RESEARCH COMMUNICATION

Radiolabelled adult *Haemonchus contortus* obtained from eggs voided in the faeces of a sheep injected with ^{59}Fe

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ABSTRACT

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The infective larvae of *Haemonchus contortus* (L3) obtained from an artificially infected sheep injected with 125 µCi of ^{59}Fe, incorporated the isotope and retained it in sufficient quantities to be detectable by autoradiography in the adult worms which developed.

This is apparently the first report of incorporation of ^{59}Fe into adult helminths, of which the L3 originated from an animal that was injected with the isotope.

Keywords: Adult worms, *Haemonchus contortus*, radiolabelling, third-stage larvae, ^{59}Fe

Unless markers are used, it is practically impossible to follow the comparative development of different generations or strains of nematodes when they occur simultaneously in the same host.

The markers developed to date, such as ^{75}Se labelling of *H.contortus* (Georgi & Le Jambre 1983), have a disadvantage in that laboratory-made faecal cultures are required in order to label infective larvae (L3). Consequently, manual distribution of larvae has to be resorted to in field investigations into the epidemiology of worm development. In the process, the L3 lose the protection of the faecal pellets or pats, and the natural development, migration and infection process cannot be investigated. What is required, is a process whereby the markers are incorporated into the organisms, while they are developing either in the host or in the faeces, without laboratory intervention.

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Unless it is lost through excessive blood or tissue loss, injected radio-iron is excreted from the body over a period of months (Finch 1964). This makes it an ideal potential marker, provided that it is incorporated into the target helminths at sufficiently high levels to be detectable.

Two 6-month-old lambs, one a ewe and the other a wether, born and raised under conditions of minimal exposure to helminths, were used in the investigation.

On day –69 of the experiment, sheep 1 (the ewe) was infected *per os* with about 2 000 L3 of a pure culture of the OP "susceptible" strain of *H. contortus*. The faecal worm egg count (epg) was 1 900 when determined on day –4. On day 0, this sheep was injected intravenously with 125 µCi of ferric ^{59}Fe citrate (Weil Organization, Amersham) in sodium chloride and sodium citrate (pH 6,8; specific activity 3–20 mCi mg^{-1} Fe; radioactive concentration 100 µCi $m\ell^{-1}$). Faeces were collected and the radioactivity of aliquots

was determined by means of a Tri-Carb 1600 CA liquid scintillation analyser.

On day +14, after it had been confirmed that the faeces were radioactive, faecal cultures were made, and L3 harvested 7 d later (Reinecke 1973). The radioactivity of these larvae was determined by the same method and had a CPMA count of 150/million.

On day +26, sheep 2 (the wether) was infected with about 2 000 L3 from the faecal cultures made on day +14 from the faeces of sheep 1. On day +61, sheep 2 had an epg of 1 650, and on day +79, it was slaughtered for worm recovery.

An aliquot of the recovered adult *H. contortus* was autoradiographed as described by Georgi & Le Jambre (1983), and left in contact with the photographic plates for 399 d before they were developed and examined for images of the worms.

From 76 female and 4 male *H. contortus* in the autoradiographic preparation, 74 (92,5%) images were obtained on the photographic plate. While all but two females produced an image (97,4% success rate), none of the males did (0% success).

It seems likely that the radioactivity in the L3 examined, originated both from incorporation of ^{59}Fe into the eggs laid by the worms in the sheep injected with the isotope and from the isotope excreted in the faeces of this sheep.

The images that formed from the radiation were not prominent. Nevertheless, given the halflife of ^{59}Fe of about 45 d, it was probably unnecessary to leave the autoradiographic preparation for longer than 6 months before development. It seems, therefore, that the amount of radioactivity in the worms in this investigation was close to the limits of detection by the technique used.

The short halflife of ^{59}Fe, together with the low levels of radioactivity, will limit the use of the technique for epidemiological field studies—for instance for estimating the relative epidemiological importance of *H. contortus* L3 that overwinter in sheep or on pasture. Because of the danger of environmental contamination, it seems unlikely that sufficient radio-iron may be injected into a sheep to overcome this limitation. On the other hand, the short halflife is advantageous as regards the dangers of radioactive contamination during field use.

This investigation was of a very preliminary nature, and it may be worth while to do a thorough investigation of the full potential of the present technique under field conditions, similar to that undertaken by Georgi & Le Jambre (1983). The fact that the helminths concerned incorporate ^{59}Fe that originates from the host, is a great potential advantage, compared to previous techniques according to which the radioisotope had to be added to the faecal cultures by hand. Now labelled, free-living stages of helminths can be deposited on pasture naturally, overcoming the problem of having to attempt to simulate the natural process while distributing faeces by hand.

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