USE OF IRRADIATION TO CONTROL INFECTIVITY OF FOOD-BORNE PARASITES

PROCEDINGS OF A FINAL RESEARCH CO-ORDINATION MEETING, MEXICO CITY, MEXICO, 24–28 JUNE 1991
ORGANIZED BY THE JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

1. Ingestion of meat
2. Meat
3. Cercaria
4. Gravid proglottids
5. Eggs
6. Ingestion of eggs
7. INTERMEDIATE HOST
8. DEFINITIVE HOST

 Icons: FAO and IAEA logos

Diagram shows the lifecycle of a food-borne parasite, starting with ingestion of meat, leading to the definitive host, which then ingests eggs, leading to the intermediate host, and the cercaria, which further develops into gravid proglottids.
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TO CONTROL INFECTIVITY 
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INTERNATIONAL ATOMIC ENERGY AGENCY 
VIENNA, 1993
FOREWORD

Food-borne parasitic diseases are common throughout the world, pose significant health problems and cause economic losses in terms of agricultural commodities and human productivity. The diseases usually occur through consumption of raw or partially cooked foods which are infected by various parasites (e.g. tapeworms, roundworms, flukes, parasitic protozoa, etc.). The problem is significant in developing countries where the population has the habit of consuming raw food of animal origin. Available data, with the exception of data on *Trichinella spiralis*, a parasitic nematode, were insufficient for the use of irradiation technology to control food-borne parasites. Therefore, a Co-ordinated Research Programme (CRP) on the Use of Irradiation to Control Infectivity of Food-Borne Parasites was implemented by the FAO/IAEA in 1986.

The results of the work carried out over five years (1986–1991) by twelve researchers participating in the programme, i.e. from Argentina, Belgium, China, Japan, the Republic of Korea, Mexico, Poland, Thailand and the United States of America were presented at the Final Research Co-ordination Meeting (RCM) of this CRP in Mexico City, Mexico from 24 to 28 June 1991. The findings of this CRP have established conclusively the potential for application of food irradiation in the control of liver flukes, tapeworms, roundworms, trichinosis, toxoplasmosis, etc. The Proceedings resulting from the CRP include the final reports on work carried out by the participating scientists. A summary report of the RCM is also included in the Proceedings. This report includes the conclusions and recommendations of the participants concerning the results obtained and need for further research.

The Scientific Secretary of this CRP was P. Loaharanu, Food Preservation Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.
EDITORIAL NOTE

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SUMMARY REPORT

A. Use of irradiation to control fish and invertebrate food-borne parasites

1. INTRODUCTION

Fish-borne, snail-borne and crustacean-borne parasitic diseases are found throughout the world, with the highest prevalence in Asia.

Liver flukes, such as *Opisthorchis viverrini*, are endemic in Thailand and neighbouring countries, with prevalence as high as 40%, while *O. felineus* is found predominantly in Russia and the eastern European countries. The Chinese liver fluke, *Clonorchis sinensis*, is reported throughout China and in Korea and Japan. Prevalence rates vary and in some areas are very high. Other trematode infections are acquired from eating raw freshwater fish, but the prevalence and distribution are not completely documented. Most infected persons experience few symptoms, but in heavy infections there may be gastrointestinal disturbances. Prolonged infections caused by *C. sinensis* and *O. viverrini* may provoke biliary cholangiocarcinoma. Gnathostome infections, although not widely distributed, are found with the highest infection rates in Thailand. *Gnathostoma spinigerum* is the species most commonly involved and may cause cutaneous and visceral larva migrans. Migration of the parasite into vital organs may lead to death. Raw, undercooked, and fermented fish are major sources of infection in Thailand.

Anisakiasis is a nematodiasis acquired from eating marine fish and cephalopods containing the larval stages of *Anisakis*. *Anisakis simplex* is the most important species, with infections in humans occurring worldwide. Most human disease occurs in Japan, where the larval stage of the parasite causes eosinophilic granulomas in the intestinal tract. Other species of marine heterocelid nematodes may infect humans but cause no disease except, for example, a minor throat irritation called ‘tickle throat’.

Intestinal capillariasis is a fish-borne parasitosis found primarily in populations in the Philippines and Thailand. Sporadic infections have been reported from Japan, China (Taiwan), Egypt and the Islamic Republic of Iran. Infected freshwater fish eaten raw transmit the parasite to humans, where it multiplies and causes a severe gastroenteritis and protein-losing enteropathy. Untreated infections lead to death.

Only a few population groups eat uncooked molluscs. Clams and snails are usually eaten cooked, but when people eat infected molluscs raw, they may acquire angiostrongyliasis or echinostomiasis. Infection with *Angiostrongylus cantonensis* is widespread but human infections are reported primarily from Thailand and China (Taiwan). Snails of the *Pila* species in Thailand and *Achatina fulica* in Taiwan are major sources of infections. Humans infected with *A. cantonensis* may experience
### TABLE I. EFFECT OF IRRADIATION ON VARIOUS FOOD-BORNE PARASITES MED^a (kGy)

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Isolated Larvae</th>
<th>Larvae in animal tissue</th>
<th>Variation among isolates/strain</th>
<th>Bioassay</th>
<th>Effect of irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Fish and invertebrate food-borne parasites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clonorchis sinensis</em></td>
<td>0.05</td>
<td>0.15</td>
<td>ND</td>
<td>Rats</td>
<td>Inhibits maturation (bile duct)</td>
</tr>
<tr>
<td><em>Opisthorchis viverrini</em></td>
<td>0.1</td>
<td>0.1</td>
<td>ND</td>
<td>Hamsters</td>
<td>Inhibits maturation (bile duct)</td>
</tr>
<tr>
<td><em>Angiostrongylus cantonensis</em></td>
<td>(2.0)</td>
<td>(2.0)</td>
<td>ND</td>
<td>Mice, rats</td>
<td>No tissue penetration (vessel in brain)</td>
</tr>
<tr>
<td><em>Angiostrongylus costaricensis</em></td>
<td>(4.0)</td>
<td>(4.0)</td>
<td>ND</td>
<td>Mice, rats</td>
<td>No tissue penetration (vessel in brain)</td>
</tr>
<tr>
<td><em>Paragonimus westermani</em></td>
<td>(0.1)</td>
<td>(0.1)</td>
<td>ND</td>
<td>Cats</td>
<td>No tissue penetration (lung or other organ)</td>
</tr>
<tr>
<td>Parasite</td>
<td>MED 1</td>
<td>MED 2</td>
<td>Infectivity</td>
<td>Host</td>
<td>Effect</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-------</td>
<td>-------</td>
<td>----------------------</td>
<td>-----------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>0.7</td>
<td>0.7</td>
<td>0.5–0.7</td>
<td>Mice, cats</td>
<td>Elimination of infectivity</td>
</tr>
<tr>
<td><em>Trichinella spiralis</em></td>
<td>0.15</td>
<td>0.15</td>
<td>0.1–0.6</td>
<td>Mice, rats</td>
<td>Elimination of infectivity</td>
</tr>
<tr>
<td><em>Taenia saginata</em></td>
<td>(0.3)</td>
<td>(0.3)</td>
<td>ND</td>
<td>Human volunteers</td>
<td>Elimination of infectivity</td>
</tr>
<tr>
<td><em>Taenia solium</em></td>
<td>ND</td>
<td>6.5 b</td>
<td>Suspected</td>
<td>Hamsters</td>
<td>Death of metacestodes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(inability to evaginate, no growth in hamsters)</td>
</tr>
</tbody>
</table>

MED = minimal effective dose. This dose was determined on the basis of experiments. In practice, regulatory authorities may set the MED higher than the one found by experiments.

Man is the only definite host. However, no experiments can be conducted in humans because of the danger of acquiring neurocysticercosis.

ND = No data.

( ) = Data have to be further verified.
eosinophilic meningitis; death is rare, except in heavy infections. *A. costaricensis*, found in Central and South America, causes eosinophilic granulomas in the intestines of humans. Transmission is by the accidental ingestion of slugs or the larvae-containing mucus of slugs on vegetation, but the incidence of disease is low.

Infected crustaceans, especially freshwater crabs and crayfish when eaten raw, poorly cooked, or pickled, are responsible for the transmission of paragonimiasis. The main species in Asia is *Paragonimus westermani*, but *P. heterotremus* is important in Thailand; other minor species are endemic in China and other parts of Asia. The prevalence of infection, although widespread, is usually low. The disease can be severe if the worm migrates to the brain or other vital organs.

Praziquantel is very effective for treating liver fluke infections. Some infections can be completely eliminated with this drug. Gnathostomiasis and anisakiasis are not currently treated with anthelmintics; surgery is usually required, and for anisakiasis, enteroscopic methods can be used. Intestinal capillariasis can be successfully treated with anthelmintics, such as mebendazole or albendazole.

Some reports indicate that mebendazole or albendazole are of some value in treating angiostrongyliasis, although anthelmintics are not usually recommended. Most cases are self-limiting if few worms are involved. Echinostomiasis is also usually self-limiting, but mebendazole will eliminate infections. Paragonimiasis can be treated with praziquantel.

These parasitoses can be controlled by not eating raw or inadequately cooked freshwater fish and molluscs. Although changing eating habits would eliminate infections, this may not be feasible in the foreseeable future. In addition, the parasitoses continue in nature in infected wild definitive and intermediate hosts. Control measures presently available have not been able to eliminate the parasites nor halt the transmission of infections in endemic areas. There is a need for a new technology either to interrupt the life cycle of the parasites or to prevent transmission of these food-borne parasitoses.

2. EFFECTIVENESS OF IRRADIATION AS A CONTROL MEASURE

To evaluate the effectiveness of irradiation as a control measure for fish-borne or other aquatic food-borne parasites, the following were considered: the minimal effective dose (MED) of irradiation needed to eliminate infectivity of each parasite, organoleptic changes in the treated food products, and the need for further verification of the MED.

As summarized in Table I, seven parasites were studied. Based on two rat bioassay studies done in China and the Republic of Korea, the MED for *Clonorchis* sp. larvae contained in fish was 150 Gy, and for isolated larvae of *C. sinensis* was 50 Gy. Differences in the MED of different geographical strains of the parasite were not recognizable. The MED for different types of food was not determined and
sensory acceptability evaluations were not performed since it was thought the results would be similar to those for irradiated fish infected with *Opisthorchis viverrini*.

Thai reports on *O. viverrini*, using a hamster bioassay, found that the MEDs for isolated larvae and larvae contained in fish were both 100 Gy. Sensory evaluation of the irradiated fish was done using 19 panel members. No significant changes in odour or taste of irradiated fish were reported. No information is available on the MED of *O. felineus*.

For *G. spinigerum*, the MED for isolated larvae and for those in fish or other hosts was not determined, although Thai studies found that irradiation of infected mice at doses of at least 7 kGy markedly reduced the worm recovery rate. Sensory evaluations should be done, especially on ‘Som Fuk’, a popular fermented fish product of Thailand.

Using a rabbit bioassay, the larvae of *A. simplex* (*Anisakis* type I larvae) were found to be highly resistant to irradiation even at high doses of 2 kGy or 10 kGy for suppressing motility of the larvae. The MED needs further study. Organoleptic tests should be performed.

From Japan, preliminary data from mice and rat bioassays on *A. cantonensis* and *A. costaricensis*, using *Achatina fulica* and *Pila* species of snails, indicated that the MED for *A. cantonensis* was 2 kGy and for *A. costaricensis* 4 kGy. Further studies need to be done.

A report from China indicated that the MEDs for *P. westermani* isolated larvae and *P. westermani* larvae in crabs were both 100 Gy, using the cat as the final host. The MED for complete inhibition of the penetration of larvae through the intestinal wall has to be further verified for each of the various *Paragonimus* species.

3. IRRADIATION FOR CONTROL OF FISH AND OTHER AQUATIC FOOD-BORNE PARASITES FOR LOCAL CONSUMPTION AND FOR EXPORT

Locally consumed fish prepared in a dish called ‘Koi Pla’ is an important source of *O. viverrini* infection in Thailand. In China, Japan and Korea, ‘sashimi’ fish dishes are sources of infection with *C. sinensis*. It would be very difficult to implement irradiation control measures in remote rural areas where fish is usually eaten raw shortly after being caught. One approach would be to have sources of irradiation in mobile units at easily accessible locations in rural areas.

There is a possibility that freshly caught fish could be irradiated at central locations before being made available to commercial markets and restaurants, and for use in cottage industries where fish dishes are prepared for market. Examples of these prepared fish products are ‘Pla Som’ in Thailand and smoked fish in Russia, which transmit opisthorchiasis, Som Fuk and ‘Pla La’ (fermented fish dishes), which harbour *Gnathostoma* larvae, and ‘Poo Kem’ (pickled fermented crab), which transmits paragonimiasis in Thailand.
<table>
<thead>
<tr>
<th>Parasite</th>
<th>MED</th>
<th>Animal tissue investigated</th>
<th>Bioassay for viability of the parasite</th>
<th>Effect of irradiation</th>
<th>Differences in radiosensitivity by different parasite strains or isolates (MED variation; kGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>0.7</td>
<td>Pig and mouse</td>
<td>Mouse inoculation test (MIT)</td>
<td>Eliminated infectivity</td>
<td>0.5–0.7</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>Pig and mouse</td>
<td>Cat feeding test</td>
<td></td>
<td>0.5–0.7</td>
</tr>
<tr>
<td><em>Trichinella spiralis</em></td>
<td>0.3</td>
<td>Pig&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Reproductive capacity (RC)</td>
<td>Eliminated infectivity</td>
<td>No information</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>Mouse</td>
<td>RC — mouse inoculation test</td>
<td>Eliminated infectivity</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>Rat</td>
<td>RC — rat inoculation test</td>
<td>Eliminated infectivity</td>
<td>0.1–0.6</td>
</tr>
<tr>
<td><em>Trichinella nativa</em></td>
<td>0.1</td>
<td>Mouse</td>
<td>RC — mouse inoculation test</td>
<td>Eliminated infectivity</td>
<td>Not recognizable</td>
</tr>
<tr>
<td><em>Trichinella&lt;sup&gt;b&lt;/sup&gt; type 3</em></td>
<td>0.2</td>
<td>Mouse</td>
<td>RC — mouse inoculation test</td>
<td>Eliminated infectivity</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td><em>Trichinella&lt;sup&gt;b&lt;/sup&gt; nelsoni</em></td>
<td>0.4</td>
<td>Mouse</td>
<td>RC — mouse inoculation test</td>
<td>Eliminated infectivity</td>
<td>0.2–0.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Recommended MED is 0.7 to allow for sufficient dose to also inactivate any *T. gondii* that may be present. (See text.)

<sup>b</sup> *T. britovi* n. sp. prop.
Similar irradiation procedures could be applied to crabs in endemic areas and to prepared products such as Poo Kem before releasing them to markets or for export.

**B. Use of irradiation to control toxoplasmosis**

1. **INTRODUCTION**

   *Toxoplasma gondii* is one of the most widespread parasitic infections. Various studies have shown that the prevalence of the antibody in humans may range up to 50% worldwide. *Toxoplasmosis* is an important cause of congenital malformations in infants. For example, in the United States of America (USA) alone, as many as 3300 infected children are born each year. The annual medical cost to treat and/or institutionalize these children is US $430 million. Toxoplasmosis also affects the production of livestock. Spontaneous abortion in sheep due to toxoplasmosis is a serious problem and the economic costs are substantial. Sources of infection for humans are cats and raw or undercooked meat. Because swine are frequently infected, pork and pork products are considered important sources of toxoplasmosis. Serological surveys of swine in various countries indicate prevalences from 10% to 53%. It is now recognized that cerebral toxoplasmosis is an important component of the AIDS related complex.

   Treatment of acute toxoplasmosis in humans is with pyrimethamine and trimethoprim. Generally, physicians advise pregnant women who are serologically negative to avoid exposure to cat faeces and to avoid eating undercooked meat. In some countries, such as France, serological testing for toxoplasmosis in pregnant women is carried out.

2. **EFFECTIVENESS OF IRRADIATION AS A CONTROL FOR TOXOPLASMOSIS**

   Over the past decade, scientists in several countries have carried out research designed to determine the effectiveness of gamma irradiation to destroy the larvae of *T. gondii* in pork. Several of them have collaborated in this effort.

   Various geographical isolates of *T. gondii* have been evaluated for radiosensitivity in pork. Irradiation studies were performed using infected mouse tissue and infected pork. The bioassays used were the mouse inoculation test and the SPF cat-feeding procedure. The results from investigations in China, Yugoslavia, and the USA were nearly identical: the MEDs for the NT isolate from China was 0.55 kGy, the TG-3 isolate from Yugoslavia was 0.7 kGy, and the ME-49 isolate from the USA was 0.5 kGy. The consensus was that the MED for *T. gondii* should be 0.7 kGy to
allow for strain variation (see Table II). Because *T. gondii* is so easily inactivated in processed pork by heating, freezing, or curing, the above investigations on irradiation dealt only with fresh pork.

Although none of the above investigations included organoleptic assessments, other studies on irradiated pork reported no significant organoleptic changes at doses up to 4 kGy.

C. Use of irradiation to control trichinellosis

1. INTRODUCTION

Although infections with *Trichinella spiralis* in swine and humans have decreased in recent decades in developed countries, trichinellosis remains a significant food safety problem in many developing countries. Regulatory costs to safeguard against trichinellosis, public health costs associated with sporadic outbreaks, and the generally depressing effect the disease has on consumer demand and exports are important.

In the USA, prevalence surveys in swine indicate that approximately one hog in 1000 is infected. Data for human infections show a minimum of 50 to 100 clinical cases occurring annually; the number of subclinical or misdiagnosed cases is undoubtedly much higher.

In the early 1970s, the percentage of infected pigs in Poland decreased markedly to 0.001-0.004%. Trichinellosis in humans decreased also. From 1970 to 1973 the number of registered cases was 1884; however, between 1978 and 1988 there were 2614 cases with 22 fatalities. In 1990, the incidence of human trichinellosis in Poland was 2.3 times higher than in 1989; in 1990 the number of registered cases was 272. One reason might be the development of small family farms and local butcheries, the purchase of pork for home use directly from small slaughterhouses, and inadequate meat inspection. Infections have appeared mainly as familial outbreaks.

In Poland, in 4.6-61.5% of the total cases and in about 35% of all clinical cases of trichinellosis, the source of infection was sylvatic, wild boar meat.

Generally, trichinellosis is treated with mebendazole, with or without corticosteroids. Current prevention strategies in various countries include meat inspection; treatment of pork by freezing, heating, smoking or curing; and improvement of swine husbandry practices to prevent introduction of *T. spiralis* into the herd. In the USA, irradiation of pork for trichina control is permitted. It should also be emphasized that the burden of maintaining costly inspection activities (trichinoscope or tissue digestion) is great. Likewise, the meat industry bears an appreciable economic burden from enforcement of rigorous meat processing regulations for the
production of ready-to-eat pork products. In a study supported by the US Department of Energy, it was estimated that the US pork industry loses more than US $400 million annually in reduced consumer demand, depressed export markets, costs of compliance with regulatory rules, etc. Although the total annual medical costs are not great, i.e. US $1.5-2.0 million, the hospitalization costs for a patient with trichinellosis are substantial. The economic burden for other countries is not well documented.

2. EFFECTIVENESS OF IRRADIATION FOR CONTROL OF TRICHINELLOSIS

In vivo studies on pork by two different laboratories produced nearly identical results. In the US investigations using rat and mouse bioassays on infected pork, it was found that a dose of 0.15 kGy of gamma irradiation was sufficient to interrupt the intestinal maturation of the L1 larvae, thereby preventing muscle invasion and disease. As a safety factor, a MED of 0.3 kGy was set by regulatory agencies. In Poland, researchers investigated the radiosensitivities of a number of pig and sylvatic isolates. The investigations utilized rat and mouse infections, and also used rats and mice in bioassays. The results showed that there was some variation in radiosensitivity between isolates and species of Trichinella (see Table II). In the rat bioassay, the radiosensitivities varied from 0.1 kGy to 0.6 kGy. In the mouse assay, the variation was from 0.1 kGy to 0.2 kGy. Host susceptibility differences between mice and rats undoubtedly account for these variations. As a general recommendation for pork, it was agreed that for the control of T. spiralis, the MED should be at least 0.7 kGy to inactivate any T. gondii present.

In vitro studies using infected pork were carried out in the USA to ascertain the importance of the tissue environment on radiosensitivity. It was determined that cooling meat, post-irradiation holding of irradiated meat, and vacuum packaging had little or no effect on the effectiveness of irradiation.

3. RESEARCH RECOMMENDATIONS

No further fundamental research on the effectiveness of irradiation on T. gondii or T. spiralis is needed.
D. Use of irradiation to control taeniasis/cysticercosis

1. INTRODUCTION

Taeniasis/cysticercosis caused by *Taenia saginata* and *T. solium* are prevalent in Mexico and in many other countries where poverty and poor hygiene prevail. Regions highly endemic for *T. saginata* include areas in Central and East Africa, the Caucasian and south-central Asian republics, Near East countries, and parts of the former Yugoslavia. Moderate infection rates are reported from Europe, Southeast Asia and South and Central America, and low rates from the USA, Canada and Australia.

Cysticercosis in cattle and taeniasis in humans are serious diseases found worldwide. The infections occur when raw or insufficiently cooked pork is consumed and where sanitary conditions are poor and human beings become infected with *T. solium* eggs. It has been reported from Mexico, Central and South America, Spain, parts of eastern Europe, the Far East, India and other tropical regions. In human patients, metacestodes of *T. solium* tend to migrate to the central nervous system, where they can cause very serious neurological problems. It was estimated that the annual economic loss in Mexico caused by cysticercosis in pigs amounts to US $43 million. Treatment for neurocysticercosis can cost from US $850 to US $2500, depending upon the diagnostic tests used and whether the patient needs to be hospitalized.

Among the reasons for the high frequency of both human and animal cysticercosis in developing countries is poor and inadequate hygiene, especially the disposal of human and animal faeces in rural areas. Meat for sale in some villages and small towns is not inspected or inadequately inspected. Also, effective regulatory measures have either not been introduced yet or are not widely enforced in many developing countries.

Scientists involved in improving the health status of populations are investigating alternatives that would interrupt the life cycle of the parasite. Studies are being undertaken to determine methods to destroy or inactivate metacestodes in live animals or in carcasses in order to prevent the development of the tapeworm in humans. Effective drugs for the treatment of neurocysticercosis in humans and cysticercosis in animals are available. However, the treatment of infected pigs in rural areas presents problems because peasants own small numbers of animals and it is difficult to detect the infection. A technical problem arises because between treatment and slaughter a holding time must be allowed for the drug to become effective and for lesions to disappear, and this would increase the cost of production.

Given the high endemic occurrence of these parasitoses, only large scale measures may be able to break the life cycle of *Taenia* sp. The measures implemented by governments, such as freezing, cooking, frying, salting, drying and mincing of parasitized meat, have been only moderately successful in the developing world.
2. EFFECTIVENESS OF IRRADIATION

Work has been done to establish the MED to evaluate the effect of irradiation on the infectivity of metacestodes of *T. solium* and *T. saginata*. It must be stressed that evagination in vitro is used as a criterion for viability but that it cannot be equated with infectivity and the ability of a parasite to maintain itself in a host.

Up to now there is no conclusive evidence that *T. saginata* and *T. solium* can mature in any host but man; for this reason, there are only few data on the infectivity of irradiated cysts in the human intestine. Preliminary observations have shown that *T. saginata* could develop for at least three weeks in gerbils treated with an immunosuppressant. However, in another study it appears that the infection rate of gerbils for *T. saginata* is very low.

In vitro trials with doses of 0.2 to 0.6 kGy did not show any difference in evagination and motility between irradiated and non-irradiated metacestodes of *T. saginata*. Based on experiments using three human volunteers, the MED was found to be 0.3 kGy. These findings should be considered preliminary and more work with animal models and human volunteers is recommended.

Infectivity trials were carried out in hamsters with metacestodes of *T. solium*. Preliminary data indicated that doses higher than 0.7 kGy were required to suppress infectivity. These data also indicated that doses of 6.5 kGy and above were sufficient to cause death of the metacestodes, judged by their inability to evaginate or grow in hamsters.

Considering that variations in the susceptibility of individual hamsters and also of the infectivity of metacestodes of different isolates seem to exist, more research to find an appropriate animal model and effect of irradiation on different isolates is recommended. It is also advisable to study the changes that occur in the DNA of irradiated metacestodes in order to recommend the MED to control the transmission of taeniasis/cysticercosis to humans.

**E. Strategies to implement food irradiation programmes in the control of food-borne parasitic infections**

Low dose irradiation to render food safe for consumption has proven to be a useful tool for the destruction of bacterial and parasitic pathogens and also for the preservation of food in general. Food-borne parasitic infections are highly prevalent among low income populations and those which consume raw food. Economics and the public health are important issues in every country. Therefore, governments have to take necessary measures to control the transmission of food-borne parasitic infections.

The following strategies were formulated to serve as guidelines for countries to implement for the control of food-borne parasitic infections.
Strategy 1. Provision of information for policy makers on:

1.1. The impact of food-borne parasitic infections in terms of economic loss to the population, including:

(a) Disease prevalence and the number of people at risk for each infection.
(b) The estimation of direct costs, such as those for chemotherapy and conventional control measures.
(c) The indirect expenses, such as hospitalization cost, work replacement cost, loss of export opportunities, loss of individual income, animal abortions.

This information may not be available or complete in all endemic countries at present. A study to obtain such information has to be conducted, analysed and presented to the policy makers.

1.2. The impact of food-borne parasitic infections on food exports, including:

(a) The quantity and value of food (meat, fish and seafood) exported.
(b) The estimated cost of rejection by importing countries of potentially infected food.
(c) Evidence that irradiation can safely solve the problem and how quality control can be enforced.

1.3. The magnitude of the health problem incurred from food-borne diseases, including:

(a) The morbidity and mortality rates among the populations at risk from the infections, especially in groups of the working population.
(b) Complications associated with disease, e.g. cholangiocarcinoma in clonorchiasis and opisthorchiasis, and nervous disorders associated with neurocysticercosis.
(c) The burden of care and costs associated with high infection rates on public health services.

