiation required for decontamination of sheep faeces using different radiation levels (0, 200, 400 and 500-Gy). In experiment 2, the γ-sterilized and oven-sterilized faeces were used for culturing a known number of *Chabertia ovina* eggs to determine the effect of γ-irradiation on the percentage larval recovery. The charcoal faecal cultures were prepared[6] and the larval counts were made by dilution techniques.

The faeces was contaminated with eggs of *Chabertia*, *Bunostomum*, *Oesophagostomum*, *Trichuris* and *Trichostrongylus* spp. nematodes. Exposure to varying levels of γ-irradiation of the contaminated faeces resulted in decreased mean percentage larval recovery, when compared with control cultures in group I ($p < 0.05$–0.001; table 1). An irradiation dose of 500–Gy was found to be optimum for perfect decontamination of faeces from nematode ova. Although the dose levels of 600 and 800–Gy were tried in the present investigation, this has not been shown in the table as the difference is not significant from group IV cultures. These findings agree with previous reports that ionizing radiation, possesses ovicidal effects on various free-living and parasitic nematode eggs[2, 3, 7–9]. However, very little information is available on the effect of γ-rays on the eggs of GI nematodes in ruminant faeces, except for an isolated report on the lethal effects of UV rays on *C. ovina* eggs[10].

Table 1 *Experimental design and mean developmental stages recovered from GI nematode infected faeces (post-irradiation) (Intestine Nos. 5, Total worms** 2080, Mean EPG* 193; estimated eggs cultured*: 3804)*

Group	Level of radiation	Larval stages recovered: DAY-7 No.	DAY-7 %	DAY-10 No.	DAY-10 %	Sterilization
I	0-Gy	8904.6	(23.4)	9869.4	(25.9)	No
II	200-Gy	819.8	(2.15)	844.0	(2.22)	No
III	400-Gy	277.6	(0.73)	313.2	(0.82)	No
IV	500-Gy	0	(0)	0	(0)	Yes

*In thousands; **Intestines at autopsy revealed *Chabertia ovina*, *Bunostomum trigonocephalum*, *Oesophagostomum columbianum*; *Trichuris ovis* and *Trichostrongylus* spp.

The results of experiment 2 reveal that irradiated faecal cultures yielded on an average, 7.4% more infective larvae of *C. ovina* than conventional oven-sterilized faecal cultures.

These findings are of practical importance in veterinary parasitology, as the conventional technique of charcoal-faecal cultures in oven-sterilized faeces is bound to denature and reduce the quantity of available nutrients in the culture medium. Hence γ-sterilized faeces seems more useful as a quick and nutritious medium for higher yield of infective larvae than the conventional oven-sterilized faecal cultures. Predisposal γ-irradiation of faecal material may also prove promising in epidemiological control of helminth infestations by preventing their further spread.

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IN VIVO SISTER CHROMATID DIFFERENTIATION IN AN AMPHIBIAN MODEL—APPLICATION OF REVERSE BANDING

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COMBINING 5-bromo-2-deoxyuridine (BUdR) incorporation with Hoechst 33258 staining[1], it is now possible to visualize sister chromatid differentiation (SCD) at the metaphase stage of cell division in which unifilarly BUdR-substituted (TB) chromatid fluoresces more intensely than its bifilarly (BB)-substituted counterpart. The mechanism of this SCD was explained as a result of quenching effect of BUdR on the fluorescence of 33258 Hoechst chromosome complex[2,3]. To avoid technical disadvantages, fluorescence plus Giemsa-stained preparation (FPG) was introduced[4]. This modified technique gives differentially Giemsa-stained chromosomes with darkly-stained TB chromatids and faintly-stained BB chromatids. The mechanism of Giemsa differential staining by FPG technique is based on the differential photolysis of BUdR-substituted DNA. Considering its importance in mutagenicity/carcinogenicity bioassay, various attempts were made to simplify the technique. Takayama and Sakanishi[5] obtained good SCD in the BUdR-incorporated chromosomes using acid extraction technique which showed reverse results in their preparation, viz. the TB chromatids stained faint while the BB chromatids stained dark. The reverse differential staining of sister chromatids has also been reported[6]. Subsequently direct staining of BUdR-substituted chromosomes has been reported[7] in a Na_2HPO_4-Giemsa solution without any fluorochrome pretreatment resulting in a reverse, i.e. B-dark type of SCD. The B-dark type SCD was also obtained[8] by immersing BUdR-substituted chromosomes into 4-Na EDTA-Giemsa solution at room temperature. But all these studies were confined to mammalian cell systems. On the other hand, the application of *in viro* SCE by differential staining in amphibian system has extended the horizon of mutagenicity and carcinogenicity study; this has special significance because of their dual habitats—land and water. The successful application of FPG technique in amphibian system was earlier reported[9,10]. Recently, we have succeeded in producing the SCD of a B-dark type in an amphibian specimen by direct staining of *in vivo* BUdR-substituted chromosomes. The use of *in vivo* SCD and SCEs in Amphibia, and its present staining modification will be of value in cytogenetic monitoring of environmental mutagens.

Specimens of *Bufo melanostictus* (Schneider; Anura; Amphibia) of either sex and weighing about 25 g received intraperitoneal injection of BUdR (Sigma, St. Louis, USA) at 100 mg/kg/h and exposed *in vivo* for 15, 20, 25, 30, 35, 40 and 45 h to allow the cells to pass at least two consecutive replication cycles. Metaphase chromosomes were prepared from intestinal epithelial cells by mitotic division inhibition technique[11] already in practice in our laboratory. Three-day-old air-dried slides were used for staining. For reverse banding, air-dried slides were trypsinized in 0.001% trypsin (Difco, Michigan, USA) solution at 30–32°C for 30–40 sec and rinsed briefly in alkaline solution of 0.1 M Na_2HPO_4 at pH 9. The slides were then stained in Giemsa stain diluted in 10% alkaline solution (0.1 M Na_2HPO_4, pH 9) for 10 min. The staining time, however, was variable depending on the age of the slide, the temperature and the quality of preparation.

The conventionally-stained metaphase complement of *B. melanostictus* consists of 22 biarmed chromosomes (NF = 44) (figure 1). SCD was clearly visible after direct staining of trypsinized air-dried slides in Na_2HPO_4-Giemsa solution (figure 2). The frequency of second cycle cells with SCD was highest (~60%)