

## THE EFFECT OF GAMMA RADIATION ON THE CYSTICERCERI OF *TAENIA SOLIUM*

ANNA VERSTER<sup>(1)</sup>, T. A. DU PLESSIS<sup>(2)</sup> and L. W. VAN DEN HEEVER<sup>(3)</sup>

### ABSTRACT

VERSTER, ANNA, DU PLESSIS, T. A. & VAN DEN HEEVER, L. W., (1976). The effect of gamma radiation on the cysticerceri of *Taenia solium*. *Onderstepoort J. vet. Res.* 43 (1), 23-26 (1976).

Cysticerceri of *Taenia solium* were exposed to gamma radiation in doses varying from 20-140 krad. Radiation had an adverse effect on the ability of the cysticerceri to evaginate *in vitro* after a time lag of 9 days. This effect was most marked at doses of 100 krad and higher, thus no cysticerceri exposed to 140, 120 and 100 krad evaginated after 12, 18 and 21 days, respectively. On Day +24, when 60% of the control cysticerceri evaginated, 55%, 50%, 30% and 40% of the cysticerceri exposed to 20, 40, 60, and 80 krad, respectively, evaginated *in vitro*.

Cysticerceri exposed to radiation doses of 20-120 krad are as infective to golden hamsters as are unirradiated cysticerceri. Cestodes resulting from irradiated cysticerceri, however, cannot maintain themselves indefinitely, and are excreted or digested at varying times from Day +12 onwards. Moreover, cestodes resulting from such irradiated cysticerceri do not grow, but are resorbed, and finally consist of only a scolex. By Day +30 the mean length of the worms resulting from the unirradiated cysticerceri is 173.8 mm, while those resulting from cysticerceri exposed to 20 and 40 krad consist of scolices only and the hamsters fed material exposed to 60 krad were negative. It appears, therefore, that radiation inhibits the ability of the cells in the neck region to divide and thus form new proglottids.

Carcasses infested with cysticercosis can possibly be rendered fit for human consumption by exposure to gamma radiation at doses between 20 and 60 krad.

### Résumé

VERSTER, ANNA, DU PLESSIS, T. A. & VAN DEN HEEVER, L. W., (1976). L'effet de l'irradiation gamma sur les cysticerques de *Taenia solium*. *Onderstepoort J. vet. Res.* 43 (1), 23-26 (1976).

Les auteurs ont exposé des cysticerques de *Taenia solium* à l'irradiation gamma aux doses de 20 à 140 krad. L'irradiation a un effet nuisible sur le pouvoir des cysticerques de dévagner *in vitro* après un délai de 9 jours. Cet effet se manifeste au maximum aux doses de 100 krad et davantage. Donc les cysticerques exposés aux 140, 120 et 100 krad n'avaient pas dévagné après 12, 18 et 21 jours respectivement. Au 24<sup>ème</sup> jour 60 p. 100 des cysticerques de contrôle ont subi la dévagination, tandis que 55 p. 100, 50 p. 100, 30 p. 100 et 40 p. 100 des cysticerques exposés aux 20, 40, 60, et 80 krad ont respectivement subi la dévagination.

L'infectivité pour le hamster syrien de cysticerques soumis à l'irradiation aux doses de 20 à 120 krad est semblable à celle de cysticerques non-irradiés. Cependant, les cestodes provenant des cysticerques irradiés sont incapable de se maintenir pendant un temps indéfini; soit ils sont excrétés, soit ils sont digérés après un délai variable à partir du 12<sup>ème</sup> jour. En plus, de tels cestodes ne croissent pas mais sont résorbés et finalement ne consistent que d'un scolex. Au 30<sup>ème</sup> jour la longueur moyenne des vers provenant des cysticerques non-irradiés est de 173,8 mm, tandis que ceux provenant des cysticerques soumis à l'irradiation de 20 à 40 krad ne consistent qu'en scolex. En plus, des hamsters infectés, avec du matériel soumis à l'irradiation de 60 krad ont été indemnes d'infestation. L'irradiation paraît donc gêner la capacité des cellules du cou à se diviser pour produire de nouveaux proglottis.