1.4. The safety and effectiveness of food irradiation. The following information is important and should be distributed as early and regularly as possible if a food irradiation programme is to be implemented:

(a) Clear evidence of the benefits of food irradiation, particularly the lack of carcinogenicity and cell mutation over a long period of consumption. The information should be based on the results of tests among consumers in advanced and developing countries.
(b) A comparison between heat pasteurization, which is effective in controlling diseases in liquid food, e.g. milk, and irradiation, which is the only effective technology for controlling diseases in raw 'solid' food, e.g. chicken, meat, and
fish. Considering the widespread problem of salmonella contaminated poultry, the policy makers should be urged to use irradiation to control salmonellosis in poultry prior to marketing to protect consumer health. Implementing this practice would improve the prospects of introducing irradiation as a public health intervention measure, in general. In countries endemic for food-borne parasitic diseases, the policy makers should also adopt the use of irradiation for meat, fish, and other invertebrate food products.

(c) Clear evidence that very low doses of irradiation can inhibit the development of parasites.

(d) A clear explanation of the terms ‘irradiated food’, ‘radioactivity’ and ‘food contaminated by radionuclides’.

Strategy 2. Provision of information for scientific communities

The scientific community is another target group that should be given information on food irradiation. The information must be based on scientific and statistical documentation and should be presented at international, regional, and local meetings, and in the scientific publications.

The information for this group should be the same as that provided to the policy makers, but it should be more precise and based on scientific data. The emphasis should also be on the applicability of food irradiation as a supplementary measure to prevent and control food-borne infections, not a radical measure to eradicate the infections.

Strategy 3. Provision of information for the public

3.1. Increase public awareness of the disease problem

People, particularly in endemic areas, should be made aware of the disease through an effective health education programme. Efforts must be made to convince them that parasitic infections are a consequence of their own behaviour, and are chronic and may eventually cost them a large amount of money or even their lives. It is also necessary to instruct them in ways they can help themselves by improving their personal hygiene and changing eating habits. They should be informed that irradiation is the most effective technology available today to render raw food safe from parasitic diseases.

3.2. Provide information on the safety of the irradiated food

This is similar to Strategy 1, except that the contents and presentation are presented in a manner that is more easily understood. It is also important that sensory evaluation of irradiated food is acceptable to the population.
Information can usually be easily disseminated to the public through the mass media. It is necessary, therefore, to provide the mass media with information on the safety and benefits of food irradiation with respect to the prevention of disease.

**Strategy 4. Pilot project to demonstrate the effectiveness of irradiated food in prevention and control of food-borne parasitic diseases**

At present, there is sufficient scientific information to ascertain the effectiveness of irradiation in inhibiting the development of a number of food-borne parasites. However, the application of such technology to control the infections in communities has to be tested for its feasibility. This kind of research is not easy to conduct and can be expensive. It should be conducted in a rather clearly defined community with good epidemiological and behavioural data back-up. If the irradiated food cannot be tested independently of other factors, then the food should be used as a measure to supplement conventional methods of parasitic disease control such as chemotherapy, improvement of environmental sanitation and health education. It is also important that the economic cost and gain of this pilot project should be recorded.

This kind of information is valuable not only from the scientific point of view, but its cost effectiveness is also useful for health policy makers.
INHIBITION OF VIABILITY AND INFECTIVITY OF Cysticercus bovis BY IRRADIATION OF MEAT

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Abstract

INHIBITION OF VIABILITY AND INFECTIVITY OF Cysticercus bovis BY IRRADIATION OF MEAT.

Viability of Taenia saginata cysticerci was studied in vitro by exposing them to gamma radiation varying from 1 to 6 kGy. It was found that a minimum of 3.7 kGy is required to devitalize Cysticercus bovis cysts. However, for complete devitalization, 6 kGy of irradiation was needed. Inhibition of infectivity of C. bovis by irradiation of meat was also investigated in gerbils and human volunteers. It was demonstrated that gerbils are not suitable experimental hosts for T. saginata infections. In human volunteers, it was concluded that 0.3 kGy could be accepted as the minimal effective dose to inhibit the development of C. bovis larvae into adult tapeworms. However, these findings should be considered preliminary and be confirmed by further research. According to the data obtained from the Council of Meat and Fisheries of Turkey, the prevalence of bovine cysticercosis was 2.6%, varying from 1.6 to 3.8% between 1980 and 1990.

1. INTRODUCTION

Cysticercus bovis is widespread in most cattle rearing countries of the world. The highly endemic areas are Central and East African countries, Caucasian and south-central Asian republics, Near East countries, and certain regions of the former Yugoslavia. Moderate infection rates are reported for Europe, southeast Asia, and
South America, while the USA, Canada, and Australia report a low incidence of *Taenia saginata* [1].

It is reported that cysticercosis in cattle and taeniasis in humans, caused by *T. saginata*, are serious and frequent diseases in Turkey. Meat inspection data showed that the prevalence of bovine cysticercosis is about 13% (0.5–1% in Western, 15–20% in Central and 20–25% in Eastern Anatolia), and prevalence of *T. saginata* infections has been reported to be 14% (varying from 0.07 to 50%) [2, 3]. Owing to the fact that these figures were obtained from slaughter inspections, and since official reports are often lacking or unreliable, whilst almost 30% of infected beef carcasses are not detected at meat inspections [4], the prevalence of the diseases is seriously underestimated.

Because of the high endemic occurrence of this parasitosis, only large scale measures may be able to break the life cycle of *T. saginata*. Carcasses lightly infected with cysticercosis are usually frozen for a prescribed period before they are released for human consumption. The researchers investigated the possibility of destroying cysticerci of *T. saginata* by irradiation. Some workers used evagination in vitro as a criterion for assessing the effect of irradiation. Van Kooy and Robijns [5] found that a dose of 3 kGy would be required for complete inactivation of *C. bovis*. A combined treatment of 3 kGy and storage for 7 days at 2°C post irradiation is fatal to cysts in beef. Pawel [6] showed that irradiated cysticerci (4–5 kGy) were smaller than non-irradiated ones, that their anatomical features were less well marked, and their mobility less.

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. Unfortunately, *C. bovis* cannot mature in any host but man, therefore there are few data about the infectivity of irradiated cysts in the human intestine. Tolgay et al. [7] reported that a dose of 10 kGy was required to devitalize all the cysticerci in beef, and a relatively low dose of 0.4 kGy rendered *C. bovis* incapable of development and, presumably, non-infectious to man. Taylor and Parfitt [8] reported that a dose of 0.1 kGy sterilized the resulting *C. pisiformis* tapeworms in dogs and stunted their growth; after 0.3 kGy of irradiation, no worms were recovered at autopsy. They also concluded that dose levels for rendering *C. bovis* non-infectious would be similar. This viewpoint was also expressed by Verster et al. [4], who stated that carcasses lightly infected with cysticerci could be rendered fit for human consumption by exposure to low doses (0.2–0.6 kGy) of gamma irradiation. However, in a controlled experiment using human volunteers conducted in Belgium [9], cysticerci irradiated at 0.2 kGy still developed into adult *T. saginata*, whereas cysticerci which received a dose of 0.6 kGy did not.

The present study reports on the viability in vitro and infectivity to gerbils and human volunteers of irradiated cysticerci of *T. saginata*. The distribution and prevalence of cysticercosis in state slaughterhouses belonging to the Council of Meat and Fisheries of Turkey were also investigated.
2. MATERIALS AND METHODS

2.1. Viability of cysticerci

The infected meat samples were obtained from the Ankara State slaughterhouse, which is central, and slaughterers animals brought from all over the country. The encapsulated cysts were isolated either from the carcasses of animals which had low infection rates or were condemned. The samples were obtained from bovines 1-3 years of age. The infected meat pieces (meat discs, 2 cm) were brought to the laboratory the same day as collected and processed. They were bundled into small packages and irradiated by a $^{137}$Cs source of 10 000 Ci$^1$ (Mark I-22 Irradiator) with a dose rate of 1.81 kGy/h (the cylindrical sample container of the source was 20 cm in height and 8 cm in diameter) as determined by Fricke dosimetry.

The cysts were exposed to radiation doses ranging from 1 to 6 kGy. Different numbers of cysts were left as controls in each treatment, depending on the total cysts available on that day. The preparation and irradiation took place at ambient temperatures. After irradiation, the vitality of the irradiated and control pieces of meat was examined. For this purpose, the cyst was first stripped from its surrounding muscle tissue, then the connective tissue encapsulation was cut open, after which the freed larvae were put immediately into diluted cow bile at 37°C. The bile was diluted with physiological saline 1:1. Observations on the vitality were carried out after 17-24 hours. The vitality of cysts was determined by means of a stereomicroscope according to the following criteria: scolex evagination, active movement of scolex, presence of suckers, and active movement of suckers. The passively moving suckers and scolices were also recorded.

2.2. Infectivity of cysticerci

Preliminary observations of Kamiya [10] have shown that $T$. $saginata$ could develop in three weeks in gerbils ($Meriones$ $unguiculatus$) treated with immunosuppressants. Therefore, these animals were chosen as the experimental model.

In the first experiments, the gerbils were divided, by sex and weight, into six groups of ten animals each. All the cysticerci used in this study were obtained from the Ankara State slaughterhouse and their diameters were at least 6 mm (i.e. the cysticerci were at least 12 weeks old). Prior to the infection of the gerbils, the viability of each batch of cysticerci was tested in vitro. For this, about five cysticerci were placed in a 1% bile salt solution (bile salt No. 3 Difco; pH7.2-7.5) at 37°C. The cysticerci were examined after one hour.

$^1$ 1 Ci = $3.7 \times 10^{10}$ Bq.
For immunosuppression, 3 mg of Depomedrol (methyl prednisolone acetate) was administered to each gerbil subcutaneously on the day of infection and then at weekly intervals.

A total of 60 gerbils were force-fed three cysticerci of *T. saginata* irradiated with doses of 0, 0.2, 0.4, 0.5, 0.6, or 0.8 kGy. The animals were housed in pairs in cages and were treated with antibiotics (oxytetracycline, 15 mg/100 mL) and vitamins in their boiled drinking water. Their feed consisted of commercial rat chow supplemented with biscuits and sunflower seeds which had been sterilized by irradiation at a dose of 10 kGy. The gerbils were examined for cestodes 25 days after infection. After the animals had been killed with ether, the small intestine was removed and opened, and the mucose stripped in physiological saline.

In the second experiment, the infectivity of irradiated cysticerci of *T. saginata* for human volunteers was studied. The cysticerci were exposed to irradiation at 0.3, 0.4, 0.5, 0.6, or 0.7 kGy. Five human volunteers ingested two irradiated cysticerci each. All volunteers were treated with 2 g Yomesan® (niclosamide) 2.5 months later.

Meanwhile, a survey was undertaken to determine the prevalence of bovine cysticercosis based on the meat inspection data obtained from the State slaughterhouses belonging to the Council of Meat and Fisheries of Turkey.

3. RESULTS AND DISCUSSION

A total of 1015 cysts were used in the viability experiments. Of these, 683 were exposed to levels of gamma irradiation ranging from 1 to 6 kGy.

The results are summarized in Table I. They show that there is an inverse relationship between gamma radiation dose used and vitality of cysts. In this experiment, it was found that neither scolex evagination nor the presence of suction cups are reliable criteria for vitality. Even at high doses of radiation, there were large numbers of non-motile evaginated scolices and suckers. The Table shows that a minimum of 3.7 kGy irradiation dose is required to devitalize *C. bovis* cysts when active movements of scolices or suckers are considered as vitality criteria, although there were some very passively moving scolices at this or higher dose levels. However, 6 kGy of irradiation is needed for the complete devitalization of cysticerci. De Aluja et al. [11] reported that 7 kGy of irradiation is required to destroy the viability of *C. cellulosae* and this agrees with our findings.

In this study, it was found that relatively high doses (3.7 kGy or more) are required to inactivate cysticerci in meat. However, objectionable organoleptic changes were induced at these dose levels. Odour, colour and texture of meat irradiated at doses of 3.7 kGy or higher did not remain good. These findings are in accordance with those of Sudarmadji and Urbain [12]; they reported that the threshold dose for beef for an organoleptically detectable off-flavour is 2.5 kGy.
<table>
<thead>
<tr>
<th>Irradiation doses (kGy)</th>
<th>No. of cysts examined</th>
<th>Scolex evaginated (No.) (%)</th>
<th>Suction cups present (No.) (%)</th>
<th>Scolex moving A (%)</th>
<th>P (%)</th>
<th>Suction cups moving A (%)</th>
<th>P (%)</th>
<th>Non-irradiated controls (No.) (%)</th>
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<tr>
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<td>59</td>
<td>53 89.8</td>
<td>55 93.2</td>
<td>52 88.1</td>
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<td>47 79.6</td>
<td>—</td>
<td>29 100</td>
</tr>
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<td>50</td>
<td>33 66.0</td>
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<td>17.4</td>
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<td>1 1.5</td>
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<td>—</td>
<td>4 100</td>
</tr>
</tbody>
</table>

* Active movement.

* Very passive movement.

* All controls were vital according to all criteria used.
<table>
<thead>
<tr>
<th>Year</th>
<th>Total number of slaughtered animals</th>
<th>Carcasses frozen</th>
<th>Carcasses condemned</th>
<th>Prevalence (%)</th>
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<td>8 860</td>
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<td>186</td>
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</tr>
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<td>1990</td>
<td>364 325</td>
<td>5 757</td>
<td>15</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*a According to the data obtained from the Council of Meat and Fisheries of Turkey.

In the infectivity experiments, none of the gerbils developed infections after being fed either irradiated or non-irradiated cysticerci of *T. saginata*; no cestodes were recovered from these animals. An additional 12 gerbils were each fed five non-irradiated cysticerci and examined for cestodes 25 days after infection. In this trial, two gerbils had one tapeworm each (infection rate of 3.3%). The lengths of the tapeworms were 6.5 and 7 cm. Preliminary observations of Kamiya [10] have shown that *T. saginata* could develop for at least three weeks in gerbils treated with immunosuppressants. However, in this study, it was shown that the infection rate of gerbils for *T. saginata* is very low and it was concluded that gerbils were not suitable experimental hosts for *T. saginata* infections.

In the second trial, no tapeworms were recovered from the volunteers who infected themselves with cysticerci irradiated with 0.3, 0.4, 0.5, 0.6, or 0.7 kGy. Geerts [9] reported that cysticerci irradiated at 0.2 kGy still developed into *T. saginata*, whereas cysticerci irradiated at 0.6 kGy did not. According to our findings and Geerts', it can be concluded that 0.3 kGy can be accepted as a minimum effective dose that inhibits the development of *C. bovis* larvae into adult tapeworms. Tolgay et al. [7], Taylor and Parfitt [8], and Verster et al. [4] reported similar findings, except that the latter suggested the effectiveness of a lower irradiation dose of 0.2 kGy for light infections [4].
INHIBITION OF C. bovis VIABILITY

According to the meat inspection data obtained from the Council of Meat and Fisheries of Turkey, the prevalence of cysticercosis is 2.6%, varying from 1.6 to 3.8% between 1980 and 1990 (Table II). The figures obtained from slaughter inspections and official reports are often misleading, however, since the wide variability by provinces may be obscured by single overall or regional prevalence statistics and figures from the Council of Meat and Fisheries of Turkey, which covers 10% of the total slaughtered cattle in Turkey. These prevalence rates, however, are not as high as those reported by other authors [2, 3].

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REFERENCES


USE OF GAMMA IRRADIATION TO PREVENT INFECTIVITY OF METACESTODES OF *Taenia solium* IN PORK

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Abstract

USE OF GAMMA IRRADIATION TO PREVENT INFECTIVITY OF METACESTODES OF *Taenia solium* IN PORK.

The importance of taeniasis/cysticercosis in humans and of cysticercosis in pigs in Mexico is briefly described. With the objective of interrupting the life-cycle of *Taenia solium*, the effect of gamma irradiation of the metacestode was studied. Larvae were irradiated with doses of 0.5, 0.7 and 4 to 11 kGy. Doses of 6.5 kGy and higher killed all larvae, as judged by 100% failure to evaginate. When larvae irradiated with 7 kGy were fed to hamsters, no taeniae developed in their intestines. Doses of 0.5 and 0.7 kGy did not affect the capacity to evaginate, but inhibited infectivity. Twenty hamsters were each fed five larvae irradiated with 0.7 kGy, and 17 stunted taeniae were recovered ten days later, none after 30 days. Ten hamsters were each fed five larvae, each irradiated with 0.5 kGy; one worm was recovered ten days later, none after 30 days. Individual differences in susceptibility of hamsters were registered and also differences in infectivity of metacestodes of different pigs. The results are discussed and more research is recommended to establish the minimum effective dose of irradiation to prevent the growth of *T. solium* in humans.

1. INTRODUCTION

Taeniasis/cysticercosis, caused by *Taenia saginata* or *Taenia solium*, are still prevalent in Mexico, as well as in many other countries where poverty and lack of hygiene prevail. In the human patient, the metacestodes of *T. solium* have a predilection for the central nervous system, where they can cause serious neurological problems, as well as considerable suffering and expenses which the family can often not afford.

The prevalence of neurocysticercosis (*T. solium*) in humans in some regions of Mexico is as high as 24.4%, while in other areas figures of 7.1% and 12.0% have
been recorded [1]. Twenty-five per cent of patients in hospitals of neurology and neurosurgery are found to suffer from neurocysticercosis [2]. In autopsies of patients who died due to unspecified causes in general hospitals, a frequency of 2.4% to 3.6% has been reported [2]. In many of these patients cysticercosis may never have been suspected, as the disease often develops without specific symptoms.

Data obtained from slaughterhouses where records on the cause of condemnation of carcasses are kept, show that in certain states of the country the frequency of cysticercosis in pigs may be as high as 10%. Figures given by health authorities on swine cysticercosis are by no means reliable, as much of the meat consumed in rural areas does not undergo inspection [3].

Among the reasons for the high frequency of both human and porcine cysticercosis in Mexico and in other developing countries is the lack of hygiene in rural areas, particularly the absence of toilets of any kind. It is still a widespread habit to have pigs feed on human faeces and some of the few existing latrines are deliberately constructed for this purpose. In many villages one sees pigs roaming freely in fields and streets, looking for food; in fact, they are useful scavengers, keeping villages free of manure and garbage.

Another important reason is corruption among meat and animal dealers. Much of the meat on sale in smaller towns and most villages is either not properly inspected or sold without any kind of control.

Effective measures to control this situation have not been introduced by government authorities in Mexico and scientists involved in the efforts to eradicate this disease are studying alternatives that would interrupt the life-cycle of the parasite. One such possibility would be a vaccine to prevent pigs from becoming infected with eggs; another one would be to find ways to destroy the metacestode in the live animal or in its carcass in order to avoid the development of the tapeworm in the human being.

Several products are available for chemotherapy of human cysticercosis and excellent results have been obtained. Work has also been published on the efficiency of these in the pig [4]. However, treatment of infected animals presents problems in view of the fact that the disease prevails in rural areas where peasants own small numbers of pigs and the disease is difficult to detect. A technical problem would also arise in view of the fact that between treatment and slaughter a certain time must be allowed for the medicine to act and for the lesions caused by the deteriorating larvae to disappear. This would increase the cost of production.

The use of low dose irradiation has proved to be a useful tool in the preservation of food and also in the destruction of bacterial and parasitic pathogens [5]. With partial financial support of the IAEA, a study was undertaken to investigate the efficiency of irradiation on the metacestode of T. solium in pork.

The study is divided into two parts. The objective of the first part was to determine the dose that is necessary to kill the metacestodes. The second objective was to find the minimum effective dose to suppress infectivity.
2. MATERIAL AND METHODS

2.1. Part one

Twenty-two infected pigs were obtained, 19 from local slaughterhouses, and three that had been experimentally infected with *T. solium* eggs three months previously. The age of the slaughterhouse pigs was approximately seven to ten months. The experimentally infected animals were six months old and had been infected at three months. Both shoulders were removed from each carcass, the right one to be irradiated, the left one for control. The right shoulder was wrapped in a polyethylene bag, and placed in a carton for irradiation. A $^{60}$Co irradiator was used (Gammabeam 651 PT). The dosimetry was determined by a modified Fricke method as described by Trevino et al. [6]. The dose rate was 2.81 kGy/h at the beginning of the study and 2.38 kGy/h at the end, approximately one year later.

After irradiation with doses ranging from 4 to 11 kGy/g, larvae were carefully dissected from the irradiated meat. Sixty of them were then placed into a Petri dish with 16 mL of a solution containing 25% bovine bile dissolved in RPMI1640 1.4%, dextrose 0.4%, sodium bicarbonate 0.2%, and distilled water 98.36%. They were incubated at 37°C for 12 h. Larvae were also dissected from the non-irradiated (NI) shoulder and received the same treatment. After 12 h of incubation, the evaginated larvae were counted; only those that had a completely distended neck and where hooks and suckers were clearly visible and motile were considered fully evaginated. The dose that caused 100% suppression of evagination was considered to be the lethal dose.

After establishing the dose that suppressed evagination of the metacestodes, inoculation trials were carried out in twelve-week-old female golden hamsters (*Mesocricetus auratus*). Coproparasitoscopic tests proved negative in all animals. Two groups (A and B) of six animals each and two (C and D) of seven animals each were used. Each animal in groups A and B was inoculated orally with five irradiated cysticerci and each animal in groups C and D with five NI larvae. All animals were immunosuppressed with 4 mg of methylprednisolone acetate (Depomedrol) 24 hours before inoculation and once weekly thereafter. Groups A and C were euthanized ten days and groups B and D 22 days after inoculation. Necropsies were performed and the small intestine was removed. Intestinal contents were flushed out and examined in a tray on a black surface. Finally each intestine was opened lengthwise and carefully examined.

2.2. Part two

Seven infected pigs were obtained in rural areas; two had been experimentally infected with eggs of *T. solium* three months earlier. The age of the rural pigs could not be determined. The experimentally infected ones were six months old. The
TABLE I. EVAGINATION OF METACESTODES (Taenia solium) FROM 22 PIGS IRRADIATED WITH DIFFERENT DOSES

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Dose (kGy)</th>
<th>Evagination</th>
<th>Volume of each larva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NI^a/T^b</td>
<td>I^b/T^b</td>
</tr>
<tr>
<td>1</td>
<td>4.0</td>
<td>24/65</td>
<td>7/65</td>
</tr>
<tr>
<td>2</td>
<td>4.9</td>
<td>97/100</td>
<td>79/100</td>
</tr>
<tr>
<td>3</td>
<td>4.9</td>
<td>99/100</td>
<td>72/100</td>
</tr>
<tr>
<td>4</td>
<td>4.9</td>
<td>25/65</td>
<td>1/65</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>108/154</td>
<td>20/154</td>
</tr>
<tr>
<td>6</td>
<td>5.6</td>
<td>69/80</td>
<td>30/80</td>
</tr>
<tr>
<td>7</td>
<td>5.7</td>
<td>121/155</td>
<td>14/155</td>
</tr>
<tr>
<td>8</td>
<td>5.9</td>
<td>44/65</td>
<td>11/65</td>
</tr>
<tr>
<td>9</td>
<td>6.0</td>
<td>131/160</td>
<td>21/160</td>
</tr>
<tr>
<td>10</td>
<td>6.0</td>
<td>91/100</td>
<td>1/100</td>
</tr>
<tr>
<td>11</td>
<td>6.5</td>
<td>56/66</td>
<td>0/66</td>
</tr>
<tr>
<td>12</td>
<td>6.5</td>
<td>63/66</td>
<td>0/66</td>
</tr>
<tr>
<td>13</td>
<td>7.0</td>
<td>42/95</td>
<td>4/95</td>
</tr>
<tr>
<td>14</td>
<td>7.0</td>
<td>49/60</td>
<td>2/60</td>
</tr>
<tr>
<td>15</td>
<td>7.0</td>
<td>60/60</td>
<td>6/60</td>
</tr>
<tr>
<td>16</td>
<td>7.6</td>
<td>75/75</td>
<td>3/75</td>
</tr>
<tr>
<td>17</td>
<td>7.7</td>
<td>44/50</td>
<td>0/50</td>
</tr>
<tr>
<td>18</td>
<td>7.9</td>
<td>98/100</td>
<td>0/100</td>
</tr>
<tr>
<td>19</td>
<td>9.7</td>
<td>0/40</td>
<td>0/40</td>
</tr>
<tr>
<td>20</td>
<td>9.7</td>
<td>15/48</td>
<td>0/48</td>
</tr>
<tr>
<td>21</td>
<td>9.7</td>
<td>9/49</td>
<td>0/49</td>
</tr>
<tr>
<td>22</td>
<td>11.8</td>
<td>40/41</td>
<td>0/41</td>
</tr>
</tbody>
</table>

^a NI = Non-irradiated.
^b T = Total.
^c I = Irradiated.
^d Pin-sized.
methodology was the same as described for the first part. The irradiation doses were 0.5 and 0.7 kGy (Table I). Hamsters were immunosuppressed only once, one day before inoculation.

3. RESULTS

3.1. Part one

3.1.1. Evagination of metacestodes

The degree of evagination with the different doses is seen in Table I. One hundred per cent of the larvae irradiated with 6.5 kGy did not evaginate. In a few larvae of the three groups that received 7 kGy, the scolexes began to show in the entrance canal but the neck did not extend and no movements of hooks or suckers could be detected. Forty-four to 100% of the NI larvae evaginated.

3.1.2. Inoculation in hamsters

In view of the fact that the dose of 7 kGy is within the limits of what is considered safe for food irradiation [7] it was chosen for hamster inoculation. In the six

<table>
<thead>
<tr>
<th>Hamster No.</th>
<th>Dose (kGy)</th>
<th>Evagination</th>
<th>10 days</th>
<th>22 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>NI</td>
<td>I</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>0</td>
<td>100%</td>
<td>0/5</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>0</td>
<td>100%</td>
<td>0/5</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>0</td>
<td>100%</td>
<td>0/5</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>0</td>
<td>100%</td>
<td>0/5</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>0</td>
<td>100%</td>
<td>0/5</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>0</td>
<td>100%</td>
<td>0/5</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>0</td>
<td>100%</td>
<td>—</td>
</tr>
</tbody>
</table>

Total 19/35 (54%) 16/30 (53%)
TABLE III. LENGTH OF TAENIAE (cm) IN HAMSTERS INOCULATED WITH NON-IRRADIATED CYSTICERCi

<table>
<thead>
<tr>
<th>Hamster No.</th>
<th>10 days</th>
<th>22 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4, 0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>0.6, 0.6, 1.1, 1.2, 1.4</td>
<td>0.4, 0.5, 0.6, 0.7</td>
</tr>
<tr>
<td>3</td>
<td>0.2, 0.3, 1.0</td>
<td>None found</td>
</tr>
<tr>
<td>4</td>
<td>0.1, 0.4, 0.5</td>
<td>Died</td>
</tr>
<tr>
<td>5</td>
<td>0.2, 0.4</td>
<td>2.3, 7.3, 15.0</td>
</tr>
<tr>
<td>6</td>
<td>0.7</td>
<td>0.5, 0.6, 0.6, 0.6, 0.9</td>
</tr>
<tr>
<td>7</td>
<td>0.6, 1.1, 1.4</td>
<td>2.1, 6.0, 9.0</td>
</tr>
</tbody>
</table>

Animals that were inoculated with five irradiated metacestodes each, no taeniae were found. In the seven that were given NI larvae, a total of 19 taeniae were collected after ten days and 16 after 22 days (Table II). The number of taeniae and their size varied considerably in each individual (Table III). A panel of experts did not find any changes in organoleptic characteristics of the meat that was irradiated with 7 kGy.

