RADIOISOTOPE TAGGING FOR STUDIES ON THE ECOLOGY OF THE SHEEP TICK, *IXODES RICINUS* (L.) [1, 2]

BY

DANIEL E. SONENSHINE [3]

ABSTRACT

This study demonstrates the feasibility of radioisotope tagging of the sheep tick, *Ixodes ricinus* (L.). Details of oviposition, hatching, and radioactivity in eggs and larvae of this species are described.

The sheep tick, *Ixodes ricinus* (L.), is a major vector of tickborne diseases throughout its range. Although the ecology of this tick has been studied extensively, (CAMPBELL, 1950 ; LEES, 1948 ; MACLEOD, 1939 ; MILNE, 1943, 1945 *a*, *b*, 1947 ; WALTON and O'DONNELL, 1969), knowledge of several ecological characteristics which may influence its ability to spread disease is still lacking. Tick population density, the degree of dispersal by domestic animals, overwinter survival, and survival of population cohorts are among the important ecological attributes of the tick which must be analyzed quantitatively in order to understand the persistence and spread of disease by *I. ricinus*. Radioecological methods (i. e., ecological tracer technique) appear to be especially suited to such fields studies. Radioisotope tagging of ticks was proved feasible for 5 species (SONENSHINE and YUNKER, 1968), and the technique was used in a study of the ecology of *Dermacentor variabilis* (Say) (SONENSHINE, 1972). Nevertheless, one cannot conclude that this technique is equally useful for all other ticks.

This paper reports the results of laboratory studies to determine whether adequate radioisotope tagging of *I. ricinus* larvae was feasible.

MATERIALS AND METHODS

Labelled ticks were reared from wild caught engorged females obtained from livestock near Cork, Ireland during February 1971. The fecund females were weighed and 45 were injected with an average dose of 6.5 microcuries of uniformly labelled ^{14}C glucose (Amersham Searle, Arlington Heights, Illinois, USA) in volumes of 6 to 8 microliters. The subcuticular inoculations were done with a 30 gauge needle, 50 microliter syringe, and micromanipulator as described by SONENSHINE and YUNKER (1968) ; from 8 to 10 minutes were allowed to elapse in each case before withdrawal of the needle, to minimize loss of inoculum by external oozing of fluids. Subsequently, the inoculated ticks were allowed to oviposit without disturbance in a climate controlled environment chamber (Aminco-Aire Type R Chamber, American Instrument Co., Silver Spring, Md., USA) held at 27°C and 95 % RH. Total egg production in a sample comprising 15 inoculated

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3. Biology Department, Old Dominion University, Norfolk, Virginia 23508.

females was estimated by determining the weight of the entire mass, and then observing the average weight of a single egg. Egg production in 4 fully engorged females which were not inoculated was also estimated.

Radioassay to determine radioactivity in eggs and larvae was done with undissolved samples held on planchets and tested with a Nuclear Chicago Model 470 gas flow detector and 8166 decade scaler (20.6 %) efficiency). Samples of eggs and larvae were subsequently assayed with a Beckman Model LS-250 liquid scintillation detector, as a check on the accuracy of the assays done with the planchet counter. Samples were hydrolyzed with 2N sodium hydroxide and solubilized in Toluene-fluor cocktails with the aid of Beckman BBS-2 solubilizer. Hatching in the egg masses also was estimated.

Results

Post-inoculation fluid loss. Exudation of a clear fluid, presumably a mixture of haemolymph and inoculum accompanied all inoculations, regardless of the length of time the needle was held in the engorged tick. The volume of this exudate varied from less than 5 *ul* to more than 30 *ul* in one instance ; it was less than 20 *ul* in most ticks.

Oviposition and hatching. Only 1 inoculated female failed to oviposit. The mean weight of the remaining 44 engorged females prior to inoculation was 0.217 grams ± 0.0014 (S. E.) (range from 0.096 to 0.324 grams). The mean weight of the egg mass produced by these ticks was 0.099 grams ± 0.009 (S.E.), or 45.7 % of the engorged body weight. The mean number of eggs was 1,597 ± 145.6 (S.E.) (range from 691 to 2243). Egg production in 4 other ticks held as controls was estimated at approximately 1700 eggs. Hatching failed completely in egg masses of 7 inoculated females, and only a few eggs hatched in eggs of 3 others (less than 7 eggs hatched). Mold was observed in 3 of these egg masses, and this agent may have been responsible for their failure to hatch. The mean hatch in all egg masses was 11.8 % ± 1.3 ; of those egg masses in which substantial hatching occurred, 15.6 % ± 1.4. The total yield of tagged larvae was estimated to be 7,750.

Radioactivity in eggs and larvae. The mean radioactivity in eggs determined by assays of whole, undissolved egg samples with the Geiger-Mueller Counter was 621.7 *uu*Ci/egg ± 2.0 (S.E.) ; as determined on the basis of dissolved samples assayed with the liquid scintillation counter, 1,039.7 *uu*Ci/egg ± 3.3. The range (based on the liquid scintillation assays) was from 9.02 *uu*Ci/egg in the egg mass with the lowest activity to 3,506.0 *uu*Ci/egg in the egg mass with the greatest radioactivity. The average radioactivity transferred to the egg mass by the inoculated female was 1.66 *u*Ci, or 25.6 % of the original dose administered. The mean radioactivity in larvae based on assays done with Geiger-Mueller counter was 309.1 *uu*Ci/larva ± 5.2, or 27.9 % of the activity in eggs ; the range was from 140.7 *uu*Ci/larvae in the hatch with least activity to 1469.6/larva in the hatch with the greatest activity.

Discussion

The feasibility of radiolabelling *Ixodes ricinus* immatures by transovarial transfer of a radioactive metabolite is demonstrated by these results. The process of inoculation of the large doses of ^{14}C labelled glucose used to rear highly radioactive larvae did not appear to greatly reduce egg production, if at all. Lees (1952) estimated the mean egg production of a sample of 3-7 females

at 1,980 eggs, or 19.3 % greater than the mean number produced by the inoculated females in this study. However, the mean weight of the engorged females studied by LEES was 18 % greater than that of the females I used, and this difference may have contributed to the higher egg production. Hatching, however, was greatly restricted. This result may be attributed to radiosensitivity. Little or no hatching occurred when the mean radioactivity/egg in an egg batch exceeded approximately 950 *uu*Ci/egg ; this occurred in 14 egg batches, or almost one-third of all those inoculated. The amount of radioactivity introduced into the unfed larvae (mean =309.1 *uu*Ci/larva) was substantially greater than that necessary for detection of all tagged individuals in the hatch of a single egg mass. This amount of radioactivity resulted in a mean count of 64 counts/min. above background with our Geiger-Mueller detector system (average background, 15 counts/min.). These results suggest that improved yields of labelled larvae might be expected by the use of much lower doses of radiochemical material without sacrificing detectability of those individuals.

The results of this study indicate that rearing of large numbers of radiolabelled immature *I. ricinus* is practical.

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