Il est donc possible de rendre propres à la consommation humaine des carcasses infestées de cysticerques en les exposant à l'irradiation gamma aux doses de 20 à 60 krad.

### INTRODUCTION

Man is the definitive host of 2 tapeworms which utilize domestic animals as their intermediate hosts, viz. *Taenia solium* the so-called "pork tapeworm", and *Taenia saginata* the "beef tapeworm". Both these tapeworms have a cosmopolitan distribution. During the last 40-50 years, however, the incidence of *T. solium* infestation has declined in most parts of the world, and its occurrence has become rare in some countries, for example, in France and Germany (Merle, 1958). In contrast to this, the incidence of *T. saginata* infestation has increased in many countries, notably in Germany and Poland (Pawlowski & Schultz, 1972). Various factors related to the life cycles of these 2 parasites are responsible for the present disparity in their incidence. The fact that 99.8% of pigs with cysticercosis are usually heavily infested (South African Livestock and Meat Industries Control Board, unpublished data, 1975) and the cysticerceri are therefore easily detected during meat inspection, has played an important part in the reduction in the incidence of *T. solium* infestation. Conversely, cattle are usually lightly infested with cysticerceri

of *T. saginata*, and such carcasses are not always detected during routine meat inspection. Urquhart (1966) states that competent meat inspection will detect only two-thirds of the carcasses that are actually infested, while Dewhirst, Cramer & Sheldon (1967) found that 27.7% of the lightly infested carcasses were not detected by routine procedures.

Carcasses lightly infested with cysticercosis are usually frozen for a prescribed period before they are released for human consumption. Taylor & Parfitt (1959) and Van Kooy & Robijns (1968) investigated the possibility of destroying cysticerceri of *T. saginata* by irradiation. Since *T. saginata* develops to sexual maturity in man only, these workers used evagination *in vitro* as a criterion for assessing the effect of irradiation. Evagination *in vitro* can be used as a criterion of viability, but cannot be equated with infectivity and the ability of a parasite to maintain itself in a host. In an attempt to overcome this difficulty, Taylor & Parfitt (1959) irradiated cysticerceri of *Taenia pisiformis* and subsequently fed them to dogs.

It has been shown that golden hamsters treated immunosuppressants are susceptible to infestation with *T. solium* (Verster, 1971; 1974). In such hamsters, these worms develop to sexual maturity but do not produce eggs. Since at least 50% of the hamsters survive this treatment for 30 days, they may be used

<sup>(1)</sup> Veterinary Research Institute, Onderstepoort

<sup>(2)</sup> Radiation Technology Subdivision, Atomic Energy Board, Pretoria

<sup>(3)</sup> Faculty of Veterinary Science, University of Pretoria, P.O. Onderstepoort

to assess the infectivity of irradiated cysticerci as well as the longevity of the tapeworms which develop from them (Verster, 1974).

The present paper reports on the viability *in vitro* and the infectivity to hamsters of irradiated cysticerci of *T. solium*, as well as the longevity of the tapeworms resulting from them.

## MATERIALS AND METHODS

### Irradiation

On 8 separate occasions, cysticerci from heavily infested pig carcasses were exposed to gamma radiation in a 50 kCi (nominal) Gammabeam 650 irradiator\* at Pelindaba, Pretoria. All irradiations were carried out at a constant dose rate of 1 Mrad.h<sup>-1</sup> as determined by Fricke dosimetry. The influence of irradiation dose was investigated over the dose range from 20–140 krad at ambient temperature (25 °C).

### Evagination of cysticerci *in vitro*

Prior to the infestation of the hamsters, the viability of irradiated and unirradiated control cysticerci was tested by their ability to evaginate *in vitro*, as described previously (Verster, 1974).

Cysticerci exposed to radiation doses varying from 20–140 krad were stored at 2–4 °C, and 20 from each radiation dose were tested every 3rd day from Day 0 to Day +24.

### Infectivity of cysticerci

In the 1st experiment, 6 groups of 5 hamsters each were infested with unirradiated control cysticerci, or cysticerci exposed to radiation doses of 20, 30, 40, 50 and 60 krad, and were examined on Day +10. Subsequently, similar groups of animals were infested with unirradiated cysticerci or cysticerci exposed to radiation doses of 60, 80, 100, 110, 120 and 140 krad. These animals were examined as they died until Day +20, when all the surviving animals were killed.