3.2. Part two

Pig No. 7 died a few days before the work was started and the metacestodes found in pigs Nos 5 and 8 were too small to be considered useful for the experiment.

3.2.1. Evagination of metacestodes irradiated with 0.7 kGy

The number of irradiated and NI larvae that evaginated was similar in the four pigs (Table IV).

3.2.2. Evagination of metacestodes irradiated with 0.5 kGy

Seventy-four per cent of the irradiated and 82% of the NI larvae evaginated (Table IV).

3.2.3. Inoculation of hamsters with larvae irradiated with 0.7 kGy

No worms were found in five hamsters inoculated with irradiated larvae from pig No. 1. The NI larvae produced seven worms in five hamsters after ten days and
TABLE IV. TAENIAE IN HAMSTERS FED IRRADIATED (I) AND NON-IRRADIATED (NI) LARVAE
(each was fed five larvae)

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Evaginated 60 larvae</th>
<th>I</th>
<th>NI</th>
<th>10 days</th>
<th>Total</th>
<th>30 days</th>
<th>Total</th>
<th>10 days</th>
<th>Total</th>
<th>30 days</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7</td>
<td></td>
<td></td>
<td>1 2 3 4 5</td>
<td>0</td>
<td>1 2 3 4 5</td>
<td>0</td>
<td>1 2 3 4 5</td>
<td>0</td>
<td>1 2 3 4 5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.7</td>
<td>2 1 3 4 0</td>
<td>10^a</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.7</td>
<td>0 3 2 1 0</td>
<td>6^a</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.7</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.5</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
<td>0 0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>308/360</td>
<td>311/360</td>
<td>40/130</td>
<td>35/130</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Pin-sized.
four after 30 days. Irradiated larvae of pig No. 2 produced ten worms after ten days; none was found after 30 days. The worms found after ten days were very small, most of them pin sized, and upon microscopic observation only the armed rostellum with a few immature segments could be identified. The irradiated larvae of pig No. 3 produced six short taenia after ten days, and none at 30 days. In the hamsters inoculated with the NI larvae, 18 and five taeniae were found at ten and 30 days, respectively.

Pig No. 4 produced no taeniae in hamsters at ten and 30 days with the irradiated larvae, and 18 and five, respectively, with the NI ones.

3.2.4. Inoculation of hamsters with larvae irradiated with 0.3 kGy

No worms were found in hamsters inoculated with the irradiated larvae of pig No. 6 at ten and 30 days. In the animals inoculated with the NI larvae, three worms were found after ten days and three after 30 days. The irradiated larvae of pig No. 9 produced one pin sized worm after ten days and none after 30 days. In the hamsters inoculated with the NI larvae of that pig, one worm was found after ten days and 13 after 30 (Table IV). The number of taeniae and their size varied considerably in each individual animal, the longest one, developed from a larva of pig No. 9, measured 50 cm 30 days after inoculation.

4. DISCUSSION

Taeniasis/cysticercosis (T. solium) is one of the parasitic zoonoses that causes serious health problems in humans for which, at present, no perfect animal model exists. The golden hamster can be infected, but in our limited experience the parasite does not develop gravid segments and produces no fertile eggs in this host. Unless a method can be found to assess if the damage produced to the DNA of an irradiated parasite will suppress infectivity in humans, one would be reluctant to recommend a dose that still produces live worms in hamsters, however stunted they may appear. Their behaviour in their natural surroundings, the human intestine, may not be the same as in the laboratory animal, which under normal conditions does not harbour this parasite.

The hamsters used in this work all came from reliable breeders; they were of the same age, sex and breeding stock. In spite of this, differences in their susceptibility to parasitic infection are evident: of 25 animals that received five metacestodes from the same pig each, one harboured none, others one, and others up to five worms (Tables II and IV). It would, therefore, seem important to study the factors that determine these differences.

Variations in the infectivity of larvae from different pigs were also noticed. Larvae obtained from pig No. 6 produced six taeniae in ten hamsters, while those
from pig No. 3 produced 23 in the same number of animals (Table IV). The irradiated larvae from pig No. 3 produced six worms in one hamster after ten days; none were found after 30 days. In general, the number of worms developing from NI larvae decreased after 30 days, a finding that has been reported already by Verster [8] and Arias Naranjo [9].

Owing to difficulties in obtaining infected pigs in the vicinity of Mexico City, the dose of 0.5 kGy could only be repeated once and more trials have to be run in order to corroborate the results.

In conclusion, results obtained in this study show that doses of 7 kGy kill the metacestodes. Doses of 0.5 to 0.7 kGy produce changes which do not seem to interfere with the viability of the larvae but suppress their infectivity. Larvae irradiated with these doses produced a few worms that appeared stunted in hamsters euthanized ten days after infection. After 30 days no worms were found.

Variations in the susceptibility of individual hamsters to infection and, also, of the infectivity of metacestodes seem to exist. Therefore, more research is necessary to find an appropriate animal model, and in the field of molecular biology, before the optimal dose to prevent taeniasis/cysticercosis in human beings can be defined.

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REFERENCES


EFFECTS OF GAMMA IRRADIATION ON THE SURVIVAL AND DEVELOPMENT OF
Clonorchis sinensis METACERCARIAE

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Soon-Hyung LEE
Institute of Endemic Diseases
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College of Medicine,
Seoul National University,
Seoul, Republic of Korea

Abstract

EFFECTS OF GAMMA IRRADIATION ON THE SURVIVAL AND DEVELOPMENT OF
Clonorchis sinensis METACERCARIAE.

The effects of gamma irradiation on the survival and development of Clonorchis sinensis metacercariae were studied to evaluate the feasibility of irradiation as a control measure for clonorchiasis. Freshwater fish, Pseudorasbora parva, were collected from a river endemic for clonorchiasis and used for irradiation of the metacercariae in two schemes. In Scheme 1, irradiation of the isolated metacercariae from the fish was followed by infection of experimental rats. In Scheme 2, irradiation of the fish was followed by isolation of the metacercariae and infection of rats. Irradiation doses varied from 5 Gy to 200 Gy. The rats were sacrificed two to six weeks after infection. In Scheme 1, all the metacercariae irradiated at 50 Gy failed to survive in the rats after two or six weeks, while 1–44% of the metacercariae irradiated at 5–30 Gy survived. The estimated LD_{50} of Scheme 1 was 16.5 Gy. The flukes irradiated by Scheme 2 survived better than those in Scheme 1. Increasing the radiation dose up to 100 Gy, however, brought a remarkably low survival rate of worms (average 1%, 0–3% individually). Moreover, the recovery rate from the group irradiated at 150 Gy or 200 Gy was zero, which means complete control of the infectivity of C. sinensis metacercariae with over 150 Gy irradiation. The LD_{50} of Scheme 2 was calculated to be 47.5 Gy; nearly all of the recovered worms from the rats were morphologically normal except for a few retarded and underdeveloped worms. The present results suggest that irradiation of fish infected with C. sinensis by 150 Gy could be effective for the control of infectivity of C. sinensis metacercariae.

1. INTRODUCTION

The Chinese liver fluke, Clonorchis sinensis, has been one of the most important human parasitic helminths in the Republic of Korea. In recent years its prevalence in endemic areas has been gradually decreasing due to the strenuous efforts in health education and wide use of an effective drug, praziquantel [1]. However, it is
still prevalent enough to draw attention, from the public health point of view. Human infection is contracted by eating raw freshwater fish which harbour the metacercariae of *C. sinensis*. Therefore, *C. sinensis* could be controlled if all the metacercariae in the fish could be killed.

Ionizing radiation was found to have various effects on helminth parasites. Only a small proportion of *Schistosoma mansoni* cercariae that were gamma irradiated at 23 Gy grew to be adults, and the majority of the grown females were sterile [2]. The migration activity of schistosomulae was also weakened by irradiation [2]. Such effects of radiation were also reported in other parasites, such as *Toxoplasma gondii* [3, 4], *Opisthorchis viverrini* [5], *Trichinella spiralis* [6], *Paragonimus* [3], *Fasciola hepatica* [7], *Toxocara canis* [8], *Angiostrongylus cantonensis* [9, 10], metacestodes of *Taenia solium* [11] and *Hymenolepis microstoma* [12]. Most of the irradiated helminths were either significantly attenuated or killed, depending on the dose of irradiation. Thus, irradiation of the infective stage is being applied as a measure for making vaccines, for example, against schistosomiasis [13]. Another application could be to control infectivity of parasites by irradiation of foods before consumption. Control of trichinosis, cysticercosis, clonorchiasis, or other food-borne parasitoses is a promising subject for investigation.

The purpose of the present study is to observe the effects of gamma irradiation on the survival and development of *C. sinensis* and ultimately to evaluate the feasibility of irradiation as a control measure for clonorchiasis.

2. MATERIALS AND METHODS

2.1. Collection of *C. sinensis* metacercariae

Hundreds of *Pseudorasbora parva*, a species of freshwater fish of 5–8 cm in length, were collected from the Nakdong River near Pusan, where clonorchiasis is highly endemic. From the digested material of the fish, the metacercariae were isolated under stereomicroscopy. They were washed and stored in cold physiological saline until use.

2.2. Experimental schemes

2.2.1. Scheme 1: Irradiation of metacercariae of *C. sinensis*

One thousand metacercariae were placed into each Petri dish (8 cm in diameter) containing 20 mL saline. The dishes were put on the rounding plate of a MK 1-68 ¹³³Cs gamma irradiator and irradiated at the rate of 3 Gy/0.79 min from 40 cm distance. The radiation dose was adjusted from 5 to 50 Gy, changing the exposure time.
2.2.2. Scheme 2: Irradiation of fish, Pseudorasbora parva

Freshly caught fish were irradiated at doses of 10–200 Gy, half on one side and half on the other. After irradiation, the metacercariae were isolated from the digested material of the fish and used for the infection of rats.

2.3. Experimental infection of rats

Albino rats (Sprague-Dawley strain, from the Laboratory Animal Centre, Seoul National University) were orally given either irradiated or non-irradiated metacercariae through a gavage needle. Each rat was infected with 100 metacercariae.

2.4. Recovery of C. sinensis from the rats

The rats infected with irradiated metacercariae were sacrificed by spinal shock two or six weeks after infection. The livers were removed and minced. Worms were recovered by groups, fixed, stained with Semichon's acetocarmine, and observed under light microscopy.

3. RESULTS

3.1. Morphology of the irradiated metacercariae

The irradiated metacercariae showed no abnormality in morphology or activity. They were active in the cysts and after excystation.

3.2. Recovery of irradiated C. sinensis from the rats

3.2.1. Scheme 1: Irradiation of metacercariae

In the control rats infected with non-irradiated metacercariae, about half of the infected C. sinensis were recovered at two weeks after the infection (Table I). In contrast, no worm was recovered from the rats infected with the metacercariae which were irradiated at 50 Gy. The recovery rate of the worms, however, increased as the radiation dose was reduced, from 1% in the 30 Gy group to 44% in the 10 Gy group (Table I and Fig. 1).

The results were not very different when the rats were sacrificed at six weeks after the infection. As against a 63% recovery rate in the control group, no worm was found in the 50 Gy irradiation group (Table I). The rate, however, increased to
8% in the 30 Gy group and to 44% in the 10 Gy group. The LD$_{50}$ by this irradiation scheme was about 16.5 Gy (Fig. 2) and the minimum lethal dose by this scheme 50 Gy.

3.2.2. Scheme 2: Irradiation of fish

In general, more worms were recovered by this irradiation scheme (Table II and Fig. 1) than in Scheme 1. The recovery rates were 28–80% in the 10–50 Gy irradiation groups. Even in the 100 Gy group 1% of the worms were recovered. However, in the 150 Gy and 200 Gy groups, no worm was recovered. The LD$_{50}$ in this scheme was calculated to be 47.5 Gy (Fig. 2) and the minimum lethal dose was 150 Gy.

### TABLE I. RECOVERY OF *C. sinensis* FROM RATS INFECTED WITH IRRADIATED METACERCARIAE (SCHEME 1)

<table>
<thead>
<tr>
<th>Irradiation dose (Gy)</th>
<th>No. of rats$^a$</th>
<th>Worm recovery rate (%)</th>
<th>No. of rats$^a$</th>
<th>Worm recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 weeks</td>
<td></td>
<td>6 weeks</td>
</tr>
<tr>
<td></td>
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<td>Range</td>
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<td>11–52</td>
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<td>6</td>
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<td>5</td>
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<td>5</td>
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<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

$^a$ Each rat was infected with 100 metacercariae.
**FIG. 1.** Recovery rate (%) of *C. sinensis* irradiated on metacercariae or fish, two or six weeks after infection by gamma-ray dose.

**FIG. 2.** Survival rate (%) of *C. sinensis* on a logarithmic scale by gamma irradiation dose: irradiation on the metacercariae or on the fish, arrowheads denote the LD$_{50}$.
TABLE II. RECOVERY OF *C. sinensis* FROM RATS SIX WEEKS AFTER INFECTION WITH THE METACERCARIAE IRRADIATED IN THE FISH (SCHEME 2)

<table>
<thead>
<tr>
<th>Irradiation dose (Gy)</th>
<th>No. of rats</th>
<th>Total No. of metacercariae infected</th>
<th>Total No. of worms recovered</th>
<th>Worm recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
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<td>80</td>
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<td>321</td>
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</tr>
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<td>28</td>
</tr>
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<td>100</td>
<td>7</td>
<td>700</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>150</td>
<td>6</td>
<td>600</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>6</td>
<td>600</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

3.3. Morphology of *C. sinensis* after irradiation

The worms which were recovered at six weeks after infection were compared by groups. Both immature and mature worms were recovered from rats infected with either non-irradiated and irradiated metacercariae. However, between the groups little difference was found in the morphology of the worms. In all of the groups, most of the surviving worms were fully grown and matured.

4. DISCUSSION

The effects of ionizing radiation on parasitic helminths are growth retardation, vacuolization of the interstitium, elevation of the tegument, malformation or under-development of the reproductive organs, failure of reproduction, and/or weakened pathogenicity in the host [14, 15]. In addition, an inhibition of migration has been found to occur in helminths that need to migrate in their host for completion of their life cycle. *Schistosoma mansoni* [2], *Fasciola hepatica* [7], *Toxocara canis* [8] or *Paragonimus ohirai* [16] had higher death rates at the migration sites or a prolonged time of migration.
The most prominent effect of gamma irradiation on *C. sinensis* metacercariae, in both Scheme 1 and Scheme 2, was the reduction of their survival rate in the rat host. The survival rate of the irradiated metacercariae in Scheme 1 decreased as the dose of radiation was increased; no worms were recovered from the 50 Gy irradiation group. The number of surviving worms did not differ much according to the duration of infection, whether two weeks or six weeks. At present, however, it is uncertain as to when the irradiated metacercariae die. At the time immediately after the exposure to radiation, morphologically they looked completely normal, but they failed to survive in the rats during their early developmental stages, within two weeks of infection.

More worms survived when the infected fish were irradiated (Scheme 2). In other words, the metacercariae of *C. sinensis*, irradiated when they were encysted in the flesh of fish, were less susceptible to irradiation than those irradiated after isolation from the fish. An average of 73% (55–82%) of the infected worms were recovered from the rats in the 30 Gy group, and as many as 28% (7–39%) were recovered in the 50 Gy group of Scheme 2. With 100 Gy irradiation by Scheme 2, however, only 1% (0–3%) of the infected flukes were recovered. Moreover, further increase of the dose to 150 Gy or 220 Gy brought about complete control of the infectivity of *C. sinensis* metacercariae. Such a difference in the radiation susceptibility of *C. sinensis* metacercariae between the two schemes may be caused by the differences of the milieu around the metacercariae. It is well known that gamma rays penetrate deeply into animal tissue with little loss. Hence, there must have been no differences in the doses of radiation that arrived at the metacercariae in Schemes 1 and 2. In Scheme 1, the metacercariae were stored in 20 mL 40°C physiological saline after isolation from the fish and were irradiated in a small Petri dish, whereas, in Scheme 2, the infected fish were irradiated. Therefore, the only difference was in the surrounding media, such as saline or flesh of the dead fish. Radiation is known to destroy DNA structures directly or by forming hydroxy radicals in the tissue, so that there may be three times more cell destruction in the oxygenated state than in the anoxic one [17]. It is suggested that the flesh of the dead fish should have been anoxic; therefore, there was less destruction of the fluke cells, and more of the irradiated metacercariae in the flesh survived.

*Clonorchis sinensis* in this study appeared to be quite sensitive to radiation, compared with other helminths. The LD$_{50}$ of *C. sinensis* metacercariae by Scheme 1 was only 16.5 Gy and the minimum lethal dose was 50 Gy. *Paragonimus ohirai* metacercariae were also sensitive to irradiation and were dead in the liver of the host after exposure to 20 Gy [16]. Unlike *C. sinensis* or *P. ohirai*, 1% of the schistosoma adults were still recovered after irradiation at a higher dose, 80 Gy [18, 19], and migrating schistosomules became undetectable in the lungs only after irradiation at a considerably large dose, 200 Gy [2]. The larvae of *Toxocara canis* were also reported radioresistant to be able to survive even after receiving 1500 Gy irradiation [8]. A remarkable difference is seen in different species of parasites: for
example, trematodes are in general more sensitive to irradiation than nematodes or cestodes. However, the difference of radiosensitivity may also be seen in the same species at various stages of development, for which the frequency of cell division is different, and it is well known that the more the cell division, the greater its sensitivity to radiation.

It is interesting to note that some of the *C. sinensis* worms irradiated at their metacercarial stage revealed their underdeveloped body and retarded growth when recovered from the rat host; their sexual organs were especially poorly developed. It was also noted that in *Schistosoma mansoni* both spermatogenesis and oogenesis were suppressed by 23 Gy radiation [2]. It is generally known that males are more sensitive to radiation than females, as observed in worms with separate sexes, such as *Angiostrongylus cantonensis* [9], and in arthropods, such as *Amblyomma* sp. [20] and *Oermanyssus* sp. [21].

**ACKNOWLEDGEMENTS**

The authors would like to express their appreciation to Charn Il Park, Sung Whan Ha, and Il Han Kim, Department of Therapeutic Radiology, College of Medicine, Seoul National University for their support in irradiation techniques and assistance in the interpretation of results.

**REFERENCES**


EFFECTS OF GAMMA IRRADIATION ON THE SURVIVAL AND INFECTIVITY OF Anisakis LARVAE

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 Soon-Hyung LEE
 Institute of Endemic Diseases
 and Department of Parasitology,
 College of Medicine,
 Seoul National University,
 Seoul, Republic of Korea

Abstract

EFFECTS OF GAMMA IRRADIATION ON THE SURVIVAL AND INFECTIVITY OF Anisakis LARVAE.

The effects of gamma irradiation on the survival and infectivity of Anisakis larvae were studied to evaluate the feasibility of irradiation as a control measure for human anisakiasis. Sea eels, Anago anago, one of the fish intermediate hosts for Anisakis, were purchased from the Noryangjin fish market and were used for collection of Anisakis larvae. Only Anisakis type I larvae were isolated and irradiated at doses between 10 and 10 000 Gy. Rats and rabbits were used for infection with either non-irradiated or irradiated larvae. In the control group, the recovery rates of Anisakis larvae averaged 25% and 17% in the rats and the rabbits, respectively. In the rats, the recovery rates of the irradiated groups with doses less than 1000 Gy were 12.5-50% and no differences were observed between the control and irradiated groups. But in the irradiated group with doses of 2000 Gy or 10 000 Gy, the infectivity of the larvae seemed to be controlled, since no larvae were recovered from the rat host. In the rabbits, however, the recovery rate of larvae from the group irradiated with 2000 Gy was 16%, no different from the control group. The worm distribution was mainly on the serosal side of the gastric wall in the control group, whereas it was mainly on the gastric luminal side in the irradiated group. It seems likely that the penetrability of Anisakis larvae to the stomach wall of the rat and the rabbit is slightly weakened after irradiation. The present data show, however, that gamma irradiation of the Anisakis larvae with a very high dose of 2000 Gy did not satisfactorily control the infectivity of Anisakis larvae.

1. INTRODUCTION

The Anisakidae are gastrointestinal parasites of fish-eating birds and mammals. If the larvae are introduced into the human stomach, acute abdominal pain and diarrhoeal symptoms can occur [1]. There are four species of causative agents of human
anisakiasis: the larvae of *Anisakis* type I, *Anisakis* type II, *Pseudoterranova decipiens* and *Contracaecum* sp. [2, 3]. In the Republic of Korea, the raw flesh of *Anago anago*, the seal eel (conger), is the main source of human anisakiasis [4]. We studied the effects of gamma irradiation on the survival and infectivity of *Anisakis* larvae to examine the feasibility of fish irradiation as a control measure for human anisakiasis.

2. MATERIALS AND METHODS

2.1. Collection of *Anisakis* type I larvae

*Anago anago* were purchased from the Noryangjin fish market. Encysted nematode (anisakid) larvae were collected from the viscera and then the sheaths surrounding the parasite were removed in physiological saline under stereomicroscopy. Only the *Anisakis* type I larvae, 2.0-2.5 cm in length, actively moving, were isolated and used for the experiment.

2.2. Irradiation of *Anisakis* larvae

A total of 440 *Anisakis* type I larvae were dispensed, 40 into each Petri dish (8 cm in diameter) containing saline, according to the experimental schemes (Tables I and II). The dishes were put on a round plate in a MK 1-68 137Cs gamma

<table>
<thead>
<tr>
<th>Irradiation dose (Gy)</th>
<th>No. of rats</th>
<th>Total No. of larvae infected</th>
<th>Total No. of worms recovered</th>
<th>Worm recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
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<tr>
<td>10 000</td>
<td>1</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
TABLE II. RECOVERY OF *Anisakis* TYPE I LARVAE FROM RABBITS 14 HOURS AFTER INFECTION WITH IRRADIATED LARVAE

<table>
<thead>
<tr>
<th>Irradiation dose (Gy)</th>
<th>No. of rabbits</th>
<th>Total No. of larvae infected</th>
<th>Total No. of worms recovered</th>
<th>Worm recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
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<tr>
<td>2000</td>
<td>5</td>
<td>100</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

irradiator and irradiated at a rate of 3.47 Gy/min from 40 cm distance. The radiation dose was adjusted between 10 and 10 000 Gy by changing the exposure time.

2.3. Experimental infection of rats and rabbits

Albino rats (Sprague-Dawley strain, from the Laboratory Animal Centre, Seoul National University) were orally infected with either irradiated or non-irradiated larvae through a gavage needle. Each rat was given ten larvae. For comparison with the results from the rat experimental infection, ten rabbits were each infected with 20 irradiated or non-irradiated (2000 Gy) larvae through a Nelaton tube.

2.4. Recovery of *Anisakis* larvae

The rats infected with either irradiated or non-irradiated larvae were sacrificed by cervical dislocation 16 hours after the infection. The larvae were collected from the stomach, intestine, omentum and abdominal cavity. The rabbits were sacrificed under anaesthesia 14 hours after the infection and the larvae were recovered also from the stomach, intestine, omentum and abdominal cavity.

3. RESULTS

3.1. Motility of the irradiated larvae

The larvae which were exposed to gamma irradiation showed no abnormality in their morphology and motility. They showed active movements in physiological saline even after irradiation with 10 000 Gy, an amazingly high irradiation dose.
3.2. Recovery of irradiated *Anisakis* type I larvae

In the control rats infected with non-irradiated larvae, a quarter of the orally infected *Anisakis* were recovered from the omentum or abdominal cavity (Table I). The recovery rates from groups irradiated with 10–1000 Gy revealed no differences from the control group. Furthermore, the recovery rates of the 10 Gy and 200 Gy groups appeared even higher than the control group. The only favourable result obtained was that no worm was recovered from the groups irradiated with 2000 Gy and 10 000 Gy despite their active movements directly after irradiation. It was

<table>
<thead>
<tr>
<th>Irradiation dose (Gy)</th>
<th>Gastric lumen</th>
<th>Gastric wall</th>
<th>Duodenal lumen</th>
<th>Omentum</th>
<th>Abdominal cavity</th>
<th>Total No. of worms recovered</th>
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</thead>
<tbody>
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<td>10</td>
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<td>1</td>
<td>7</td>
<td>2</td>
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<td>5</td>
<td></td>
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<td>200</td>
<td>1</td>
<td>6</td>
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<tr>
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<td>4</td>
<td>2</td>
<td>3</td>
<td>10</td>
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</tr>
</tbody>
</table>

* Total No. of infected larvae is 40.

<table>
<thead>
<tr>
<th>Irradiation dose (Gy)</th>
<th>Gastric lumen</th>
<th>Gastric wall</th>
<th>Jejunal wall</th>
<th>Ileal wall</th>
<th>Abdominal cavity</th>
<th>Total No. of worms recovered</th>
</tr>
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<td>6</td>
<td>1</td>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Total No. of infected larvae is 100.
interesting, however, that the larval distribution in the rats of the control group was mainly at the omentum rather than in the stomach, while in the irradiated group, many larvae were located in the stomach and duodenum (Table III).

In the control rabbits infected with non-irradiated larvae, 17% of the infected *Anisakis* were recovered from various sites (Table II). Also, the recovery result was almost the same in the group irradiated with 2000 Gy, where 16% of the infected larvae were recovered. Thus there were no differences in the recovery rates between that group and the control. The main location of *Anisakis* larvae in the control rabbits was the gastric wall, especially the serosal side, which may imply that the activity and penetrability of the larvae was strong. On the other hand, in the irradiated group, the larvae were recovered mainly from the gastric lumen and sometimes the gastric wall of the luminal side (Table IV), which suggests some deterioration or loss of penetrability and invasion ability of the larvae. The morphology and motility of the worms recovered from the rats and rabbits were both normal.

4. DISCUSSION

The *Anisakis* larvae irradiated with a dose from 10 Gy to 1000 Gy were still infective to the rat host, just like the non-irradiated worms. This means that control of the infectivity of *Anisakis* larvae by irradiation is not a promising subject of investigation. However, it seems likely that the penetration force or infectivity of the irradiated larvae are somewhat weakened by irradiation, since their locality in the rats given them was mainly at the gastric lumen and wall rather than the abdominal cavity or omentum, as seen in the control group. Although the irradiated larvae were all active even in the 2000 Gy and 10 000 Gy groups the infectivity of these worms to the rats appeared to have been completely lost. This finding led us to do another experiment using a different host. However, disappointing results were observed when we used rabbits as the experimental host. The worm recovery rate from the rabbits given larvae irradiated with 2000 Gy was not different from that of the control group. The only notable finding was that, in the irradiated group, the larval locations were mainly on the luminal side of the gastric wall rather than the serosal side. This strongly suggests that the penetration ability and infectivity of the *Anisakis* larvae are only partially reduced even after high doses of radiation.

Our results showed that *Anisakis* type I larvae were more resistant to irradiation than the metacercariae of *Clonorchis sinensis*. We thought that the differences of basic metabolism and oxygen content between the two parasites might be responsible for the results. In the future, a study is needed on the effect of radiation on *Anisakis* larvae encysted in live fish and to determine the reasons for the greater resistance of *Anisakis* to irradiation compared to trematode metacercariae.
REFERENCES


SUSCEPTIBILITY OF *Taenia saginata* METACESTODES TO GAMMA IRRADIATION AND SHELF-LIFE EXTENSION OF THE TREATED MEAT

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Abstract

SUSCEPTIBILITY OF *Taenia saginata* METACESTODES TO GAMMA IRRADIATION AND SHELF-LIFE EXTENSION OF THE TREATED MEAT.

*Taenia saginata* metacestodes from experimentally infected calves were irradiated at levels varying from 0.2 to 0.6 kGy. Human volunteers who infected themselves with the irradiated cysticerci showed that a minimum irradiation dose level of 0.3 kGy is required to inhibit the development into adult tapeworms. Irradiation of infected carcasses by 0.5 kGy to 0.6 kGy seems to be effective. The evagination reflex and the in vitro maintenance did not show any difference in behaviour of irradiated and non-irradiated cysticerci. Even this relatively low irradiation dose produced a lower microbiological contamination in hygienically produced minced veal.

1. INTRODUCTION

The elimination of *Cysticercus bovis* in beef is traditionally achieved by freezing, cooking, cutting or passing through a meat mincer with a maximum mesh size of 1.5 mm, salting and drying.

To date, only a few studies have been carried out to evaluate the effect of irradiation on the infectivity of metacestodes.

The development of irradiated *C. pisiformis* and *cellulosae* was examined, respectively, in dogs and in hamsters [1, 2]. No data are available, however, on the fate of irradiated cysticerci of *Taenia saginata* or *T. solium* subsequently to arriving in man. Between June 1988 and March 1990 some observations were carried out on *T. saginata*. 
2. MATERIALS AND METHODS

2.1. Production of *T. saginata* cysticerci and irradiation

Three calves were experimentally infected with 50,000 to 60,000 *T. saginata* eggs and slaughtered at 10, 12 and 16 weeks, respectively, after infection. Living cysticerci were dissected out of the carcasses with some surrounding tissue, measuring about 0.5 cm\(^3\), and were packed in small plastic bags, each containing three to ten samples.

Gamma irradiation was carried out at the National Institute for Radio-elements (IRE) at Fleurus, Belgium. Within 24 hours after the dissection, the cysticerci were exposed to a \(^{60}\)Cu source (Gammacell 220) (radiation level = 5.18 kGy/h) at different doses: 0.2, 0.3, 0.4 and 0.6 kGy. The cysticerci were maintained at 4°C until subsequent experimental infection.

2.2. Experimental infection of human volunteers

Since there is no alternative definitive host for *T. saginata* [3], the study of infectivity of the irradiated cysticerci required human volunteers. The volunteers, who agreed to consume only well cooked meat during the course of the study to avoid any *T. saginata* infection from unknown origin, were selected after informed consent was obtained. About four hours after irradiation of the cysticerci, the volunteers infected themselves by swallowing three cysticerci (in some minced meat previously deep frozen). The main features are summarized in Table I.

All volunteers were treated with 2 g niclosamide (Yomeson\textsuperscript{®}, Bayer) shortly after they started eliminating the proglottids with the faeces or, if this was not the case, about four months after the infection.

2.3. In vitro evagination and cultivation of the cysticerci [4]

Given the difficulties in relation to the use of human volunteers, which some may call unethical, we tried to find an alternative using an in vitro system. Therefore, a large number of cysticerci were aseptically dissected and evaginated in 10% ox bile for one hour.

Afterwards, the cysticerci were washed with and transferred to RPMI 1640 medium, supplemented with 20% foetal calf serum and 0.1 mg/mL gentamicin. Metacestodes were incubated at 37°C in a 5% CO\(_2\) atmosphere at two larvae per mL medium.

Three times a week the medium was changed. The in vitro culture was maintained for two to three weeks. For each irradiation dose, a batch of five to ten cysticerci was used and their behaviour was compared with the same number of non-irradiated cysticerci maintained in vitro.
2.4. Microbiological evaluation of irradiated carcasses

Owing to the limited capacity of the irradiation unit, it was impossible to irradiate the whole carcass. Instead, randomly selected parts of the muscles of calf No. 2 were minced and divided into eight plastic bags containing 200 g meat each. The bags were brought to the irradiation unit and exposed to 0, 0.4, 0.6 and 2.0 kGy. They were stored at 2°C for one to three weeks and the number of microorganisms determined by successive dilution and inoculation on plate count agar, while organoleptic characteristics were estimated (Table II).

3. RESULTS

3.1. Infection of volunteers (Table I)

The volunteers in experiment 1, who ingested the non-irradiated cysticerci (A) and those irradiated at 0.2 kGy (B), eliminated their first proglottids on day 78 and day 100, respectively, after infection. Volunteer A first expelled a chain of immature proglottids on day 78, and 55 days later a large number of gravid proglottids. A first treatment with niclosamide appeared to be unsuccessful, since 30 days later proglottids reappeared in the faeces. Two months later a second treatment with niclosamide resulted in the expulsion of two tapeworms. A third treatment with praziquantel (10 mg/kg) did not reveal any other tapeworms. Following treatment of volunteer B only one tapeworm was evacuated with the faeces, whereas no proglottids were detected before or after treatment in the case of the volunteer infected with cysticerci irradiated at 0.6 kGy.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Age of cysticerci (weeks)</th>
<th>Number of positivea volunteers at irradiation doseb (kGy)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>1/1(A)</td>
<td>1/1(B)</td>
</tr>
<tr>
<td>2</td>
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<td>—</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>1/2</td>
<td>—</td>
</tr>
</tbody>
</table>

a Number positive/number infected positive: i.e. presence of adult tapeworm three to four months after infection.
In experiment 2, cysticerci of ten weeks of age appeared to be unable to develop into adult tapeworms in vivo.

The viability of the cysticerci in experiment 3 appeared to be normal because one of the two persons who ingested non-irradiated cysticerci observed an adult worm 86 days after ingestion. Irradiation with 0.3 kGy and more confirmed the presumption derived from experiment 1.

3.2. Evagination and culture in vitro

In experiment 1, none of the three batches of five to six cysticerci irradiated at 0.2 kGy and 0.6 kGy or non-irradiated evaginated. No difference was observed in the behaviour of the different batches of cysticerci during the culture in vitro. They were all slightly mobile.

In experiment 2, ten-week-old cysticerci were used. All the three batches, containing ten cysticerci each either irradiated at 0.4 kGy and 0.6 kGy or non-irradiated, showed 100% evagination. During the first week of the culture in vitro, the behaviour and motility of the different batches of cysticerci were very similar. On day 8, however, two scolices out of ten separated from the bladder in both the non-irradiated and the 0.4 kGy batches. On day 13, a third scolex became free from the bladder in the non-irradiated batch. Twenty days after the start of the culture in vitro, all cysticerci, including the free scolices, were still viable, except one in the non-irradiated batch and one in the 0.6 kGy batch. No free scolices were present in this latter batch.

Experiment 3 gave nearly the same results, except a lower evagination reflex and a lower separation tendency.

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Irradiation level (kGy)</th>
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<tbody>
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</tr>
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<td>21</td>
<td>$10^7$</td>
</tr>
</tbody>
</table>

TABLE II. MICROBIOLOGICAL EVOLUTION OF EIGHT SAMPLES OF IRRADIATED MINCED VEAL (STORAGE AT 2° TO 4°C) (total aerobic count/g)
3.3. Shelf-life extension (Table II)

The small number of samples and the low microbial count (100 bacteria/g) of the minced veal hinder firm conclusions. It appears, however, that irradiation at 0.4 kGy decreased the total bacterial count by a factor of 10, whereas a dose of 0.6 kGy resulted in a decrease by a factor of 1000 after three weeks of storage at 2°C.

After the same time span, the total bacterial count of meat which was non-irradiated or irradiated at 2 kGy, was 107 and less than 100 microorganisms per gram, respectively. Coliforms and staphylococci were absent in all samples. Minced meat, irradiated at 0.4 kGy or more, tended to become paler than non-irradiated meat.

4. COMMENTS AND CONCLUSIONS

4.1. Effect on the development of *Taenia saginata* in vivo

An irradiation dose of 0.2 kGy was not sufficient to inhibit the development of a *Cysticercus bovis* into an adult tapeworm. Even this dose level might have affected the cysticerci, resulting in a longer prepatent period; furthermore, only one of three cysticerci developed into a sexually mature tapeworm.

Irradiation of the isolated *C. bovis* at 0.3–0.4 kGy probably is sufficient to inhibit its development (see Table I). An irradiation dose of 0.6 kGy undoubtedly inhibits the development in the intestine of man under experimental conditions.

If a security factor of 1.6 can be applied, a dose between 0.5 kGy and 1.0 kGy should practically be safe to control infectivity of metacestodes in bovine carcasses.

Because cysticercosis (infection with several hundreds of larvae per host) is extremely rare in Belgium, it was not possible to confirm these data on naturally infected carcasses.

4.2. Extension of the shelf-life of irradiated meat

In general, the preservation capacity of healthy carcasses or vacuum packed pieces of beef at 2°C to 4°C will be about three to ten weeks. There is no need to extend beyond these periods.

Nevertheless, the observation of decreased bacterial growth by a factor of 10 to 1000 at an irradiation level of 0.4–0.6 kGy, is attractive for the butcher preparing minced meat. These doses do not clean up meat which is already contaminated, but may help to keep a clean lot at an acceptable contamination level for a longer time.

A disadvantage of irradiation by 0.4 kGy and three week storage of the unsalted or unspiced minced meat is the loss of the typical pink colour of fresh meat in comparison with the non-irradiated control.
4.3. Culture in vitro

The évagination and culture in vitro did not show any difference in behaviour between irradiated and non-irradiated batches. Even at a dose of 0.6 kGy, the cysticerci were able to evaginate and their motility during the culture in vitro was similar to that of non-irradiated cysticerci. This confirms the earlier observations [2] that there is no correlation between the évagination reflex of irradiated cysticerci and their infectivity.

4.4. Note

It has to be noted that none of the ten-week-old cysticerci in experiment 2, including the non-irradiated ones, developed into adult tapeworms after ingestion. This is in contradiction to the observations of McIntosh and Miller [5], who predicted (on the basis of morphological characteristics) that ten-week-old cysticerci of *T. saginata* should also be infective.

ACKNOWLEDGEMENTS

The authors thank the authorities at the International Atomic Energy Agency for their support and interest in these research activities. The authors also wish to thank the volunteers who kindly agreed to collaborate, J.P. Lacroix and P. Dardenne of the IRE, Fleurus, for the irradiation facilities, and the personnel of the Veterinary Department for their assistance.

REFERENCES

EFFECT OF LOW DOSE IRRADIATION ON Trichinella ISOLATES

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Abstract

EFFECT OF LOW DOSE IRRADIATION ON Trichinella ISOLATES.

Irradiation should be an effective and safe method for reducing the risk of human trichinellosis. With the existence of different Trichinella phenotypes, however, parasite strains with different gene pools may exhibit different radiosensitivity. Studies were performed using the rat as a laboratory animal for testing Trichinella spiralis isolated from different hosts from one geographic region. The results showed no unique radioresistance of the strains. Although the effective dose of irradiation (i.e. the dose required for total blocking of development of muscle larvae) for most isolates was 0.6 kGy, that dose did not affect the viability of Trichinella larvae of all strains. Two strains, tested at doses only up to 0.6 kGy, had their reproductive capacities reduced by more than 10 000-fold as a result of exposure to irradiation. On the other hand, strains of Trichinella isolated in a different region from different hosts and belonging to different taxa (T. spiralis, T. nativa, T. nelsoni, putative European T. nelsoni = T3) were similarly more radiosensitive when tested in mice. A dose of 0.2 kGy prevented the production of larval progeny of all but one strain. The results of the experiments performed with the same T. spiralis strain on two different laboratory hosts (rat and mouse) showed unequivocally that the different results of the former experiments were attributable to the laboratory host used. The laboratory rat was found to be more sensitive in a bioassay for monitoring Trichinella larvae viability. It is concluded that low dose irradiation, 0.3–0.6 kGy, of hog carcasses can provide a substantial margin of safety for human consumption of pork heavily infected with Trichinella. However, irradiation procedures should be complemented by health education, improvement in sanitary measures on farms raising pigs and improvement in diagnosing infections in animals. Isolation of the domestic transmission cycle from the wildlife transmission cycle is also important.
Meat plays a very important role in the transmission of parasites to man, and certain food habits increase the risk of infection. The transmission of *Trichinella spiralis* provides an excellent example of this.

The genus *Trichinella* is comprised of parasite populations transmitted over large areas, and has a wide range of hosts. The principal source of infection in humans is the pig; therefore, this should permit easy control of human trichinellosis. However, this is not the case. The wild (or sylvatic) source of infection for man (bear, wild boar, fox, walrus, camel) is also responsible for outbreaks. Despite the low infectivity of the sylvatic types for pigs and rats [1], the danger of transmission of sylvatic isolates to pigs is sufficient to require consideration in any control strategy [2].

Since irradiation of food is effective for many pathogens, it should be considered in designing effective control strategies for trichinellosis. The results of extensive experimental studies by Brake et al. [3] suggested a possible application of low dose irradiation for control of trichinellosis. However, the existence of different *Trichinella* genotypes indicates high genetic variation among the isolates of this parasite. Recently, different unrelated patterns of DNA were also found [4–7]. The isoenzymatic differences found by Pozio [8] and the multivariate analysis of both biological and biochemical features made recently by La Rossa et al. [9] support the existence of different gene pools of *Trichinella* populations. It may be presumed that strains with different gene pools could exhibit different radiosensitivities. Therefore, since 1987, we have performed studies, sponsored by the IAEA, on the effect of low dose irradiation on *Trichinella* isolates from different hosts and from different regions. Our work complemented earlier studies which showed a possibility of controlling trichinellosis by irradiation.

### 2. OBJECTIVE

The aim of the project was to perform studies on the radiosensitivity of various *Trichinella* isolates from different hosts and regions; the pathomorphological changes in intestinal wall, muscle, heart, liver, kidney and spleen caused by *Trichinella* infections developed from irradiated larvae; the antigenicity potential of worms developed from irradiated *Trichinella* larvae; and the radiosensitivity of the same *Trichinella* isolate in different laboratory animals (rat and mouse).
3. MATERIAL AND METHODS

3.1. Trichinella strains

3.1.1. Trichinella strains isolated from one region (Poland)

In phase 1 of investigations ten *T. spiralis* strains were used:

(a) Three strains (P/Kr, P-1/Po, P-2/Po) isolated from domestic pigs in 1960, 1987 and 1986, respectively; the P/Kr strain is the reference strain of *T. spiralis* 5.6 used by Istituto Superiore di Sanità, Rome, Italy.

(b) Two strains (H-1/Po and H-2/Po) isolated from patients with severe trichinellosis (Clinic for Parasitic and Tropical Diseases, Academy of Medicine, Poznań, Poland) in 1987 and 1988.

(c) Four strains (WB-1/Po, WB-2/Po, WB-3/Po, WB-4/Po), isolated from wild boars in 1987 and 1988.

(d) One strain (C-1/Po) isolated from a coypu in 1988.

In phase 3 of the study, performed with two different laboratory animals (rat and mouse), the same *T. spiralis* strain (H-2/Po) was used.

3.1.2. Trichinella strains of different taxa isolated from different hosts in different regions of the world

In phase 2 of the experiments, eight *Trichinella* isolates from different hosts in different regions of the world were used. The isolates were supplied by the *Trichinella* Reference Centre (TRC) of the Istituto Superiore di Sanità, Laboratorio de Parassitologia, in Rome, where they were preserved in liquid nitrogen:

(a) Two *T. spiralis* sensu stricto strains (MSUS/US/50/ISS4, MSUS/CH/80/ISS146) isolated from domestic pigs in 1950 in the United States of America and in 1980 in Chile.

(b) Two *T. nativa* strains (MURS/NO/84/ISS10, MCAN/SU/87/ISS70) isolated in 1984 from a polar bear in Norway and in 1987 from a wolf in the Union of Soviet Socialist Republics.

(c) Two European strains of putative *T. nelsoni* (MVUL/IT/82/ISS2, MVUL/SW/76/ISS55), designated as T-3, isolated from foxes in 1982 in Italy and in 1976 in Sweden.

3.2. The maintenance of the *Trichinella* isolates

The *T. spiralis* isolates were maintained by passage in Wistar rats (items (a)-(d) in Section 3.1.1.) or in Swiss mice (items (a)-(d) in Section 3.1.2.) at the Department of Biology and Medical Parasitology, Academy of Medicine, Poznań. Infective muscle larvae were freed from infected rats or mice by digestion of the whole carcass in a solution of 1% pepsin and 0.7% HCl for two hours at 37°C. The settled larvae were washed in warm saline and counted microscopically in a Petri dish. The inocula containing 7000 muscle larvae per rat or 700 muscle larvae per mouse in phosphate buffered saline (PBS) were administered intragastrically to the animals, which were lightly anaesthetized with ether.

The cryopreserved newborn larvae supplied by the TRC were passed a number of times in Swiss mice to increase their numbers.

3.3. Isoenzyme patterns testing

The gene pool of *Trichinella* isolates was determined by using starch gel electrophoresis, described elsewhere [8], for twenty-five gene enzyme systems: α-GPDH (1.1.1.8), LHD (1.1.1.27), MHD (1.1.1.37), ME (1.1.1.40), IDH (1.1.1.42), 6PGDH (1.1.1.44), G6PDH (1.1.1.49), G3PDH (1.2.1.12), GLDH (1.4.1.3), SOD (1.15.1.1), GOT (2.6.1.1), ALAT (2.6.1.2), CK (2.7.3.2), AK (2.7.4.3), PGM (2.7.5.1), EST (3.1.1.1), ACPH (3.1.3.2), PEP-A (3.4.11), PEP-B (3.4.11), ADA (3.5.4.4), ALDO (4.1.2.13), CA (4.2.1.1), FUM (4.2.1.2), GPI (5.3.1.8) and MPI (5.3.1.9).

3.4. Experimental animals

The outbred Wistar rats and Swiss mice, worm-free for several generations and of mixed sex, were reared at the Department of Biology and Medical Parasitology. The rats, weighing between 200 and 300 g at the beginning of the experiment, were housed three to a cage and the mice, weighing between 20 and 25 g at the beginning of the experiment, were housed ten to a cage. The animals were maintained by providing a 12 hours light/dark cycle, constant temperature and ad libitum access to standard pellet food and water.

3.5. Irradiation procedure of *Trichinella* infected muscle tissue and preparation of inocula

The animals infected with *Trichinella* were killed with ether at 40 days post infection (DPI), skinned and eviscerated. The carcasses were split into halves and minced in a meat grinder; one half was irradiated within two hours after killing of the animal, and the second half served as the control with unirradiated larvae.
Minced carcasses of infected rats or mice were irradiated in a $^{60}$Co facility at the Laboratory of Nuclear Methods in Agriculture, University of Agriculture, in Poznań. The laboratory type source RCHM-Gamma-20 contained nine elemental pencil sources in a circumferential arrangement. The samples were placed on a rack in the working chamber at the geometrical centre of the source. They were irradiated with doses from 0.1 kGy to 0.8 kGy, at a dose rate of 1.4 kGy/h. In order to measure the absorbed dose in samples, a Fricke dosimeter was used.

The samples were subjected to bioassay within two hours after irradiation by digestion in 1% pepsin and 0.7% HCl by continuous agitation with an electric stirrer for two hours at 37°C. After washing with warm saline, the concentration of the larvae was assessed microscopically by taking three 100 μL samples of the stirred suspension. The inocula were prepared by suspending the entire number of larvae in saline. Inocula sizes of 7000 larvae per rat and 700 larvae per mouse were administered intragastrically.

3.6. Bioassay for viability of *Trichinella* larvae

The viability of *Trichinella* larvae in inoculated rats was determined in phase 1 of the experiments on rats by controlling two viability endpoints according to Brake et al. [3]: (1) maturation of *Trichinella* in the rat intestine at 6 DPI and (2) production of progeny larvae in rat or mouse muscle tissue at 40 DPI.

In phases 2 and 3 of the experiments the viability of *Trichinella* larvae in inoculated animals was determined by controlling only one viability endpoint on day 40 post infection, namely the ‘reproductive capacity’.

Eight hours before the infected rats were killed, food was removed to reduce the amount of semi-digested intestine contents. The rats were killed with ether, bled by cardiac puncture and skinned. The sera were used for serological examinations. The entire small intestine was removed, slit longitudinally, placed on a plastic screen and immersed in saline bath for two hours at 37°C to recover adult worms. After removing the intestine pieces, the sedimented worms were washed with warm saline and quantified microscopically; the maturity and sex of the parasites were recorded. Part of the intestines was preserved for pathomorphological study.

Muscle larvae numbers were assessed in whole, skinned and eviscerated carcasses after digestion in pepsin/HCl solution as described above. The results were estimated in terms of the percentage recovery of adult worms and reproductive capacity according to Brake et al. [3]:

\[
\text{Adult worms recovery} = \frac{\text{number of adult worms at 6 DPI}}{\text{number of inoculated muscle larvae}} \times 100
\]

\[
\text{Reproductive capacity} = \frac{\text{number of muscle larvae at 40 DPI}}{\text{number of inoculated muscle larvae}}
\]
3.7. Histopathological techniques

The histopathological study was performed in phase 1 of the experiments on laboratory rats at the Department of Pathomorphology, Academy of Medicine, Poznań. For general morphology, muscle tissue, heart, liver, kidney, spleen and a ‘Swiss roll’ of the jejunum (i.e. a 10 cm portion of the proximal jejunum opened longitudinally and rolled up from the posterior end with the mucosa outward on a wooden stick) were fixed in buffered formalin, and embedded in paraffin. Sections (5 μm thick) were stained with hematoxylin and eosin.

3.8. Immunological assay

The immunological assay was performed only in phase 1 of the experiments with six *T. spiralis* strains (WB-2/Po, WB-3/Po, WB-4/Po, H-2/Po, C-1/Po, P-2/Po).

The blood samples of infected rats for antibody determination were collected by exsanguination 40 DPI. Rat sera stored at −20°C were tested at the Military Institute of Hygiene and Epidemiology, Warsaw. *Trichinella spiralis* excretory-secretory (ES) antigen was used in the enzyme linked immunosorbent assay (ELISA).

3.8.1. Preparation of *T. spiralis* ES antigen

Muscle larvae were freed from the infected rat muscles by digestion, and thoroughly washed with sterile PBS. They were incubated in a proportion of 1 mL pellet of the larvae in 15 mL of the medium containing PBS supplemented with 300 μg penicillin and 300 U streptomycin per mL. After 48 h at 37°C, the larvae were removed after centrifugation of the medium. The supernatant was filtrated through a 0.45 μm Millipore filter.

3.8.2. Enzyme linked immunosorbent assay (ELISA)

Each well of polystyrene plate (Plastomed, Warsaw) was coated with 200 μL of ES antigen in 0.05 M carbonate buffer pH9.6 (diluted 1 : 200) and incubated at 37°C for 18 h. The excess antigen was removed and the plates washed three times with 0.05% Tween-20 in PBS pH7.4 (PBS-T). Rat sera for testing were diluted in PBS-T-A (PBS-T containing 0.05% of bovine albumin), and 150 μL of the appropriate dilution was dispensed into each well. A fourfold dilution scheme from 1 : 10 to 1 : 40960 was used. After incubation at 37°C for two hours the plates were washed three times with PBS-T. Anti-rat gamma globulin rabbit antibodies (Laboratory of Serum and Vaccines, Warsaw) in dilutions of 1 : 200 were added and incubated for one hour at 37°C. After washing, horseradish peroxidase-labelled goat
anti-rabbit globulin (Heintel, Austria) in PBS-T-A was added and incubated for one hour at 37°C. After the next washing, 150 µL/mL of freshly prepared solution of o-phenylenediamine-HCl (0.34 mg/mL) and H₂O (0.01% in a 0.1M phosphate–citrate buffer, pH5.0) were applied to each well and incubated for 30 min at 37°C. The enzyme reaction was stopped by adding 75 µL of 4N H₂SO₄.

3.8.3. Estimation of antibody amount

The reaction product was measured by reading the absorbance at 490 nm in a Minireader II (Dynatech, Cambridge, MA). The amount of antibodies was estimated on the basis of a protocol described by Gripenberg and Gripenberg [10] and by using a computer program [11]. The resulting amounts of antibodies were expressed as the mean of the two measures. The upper limit of normal mean values for non-infected rats was 163. The amount of antibodies calculated as described above is correlated with an ELISA titre. The coefficient of variability (standard deviation: geometric mean × 100) for the ELISA used was 0.4%.

3.9. Statistical analysis

Cluster analysis for groups was used to examine the nearest distance between objects, i.e. animals infected with Trichinella irradiated with the dose of 0.1 kGy versus a control group of animals (infected with non-irradiated parasites). The comparison of isoenzyme patterns of the isolates was carried out with the Jaccard coefficient index.