### Longevity and growth of tapeworms

Hamsters were infested with unirradiated cysticerci and cysticerci exposed to radiation doses of 20, 40 and 60 krad. The animals were examined at various times from Day +10 – Day +30 for a comparison of the longevity of the resultant cestodes.

The growth of cestodes, varying in age from 10–30 days and resulting from unirradiated control cysticerci, was compared with that of cestodes resulting from cysticerci exposed to radiation doses of 20, 40 and 60 krad.

### Infestation and treatment of hamsters

Groups of 5–50 hamsters, housed, fed and maintained as previously described (Verster, 1974), were each force-fed 3 cysticerci which had been exposed to various irradiation intensities. Immediately before infestation, and at weekly intervals thereafter, the hamsters were injected subcutaneously with 5 mg methyl-prednisolone acetate†.

### Recovery of the cestodes

The hamsters were examined for cestodes when they died, or at predetermined times from 10–30 days after infestation.

After the animals had been killed with ether, the small intestine was removed, opened, and the mucosa stripped off in physiological saline. The cestodes were killed by being repeatedly dipped in physiological saline heated to 60 °C, and were thereafter fixed in 10% formol-saline. When a specimen was recorded as negative following macroscopic examination, the entire intestinal contents were heated to 60 °C in a waterbath, formalin was added to give a final concentration of 10% and the specimen was then re-examined microscopically.

## RESULTS

### Viability *in vitro* of irradiated cysticerci

The number of cysticerci which evaginated *in vitro* in each of 8 groups is recorded as a percentage in Table 1.

TABLE 1 Evagination (%) of cysticerci *in vitro* after irradiation

Day	Unirradiated cysticerci	Irradiated cysticerci						
		Irradiation dose (krad)						
		20	40	60	80	100	120	140
0.....	90.....	100	100	100	100	100	100	100
3.....	95.....	100	100	80	75	90	90	15
6.....	100.....	100	95	90	80	75	80	60
9.....	76.....	100	60	65	20	30	5	15
12.....	85.....	100	100	65	80	70	25	15
15.....	81.....	95	100	63	45	37	5	0
18.....	—	80	90	100	85	15	20	0
21.....	40.....	85	45	40	40	15	0	0
24.....	60.....	55	50	30	40	0	0	0

Initially, radiation had no effect on the ability of cysticerci to evaginate *in vitro*. From Day 6 onwards, however, there was a reduction in the number which evaginated. The time lag for this effect to become apparent was directly dependent on the radiation dose, particularly at doses of 100 krad and higher.

### Infectivity of irradiated cysticerci

In the initial experiment, 5 groups of 5 hamsters each were infested with cysticerci exposed to doses of 20, 30, 40, 50 and 60 krad, while a similar group was infested with unirradiated control cysticerci. When the animals were examined on Day +10, the majority were found to be infested with *T. solium* (Table 2).

In a subsequent experiment (Table 2), similar groups of hamsters were infested with cysticerci exposed to doses of 60, 80, 100, 110, 120 and 140 krad. When this experiment was planned, the intention was to examine these animals on Day +20. On Day +10, however, 7 hamsters died. Mortality continued and by Day +20 there were only 17 survivors. *T. solium* was recovered from 4 of the hamsters infested with the control cysticerci. Cestodes were also recovered from single hamsters in each of the groups infested with cysticerci exposed to doses of 60 (Day +11), 80 (Day +10), 100 (Day +11), 110 (Day +12) and 120 krad (Day +12). No cestodes were recovered from those infested with cysticerci exposed to 140 krad. One hamster from the latter group survived to Day +20, while 2 died on Day +10, and 1 each on Day +12 and Day +13.

\* Supplied by Atomic Energy of Canada, Limited

† Depo-Medrol, Upjohn

The number of animals from which cestodes were recovered in these two trials is summarized in Table 2.