4. EXPERIMENTAL DESIGN

Twenty experiments were performed by using 18 different Trichinella isolates (items (a)–(d) in Sections 3.1.1 and 3.1.2). In every experiment four different irradiation doses were tested: 0.1, 0.2, 0.4 and 0.6 (or 0.8) kGy. Each experiment was completed by a control infection with non-irradiated Trichinella larvae. Three rats or six to ten mice for each strain and each dose point, and control animals infected with non-irradiated larvae, were tested. Additional control experiments were performed by irradiation of the non-infected animal carcasses to compare the pathological changes found in muscle.
5. RESULTS

5.1. *Trichinella* genotypes

The *Trichinella* strains from one region (Poland), used in phase 1 experiments of the study, belonged to the same gene pool. The strains, isolated from different hosts (domestic pig, wild boar, human and coypu), were identified as *T. spiralis* sensu stricto (T1 according to the isolate type adopted by Pozio et al. [12]). The strains used in phase 2 of the study belonged to four different gene pools [9, 12]; two were identifiable with *T. spiralis* sensu stricto, two with *T. nativa* (T2 according to the isolate type adopted by Pozio et al. [12]), two with *T. nelsoni* (T7), whilst two strains were not identifiable with known taxa (putative European *T. nelsoni* = T3). The strains, isolated from different mammals (domestic pig, polar bear, wolf, red fox, spotted hyena, and wart-hog) and in different parts of the world (the Americas, Europe, Asia and Africa), satisfactorily represented genetic differentiation of the genus.

The isoenzymatic patterns differentiated the isolates into types [12] that corresponded with the taxa classified by Zarlenga and Murrell [7] by cloning of rDNA sequences (T1 = *T. s. spiralis*, T2 = *T. nativa* type 1, and T7 = *T. s. nelsoni*). We accepted the isoenzyme patterns and rDNA sequences for isolate classification and, therefore, refrained from using DNA probes in determining the genetic composition of the parasite’s strain (indication of the protocol in the Report of the 2nd Research Co-ordination Meeting, Bangkok, 1989; IV, paragraph 7, p. 39).

5.2. *Trichinella* bioassay

5.2.1. Bioassay performed with rats on *Trichinella spiralis* from one region

The *Trichinella* bioassay in phase 1 of the study was performed on ten strains of the parasite, isolated in one geographical area but from different hosts, and given four irradiation doses. Non-irradiated controls were also used. The criteria of larvae viability was determined by both the maturation of ingested larvae and the reproductive capacity. Nine strains were isolated in the mid-western part of the country from three different hosts (human, wild boar and coypu), and one strain was isolated from a domestic pig in the eastern part of the country, where trichinellosis is endemic.

The results (summarized in Table I) show that the encysted larvae of all *T. spiralis* isolates differed in their radiosensitivity. The differences were more distinct when the viability values by maturation and the lowest dose of irradiation (0.1 kGy) were compared. Most resistant was the human H-1/Po strain. Following inoculation of the larvae obtained from rat muscles irradiated with a dose of 0.1 kGy, the adult worm recovery of *T. spiralis* was 9.0.
### TABLE I. VIABILITY OF *T. spiralis* LARVAE IRRADIATED IN RAT MUSCLES

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<thead>
<tr>
<th>Radiation dose (kGy)</th>
<th>Adult worm recovery</th>
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<td>P-2/Po</td>
<td>H-2/Po</td>
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<td>WB-3/Po</td>
<td>WB-4/Po</td>
<td>C-1/Po</td>
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#### Reproductive capacity

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<sup>a</sup> 3 rats per data point.

<sup>b</sup> Not done.
Irradiation doses of 0.2 kGy and higher reduced the number of adults to single individuals, irrespective of the isolate used. Most resistance was found in the WB-4/Po strain isolated from wild boar. Following inoculation of the larvae irradiated with a dose of 0.4 kGy, the adult worm recovery was 0.07. The values for adult worms recovered at this irradiation dose from *T. spiralis* from domestic pig, human, wild boar, and from coypu were lower and varied from 0.0 to 0.04. We observed complete blocking of the maturation of all isolates only when doses of 0.6 or 0.8 kGy were applied. We found that the reproductive capacity, which is considered to be a more radiosensitive criterion for viability of *Trichinella* [3], did not correlate in all isolates with the adult worm recovery (e.g. in the case of P/Kr pig, WB-1/Po and WB-4/Po wild boar and H-2/Po human isolates). However, by using this criterion, the radiosensitivity of the isolates seemed to be similar. Although the effective dose of irradiation, i.e. a dose required for total absence of muscle larvae, for most of isolates studied was 0.6 or 0.8 kGy, the viability of the larvae of the two isolates, the WB-4/Po from a wild boar and H-2/Po from man, was not tested at 0.8 kGy. At 0.6 kGy, however, reproductive capacity of H-2/Po was reduced 14 000-fold and that of WB-1/Po was reduced 27 500-fold.

The effective dose of irradiation using the adult worm recovery criterion for P-1/Po and H-1/Po isolates was 0.8 kGy, whereas at doses of 0.4 and 0.2 kGy, respectively, we found a complete destruction of larvae viability when using the second criterion, reproductive capacity. We have to accept that, while adult worms of these last two isolates were recovered at these doses, the single mature survivors found in the gut must be infertile. We therefore came to the conclusion that adult worm recovery is not sensitive enough for the detection of infection.

The explanation of the development of muscle larvae in the absence of adult worms of P/Kr, WB-1/Po, WB-4/Po and H-2/Po isolates raises several questions. Either the larvae had been produced before the time of the first bioassay (adult worm recovery at 6 DPI) or the worms matured from irradiated larvae. They might not have left the intestinal wall in the saline bath used to monitor viability. The latter interpretation indicates that the adult worm recovery might not be sensitive enough to detect all mature worms.

The cluster analysis showed a relative difference of radiosensitivity among *T. spiralis* isolates investigated at the 0.1 kGy dose point (Table II). By controlling the maturation of the parasites in the rat intestine (adult worm recovery) we found three clusters (*p < 0.007*), while the production of progeny larvae in rat muscle (reproductive capacity) revealed five clusters (*p < 0.00001*). The results obtained by using the two viability criteria did not correspond with one another. If we accept the reproductive capacity as a more significant and more sensitive viability test (finding muscle larvae in digested whole rat carcass is more plausible and, on the other hand; the muscle larvae may be found in cases where adult worms are absent), then the cluster analysis showed the existence of more groups of *T. spiralis* which differ in their radiosensitivity. We did not find any correlation between this sensitivity and the species of host from which the parasites were isolated.
TABLE II. CLUSTER ANALYSIS FOR GROUPS
(Nearest distance between group of rats infected with T. spiralis larvae irradiated by a dose of 0.1 kGy and group of control rats injected with non-irradiated worms)

Using the 'adult worm recovery' criterion

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>df</th>
<th>F statistics</th>
<th>Sig. level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>0.04714</td>
<td>2</td>
<td>10.834</td>
<td>0.0072</td>
</tr>
<tr>
<td>Within groups</td>
<td>0.01523</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.06237</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cluster 1: P/Kr², P-1/Po
Cluster 2: H-1/Po, P-2/Po, WB-2/Po, WB-3/Po, WB-4/Po, C-1/Po
Cluster 3: WB-1/Po

Using the 'reproductive capacity' criterion

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>df</th>
<th>F statistics</th>
<th>Sig. level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>0.003 301 3</td>
<td>6</td>
<td>521.263</td>
<td>0.0001</td>
</tr>
<tr>
<td>Within groups</td>
<td>0.000 003 2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.003 304 5</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cluster 1: P/Kr, WB-1/Po
Cluster 2: P-1/Po
Cluster 3: H-1/Po
Cluster 4: P-2/Po, H-2/Po, WB-4/Po
Cluster 5: WB-2/Po, WB-3/Po, C-1/Po

* T. spiralis isolate denotation.

5.2.2. Bioassay performed on mice with strains of different taxa isolated from different hosts in different regions

The results of phase 2 of the study showed that the encysted larvae of all eight Trichinella strains were similar in their radiosensitivity (Table III). The lowest dose of irradiation (0.1 kGy) considerably reduced the number of larvae of four Trichinella strains (two strains of T. nativa, one strain of T. nelsoni, and one T3).
TABLE III. VIABILITY (REPRODUCTIVE CAPACITY) OF Trichinella spiralis LARVAE IRRADIATED IN MOUSE MUSCLES
(The results are presented as the mean number of larvae found in mice infected with 700 larvae/mouse)

<table>
<thead>
<tr>
<th>Radiation dose (kGy)</th>
<th>Trichinella isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ISS4\textsuperscript{a}</td>
</tr>
<tr>
<td>0 (control)</td>
<td>34.6\textsuperscript{b}</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>0.6</td>
<td>0.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The abbreviated code (last part) was quoted.
\textsuperscript{b} 7 to 10 mice per data point.

The muscle larvae of the remaining four strains (two of *T. spiralis*, sensu stricto, one of T3, and one of *T. nelsoni*) were completely destroyed by irradiation with 0.1 kGy. We observed a total absence of muscle larvae of all but one (*T. nelsoni*) strains of *Trichinella* when 0.2 kGy doses of irradiation were applied. We decided not to estimate adult worm recovery at 6 DPI as a viability endpoint determination and, therefore, it was not possible to prove whether or not the low dose irradiation inhibited the maturation of larvae or whether it blocked the fecundity of adult worms.

The radiosensitivity of *Trichinella* larvae was irrespective of the host and geographical source of parasite isolation. The least susceptible was the *Trichinella* T3 strain isolated from a fox in Sweden (MVUL/SW/76/ISS55) and both the *T. nelsoni* strains isolated from a spotted hyena and a wart-hog in Africa (Kenya and United Republic of Tanzania) (MCRO/KE/75/ISS29 and MPHA/TZ/82). Those differences were not statistically significant.

5.2.3. Comparison of radiosensitivity of the same isolate performed on two different laboratory animals (rat and mouse)

The results of phase 2 of the study performed with mice did not correspond with the results of the experiments performed with laboratory rats in phase 1. The strains of *Trichinella* used in the two phases of the study differed in their place of origin, gene pools, and natural hosts. The explanation of this non-conformity was difficult: either there were some actual differences in radiosensitivity of the parasite
TABLE IV. VIABILITY (REPRODUCTIVE CAPACITY) OF *T. spiralis* LARVAE: ISOLATE H-2/Po (MHOM/PO/88/ISS167) IRRADIATED IN RAT OR MOUSE MUSCLES
(The results are presented as the mean number of larvae found in rats infected with 7000 larvae/rat and in mice infected with 700 larvae/mouse)

<table>
<thead>
<tr>
<th>Radiation dose (kGy)</th>
<th>Reproductive capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Using rats</td>
</tr>
<tr>
<td>0 (control)</td>
<td>180.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>0.2</td>
<td>0.004</td>
</tr>
<tr>
<td>0.4</td>
<td>0.0034</td>
</tr>
<tr>
<td>0.6</td>
<td>0.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> 3 rats per data point.
<sup>b</sup> 7 to 10 mice per data point.

strains used in both experiments, or the difference depended on the laboratory host used (rat versus mouse). Observations by others [13, 14] support the concept that *Trichinella* strains may behave differently in different hosts.

The results of the experiments performed with the same *T. spiralis* strain (H-2/Po) on two different laboratory hosts (rat versus mouse) showed unequivocally that the different results of the former experiments depend on the laboratory host used (Table IV). Although the difficulties in explaining this nonconformity remained, the laboratory rat is considered a more sensitive animal to perform bioassay for monitoring *Trichinella* larvae viability.

5.3. Histopathological study

The differences in general pathomorphology caused by the *T. spiralis* isolates seem to be responsible for a clinical course of trichinellosis described earlier [15, 16]. Our histopathological observations on rats confirmed the different histological changes in jejunum, muscles, heart and kidney, caused by *T. spiralis* isolates.

The jejunum wall in rats infected with the human H-1/Po isolate appeared severely damaged. At 6 DPI, most villi were shortened and deformed and the lamina propria were infiltrated with numerous mononuclear lymphocyte-like cells, eosinophils and mucosal mast cells. These alterations were similar in rats inoculated
TABLE V. EFFECTIVE AMOUNT OF ANTIBODIES (E) FOR *T. spiralis* TESTED BY ELISA ON 40th DPI IN RATS INFECTED WITH *T. spiralis* LARVAE IRRADIATED WITH DIFFERENT DOSES (kGy) (Three rats for each irradiation dose point)

<table>
<thead>
<tr>
<th><em>T. spiralis</em> isolate</th>
<th>Irradiation dose (kGy)</th>
<th>Rat 1</th>
<th>Rat 2</th>
<th>Rat 3</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB-2/Po</td>
<td>0 (control)</td>
<td>360</td>
<td>2714</td>
<td>401</td>
<td>1158</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>127</td>
<td>161</td>
<td>176</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>193</td>
<td>225</td>
<td>225</td>
<td>214</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>247</td>
<td>287</td>
<td>283</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>126</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>WB-3/Po</td>
<td>0 (control)</td>
<td>991</td>
<td>507</td>
<td>293</td>
<td>597</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>164</td>
<td>186</td>
<td>190</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>908</td>
<td>180</td>
<td>283</td>
<td>457</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>270</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>219</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>WB-4/Po</td>
<td>0 (control)</td>
<td>1380</td>
<td>960</td>
<td>1180</td>
<td>1173</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>137</td>
<td>97</td>
<td>97</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>91</td>
<td>99</td>
<td>63</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>476</td>
<td>1120</td>
<td>1007</td>
<td>871</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>85</td>
<td>138</td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td>H-2/Po</td>
<td>0 (control)</td>
<td>1735</td>
<td>1011</td>
<td>2679</td>
<td>1808</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>98</td>
<td>150</td>
<td>122</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>88</td>
<td>88</td>
<td>130</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>528</td>
<td>53</td>
<td>50</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>200</td>
<td>126</td>
<td>201</td>
<td>176</td>
</tr>
<tr>
<td>C-1/Po</td>
<td>0 (control)</td>
<td>240</td>
<td>2365</td>
<td>ND</td>
<td>1302</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>138</td>
<td>180</td>
<td>61</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>57</td>
<td>82</td>
<td>142</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>210</td>
<td>100</td>
<td>100</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>107</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>
with muscle larvae irradiated with every dose, although worms were not found at a dosage of 0.8 kGy. At this dosage, even an increase in cellular infiltration was found. At 40 DPI the architecture of the villi was normal; however, numerous mucosal mast cells were observed. This was also true in rats inoculated with larvae irradiated with the highest dose. The human H-1/Po isolate caused the most intensive change in muscle cells of rats. At 6 DPI, intensive infiltration with mononuclear lymphocytes and early stage of basophilic transformation (disintegration of myofibrils, increasing affinity for basic dyes, nuclei enlargement and migration to the centre of cell, enlargement and strong staining with pyroninophilic dyes of nucleoli) were found. At 40 DPI no alteration of muscle tissue was observed.

The WB-1/Po isolate of T. spiralis from a wild boar caused similar well-marked changes in the jejunal wall in rats infected with non-irradiated and irradiated worms (shortened villi, desquamation of epithelial cells, infiltration with mononuclear lymphocyte-like cells and mucosal mast cells). The most apparent change was the increase of mucosal mast cells. These changes persisted in rats at each irradiation dosage. In animals inoculated with irradiated worms a slight morphological change of muscle tissue (signs of basophilic transformation and infiltration with mononuclear lymphocytes, plasmacytes, eosinophils and mast cells) was found at the 0.2 kGy dose point at 6 DPI and at 0.1 kGy at 40 DPI.

The two other wild boar T. spiralis isolates (WB-3/Po and WB-4/Po) as well as the strain isolated last year from a pig (P-2/Po) also caused changes in the jejunal wall, but of lesser intensity (focal or single cellular infiltrations and destruction of some villi architecture). These changes were observed at 6 DPI in rats infected with the larvae irradiated with the lowest dose (0.1 kGy). On the other hand, at 40 DPI, no changes were observed in muscles of rats infected with irradiated worms, even with the lowest dose.
The remaining isolates of *Trichinella* from pig (P/Kr, P-1/Po), wild boar (WB-2/Po), human (H-2/Po), and coypu (C-1/Po) produced fewer histological changes in the jejunal wall and in muscle tissue.

Striking changes were observed in rats in which no worms, adult or larval, were observed after inoculation with irradiated larvae; most apparent was a desquamation of jejunal epithelium and a great number of mucosal mast cells, observed also at 0.8 kGy dose after 40 DPI. We performed the investigation with additional controls (II) in rats fed with irradiated and non-infected rat meat, and did not find any changes in the jejunum and muscles.

Although morphological changes of other organs (heart and kidney) varied and were generally mild, more intensive alterations were found in the hearts (cellular infiltrations) of the control rats infected with the non-irradiated wild boar (WB/Po, WB-2/Po, WB-3/Po, WB-4/Po), from a pig (P-2/Po), and from human (H-2/Po) isolates. The same isolates caused changes in the kidney (cellular infiltrations, glomeruli lobulation, swelling of endothelial cells of glomeruli). Except for changes caused by a wild boar isolate WB-3/Po, there were no substantial alterations in heart and kidney of rats inoculated with irradiated specimens, either at 6 or 40 DPI.

In general, the histopathology of rats infected with the *T. spiralis* isolates did not always correlate with observations on worm survival to irradiation. Most intensive and overall changes were caused by a human radiosensitive *T. spiralis* and by the most radioresistant isolate from wild boar.

5.4. Immunological study

The ELISA carried out with sera collected from rats at 40 DPI was highly positive, but different reactions were observed in all control groups of rats infected with all six *Trichinella* strains in phase 2 of the study (Table V). The highest level of antibodies was observed in rats infected with the recently isolated pig strain (P-2/Po). Wassom et al. [17] concluded that the differences observed by using different *Trichinella* isolates are due to differences in the expression of functionally relevant antigens by different isolates used. In this study, inoculation of irradiated worms resulted in the absence of an immune response of the rats, or a weak one. Only in a few cases was the level of antibodies elevated over the normal limit of values. However, more pronounced reactions were observed at each irradiation dose point in rats infected with a wild boar isolate (WB-3/Po) of *Trichinella*.

6. CONCLUSIONS

Studies performed in rats showed no difference in radiosensitivities of strains of *T. spiralis* isolated from different hosts in one geographic region. The assay for monitoring worm viability showed that the low irradiation dose of 0.1 kGy reduced
the number of adult *T. spiralis* to single individuals. However, for most *T. spiralis* isolates, irrespective of their hosts, higher doses of irradiation, over 0.4 kGy, were required for total destruction of encysted muscle larvae.

Studies with mice showed the existence of a unique radioresistance of *Trichinella* isolates from different hosts in different geographic regions and belonging to different taxa (*T. spiralis*, *T. nativa*, *T. nelsoni*, putative European *T. nelsoni* = T3). The assay for monitoring worm viability showed that the lowest irradiation dose of 0.1 kGy reduced the number of progeny muscle larvae to single individuals or totally destroyed their potential of maturation and/or fecundity. In all cases of strains studied in mice, irrespective of their hosts, doses of irradiation of 0.2 kGy caused total destruction of encysted muscle larvae.

To explain this host difference, experiments were performed with the same *T. spiralis* strain on two different laboratory hosts (rat versus mouse). The results showed unequivocally that the results of the former experiments depended on the laboratory host used. The laboratory rat was a more sensitive animal for monitoring *Trichinella* larvae viability.

The assay for monitoring worm viability showed that the lowest irradiation dose of 0.1 kGy reduced the number of progeny muscle larvae to single individuals or totally destroyed their potential of maturation and/or fecundity. In all cases of strains studied in mice, irrespective of their hosts, doses of irradiation of 0.2 kGy caused total destruction of encysted muscle larvae.

To explain this host difference, experiments were performed with the same *T. spiralis* strain on two different laboratory hosts (rat versus mouse). The results showed unequivocally that the results of the former experiments depended on the laboratory host used. The laboratory rat was a more sensitive animal for monitoring *Trichinella* larvae viability.

The histopathology of the jejunal wall of rats showed that the most virulent were the *T. spiralis* H-1/Po human isolate and the wild boar WB-1 radioresistant isolate. The most apparent histological change was the increased number of mast cells (mucosal and connective tissue) in rats inoculated with irradiated larvae. In animals inoculated with irradiated worms a slight morphological change of muscle tissue at 6 DPI was found. However, at 40 DPI, no changes in muscles were observed. The morphological changes in other organs of rats were variable and generally mild.

The development of muscle larvae in the absence of mature worms indicates that the adult worm recovery is not sufficiently sensitive as a *Trichinella* viability criterion.

The histopathology of the jejunal wall of rats showed that the most virulent were the *T. spiralis* H-1/Po human isolate and the wild boar WB-1 radioresistant isolate. The most apparent histological change was the increased number of mast cells (mucosal and connective tissue) in rats inoculated with irradiated larvae. In animals inoculated with irradiated worms a slight morphological change of muscle tissue at 6 DPI was found. However, at 40 DPI, no changes in muscles were observed. The morphological changes in other organs of rats were variable and generally mild.

The results of the immunological study showed that irradiation of *T. spiralis* larvae of most strains isolated from different hosts decreased their immunogenicity at each irradiation dose point; an exception was the more radiosensitive wild boar isolate, which induced an immune response in rats manifested by a relatively high level of antibodies.

Irradiation should be an effective and safe procedure to prevent the exposure of humans to trichinellosis. Low dose irradiation, 0.3 to 0.6 kGy, of hog carcasses can provide a substantial margin of safety for human consumption of heavily infected pork. It should be emphasized, however, that we did not assess the radiosensitivity of these strains in pork. However, the procedure should be complemented by health education, improvement in sanitary conditions on farms and in the diagnosis of infections in animals. The eradication of sylvatic foci of infections or at least the isolation of the domestic transmission cycle from the wildlife transmission cycle should also be considered. Irradiation procedures should be accepted by the consumer. Unfortunately, most people associate irradiation with the noxious effects of nuclear energy. A programme of public education should be a substantial part of the control measures.
REFERENCES


EPIDEMIOLOGY AND CONTROL OF TRICHINELLOSIS AND TOXOPLASMOSIS

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Abstract

EPIDEMIOLOGY AND CONTROL OF TRICHINELLOSIS AND TOXOPLASMOSIS.

Investigations of the effectiveness of $^{137}$Cs irradiation to inactivate Trichinella spiralis and Toxoplasma gondii in pork were carried out. The results demonstrate that gamma irradiation is highly effective. Muscle larvae of T. spiralis were rendered completely incapable of further development in the exposed test animal at $^{137}$Cs doses less than 0.2 kGy. Toxoplasma gondii infectivity was destroyed at 0.5 kGy. Coincident with these studies, a national seroprevalence study for toxoplasmosis in swine was carried out. Serum samples from 11 842 commercially slaughtered swine were tested using an agglutination test. Anti-T. gondii antibodies were found in 24% of the samples. The prevalence was higher in breeder pigs (42%) than in market pigs (23%). These results show that swine toxoplasmosis is widespread in the US national swine herd. National surveys for swine trichinosis were also carried out. The results indicate that, while the national prevalence is low (0.1%), prevalence in some regions is high (0.5 to 1.0%). A serological test for swine trichinellosis was developed using a purified excretory–secretory antigen. The test, highly specific and sensitive, is now being commercially produced. A vaccine for swine was also developed and is under further development in Yugoslavia.

1. CONTROL OF PORCINE TRICHINELLOSIS AND TOXOPLASMOSIS BY IRRADIATION

In collaboration with the Los Alamos National Laboratory, a series of studies were carried out to assess the feasibility of using $^{137}$Cs to treat pork infected with Trichinella spiralis and Toxoplasma gondii [1, 2]. These extensive studies are summarized below:

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Gamma irradiation of *T. spiralis*-infected pork with a dose of 0.15 to 0.3 kGy blocks maturation of ingested larvae in the host gut and prevents production of larval progeny. Experiments with freshly slaughtered (prerigor) hog carcasses indicate that larvae distributed throughout the skeletal muscles have similar radiosensitivities. Neither the age of the encysted muscle larvae nor vacuum packaging of the meat significantly affected this radiosensitivity. Post-irradiation holding of irradiated meat leads to little, if any, recovery of *Trichinella* viability. The data indicate that 0.3 kGy gamma radiation can be delivered to split market-weight hog carcasses with acceptable uniformity, and that under all conditions tested such a dose can provide a substantial margin of safety for human consumption of heavily infected meat.

Muscles from tongue, heart and limbs of 14 pigs inoculated orally with *T. gondii* oocysts were irradiated with 0.1, 0.2, 0.25 and 0.3 kGy of gamma (*¹³⁷Cs and *⁶⁰Co*) irradiation. Viability of *T. gondii* cysts was assayed by feeding porcine muscles to *T. gondii*-free cats and/or by inoculation of sediment from acid-pepsin digested porcine muscle into mice. Cats fed 500 g samples of muscles irradiated with up to 0.2 kGy shed *T. gondii* oocysts. Cats fed muscles irradiated with 0.25 or 0.3 kGy did not shed oocysts. Mice were inoculated with eight isolates of *T. gondii*, and tissue cysts in their brains irradiated with up to 0.4 kGy were infective to mice; however, there was a 10 000-fold reduction in the viability of organisms in tissue cysts irradiated at this dose, compared with that in non-irradiated cysts. At 0.5 kGy of gamma irradiation, there were no detectable infective organisms in infected mouse brains.

2. EPIDEMIOLOGICAL INVESTIGATIONS ON TRICHINELLOSIS AND TOXOPLASMOSIS

National prevalence surveys for swine toxoplasmosis and trichinellosis, and on-farm epidemiological investigations of transmission, were carried out. These studies are summarized below:

2.1. Trichinellosis

Digests of diaphragms from 33 482 hogs slaughtered in the mid-Atlantic states were examined for the presence of *T. spiralis* larvae. The samples were obtained from seven slaughterhouses, ranging in slaughter capacity from less than 50 per day to more than 4000 per day. The sources of the hogs varied from 'backyard' operations (raising hogs for home use) to commercial farms. The means by which hogs were brought to the slaughterhouse also differed: the larger slaughterhouses often purchased directly from the producer, while the smaller slaughterhouses (1000 hogs per day or fewer) usually purchased through dealers or brokers. Infected hogs were detected more frequently than was expected from previously published prevalence
TRICHINELLOSIS AND TOXOPLASMOsis

studies; overall, 0.58% of samples examined contained *T. spiralis* larvae. All the infected hogs were marketed through the smaller slaughterhouses (fewer than 1000 per day) and nearly all were marketed through brokers. The mean number of larvae per gram of diaphragm, determined by slaughterhouse type, ranged from 0.5 to 74.6; most infections were light, although five had counts of 1000–2480. Most of the positive samples were obtained from one slaughterhouse, the data from which exhibited marked differences in the frequency of infection by day of the week; 128 positive samples of the total of 190 found were obtained on Fridays, although only 10% of all samples were obtained on that day. Analysis of the geographic origin of shipments containing infected hogs revealed that most originated in New Jersey and Pennsylvania, although the hogs shipped from the latter state might have originated in New England or Maryland. Attempts to trace back infected hogs for further epidemiological investigation were largely futile; owing to the absence of an identification system [3].

Diaphragms of 5315 slaughter hogs from the New England states were examined for *T. spiralis* infection between 22 June and 22 December 1983. Thirty-nine hogs (0.73%) were infected. The infections were relatively light, none exceeding 30 larvae/g of tissue, with an arithmetic mean of 4.7 larvae/g. Infected hogs originated from five of the six New England states. The widespread occurrence of porcine trichinosis in New England indicates that the small or part-time hog farming operations frequently found in this region may be especially vulnerable to the introduction and maintenance of the parasite [4].

A hog found infected with *T. spiralis* at slaughter was traced to its farm of origin, where an epidemiological investigation found the infection prevalent in swine and rats. Garbage feeding was not responsible for maintaining the high prevalence of infection on this farm, although it may have been responsible historically for the introduction of the infection. Poor husbandry, malnutrition, and intercurrent disease resulted in frequent death and the availability of porcine carcasses for cannibalism. Tissue samples from partly devoured carcasses contained *T. spiralis* larvae, implicating cannibalism as a major vehicle for the spread of *T. spiralis* in the herd. Rats also fed on these carcasses, and their rate of infection increased markedly during the first five months of observation. Experimental investigation indicated that rats could also be important in maintaining a high prevalence of infection in swine. For the purpose of investigation, the farm was depopulated of swine and restocked with parasite-free, sentinel pigs confined in three groups exposed to increasing degrees of contact with rats. Pigs exposed to large numbers of rats acquired infections rapidly, whereas pigs with strongly limited rat exposure failed to acquire infection during a twelve month period. These results indicate that when rats are available to swine and the prevalence of *T. spiralis* infection in the former is high, predation (or scavenging) on rats may be as effective as cannibalism in maintaining a high prevalence of porcine *T. spiralis* infection [5].
Other epidemiological investigations established that the parasite also occurred in rats, skunks, opossums and raccoons associated with the farm. Because considerable uncertainty exists regarding the role of sylvatic trichinellosis as a reservoir for the synanthropic cycle, studies were conducted to determine the genetic nature of the various isolates from this agro-ecosystem. Pig infectivity trials, isoenzyme analyses, and repetitive DNA sequence analyses were performed. The results showed that all isolates from the farm environs were genetically similar and that they are related to *T. spiralis* isolated from domestic pigs. The implication of these findings, in contrast to studies on isolates from wildlife captured elsewhere, is that this parasite is transmitted from domestic swine to sylvatic hosts and that any control or eradication efforts must take into account the potential for reinfection of hogs from wild animals [6].

As part of this epidemiological study on the transmission of *T. spiralis* in an agricultural ecosystem, resident wild and feral animals were trapped to determine the extent of the involvement in the natural, on-farm cycling of the parasite among swine. During a 21-month study, seven of 15 skunks (*Mephitis mephitis*), one of the three opossums (*Didelphis virginiana*), two of two feral domestic cats and a raccoon (*Procyon lotor*) were found to be infected, while five shrews (*Blarina brevicauda*) and 18 deer mice (*Peromyscus* spp.) were uninfected. Most of the former hosts probably became infected by scavenging dead infected swine or rats (*Rattus norvegicus*). However, infections obtained through predation of living rats, particularly with regard to the cats, cannot be excluded. Our observations do not suggest that there was transmission of *T. spiralis* from the wild animals to swine. Therefore, transmission of *T. spiralis*, in this instance, appeared to occur only from the farm's swine and rats to the associated wild feral animals [7].

In another herd of approximately 1000 hogs, evaluation of muscle specimens collected at various intervals during a twelve year period (1973 to 1985) indicated continuous transmission of *T. spiralis*. The farm's rat population and the incidence of trichinosis in the rats was high during 1974, but diminished markedly by 1978. In January 1984, a longitudinal investigation, using tracer pigs, was performed to determine whether rodents and/or other wild animals were involved in transmission of *T. spiralis* on this farm. Tracer pigs exposed to rodents and wild animals did not become infected with *T. spiralis*. The rodent population on the farm was small and none of the rodents trapped and examined were found to be infected. Hog cannibalism also was evaluated as a mode of *T. spiralis* transmission. The results indicated that hog cannibalism was the primary mode of transmission for trichinellosis in the herd [8].

An enzyme immunoassay (EIA) has been developed for identification of antibodies to *T. spiralis* in hogs and adopted for testing in a modern slaughterhouse. The test utilizes a specific excretory–secretory antigen from muscle larvae and is applicable to whole blood samples from hogs obtained at the slaughterline. Using a robotic sample handler and conventional laboratory equipment, one testing system
provides up to 400 analyses per hour. The run-to-run precision of a positive sample 
(N = 1052, x = 65% EIA) tested over four months showed a 6% coefficient of vari­
ation. Comparison of a EIA analyses for over 20 000 market hogs indicated excellent 
sensitivity in detecting animals with greater than 0.2 larvae/g [9].

2.2. Toxoplasmosis

Serum samples from 11 842 commercial pigs killed in 1983–1984 throughout 
the USA were tested for anti-T. gondii antibodies by the agglutination test in dilutions 
of 1 : 25, 1 : 50 and 1 : 500. Anti-T. gondii antibodies were found in 23.9% of pigs. 
At dilutions of 1 : 25, 1 : 50 and 1 : 500, 13.5%, 6.9% and 3.5% were serologically 
positive, respectively. The prevalence of anti-T. gondii antibodies was higher in 
breeder pigs (42%) than in market pigs (23%). These results indicate that anti-
T. gondii antibodies are widespread in the national swine herd because the rates were 
consistent by region [10].

A farm in Illinois had swine with enzootic T. gondii infections. Ninety-five of 
99 pigs had antibody against T. gondii by the modified agglutination test; modified 
agglutination test titres were < 1 : 10 (4 pigs), 1 : 10 (15 pigs), 1 : 20 (12 pigs), 
1 : 40 (10 pigs), 1 : 80 (20 pigs), 1 : 160 (11 pigs), 1 : 320 (12 pigs), 1 : 640 (9 pigs) 
and > 1 : 1280 (6 pigs). To trace the route of infection, Toxoplasma-free pigs were 
introduced into the farm and were evaluated serologically at various intervals. 
Analysis of data derived from these tracer pigs indicated that cannibalism was a 

3. VACCINATION STUDIES

Attempts are being made to develop vaccines for both swine trichinellosis and 
toxoplasmosis. The potential of crude T. spiralis newborn larval antigens for pig 
immunization was investigated. A preparation of whole newborn larvae killed by 
freezing and thawing, and combined with Freund's complete adjuvant, induced a 
high level of protection against challenge (78%), compared to a 40% resistance level 
in pigs immunized with excretory-secretory antigens of muscle larvae. Sera from 
pigs immunized with newborn larvae contained antibodies which bound to the sur­
face of the newborn larvae, as determined by immunofluorescence. In a second trial, 
the freeze thawed newborn larvae preparation was compared with a soluble and 
insoluble fraction prepared by sonication of whole newborn larvae. Pigs receiving 
whole newborn larvae or the insoluble fraction developed strong immunity to 
challenge (88.2% and 85.5%, respectively); the soluble fraction was ineffective. 
Immunization with all preparations induced antibody to newborn larval antigens, but 
not to adult or muscle larvae excretory-secretory antigens. Polyacrylamide gel elec­
trophoresis of the soluble and insoluble fractions indicated that sonication was
ineffective in solubilizing the larger molecular weight components. These results demonstrate that newborn larval antigens are highly protective in pigs, but that their further development as a vaccine will require more efficient procedures for antigen solubilization and large scale production [12]. A field trial of this vaccine is in preparation with several institutes in Yugoslavia.

The RH strain of *T. gondii* is highly virulent; one infective organism is uniformly lethal to mice. Three pigs inoculated subcutaneously with 103 tachyzoites of the RH strain developed fever but otherwise remained normal, and *T. gondii* was not demonstrable in pig tissues by bioassays into mice. To determine if vaccination with the RH strain can induce protective immunity to a lethal oral challenge with *T. gondii* oocysts, 12 pigs were divided into three groups (A, B, C) of four pigs each. Pigs in groups A and B were inoculated intramuscularly with $10^6$ tachyzoites of the RH strain and four pigs in group C served as uninoculated controls. Except for fever, the pigs remained clinically normal after inoculation with the RH strain and *T. gondii* was not found (by bioassays in mice) in tissues of four pigs killed on post-inoculation day (PID). Pigs in groups B and C were challenge-inoculated orally with $10^4$ (four pigs) or $10^5$ (four pigs) *T. gondii* oocysts. The previously uninoculated pigs developed fever, anorexia, and diarrhoea from oocyst challenge from PID 3 to 8. One of the two pigs given $10^5$ oocysts became moribund because of toxoplasmosis and was killed on PID 9. Pigs vaccinated with the RH strain remained free of clinical signs after challenge with oocysts. Judging from the results of bioassay, fewer tissue cysts developed in the RH strain vaccinated pigs than in the previously uninoculated pigs [13]. Further studies on this live vaccine approach are in progress. Further investigations of purified antigens for vaccination are planned.

REFERENCES


RADIATION SENSITIVITY OF Angiostrongylus cantonensis AND A. costaricensis INFECTIVE LARVAE

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Abstract

RADIATION SENSITIVITY OF Angiostrongylus cantonensis AND A. costaricensis INFECTIVE LARVAE.

Infective larvae (third stage larvae) of both Angiostrongylus cantonensis and A. costaricensis from snail intermediate hosts were subjected to either X ray or gamma ray irradiation. The viability of the irradiated larvae was assayed by oral inoculation of the larvae into rodents. A. cantonensis larvae viability was assayed in mice and rats, while A. costaricensis was assayed in mice only. From the results of worm recovery, the minimum dose that inhibits the infectivity of the third stage larvae of A. cantonensis and A. costaricensis appears to be 2 and 4 kGy, respectively.

1. INTRODUCTION

Angiostrongylus cantonensis is a causative agent of human eosinophilic meningoencephalitis in Asia and the South Pacific area [1]. Infection occurs through the ingestion of raw or improperly cooked snails or slugs which harbour the infective third stage larvae. Since the worm migrates to the brain, the infection can be fatal. A. cantonensis is an important food-borne parasite.

Another zoonotic angiostrongylid disease is caused by A. costaricensis, which induces abdominal granuloma and clinical symptoms which resemble those of appendicitis, especially in children in Latin American countries [2].

Since there is no satisfactory treatment available for either parasitosis, prophylaxis is the logical strategy in the control of transmission. Irradiation of molluscs, especially of commercially available food snails, such as Pila spp. or Achatina fulica, provides a method of killing the infective larvae or inhibiting infectivity to consumers.

The aim of the present investigation is to determine the radiation sensitivity of the infective larvae of these two angiostrongylid species so as to contribute to the potential use of irradiation in the control of these food-borne parasites.
2. MATERIALS AND METHODS

2.1. Parasites

The Hawaiian strain of *A. cantonensis* was maintained in our laboratory by passage between *Biomphalaria glabrata* as the intermediate host and the Wistar rat as the final definitive host. Infective third stage larvae were obtained from the laboratory reared snails which had been exposed six weeks prior to infection with the first stage larvae from the faeces of the definitive host. Infective third stage larvae of *A. costaricensis* were obtained in the same way except that ICR mice were used as the definitive host.

2.2. Irradiation protocol

2.2.1. X ray irradiation

Infected snails were placed between two glass Petri dishes, squashed, and their shells washed off in a breaker of saline. The snails were then macerated into small pieces and vigorous pipetting was done to release the larvae from the snail. The larvae were then suspended in 3 mL of saline contained in a 30 mL glass vial. The vial was then irradiated using an X ray machine operating at 170 kV and 250 mA, without any filter. The dose rate was determined by Fricke dosimetry.

2.2.2. Gamma ray irradiation

Infective third stage larvae in the snail were irradiated in situ with gamma rays from a $^{60}$Co source mounted on a Toshiba 1007 machine. After irradiation, the larvae were released from the flesh of the snail by vigorous pipetting followed by oral inoculation of larvae into the rodents.

2.3. Viability studies

2.3.1. X ray irradiated larvae

The X ray irradiated infective third stage larvae were orally inoculated into two to three-month-old male rats for *A. cantonensis* and into two-month-old male mice for *A. costaricensis*. The rats were sacrificed one week post infection (PI) and their brains and spinal cords examined for the presence of *A. cantonensis* worms under a dissection microscope. The mice were sacrificed on day 6 PI and their intestines and mesenteries examined for the presence of *A. costaricensis*. 
2.3.2. **Gamma ray irradiated larvae**

Gamma ray irradiated infective larvae of *A. cantonensis* were orally inoculated into eleven-month-old female mice which were sacrificed one week PI. *A. costaricensis* mice were similarly inoculated and sacrificed on day 15 PI.

3. **RESULTS**

3.1. **Preliminary experiments**

In a preliminary experiment, worm recovery from the brains of rats inoculated with 100 X ray irradiated infective larvae of *A. cantonensis* at radiation doses of 0.3, 0.2, 0.1, 0.05 or 0 kGy was 10, 13, 27, 33 and 46, respectively. Similarly, worm recovery from mice inoculated with 100 X ray irradiated *A. costaricensis* infective larvae at radiation doses of 2.0, 1.0, 0.5 or 0 kGy was 3, 20, 31 and 70, respectively. These results showed that infective larvae of *A. cantonensis* are resistant to a radiation dose of 0.3 kGy and those of *A. costaricensis* to a dose of 2 kGy.

3.2. **Minimal effective radiation dose**

The minimal effective radiation dose of X rays (Table I) and gamma rays (Table II) for the inhibition of the infectivity of *A. cantonensis* is 2 kGy. Worms which were irradiated with 1.5 kGy showed retarded growth as compared to the non-irradiated control.

### TABLE I. WORM RECOVERY ON DAY 7 POST INFECTION FROM RATS INOCULATED WITH 100 X RAY IRRADIATED *Angiostrongylus cantonensis* INFECTIVE LARVAE

<table>
<thead>
<tr>
<th>Irradiation dose (kGy)</th>
<th>No. of positive rats</th>
<th>No. of worms recovered from each individual rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of inoculated rats</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3 / 3</td>
<td>62, 54, 13</td>
</tr>
<tr>
<td>0.5</td>
<td>2 / 3</td>
<td>3, 1, 0</td>
</tr>
<tr>
<td>1.0</td>
<td>1 / 3</td>
<td>1, 0, 0</td>
</tr>
<tr>
<td>2.0</td>
<td>0 / 3</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td>3.0</td>
<td>0 / 3</td>
<td>0, 0, 0</td>
</tr>
</tbody>
</table>
TABLE II. WORM RECOVERY ON DAY 7 POST INFECTION FROM MICE INOCULATED WITH 100 GAMMA RAY IRRADIATED Angiostrongylus cantonensis INFECTIVE LARVAE

<table>
<thead>
<tr>
<th>Irradiation dose (kGy)</th>
<th>No. of positive mice</th>
<th>No. of worms recovered from each individual mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of inoculated mice</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3 / 3</td>
<td>20, 12, 7</td>
</tr>
<tr>
<td>0.5</td>
<td>3 / 3</td>
<td>4, 3, 2</td>
</tr>
<tr>
<td>1.0</td>
<td>3 / 3</td>
<td>4, 2, 1</td>
</tr>
<tr>
<td>1.5</td>
<td>2 / 3</td>
<td>1, 1, 0</td>
</tr>
<tr>
<td>2.0</td>
<td>0 / 3</td>
<td>0, 0, 0</td>
</tr>
</tbody>
</table>

TABLE III. WORM RECOVERY ON DAY 6 POST INFECTION FROM MICE INOCULATED WITH 100 X RAY IRRADIATED Angiostrongylus costaricensis INFECTIVE LARVAE

<table>
<thead>
<tr>
<th>X ray dose (kGy)</th>
<th>No. of positive mice</th>
<th>No. of worms recovered from each individual mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of inoculated mice</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3 / 3</td>
<td>48, 40, 36</td>
</tr>
<tr>
<td>1.0</td>
<td>3 / 3</td>
<td>16, 7, 3</td>
</tr>
<tr>
<td>2.0</td>
<td>3 / 3</td>
<td>8, 7, 3</td>
</tr>
<tr>
<td>3.0</td>
<td>3 / 3</td>
<td>9, 8, 2</td>
</tr>
<tr>
<td>4.0</td>
<td>0 / 3</td>
<td>0, 0, 0</td>
</tr>
</tbody>
</table>

The minimal effective radiation dose of X rays (Table III) for the control of the infectivity of A. costaricensis is probably 4 kGy. Recovery of A. costaricensis from the various organs of inoculated mice is shown in Table IV. In the gamma ray experiment, as shown in Table V, no worm was recovered even at a radiation dose of 1.5 kGy because the mice were sacrificed at 15 days PI. Probably the irradiated larvae were not able to survive for more than two weeks in the mice and might have been eliminated.
**TABLE IV. WORM RECOVERY FROM THE VARIOUS ORGANS OF MICE INOCULATED WITH 100 X RAY IRRADIATED Angiostrongylus costaricensis INFECTIVE LARVAE**

<table>
<thead>
<tr>
<th>X ray dose (kGy)</th>
<th>Mesentery</th>
<th>Small intestine</th>
<th>Caeco-colon</th>
<th>Total worms per mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>0</td>
<td>45</td>
<td>48</td>
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<tr>
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<td>39</td>
<td>40</td>
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<td>0</td>
<td>35</td>
<td>36</td>
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<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>13</td>
<td>16</td>
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<td>0</td>
<td>2</td>
<td>5</td>
<td>7</td>
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<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
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<td>3</td>
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<td>3</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**TABLE V. WORM RECOVERY ON DAY 15 POST-INFECTION FROM MICE INOCULATED WITH 50 GAMMA RAY IRRADIATED Angiostrongylus costaricensis INFECTIVE LARVAE**

<table>
<thead>
<tr>
<th>Gamma ray dose (kGy)</th>
<th>No. of positive mice</th>
<th>No. of worms recovered from each individual mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3 / 3</td>
<td>16, 12, 11</td>
</tr>
<tr>
<td>1.5</td>
<td>0 / 3</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td>3.0</td>
<td>0 / 3</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td>3.5</td>
<td>0 / 3</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td>4.0</td>
<td>0 / 3</td>
<td>0, 0, 0</td>
</tr>
</tbody>
</table>
Irradiation of *A. cantonensis* third stage larvae with the aim of establishing an attenuated vaccine [3] and studying the development and fecundity of the adults developing from the irradiated larvae [4] has been reported. The present investigation differed from these studies by emphasizing irradiation doses which were sufficient to prevent the migration of the larvae to the central nervous system in the mammalian definitive host. Clinical symptoms and severe pathologic lesions in humans occur only when infective larvae are able to migrate to the brain.

The present study shows that a higher radiation dose of 4 kGy is required for the control of the infectivity of *A. costaricensis* than for *A. cantonensis*, which requires 2 kGy. As far as the authors are aware, this is the first irradiation study on *A. costaricensis* infective larvae. Nevertheless, these radiation doses are comparatively much lower than those required for completely preventing the migration of the second stage *Toxocara canis* larvae, 10 kGy [5]. Generally, the nematode can withstand a comparatively higher irradiation dose, as shown by the high percentage of survival of anisakid larvae even after irradiation at 6 kGy [6]. However, on the other end of the radiation sensitivity spectrum lie *Trichinella spiralis* muscle larvae, which are highly sensitive to irradiation and can be sexually sterilized at a dose of 0.3 kGy.

A dose of 0.1 kGy of gamma ray irradiation on the first stage larvae of *A. can- tonensis* has been reported to have a deleterious effect on the development of the larvae to third stage and also to result in reduced recovery of adult worms from rats [7]. This report supports our observation on the retarded growth of irradiated third stage larvae in the definitive host. Since the worm recovery of X ray and gamma ray irradiated *A. cantonensis* larvae correlate well, there seems to be no significant difference between irradiating free larvae in saline solution and larvae still in the flesh of the snail.

ACKNOWLEDGEMENTS

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REFERENCES


Angiostrongylus LARVAE


STUDIES ON THE USE OF ⁶⁰Co GAMMA IRRADIATION TO CONTROL INFECTIVITY OF Clonorchis sinensis METACERCARIAE

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Abstract

STUDIES ON THE USE OF ⁶⁰Co GAMMA IRRADIATION TO CONTROL INFECTIVITY OF Clonorchis sinensis METACERCARIAE.

The purpose of the present study is to observe the survival and development of Clonorchis sinensis metacercariae in their final hosts after ⁶⁰Co gamma irradiation of isolated metacercariae or in fish. Guinea pigs or albino rats were orally infected with metacercariae by gavage. Bioassay, faecal examination for ova and dissection of infected animals were the methods used for the estimation of minimal effective dose (MED) of gamma irradiation to control infectivity of C. sinensis metacercariae. Results showed that the MED for isolated metacercariae was 0.05 kGy. The LD₉₀ of the irradiation dose for metacercariae in fish was 0.05 kGy, and the MED was 0.15 kGy. No significant difference in radiation susceptibility to ⁶⁰Co gamma irradiation was found among C. sinensis metacercariae in fishes collected at different localities in northern, central or southern parts of China. The present finding suggests that irradiation of fish by 0.15 kGy could control infectivity of C. sinensis metacercariae and thus be adopted as a control measure in preventing infections.

1. INTRODUCTION

Clonorchiasis, a food-borne zoonosis, has been prevalent mainly in China, Japan, Korea, Viet Nam, Laos and other southeast Asian nations. Clonorchis sinensis is widely distributed in China, in more than 200 prefectures, counties or towns. The prevalence among human populations in endemic areas varies, with the highest infection rate being 10.59% [1, 2]. Human infections are contracted from eating raw or half-cooked freshwater fishes, shrimp, or sliced fish flesh mixed with hot porridge. The disease has been one of the most important in China. Worldwide studies are currently under way on the application of irradiation techniques for disinfection and preservation of foodstuffs. The present study was carried out from October 1987 to April 1991 to evaluate the effects of irradiation of C. sinensis metacercariae.
2. MATERIALS AND METHODS

2.1. Collection of C. sinensis metacercariae

Freshwater fish, *Pseudorasbora parva*, *Abbottina* sp. and *Ctenopharyngodon idella* were collected in endemic areas. Minced fish flesh was added to five volumes of artificial digestive juice (1% pepsin) and digested at 37°C for 3–5 hours, followed by sieving to remove coarse pieces of fish. The metacercariae were isolated under stereomicroscopy. The isolated metacercariae were stored in Alsever's solution until ready for use. In the experiments on metacercariae in fish, the isolation procedure was omitted.

2.2. Experimental animals and source of radiation

Normal guinea pigs weighing 250–500 g and albino rats of 150–200 g body weight were used. The radiation source was *60Co* with an activity of 10 kCi\(^1\). Irradiation doses varied from 0 to 0.20 kGy and the dose rate was 5 Gy/min. The radiation dose was adjusted at a fixed distance, changing the exposure time. The radiation source was provided by the Institute of Atomic Energy and Technology, Zhejiang Academy of Agricultural Sciences, and irradiation was conducted with the help of the Institute.

2.3. Experimental schemes

*Scheme 1. Observation on minimal effective dose (MED) of gamma irradiation to control infectivity of isolated C. sinensis metacercariae*

Isolated *C. sinensis* metacercariae were divided into seven groups. Six groups were irradiated at doses of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.10 kGy, respectively. The seventh group served as control. One hundred and fifty metacercariae were dispensed into test tubes containing 2 mL of Alsever's solution. Guinea pigs were orally infected by gavage with either irradiated or non-irradiated metacercariae within four hours of irradiation. Each guinea pig was given 30 metacercariae. Faecal examination for *C. sinensis* ova by means of simple sedimentation was carried out on alternate days starting 19 days after infection. The animals were sacrificed 30 days after infection and livers, bile ducts and peritoneal cavities were washed with normal saline. Livers were dissected and the recovered worms counted.

\(^1\) 1 Ci = 3.7 \times 10^{10} \text{ Bq}.
Scheme 2. Observation on the MED of gamma irradiation to control infectivity of C. sinensis metacercariae in fish

Freshwater fish 5-15 cm in length were collected from endemic areas, placed in a box and irradiated in the same manner as in Scheme 1. On the basis of preliminary experiments, the fish in four groups were irradiated at doses of 0.05, 0.10, 0.15 and 0.20 kGy, respectively, with the control group non-irradiated. After irradiation the metacercariae were isolated from the digested material of the fish and used for infecting guinea pigs. Each guinea pig was given 100 metacercariae orally. Comparative studies were also conducted on the infectivity of irradiated metacercariae for different animal hosts.

Bliss' weighted linear regression [3] was adopted to estimate LD_{50}, and maximal and minimal lethal doses. The maximal lethal dose (MCD) was regarded as the MED.

Scheme 3. Comparative studies on radiosusceptibility of metacercariae collected from different regions

Freshwater fishes were collected at Liao River near Shenyang City in the northeast, Huzhou City of the Taihu Lake hydrographic net in Zhejiang Province,

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Animals (No.)</th>
<th>Dissection</th>
<th>Faecal examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean No. of worms</td>
<td>Recovery rate (%)</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>18.0</td>
<td>60.0</td>
</tr>
<tr>
<td>0.01</td>
<td>3</td>
<td>20.0</td>
<td>66.7</td>
</tr>
<tr>
<td>0.02</td>
<td>3</td>
<td>6.7</td>
<td>22.2</td>
</tr>
<tr>
<td>0.03</td>
<td>3</td>
<td>1.0</td>
<td>3.3</td>
</tr>
<tr>
<td>0.04</td>
<td>3</td>
<td>1.7</td>
<td>5.6</td>
</tr>
<tr>
<td>0.05</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.10</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Each guinea pig was infected with 30 metacercariae.
and Sanshui County of the Pearl River system in Guangdong Province. The fish infected with *C. sinensis* were irradiated and their radiosusceptibility compared. Irradiation doses varied from 0 (control) to 0.05, 0.10 and 0.15 kGy.