TABLE 2 Infectivity of irradiated and unirradiated cysticerci to hamsters (5 animals per group)

Irradiation (krad)	Age of infestation (days)	No. of hamsters positive
Unirradiated.....	10	5
20.....		4
30.....		5
40.....		5
50.....		3
60.....		5
Unirradiated.....	20	4
60.....		1**
80.....		1*
100.....		1**
110.....		1***
120.....		1***
140.....		0

\*Recovered on Day 10  
 \*\*Recovered on Day 11  
 \*\*\*Recovered on Day 12

*Longevity and growth of T. solium resulting from irradiated cysticerci*

Since cestodes were recovered from hamsters infested with irradiated cysticerci up to Day +12 only, it was concluded, therefore, that cestodes developing from such cysticerci cannot maintain themselves indefinitely. Subsequent experiments were carried out therefore, to determine the longevity of such worms.

In a pilot trial, 30 hamsters were infested with cysticerci exposed to a dose of 60 krad. Five of these hamsters were examined daily from Day +10–Day +13, 3 on Day +14 and Day +15 and 4 on Day +16. The majority of the hamsters examined from Day +10 to Day+15 were infested, but those examined on Day +16 were negative.

In 3 subsequent experiments, hamsters were infested with cysticerci exposed to doses of 20, 40 and 60 krad and examined at various times from 10–30 days after infestation. The data on the longevity of cestodes resulting from the cysticerci used in these 3 experiments are combined and summarized in Table 3.

On Day +10 there was no difference in the survival of the cestodes resulting from irradiated or unirradiated control cysticerci, but after this there was a fluctuation in the number of positive hamsters in the groups fed irradiated cysticerci. From Day +20 onwards, however, there was a decrease in the number of positive hamsters in the 3 experimental groups. Thus on Day +30, when 80% of the control hamsters harboured worms, only 20% and 40% of those fed cysticerci exposed to 20 and 40 krad, respectively, were positive; all the hamsters fed material exposed to 60 krad were negative.

In Table 4 the mean length of the cestodes recovered from hamsters infested with cysticerci exposed to doses of 20, 40 and 60 krad is compared with that of cestodes resulting from unirradiated cysticerci. On Day +20, the majority of cestodes resulting from irradiated cysticerci consisted of a scolex only, but some of them had a short strobila. On Day +30, only scolices were recovered from those hamsters infested with cysticerci exposed to 20 and 40 krad; no worms were recovered from hamsters fed material exposed to 60 krad. The worms that consisted of a scolex only were not measured because it was difficult to mount them in a lateral position; they invariably rested on the posterior end of the scolex.

TABLE 4 Mean length (mm) of *T. solium* resulting from irradiated cysticerci

Age of infestation (Days)	Treatment			
	Unirradiated controls	20 krad	40 krad	60 krad
10.....	9,3	5,9	4,6	4,8
15.....	25,8	3,1	2,5	2,4
20.....	61,6	(10–12)*	(7–8)*	(4)*
25.....	193,9	(4–15)*	Scolices	Scolices
30.....	173,8	Scolices	Scolices	—

\*Length of the largest worms recovered; remainder consist of scolices only

TABLE 3 Longevity of *T. solium* resulting from irradiated cysticerci

Age of infestation (Days)	Treatment of cysticerci							
	Unirradiated		20 krad		40 krad		60 krad	
	Hamsters		Hamsters		Hamsters		Hamsters	
	No. infested	Positive (%)	No. infested	Positive (%)	No. infested	Positive (%)	No. infested	Positive (%)
10.....	10	70,0	10	90,0	10	70,0	10	70,0
12.....	8	87,5	18	72,2	20	55,0	10	100,0
15.....	19	63,1	29	48,3	30	43,3	30	70,0
18.....	8	75,0	19	21,0	20	55,0	20	50,0
20.....	10	80,0	10	100,0	10	60,0	10	80,0
21.....	10	70,0	9	33,3	9	33,3	10	10,0
25.....	10	100,0	10	40,0	10	50,0	10	40,0
30.....	10	80,0	10	20,0	10	40,0	10	0

## DISCUSSION

Taylor & Parfitt (1959) found that cysticerci of *T. pisiformis* that had been exposed to radiation doses varying from 10–100 krad, evaginated *in vitro*. When they treated cysticerci of *T. saginata* similarly, the results were variable. The percentage evagination of 2 control batches of unirradiated *T. saginata* cysticerci was 56.5% and 16.2%, respectively, while the percentage evagination of irradiated cysticerci was 26.3% at 10 krad, 38.6% at 20 krad, 25.5% at 50 krad, 39.7% at 75 krad and 14.9% at 100 krad. The conclusion was reached that this variation was caused by differences in the viability of the cysticerci.