### 2.4. Determination of infectivity of metacercariae

This was assessed by faecal examination for detection of eggs and dissection of infected animals for recovery of worms. If ova and/or *C. sinensis* were detected in the control rats, but not in the rats infected with metacercariae gamma irradiated at a certain dose, the corresponding dose was regarded as the effective one. The MED was obtained through repeated experiments.

### 2.5. Morphological observation of *C. sinensis* metacercariae

Mobility of the worms was observed under light microscopy and possible damage of structure caused by irradiation was observed under electron microscopy.

### 3. RESULTS

#### 3.1. Effect of **Co gamma irradiation on isolated *C. sinensis* metacercariae

The infectivity of irradiated *C. sinensis* metacercariae for guinea pigs decreased as the irradiation dose increased. The worm recovery rate was 66.7% in the 0.01 kGy group, similar to that in the control group. When the doses were increased to 0.03 and 0.04 kGy, the worm recovery rates decreased to 3.3% and 5.6%, respectively, presenting a significant difference from that of the control group (p < 0.05). When the irradiation dose was increased to 0.05 kGy or more, metacercariae failed to develop into adult worms. The MED to control infectivity of *C. sinensis* metacercariae, therefore, was close to 0.05 kGy (Table I).

#### 3.2. Effect of **Co gamma irradiation on *C. sinensis* metacercariae in fish

#### 3.2.1. Gamma irradiation to control infectivity of *C. sinensis* metacercariae

Coinciding results were obtained through seven repeated experiments. Both ova and worms were found in all control guinea pigs (30/30), and the worm recovery rate was 59.2%. Similar results were obtained in the 0.05 kGy irradiation group, but the worm recovery rate was remarkably lower (30%) than that in the control group. Positivity of both eggs and worms was 66.7% (22/33) in the 0.10 kGy group; however, the average worm recovery rate was only 1.4% (45/3300). Neither eggs nor worms were detected in the 0.15 and 0.20 kGy groups (Table II).
### TABLE II. EGG DETECTION AND RECOVERY OF *C. sinensis* FROM GUINEA PIGS INFECTED WITH IRRADIATED METACERCARIAE IN FISH

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Faecal examination</th>
<th>Dissection (worms/animal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animals positive for eggs</td>
<td>Recovery rate (%)</td>
</tr>
<tr>
<td></td>
<td>(No.)</td>
<td>(%)</td>
</tr>
<tr>
<td>0</td>
<td>30</td>
<td>100.0</td>
</tr>
<tr>
<td>0.05</td>
<td>22</td>
<td>100.0</td>
</tr>
<tr>
<td>0.10</td>
<td>33</td>
<td>66.7</td>
</tr>
<tr>
<td>0.15</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>0.20</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Each guinea pig was infected with 100 metacercariae.

### TABLE III. RESULT OF EXAMINATIONS OF DIFFERENT ANIMALS INFECTED WITH *C. sinensis* METACERCARIAE IN FISH IRRADIATED AT DIFFERENT DOSES OF ⁶⁰Co GAMMA IRRADIATION

<table>
<thead>
<tr>
<th>Animals</th>
<th>Dose (kGy)</th>
<th>Faecal examination</th>
<th>Dissection (worms/animal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Animals positive for eggs</td>
<td>Recovery rate (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(No.)</td>
<td>(%)</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Albino rats</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0.95</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Each animal was infected with 100 metacercariae.
TABLE IV. EGG DETECTION AND WORM RECOVERY OF *C. sinensis* FROM GUINEA PIGS INFECTED WITH IRRADIATED METACERCARIAE IN FISH COLLECTED IN DIFFERENT PARTS OF CHINA

<table>
<thead>
<tr>
<th>Locality</th>
<th>Climate</th>
<th>North latitude</th>
<th>Mean annual temperature</th>
<th>Dose (kGy)</th>
<th>Animals (No.)</th>
<th>Faecal examination</th>
<th>Dissection (worms/animal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Animals positive for eggs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(No.) (%)</td>
<td>X ± SD (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>5</td>
<td>5 100.0</td>
<td>61.2 3.899 61.2</td>
</tr>
<tr>
<td>Shenyang, Liaoning Province</td>
<td>Temperate</td>
<td>41°43'</td>
<td>7.3°C</td>
<td>0.05</td>
<td>4</td>
<td>4 100.0</td>
<td>30.3 6.702 30.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.10</td>
<td>5</td>
<td>2 40.0</td>
<td>0.6 0.894 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.15</td>
<td>5</td>
<td>0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Huzhou, Zhejiang Province</td>
<td>Subtropical</td>
<td>30°90'</td>
<td>15.8°C</td>
<td>0.05</td>
<td>8</td>
<td>8 100.0</td>
<td>59.3 16.333 59.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.10</td>
<td>10</td>
<td>10 100.0</td>
<td>28.5 6.654 28.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.15</td>
<td>10</td>
<td>6 60.0</td>
<td>1.0 1.054 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Sanshui, Guangdong Province</td>
<td>Tropical</td>
<td>23°11'</td>
<td>21°C</td>
<td>0.05</td>
<td>5</td>
<td>5 100.0</td>
<td>57.0 19.365 57.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.10</td>
<td>5</td>
<td>4 80.0</td>
<td>1.6 1.140 1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.15</td>
<td>5</td>
<td>0 0</td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

Each animal was infected with 100 metacercariae.
3.2.2. Calculation of MED to control infectivity of *C. sinensis* metacercariae in fish

Forty guinea pigs were divided into five groups, eight animals in each. The irradiation doses were 0 (control), 0.044, 0.066, 0.10 and 0.15 kGy, respectively. The following results were calculated according to Bliss' weighted linear regression: The LD<sub>50</sub> was 0.0501 kGy; The MLD (LD<sub>5</sub>) was 0.0279 kGy; The MLD (LD<sub>95</sub>) was 0.1294 kGy. The latter could be considered as the MED.

3.2.3. Susceptibility of two different hosts (guinea pig and albino rat) to gamma irradiated *C. sinensis* metacercariae

Both guinea pigs and albino rats were 100% infected when non-irradiated metacercariae were given; the worm recovery rates were 57.0% and 48.8%, respectively. If they were infected with gamma irradiated metacercariae, both infection rate and worm recovery rate decreased as the irradiation dose increased. No worms were recovered from animals infected with metacercariae irradiated at 0.15 kGy or more. These findings revealed that the MED to control infectivity of *C. sinensis* metacercariae was close to 0.15 kGy (Table III).

3.3. Comparative studies on radiosusceptibility of metacercariae collected from different regions to 60Co gamma irradiation

Infection rates of guinea pigs infected with *C. sinensis* metacercariae collected from three different regions were 100%. The worm recovery rates were also comparable, 61.2%, 59.3% and 57.0%, respectively. Both infection rates and worm recovery rates decreased as the irradiation dose increased. The infection rates of guinea pigs infected with metacercariae collected from Shenyang, Huzhou and Sanshui were 40%, 60% and 80%, respectively, and the corresponding worm recovery rates were 0.6%, 1.0% and 1.6% in the 0.10 kGy group. Increasing the dose up to 0.15 kGy brought the infection rates and worm recovery rates to 0% for metacercariae collected from the three regions (Table IV).

3.4. Morphology of the irradiated metacercariae

The metacercariae which were exposed to gamma irradiation at doses of 0.10–0.20 kGy showed no apparent difference from non-irradiated ones. They showed weak movements within their cysts. The outlines of the excretory bladder and caeca were clearly discernible. Observation under electron microscopy revealed no structural damage of the metacercariae irradiated with the above stated doses of gamma rays.
Findings in this study clearly suggest that $^{60}$Co gamma irradiation has an effect on the infectivity of *C. sinensis* metacercariae. The infectivity decreased as the irradiation dose increased. The estimated LD$_{50}$ of $^{60}$Co gamma irradiation for metacercariae in fish was 0.05 kGy. The average worm recovery rate in guinea pigs infected with non-irradiated metacercariae was 59.2%, compared with 30.0% and 1.4% when infected with metacercariae irradiated at doses of 0.05 and 0.10 kGy, respectively. No worms were recovered when the irradiation dose was 0.15 or 0.20 kGy (Table II). These data suggest that 0.15 kGy, but not 0.10 kGy, should be regarded as strong enough to control the infectivity of *C. sinensis* metacercariae completely. A theoretical value of the MED calculated on the data of Scheme 2 is 0.13 kGy, very close to 0.15 kGy obtained from practical experiments. It is evident that 0.15 kGy can be regarded as the MED to control infectivity of metacercariae in fish. This conclusion was based on seven repeated experiments on the metacercariae collected from northern, central and southern parts of China, representing different climates and hydrographic nets. The reliability of the results is reflected by their consistency. Therefore, irradiation by gamma rays at a dose of 0.15 kGy can be used in practical operations as the MED to control infectivity of *C. sinensis* metacercariae in fish.

Scheme 1 demonstrated that isolated metacercariae exposed to gamma irradiation at a dose of 0.05 kGy were not able to survive in guinea pigs (Table I), but the MED to control infectivity of metacercariae in fish was 0.15 kGy (Table II). The reason for this can be attributed to the milieu of tissue around the metacercariae and treatment before and during the irradiation. Our results are similar to those reported by Lee et al. [4], who suggested in their paper that the flesh of the dead fish should have been anoxic; therefore, there was less destruction of cells, and the irradiated metacercariae in the flesh were able to survive more easily. Our results support this point of view.

The effects of irradiation on the infectivity of parasites have been found to be very interesting. Many reports [5-10] suggest that this is a new and effective measure to prevent food-borne parasitoses. Our results support the past findings and confirm that no difference in radiation susceptibility exists among *C. sinensis* metacercariae collected from northern, middle or southern parts of China. This provides support for a nationwide programme for the treatment of *C. sinensis* infections of fish. Lee et al. [4] reported that the LD$_{50}$ of $^{137}$Cs gamma irradiation in treating *C. sinensis* metacercariae in fish was 0.475 kGy, and the worm recovery rate in the 0.10 kGy group was 1%. These findings are similar to ours (Table II). Despite the differences in geographical environment and radiation source used, the results are similar. This provides a scientific basis for international acceptance of irradiation techniques to control infectivity of the Chinese liver fluke. Although the effective radiation dose to prevent *C. sinensis* metacercariae from developing into adults was
obtained, it is yet uncertain whether the worms died immediately after exposure to the radiation or later. This problem, together with the mechanism of death, merits further investigation.

ACKNOWLEDGEMENTS

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REFERENCES

STUDIES ON THE EFFECT OF $^{60}$Co IRRADIATION ON THE INFECTIVITY OF 
*Paragonimus westermani* METACERCARIAE

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Zhejiang Academy of Medical Sciences,
Hangzhou, China

Abstract

STUDIES ON THE EFFECT OF $^{60}$Co IRRADIATION ON THE INFECTIVITY OF 
*Paragonimus westermani* METACERCARIAE.

Using $^{60}$Co as source of radiation, a study was made to observe the effect of irradiation on the viability of *Paragonimus westermani* metacercariae in *Potamon* sp. crabs. The crabs were collected in mountain regions in Zhejiang Province of China endemic for paragonimiasis. Adult cats and albino mice were infected with the metacercariae irradiated at different doses. Dissection of the animals was conducted 90 or 30 days, respectively, after infection for recovery of the lung fluke from infected cats, and antimetacercaria antibody was tested with sera from infected mice by enzyme linked immunosorbent assay (ELISA). Results showed that metacercariae were unable to grow into adult worms in cats after exposure to gamma irradiation at a dose of 0.10 kGy. However, a small number of metacercariae exposed to a dose of 2.0 kGy was still able to encyst and survive for 30 days. No young or adult worms were recovered from mice when the metacercariae were irradiated at a dose of 2.5 kGy. Seropositive results by ELISA were obtained when the mice were infected with metacercariae irradiated at doses ranging from 2.0–3.5 kGy. The susceptibility of *P. westermani* metacercariae to irradiation is discussed.

1. INTRODUCTION

Paragonimiasis, a food-borne zoonosis, is an important parasitic disease of humans. The disease has been reported in 22 provinces (municipalities, autonomous regions) in China. The main pathogen of the disease is *Paragonimus westermani*. Humans become infected when they consume raw or half-cooked crustacean intermediate hosts, *Potamidae* or *Astacidae*, infected with metacercariae of *P. westermani* [1]. Killing the metacercariae should be an effective measure to control the transmission of the disease.
2. MATERIALS AND METHODS

2.1. Parasite

*P. westermani* metacercariae were isolated from freshwater *Sinopotamon chekiangense* collected in a mountainous region of Zhejiang Province endemic for paragonimiasis.

2.2. Animal

The Kunming strain of albino mice, 20–25 g in body weight, was used to test the viability of metacercariae and for serological testing. Healthy cats born in non-endemic areas, one year old or slightly older, were used to study the development of metacercariae into adult worms.

2.3. Preparation of specimens and source of radiation

Live crabs were bound with thread to restrict the movement of their legs, placed in a paper box, and irradiated at various doses. The crabs were then crushed and minced, washed and sifted, and the metacercariae isolated from the sediment under stereomicroscopy. The isolated metacercariae were used for the experimental infection of cats.

Alternatively, to test the viability of metacercariae, the crabs were first crushed and washed. Metacercariae were collected, placed into test tubes with 2 mL Alsever’s solution, irradiated, and then used to infect the mice.

The irradiation procedure was conducted with the help of the Institute of Atomic Energy and Technology, Zhejiang Academy of Agricultural Sciences, where $^{60}$Co was provided as the radiation source, with an activity of $37 \times 10^{13}$ Bq and a uniform dosage of 5–13 Gy/min.

2.4. Evaluation of viability and infectivity of metacercariae

Each mouse was given 30 metacercariae by gavage. The mice were dissected 30 days after infection. Abdominal and thoracic cavities were rinsed with normal saline. Diaphragm, liver, intercostal and abdominal muscles were minced. The parasites were recovered and counted.

Serum antibody of infected mice was tested by indirect enzyme linked immunosorbent assay (ELISA). An infusion of metacercariae was used as the antigen with a protein content of 3 $\mu$g/mL. The method was in accordance with Voller with some modifications [2, 3]. A value of optical density (OD) $\geq 0.3$ was regarded as a positive reaction.

Infected cats were dissected three months after infection and their lungs and thoracic cavities searched for adult *P. westermani* worms.
3. RESULTS

3.1. Infection of mice with metacercariae irradiated at various doses

Worms were recovered from every mouse of the control group 30 days after infection, with an average of 5.2 worms per mouse. One parasite was found in one of the five mice in the 2.0 kGy dose group. No worms were found in the other groups infected with metacercariae irradiated at a dose of ≥ 2.5 kGy (Table I).

A positive reaction for anti-metacercariae antibody in mouse sera was obtained by ELISA in all groups except for the 4 kGy dose group. The OD value was, however, inversely proportional to that of the dose. The OD value decreased with increasing dosage (Table I).

3.2. Infection of cats

Both adult worms and ova were found in the two cats of the control group. The mean number of worms found per cat was 49.5. Adult worms were also found in

### TABLE I. WORM RECOVERY IN MICE 30 DAYS AFTER INFECTION WITH METACERCARIAE GAMMA IRRADIATED AT VARIOUS DOSES AND ANTIBODY DETECTION IN MOUSE SERA BY ELISA

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>No. of Mice†</th>
<th>No. of worm-positive mice</th>
<th>No. of worms recovered</th>
<th>No. of worm-positive mice</th>
<th>OD Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>5</td>
<td>26</td>
<td>5.2</td>
<td>5</td>
</tr>
<tr>
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<td>1</td>
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<td>5</td>
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<table>
<thead>
<tr>
<th>ELISA b</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
</tr>
<tr>
<td>0.63</td>
</tr>
<tr>
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</tr>
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<td>0.50</td>
</tr>
<tr>
<td>0.42</td>
</tr>
<tr>
<td>0.36</td>
</tr>
<tr>
<td>0.21</td>
</tr>
</tbody>
</table>

† Each mouse was fed 30 Paragonimus westermani metacercariae.

b OD value in normal mouse sera was 0.19 ± 0.02.

OD value 0.30 was regarded as positive reaction.
TABLE II. DISSECTION OF CATS ON 90th DAY AFTER INFECTION WITH METACERCARIAE ISOLATED FROM *Potamon* CRABS GAMMA IRRADIATED AT VARIOUS DOSES

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>0</th>
<th>0.05</th>
<th>0.10</th>
<th>0.25</th>
<th>0.50</th>
<th>1.00</th>
<th>2.00</th>
<th>3.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cats</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Number of cats positive for egg and worm</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total number of worms recovered</td>
<td>99</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Each cat was fed 100 *Paragonimus westermani* metacercariae.

the 0.05 kGy dose group, but the mean number was only 3.5. No worms were recovered in the other groups (dose range 0.10–3.0 kGy) (Table II).

4. DISCUSSION

Adult lung flukes were detected in the two cats infected with metacercariae gamma irradiated at a dose of 0.05 kGy, indicating an insufficient dosage for controlling infectivity of metacercariae. The number of worms (3.5) recovered, however, was far lower than that of the control group (49.5) (Table II), denoting an apparent effect of irradiation on the growth and development of the worm. No worms were found in other groups receiving a dosage of ≥0.1 kGy. This showed that 0.1 kGy appeared to be an effective dose for inhibiting metacercariae development in adult worms. Since the cat is a common reservoir of the parasite and not even one worm was recovered in cats in the 0.1–3.0 kGy dose groups, it is believed that the present results are valid.

A new problem has arisen from the experimental use of mice infected with gamma irradiated metacercariae. As seen in Table I, a worm was found in one mouse 30 days after it had been infected with metacercariae irradiated at a dose of 2.0 kGy. Evidently the excysted metacercaria penetrated the intestinal wall, entered the body cavity of its host and survived somewhere in its tissues. This suggests that not all of the metacercariae were killed when irradiated at a dose of 2.0 kGy. Furthermore, the sera of mice reacted negatively to ELISA only when the radiation dose reached 4.0 kGy. In the 3.5 kGy and lower dose groups all the sera were reactive, although the OD value decreased with the increasing dosage, implying a previ-
ous infection. It was reported that anti-metacercariae antibody began to emerge ten days after albino mice were infected with *P. westermani* metacercariae, and peaked 30 days post infection [4]. ELISA is a sensitive technique used in detecting specific antibodies in serum; therefore, positive sera in our experiment can be considered as a result of metacercarial infection. Thus, only 4.0 kGy can be held as an effective dose to inhibit the infectivity of *P. westermani* metacercariae.

There seems to be a distinct disparity between the latter data and those in the former experiment where an irradiation dose of 0.10 kGy was strong enough to prevent metacercariae from growing into adult worms. No doubt the metacercariae irradiated at a dose of 0.10 kGy did not develop into adult worms, but they survived for a certain period, with the result that sera were positive by ELISA. It is common knowledge that *Paragonimus* species flukes are noted for their larva migration, which may cause multiple pathological changes within their host [1]. No larva was found 30 days after the mice had been infected with metacercariae irradiated at a dose of 2.5 kGy or higher. Thus 2.5 kGy is regarded as an effective dose to control infectivity of *Paragonimus* metacercariae from host crabs. Further investigation of this finding is under way.

**ACKNOWLEDGEMENTS**

This study was supported in part by a grant from the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Research Contract No. 4696RB. We would like to express our thanks also to P. Loaharanu for his encouragement. Cordial thanks are also given to Fu Jinkang and Zhang Qiuye for their instruction and help with the irradiation technique.

**REFERENCES**

STUDIES ON THE USE OF $^{60}$Co IRRADIATION TO CONTROL INFECTIVITY OF $T. gondii$

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Abstract

STUDIES ON THE USE OF $^{60}$Co IRRADIATION TO CONTROL INFECTIVITY OF $T. gondii$.

$T. gondii$ cysts in minced pig brain, tongue, liver, heart and muscle were placed into plastic vials. These materials were irradiated at various doses. The irradiated tissue was then treated with artificial digestive juice and the suspension of bradyzoites prepared. The suspension was used to infect mice intraperitoneally and kittens orally. Unirradiated tissue with $T. gondii$ was given to mice and kittens to serve as controls. Determination of infectivity was done on the 50th day after the mice were infected or 3–15 days after the kittens were infected. Loss of infectivity was confirmed according to the following criteria: no cyst in mouse brain impression smears; no oocyst in faeces of kittens; subinoculation in mice and indirect haemagglutination tests were negative, while all bioassay, parasitological examinations and serological tests in the control group were positive. Repeated experiments were done in order to calculate the minimal effective irradiation dose (MED). Activity of radiation source: $37 \times 10^{13}$ Bq; uniform dosage: 12.38 Gy/min. Results showed that (1) the MED of gamma rays to control infectivity of the Chinese NT strain and the US ME-49 and TS-2 strains of $T. gondii$ cysts in pork was approximately 0.6 kGy; and (2) infectivity for mice of NT strain bradyzoites released from cysts irradiated with gamma rays at a dose of 0.45 kGy decreased to 1/10 000. These data could be valuable in practical operations to control $T. gondii$ in pork products.

1. INTRODUCTION

Toxoplasmosis is a widespread disease that is a problem in human health and animal husbandry. It has been reported that 50% of the adult population of the United States of America has antibodies to $T. gondii$ [1]. The disease is currently found in 28 provinces, municipalities, and autonomous regions in China [2] and the average seropositive rate in the human population is around 10%, with the highest over 30%. In Hangzhou, Zhejiang, 21% of the population are seropositive; 63.4% of the butchers and 52.8% of the pigs surveyed were seropositive [3, 4]. Toxoplasma cyst in pork is known as a source of the infective stage of the parasite, and gamma
irradiation is known to kill parasites in porcine tissues [1, 5]. In this study, carried out between 1987 and 1990, $^{60}$Co irradiation was used to control infectivity of the Chinese NT strain of *T. gondii*. A comparative study was also done on the radiosusceptibility between different geographical strains of the parasite from China and the USA.

2. MATERIALS AND METHODS

2.1. Parasites

The NT strain of *T. gondii* was isolated from an infected pig at the Nantong Animal Husbandry and Veterinary Station in Jiangsu Province. The US ME-49 and TS-2 strains were provided by J.P. Dubey of the US Department of Agriculture, Beltsville, Maryland, USA.

2.2. Animals

Female NIH mice, 20–25 g in body weight, were provided by the Zhejiang Centre for Experimental Animals. Kittens aged 1–2 months with negative indirect haemagglutination (IHA) reactions were also used in the experiment. Piglets of York-Durock hybrid, aged two months, were provided by the Animal Husbandry and Veterinary Department, Zhejiang Agricultural University.

2.3. Preparation of specimens

(a) Mouse brains harbouring *T. gondii* cysts were triturated in two volumes of normal saline and passed through a 100 mesh brass wire sieve to make homogenates. Aliquots of 12 mL were placed into vials (2.5 x 5.0 cm) and kept for irradiation experiments.

(b) In the studies on *Toxoplasma* bradyzoites to assess the effect of gamma irradiation on controlling infectivity, the irradiated homogenates were added to equal parts of 0.4% trypsinase solution and incubated at 37°C for 20 min. Normal saline was added and centrifuged to wash away the digestive juice. Normal saline was added to the sediment of released bradyzoites to make a $10^{-1}$ suspension. Another five successive tenfold dilutions were made to obtain $10^{-2}$–$10^{-6}$ suspensions. The whole procedure of digestion, washing and inoculation of mice was completed within two hours.

(c) In the comparative study on different geographical strains, the procedures were the same except that the digestion and tenfold dilution were omitted. The density of cysts in the homogenate was adjusted to about 12 cysts/mL.
Artificially infected pigs were used as experimental controls for infectivity of Toxoplasma cysts in pork. To obtain cysts, brain, tongue, heart, liver, spleen, kidney, diaphragm and muscle tissues from infected pigs were minced and mixed together; 50 or 100 g portions were placed in plastic vials. In order to raise the density of cysts in pork, intact mouse brains harbouring numerous Toxoplasma cysts were mixed with the pig specimens.

2.4. Radiation source and dosage

Cobalt-60 was used as radiation source, with an activity of $37 \times 10^{13}$ Bq, providing a uniform dosage of 12.38 Gy/min. Doses of irradiation at different locations were measured with a Fricke ferrous sulphate dosimeter. The dosages in the experiments ranged from 0.1 to 1.0 kGy.

2.5. Determination of infectivity

The infectivity of T. gondii was confirmed by bioassay in mice and kittens, and/or by the IHA test. Infectivity of Toxoplasma cysts in pig tissue was determined as described by Dubey et al. [1]. Pig tissue was digested with trypsinase solution to make a suspension of bradyzoites. One millilitre of the suspension was injected intraperitoneally into each mouse. The mice were necropsied on the 50th day after inoculation and impression smears of the cerebral cortex were made and examined for cysts. The IHA test was carried out on the sera; a titre $\geq 1:64$ was considered positive. If kittens were used in the bioassay, 4 mL of mouse brain homogenate or 100 g of pig tissue irradiated at various doses and unirradiated specimens were fed to each animal. Examination of kitten faeces for oocysts was started on the third day after feeding and continued for 15 days. The IHA test was done on the seventh day after inoculation. Blind subinoculations were done if the above mentioned results were all negative. Loss of infectivity was confirmed if the subinoculations were negative but the results of the control group were all positive. The relevant minimal effective dose (MED) was calculated accordingly.

3. RESULTS

3.1. Experiment 1: Effective dose of gamma rays to control infectivity of cysts of the NT strain of T. gondii

After mouse brain homogenates containing cysts of the NT strain of T. gondii (neither treated with artificial digestive juice nor diluted) had been irradiated at doses of 0.10, 0.25, 0.50, 0.75 and 1.00 kGy separately, they were inoculated intraperitoneally into mice of different groups (eight in each) or fed orally to kittens (one
each). No cysts were found in the 0.75 and 1.00 kGy groups 50 days after inoculation. IHA tests and blind subinoculations were also negative. Cysts were found in all other groups, and the IHA tests were also positive. The number of positive mice and the number of cysts detected per mouse were related to the doses applied. The geometric mean of reversion titre (GMRT) of IHA tests for dose groups of 0, 0.10, 0.25 and 0.50 kGy was 203, 161, 90 and 64, respectively. Results of experiments on kittens were consistent with those in mice. No oocysts were found in kitten faeces of the 0.75 and 1.00 kGy dose groups, and the IHA tests were also negative. The controls were positive.