The results obtained in the present investigation show that radiation doses of up to 140 krad do not immediately affect the ability of cysticerci of *T. solium* to evaginate.

The percentage evagination of the larval stage of *Echinococcus granulosus* showed a decrease 3 days after exposure to doses of 60 krad, 6 days after exposure to 40 krad and later at lower radiation doses (Movsesijan, Sokalić & Mladenovic, 1967). When *T. saginata* cysticerci were exposed to doses of 200 and 300 krad, respectively, there was a slight reduction in the percentage which evaginated after a week, but 14 days after irradiation 40% and 33%, respectively, evaginated (Van Kooy & Robijns, 1968). Cysticerci of *T. solium* show a similar time lag before the percentage evagination *in vitro* is adversely affected, and the time lag for this to occur is dependent on the irradiation dose, particularly at doses of 100 krad and more.

Taylor & Parfitt (1959) showed that cysticerci of *T. pisiformis* that had been exposed to radiation doses of 10 and 20 krad were infective to dogs. The worms that resulted were, however, stunted and sterile 42 days after infestation, whereas worms resulting from unirradiated control cysticerci were gravid. At 42 days the 2 dogs infested with cysticerci exposed to radiation doses of 30 and 100 krad did not harbour *T. pisiformis*. When the dog infested with cysticerci exposed to 50 krad was examined 11 days after infestation, it contained small, immature worms.

In this investigation, cysticerci of *T. solium* that were exposed to radiation doses varying from 20–120 krad were infective to hamsters, but were unable to maintain themselves indefinitely. As these worms aged, they were resorbed until only the scolex remained. The rate at which they decreased in size showed some correlation with the radiation dose to which the cysticerci were exposed, but this was not constant for the different batches of cysticerci used in the various experiments. In one experiment, the worms resulting from cysticerci exposed to doses of 20, 40 and 60 krad consisted of scolices only on Day +18. In another experiment, some of the worms resulting from cysticerci exposed to 40 and 60 krad were reduced to scolices by Day +20, but they had all been reduced to this size by Day +25. Worms resulting from cysticerci exposed to 20 krad were similarly affected, but it was only on Day +30 that they had all been reduced to scolices. This variation in the effect of radiation on different batches of cysticerci is probably due to differences in the age of the cysticerci, as young organisms are more susceptible to the effects of radiation (Van Kooy & Robijns, 1968).

According to Van Kooy & Robijns (1968), Pawel & Janicek (1963) found that radiation doses of 400–500 krad were required for the complete inactivation

of the cysticerci of *T. saginata*. Irradiation at these doses, however, affects the taste of meat as it denatures the proteins and causes chemical changes (Evans, 1970). Investigations on *Trichinella spiralis* have shown that radiation doses of this magnitude are not required to sterilize meat infested with this parasite. Larvae of *T. spiralis*, although not killed by irradiation at 20 krad, cannot produce new cells. At lower radiation doses, the resulting adults produce few larvae, as the radiation suppresses the ability of cells to proliferate and thus to produce larvae.

In the present investigation, the cestodes which resulted from irradiated cysticerci did not increase in size as did those resulting from the control cysticerci. Moreover, as they aged, the worms from the irradiated cysticerci became smaller until eventually only the scolices remained. It would appear, therefore, that radiation affects the ability of the cells in the neck region to proliferate and thus form new proglottids. The fore-body and hind-body of the newly-evaginated cestodes are gradually resorbed until only the scolices remain. The scolex can thus maintain itself until it has metabolized its reserve food material; it is then excreted.

From this investigation it would appear that carcasses infested with cysticercosis can possibly be rendered fit for human consumption when exposed to gamma radiation at doses between 20 and 60 krad. Radiation at these relatively low doses should not affect the taste or quality of the meat, as is the case when sterilizing doses of radiation are used.

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