3.2. Experiment 2: MED of gamma rays to control infectivity of cysts of the NT strain of *T. gondii*

On the basis of the preceding experiment, gamma rays were applied at doses of 0.50, 0.55, 0.60 and 0.65 kGy to mouse brain homogenates containing *T. gondii* cysts, then inoculated into healthy NIH mice. Mice of the control group were inoculated with unirradiated cysts. On the 50th day post infection, cysts were detected in every mouse of the control group and the IHA tests were all positive. Cysts were found in one out of seven mice in the 0.50 kGy group, and the IHA tests were also

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>No. of mice inoculated</th>
<th>Cyst-positive</th>
<th>IHA-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of mice</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>8</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>0.50</td>
<td>7</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>0.55</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.60</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.65</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.70</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.75</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
positive, consistent with the preceding experiment. No cysts were found in mice of the 0.55 kGy dose group nor in animals given higher doses. IHA tests in these groups were all negative (Table I). These data suggest that the MED of gamma irradiation to control infectivity of cysts of the NT strain of *T. gondii* appears to be 0.55 kGy.

### 3.3. Experiment 3: Effect of various doses of gamma rays on infectivity of bradyzoites of the NT strain of *T. gondii*

Mice were inoculated with bradyzoite-containing suspensions prepared with cyst-containing mouse brain homogenates which were irradiated with gamma rays at various doses, treated with artificial digestive juice and diluted at different multiples. Results showed that mice in the control group were infected when inoculated with unirradiated bradyzoite suspension diluted up to $10^{-5}$. When the irradiation dosage was increased from 0.10, 0.25 and 0.35 to 0.45 kGy, the corresponding maximal dilution multiples which were capable of infecting the mice were in the following order: $10^{-5}$, $10^{-3}$, $10^{-2}$ and $10^{-1}$ (Table II and Fig. 1). The ratio of the numbers of bradyzoites needed to infect the mice between the 10 and 0.45 kGy dose groups was $10^{4}$, suggesting that the infectivity of bradyzoites irradiated at a dose of 0.45 kGy decreased by 10,000 times.

### TABLE II. INFECTION OF MICE INOCULATED WITH BRADYZOITES-CONTAINING SUSPENSION IRRADIATED WITH GAMMA RAYS AND DILUTED AT DIFFERENT MULTIPLES

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>$10^{-1}$</th>
<th>$10^{-2}$</th>
<th>$10^{-3}$</th>
<th>$10^{-4}$</th>
<th>$10^{-5}$</th>
<th>$10^{-6}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5(7.5)</td>
<td>5(2.6)</td>
<td>5(0.7)</td>
<td>4(0.5)</td>
<td>2(0.4)</td>
<td>0</td>
</tr>
<tr>
<td>0.10</td>
<td>5(3.2)</td>
<td>5(2.0)</td>
<td>5(0.3)</td>
<td>2(0.2)</td>
<td>1(0.2)</td>
<td>0</td>
</tr>
<tr>
<td>0.25</td>
<td>3(2.4)</td>
<td>2(1.0)</td>
<td>2(1.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.35</td>
<td>2(2.0)</td>
<td>1(1.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.45</td>
<td>1(1.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.55</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Five mice in each group.

Data are expressed as number of mice positive for *T. gondii*.

Density is shown in brackets.
3.4. Experiment 4: Comparison of radiosusceptibility of *Toxoplasma* strains of different geographical origin to $^{60}$Co irradiation

The NT strain of *Toxoplasma* cysts from China and the ME-49 and TS-2 strains from the USA were irradiated at doses of 0.4, 0.5, 0.6, 0.7 and 1.0 kGy, and introduced into mice and kittens. Unirradiated cysts were used in the control group. No cysts were found in mouse brain tissues nor in faeces of kittens when the dose was $\geq 0.6$ kGy, whereas large numbers of cysts and oocysts were recovered from control animals. Although cysts and oocysts were found in animals of the 0.5 and 0.4 kGy groups, the numbers recovered were lower than those in the control group. A second experiment was carried out in order to determine the MED. The results showed the MED for the NT strain to be 0.55 kGy, and for the ME-49 and TS-2 strains, 0.60 kGy (Table III). This suggests that the difference in radiosusceptibility between the Chinese NT strain and US ME-49 and TS-2 strains is not significant.
TABLE III. EXAMINATION OF MOUSE BRAIN FOR CYSTS ON THE 50TH DAY AFTER INOCULATION WITH THREE STRAINS OF *Toxoplasma gondii* CYSTS IRRADIATED AT VARIOUS DOSES

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>No. of mice inoculated</th>
<th>Cyst-positive</th>
<th>No. of mice</th>
<th>%</th>
<th>Densitya</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.40</td>
<td>10</td>
<td>6</td>
<td>2</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>0.50</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>0.60</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.70</td>
<td>10</td>
<td>0</td>
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</tr>
<tr>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.50</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>0.55</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td>0.60</td>
<td>8</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>0.65</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a Mean number of cysts in 400 mm² mouse brain impression smear.

b No observation.

3.5. Use of gamma irradiation to control infectivity of *Toxoplasma* cysts of different geographical strains in pig tissue

Infected mouse brain was wrapped with pig tissue in order to increase the density of cysts in the latter. Results showed that none of the NT, ME-49 or T5-2 strain cysts was capable of infecting kittens and/or mice when irradiated at a dose ≥0.6 kGy. However, animals were infected when the radiation dose was ≤0.5 kGy. This suggests that the radiosusceptibility of different geographical strains to gamma rays is similar. The MED is close to 0.6 kGy.
TABLE IV. BIOASSAY FOR INFECTIVE *T. gondii* CYSTS IN GAMMA IRRADIATED PIG TISSUES IN MICE AND KITTENS* 

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dose (kGy)</th>
<th>Pig tissue</th>
<th>Pig tissue and mouse brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cyst-positive</td>
<td>Oocyst^b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of mice</td>
<td>% Density^c</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>9</td>
<td>90</td>
</tr>
<tr>
<td>0.4</td>
<td>0.4</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NT</td>
<td>0.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(China)</td>
<td>0.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.7</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ME-49/</td>
<td>0</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>TS-2</td>
<td>0.4</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>(USA)</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Ten mice and one kitten in each group.
^b +: oocyst-positive; -: oocyst-negative.
^c Mean number of cysts in 400 mm^2 impression smear.

4. DISCUSSION

Successive studies have been made since 1987 on the use of gamma irradiation to control infectivity of *T. gondii*. The results demonstrated that gamma irradiation exerted an apparent effect on the infectivity of the parasite, but infectivity was lost when irradiation was at a dose of 0.55 kGy [6]. The present study revealed that the MED to control infectivity of the Chinese NT strain and the US ME-49 and TS-2 strains of *Toxoplasma* cysts in pig tissue (including brain, heart, liver, spleen, kidney, diaphragm and muscle) was 0.60 kGy (Tables III and IV). Determination of infectivity was based on bioassay and, in part, on IHA testing. Kittens and mice used
in the experiments were susceptible to *Toxoplasma* infection. Mice and kittens, as controls, were given organisms digested from pig tissue and all developed infections, but those given the MED irradiated tissue did not. The IHA tests in the controls were positive, while titres in the experimental animals were negative. Parasites buried in pork meat subjected to irradiation of 0.60 kGy were also susceptible and failed to infect experimental animals. Repeated experiments provided consistent results, confirming the reliability of the studies. Comparison of radiosusceptibility of strains of *T. gondii* suggested that strains of the parasite from different geographical areas are also susceptible to 0.60 kGy irradiation.

Dubey et al. [1] reported that a dose of 0.5 kGy, using $^{137}$Cs and $^{60}$Co as radiation sources, was able to eliminate infectivity of *Toxoplasma* cysts in a mixture of eight strains (GT-1, TS-1, ME-49, 740-2 and C1, 2, 3, 4). Wikerhauser et al. [7] reported that irradiation of *T. gondii* cysts at a dose of up to 0.5 kGy might not be sufficient to control infectivity. These data differ somewhat from our results (MED: 0.60 kGy). It is uncertain whether this is attributable to the difference of the source of radiation. According to the criterion approved by FAO/IAEA/WHO, the safety dose for irradiation of foodstuffs should be under 10 kGy. The MED in the present study for controlling infectivity of *T. gondii* was only 0.60 kGy, well within the safety range.

ACKNOWLEDGEMENTS

Research was carried out with the financial support of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture under Research Contract No. 4696/RB. The authors wish to thank the International Atomic Energy Agency for supporting this research financially and particularly P. Loaharanu for his encouragement. The authors also wish to express their gratitude to J.P. Dubey and K.D. Murrell for providing strains of *T. gondii* and valuable instructions.

REFERENCES


IRRADIATION OF FISH TO CONTROL THE INFECTIVITY OF THE LIVER FLUKE

*Opisthorchis viverrini*

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Abstract

IRRADIATION OF FISH TO CONTROL THE INFECTIVITY OF THE LIVER FLUKE *Opisthorchis viverrini*.

The experiment was designed to find the minimal effective dose (MED) of irradiation that can inhibit the maturation of *Opisthorchis viverrini*. The parasite life-cycle was established and maintained in the laboratory and the animal model for bioassay was investigated. The hamster was found to be the appropriate model and was used in all experiments. Pure metacercariae (metacercariae dissected from fish before irradiation) were exposed to 50, 100, 200, 300 and 500 Gy. No physical change was observed in the exposed metacercariae. The MED was found to be 100 Gy. When whole infected fish were irradiated and metacercariae fed to hamsters, the MED was also 100 Gy. An organoleptic test was performed by a panel of 19 members to evaluate odour and taste of Koi Pla dishes prepared from irradiated and non-irradiated fish. The only difference that the panel could detect was the stronger odour of the dish made from irradiated fish. It was concluded that the MED for fish to inhibit development of *O. viverrini* metacercariae is 100 Gy and the food prepared from the irradiated fish should be palatable to humans. The applicability of irradiation as a supplementary measure to control liver fluke infection is discussed.

1. INTRODUCTION

Fresh raw food infected with parasitic materials is a problem worldwide. Several diseases of parasite origin have high morbidity. Opisthorchiasis, gnathostomiasis and cysticercosis are good examples of food-borne diseases caused by eating raw fish and pork, and produce high morbidity. Angiostrongyliasis and trichinosis can be fatal to those who have eaten raw Pila snails or wild boar. Attempts have been made to prevent these parasitic diseases by proper food cooking.
Nevertheless, measures such as boiling, grilling and frying may not be accepted by people who prefer the taste of raw meat. This is very true in the northeast of Thailand, where the indigenous population is very fond of raw fish. Carp caught in the rice fields, chopped and mixed with spices and eaten with glutinous rice, a dish called ‘Koi Pla’, is a common food consumed almost daily, at every festival or whenever people get together. Another common dish is ‘Som Pla’ in which the fish is mixed with boiled rice, salt and pepper, and left overnight before consumption. Suggestions to cook the fish either by grilling or frying were rejected on grounds of the taste, and because such cooking is not considered part of the local culture or tradition.

In Thailand, irradiation has been used in fish preservation and fish sterilization with success [1, 2]. However, the technique is aimed at the control of bacterial contamination. Studies on the inhibition of helminthic maturation or destruction of infective stages have so far not been elaborated. Among the various parasitic infections, trichinosis has drawn the most attention, while infections in fish which are consumed regularly by a large number of people, particularly the rural poor, have received less attention.

This study was designed to investigate the usefulness of irradiation on freshwater fish harbouring metacercariae of Opisthorchis. Should the treatment of infected fish by irradiation prove effective, it could be applied to the control of liver fluke infection in Thailand and elsewhere.

2. OBJECTIVES

(1) To find the minimal effective dose (MED) of irradiation that can inhibit the development of Opisthorchis viverrini metacercariae in fish.
(2) To test the organoleptic properties of the dish prepared from irradiated fish.

3. MATERIAL AND METHODS

This study was divided into three phases.

3.1. Phase 1: Preparation of parasitic materials for study

This stage included the establishment of the O. viverrini life cycle in the laboratory and identification of good laboratory animals that could be used to test the viability and infectivity of metacercariae.
3.1.1. Culture of Bithynia snails

Mature *B. siamensis goniomphalus* and *B. siamensis siamensis* were collected from the field and reared in a plastic aquarium six inches wide, ten inches long and four inches deep. Diatoms from a culture were used as food. One inch square plastic sheets were floated on the water surface for snails to lay eggs on. The eggs on the plastic sheets were harvested and transferred into another aquarium for baby snails to hatch out. After four weeks the baby snails hatched from the eggs and they were fed diatoms. When they were 4-5 mm in size, they were exposed to cercariae of *O. viverrini*.

3.1.2. Maintenance of the *O. viverrini* life cycle

*Opisthorchis viverrini* eggs collected from the stools of patients were washed employing the sedimentation technique, then transferred to a small Petri dish for infection of snails. The two species of *Bithynia* snails were tested for their susceptibility to *O. viverrini* by exposure to 12-15 eggs for a period of 3-4 hours or until all the eggs had been eaten. The infected snails were kept for six weeks and then were checked 2-3 times a week for shedding of cercariae. It was found that the prepatent period of infection in snails ranged between 60 and 65 days; *B.s. siamensis* had a higher infection rate (7-12%) than *B.s. goniomphalus* (1-7%). *B.s. siamensis* was subsequently chosen as the experimental snail host for maintenance of the life cycle of *O. viverrini* in the laboratory.

Young cyprinoid fish, Pla Ta Pien, Pla Tapak, Pla Nil and Pla Siew, 3-4 inches in length, were bought from a fish farm. They were exposed individually to 50-100 newly shed cercariae and kept in aquaria. After a few weeks, the fish were killed and the flesh examined for the presence of *O. viverrini* metacercariae. It was found that Pla Tapak had the highest infection rate (30-32%). This fish was selected as the source to produce metacercariae for this study. However, sometimes the fish were not available from the fish farm, therefore, Pla Kao, Pla Mae Sadang and other cyprinoid fish were bought from the endemic area in Khon Kaen Province and also used as sources of metacercariae.

3.1.3. Selection of laboratory animals for bioassay

Hamsters, rabbits and cats were tested for their susceptibility to *O. viverrini* infection by feeding them with metacercariae from fish. It was found that all animals were susceptible to infection. However, the hamster had advantages over the other

---

1 inch = 2.54 cm.
two animals in this experiment, being small, easy to handle, and relatively inexpensive to purchase. With 50 metacercariae per hamster, the prepatent period was 30 days and the average worm recovery was 40%. It was then decided to use hamsters to determine the infectivity of irradiated metacercariae.

3.2. Phase 2: Determining the MED that can inhibit the development of metacercariae

3.2.1. MED of pure metacercariae

Pure metacercariae of *O. viverrini* or metacercariae dissected from the flesh of infected fish were exposed to irradiation from $^{60}$Co at doses of 0, 100, 200, 300 and 500 Gy. The irradiation treatment was performed in the laboratory of Atomic Energy for Peace, Bangkok. After the treatment, all the metacercariae, including the non-irradiated ones, were taken back to the faculty laboratory and fed to hamsters by stomach tube, 46 metacercariae per hamster. The infectivity of each dose was tested in a group of ten hamsters and each experiment was done in triplicate. The animals were kept for five weeks, at which time the control groups were also positive for eggs in stools. Then all animals were sacrificed and the livers and gall-bladders were examined under a dissecting microscope for the presence of adult *O. viverrini*.

3.2.2. MED of metacercariae in irradiated whole fish

In this experiment, whole fish which were already known to have infections were exposed to irradiation with doses of 0, 100, 200, 300 and 500 Gy and then dissected. Metacercariae were recovered from the dissected fish and fed to the hamsters in the same manner as in the earlier experiments. Each dose was tested in a group of ten hamsters, each of which received 46 metacercariae, and the tests were performed in triplicate. All animals were sacrificed five weeks post infection and were examined for the presence of adult worms and eggs in the livers and gall-bladders.

Results of this experiment indicated that there may have been an experimental error. Therefore, another experiment was set up to confirm the MED by repeating the doses and lowering the exposure dose to 50 Gy.

3.3. Phase 3: Organoleptic testing

After the MED of irradiation was confirmed, an organoleptic test to check the difference in taste and odour of the food prepared from the irradiated fish was organized. A two sample test and a double blind technique were employed in this study.
Two portions of Koi Pla were prepared by the same cook; one with fish irradiated at 100 Gy and the other with non-irradiated fish. Apart from the irradiation, the two portions of fish were treated similarly in terms of freshness; i.e., the period between death and making the food. The two portions were coded and the cook, the organizer and the panel members did not know which one had been irradiated. After the test, the dishes were decoded and the result was analysed. Of the 19 panel members who were asked to evaluate the odour and taste, ten were from the northeast region of the country and were familiar with this dish.

TABLE I. NUMBER OF ADULT *Opisthorchis viverrini* RECOVERED FROM 30 HAMSTERS FED 46 NON-IRRADIATED METACERCARIAE EACH

<table>
<thead>
<tr>
<th>Hamster No.</th>
<th>No. of <em>O. viverrini</em> recovered</th>
<th>Hamster No.</th>
<th>No. of <em>O. viverrini</em> recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>24</td>
<td>17</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>25</td>
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</tr>
<tr>
<td>11</td>
<td>19</td>
<td>26</td>
<td>15</td>
</tr>
<tr>
<td>12</td>
<td>17</td>
<td>27</td>
<td>19</td>
</tr>
<tr>
<td>13</td>
<td>20</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>14</td>
<td>18</td>
<td>29</td>
<td>20</td>
</tr>
<tr>
<td>15</td>
<td>33</td>
<td>30</td>
<td>19</td>
</tr>
</tbody>
</table>

Total

Mean and standard deviation 18.6 ± 4.2

*a* This group was used as the control for the next two experiments.
4. RESULTS

4.1. MED of irradiated pure metacercariae

4.1.1. Control non-irradiated metacercariae

All 30 hamsters fed metacercariae (46 per hamster) developed infections. Eggs of *O. viverrini* were found in their faeces after 30 days. The average number of adult worms recovered was 18.6 ± 4.2 (Table I). The livers of the infected animals became enlarged, with dilation of bile ducts. Adult worms were found in the bile duct and the main bile canaliculi in the livers. Eggs of *O. viverrini* were also found in the gall-bladders of these animals.

4.1.2. Irradiated pure metacercariae

Metacercariae recovered from the flesh of fish were irradiated at doses of 100, 200, 300 and 500 Gy and then examined microscopically for the immediate effects of irradiation. There were no significant changes in the movement and colour of gall-bladders of the larvae in the cysts. Neither was degeneration of other internal structures of the larvae observed in these metacercariae.

The irradiated and non-irradiated metacercariae were fed to hamsters. All animals were kept for five weeks and then sacrificed. No young or mature *O. viverrini* worms were found in the hamsters (Table II). No changes in the gross

### TABLE II. INFECTIVITY OF *Opisthorchis viverrini* METACERCARIAE DISSECTED FROM FISH AND EXPOSED TO VARIOUS DOSES OF IRRADIATION

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>No. of hamsters fed 46 metacercariae each</th>
<th>Hamsters infected <em>(O. viverrini</em> worms/hamster)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10 × 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>10 × 3</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>10 × 3</td>
<td>0</td>
</tr>
<tr>
<td>300</td>
<td>10 × 3</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>10 × 3</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Groups of ten hamsters were used; the experiment was repeated three times.

<sup>b</sup> Mean and standard deviation = 18.6 ± 4.2.
pathology of the liver were seen in the irradiated groups, though a few blackish particles were found in the gall-bladders of some hamsters. When these particles were crushed and examined under the microscope, no worm or egg fragments or debris were identified.

In contrast, all hamsters in the group receiving non-irradiated metacercariae were found to harbour adult *O. viverrini* (Table III). The number of recovered adult worms ranged from 12 to 33 per animal, with an average of 18.7 ± 4.2. The gross pathology of the livers and gall-bladders of this group is described above.

### 4.2. MED of metacercariae from irradiated whole fish

When metacercariae dissected from fish which had been exposed to 0, 100, 200, 300 and 500 Gy were examined for the immediate effects of irradiation, no significant change in morphology was observed. These metacercariae were fed to hamsters and the infectivity was determined five weeks later.

No adult worms were recovered from hamsters in the groups that were fed with metacercariae exposed to 100 and 500 Gy. In the first experiment, one hamster in each group of animals fed with metacercariae exposed to 200 and 300 Gy was found to harbour one adult worm. In the hamster fed metacercariae irradiated with 200 Gy, adult *O. viverrini* were found in the small bile duct in the liver, and in the hamster fed metacercariae irradiated with 300 Gy an adult worm was found in the gall-bladder.
FIG. 1. Eggs of *Opisthorchis viverrini* from non-irradiated worm.

FIG. 2. Eggs of *Opisthorchis viverrini* from partially irradiated worm.
No significant change in the gross pathology of the liver and gall-bladder was observed in these two positive hamsters. The worms were fixed in formalin, stained with eosin and examined under the microscope. Unfortunately, the worm recovered from the hamster fed metacercariae irradiated with 200 Gy was partly destroyed during the liver examination process. Another worm from the hamster fed metacercariae irradiated with 300 Gy was complete and showed no difference in gross morphology from those developed from non-irradiated metacercariae. The testes and ovaries were fully developed and many eggs were present in the uterus. When the eggs in the upper part of the uterus were carefully examined, it was found that most of them stained pink and the miracidium inside was not well developed. This was in contrast to the eggs in the uterus of a normal worm, which stain blackish brown and in which fully developed miracidia can be seen.

The gall-bladder content of the two positive hamsters was examined and *O. viverrini* eggs were found. However, these eggs were poorly developed and were morphologically different from normal ones. No well-formed miracidium could be seen inside these eggs (Figs 1 and 2).

### 4.2.1. Confirmation of MED for metacercariae from irradiated whole fish

Because the results of the experiment to determine the MED for metacercaria from irradiated whole fish showed that two worms recovered after exposure to irradiation doses of 200 Gy and 300 Gy, the MED had to be confirmed. The metacercariae from whole fish irradiated with doses of 100 Gy and 200 Gy did not develop into adult worms in experimental hamsters. When the irradiation dose was 50 Gy, 80% of the hamsters were found to be infected. However, the mean worm recovery per hamster from the exposed groups was 4.36 ± 2.4 and 3.7 ± 2.0, whereas the equivalent value for the non-irradiated groups was 19.4 ± 4.4 (Table IV).

These results confirmed that the MED of irradiation for whole fish was 100 Gy.

### 4.3. Organoleptic test

The results of the organoleptic test are shown in Table V. Thirteen of 19 (68.4%) panel members detected a difference in odour between the dishes prepared from the irradiated fish and the non-irradiated fish. Eleven of them reported that the portion made with irradiated fish had a stronger fish smell; two panel members said it was weaker.

Only 16 of the 19 panel members tasted the food. Nine (56.3%) panellists said the taste of both dishes was similar and seven (43.7%) said there was a difference. Of these seven, three said the dish made with irradiated fish tasted stronger, while four said it tasted weaker.
TABLE IV. CONFIRMATION OF MINIMAL EFFECTIVE DOSES OF IRRADIATION FOR *Opisthorchis viverrini* METACERCARIAE IN WHOLE FISH

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>No. of hamsters fed 46 metacercariae each</th>
<th>No. and % of hamsters infected</th>
<th>No. of worms recovered (Range)</th>
<th>Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>10 (100%)</td>
<td>194 (12-26)</td>
<td>19.4 ± 4.4</td>
</tr>
<tr>
<td>50</td>
<td>15</td>
<td>12/15* (80%)</td>
<td>50 (1-9)</td>
<td>4.16 ± 2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>100</td>
<td>10</td>
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</tr>
<tr>
<td>200</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Two hamsters in this group had abnormally small bile ducts.

TABLE V. ORGANOLEPTIC TEST OF KOI PLA PREPARED FROM IRRADIATED AND NON-IRRADIATED FISH

<table>
<thead>
<tr>
<th></th>
<th>No. of panel members</th>
<th>Rated similar</th>
<th>Rated different</th>
<th>Rated irradiated stronger*</th>
<th>Rated irradiated weakerb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour</td>
<td>19</td>
<td>6 (31.6%)</td>
<td>13 (68.4%)</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Taste</td>
<td>16</td>
<td>9 (56.3%)</td>
<td>7 (43.7%)</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

* Stronger odour/taste than dish prepared with non-irradiated fish.
** Weaker odour/taste than dish prepared with non-irradiated fish.

5. DISCUSSION

It is obvious that irradiation can control development of liver flukes in fish. With an irradiation dose of 100 Gy, pure metacercariae and metacercariae in the flesh of fish do not continue their development. At this low dose, and as high as 500 Gy, no morphological changes in metacercariae were seen. Bladder movement,
which is usually a sign of the vitality of metacercariae, was normal. This experiment
did not attempt to study the dose that would stop the motility of larvae inside metacer-
cariae. Its objective was limited to finding the effective dose of irradiation to inhibit
maturation of the parasite..

No worms were recovered from hamsters fed metacercariae irradiated with a
dose of 100 Gy. However, two hamsters were found to harbour adult *O. viverrini*
after being fed metacercariae irradiated with doses of 200 and 300 Gy. It was felt
that this was probably due to a technical error or a problem with the source of irradiation.
The evidence indicated some type of contamination during that phase of the
experiment. The observation of pinkish and undeveloped eggs in the uterus of the
fluke and in the gall-bladders of hamsters in the 300 Gy irradiated group indicated
that this fluke was partially exposed to irradiation. The dose may not be enough to
inhibit maturation, but it probably had an effect on the gonadal tissues, which are
known to be especially sensitive to irradiation [3]; irradiated flukes produce imma-
ture or poorly developed eggs. This question was resolved by repeating the experi-
ments. The results of the repeated experiments confirmed the MED for irradiation
of whole fish to be 100 Gy.

Irradiation of *O. viverrini* or other species of *Opisthorchis* flukes has not been
previously documented. The nearest species with documented irradiation studies is
the sheep liver fluke, *Fasciola hepatica*. After exposure to an X ray dose of
19–28 Gy, the cysts of this parasite were unable to develop into adult worms [3, 4].

Research is being undertaken by a group of scientists in the Joint FAO/IAEA
Division of Nuclear Techniques in Food and Agriculture. Preliminary data has indi-
cated that the MED for metacercariae of *Clonorchis sinensis* in China and Korea is
0.05 and 0.2 kGy, respectively [5]. Based on this information, it can be concluded
that the MED for the fluke genus *Opisthorchidae* would not exceed 200 Gy. To com-
plete the information, another study is needed to find the MED of *O. felineus*, the
*Opisthorchis* species common in Russia. It is highly prevalent in that area, with rela-
tively mild clinical manifestations.

The MED to inhibit the development of *Opisthorchis* larvae at the level
≤ 200 Gy is very low in comparison to the usual dose of irradiation to preserve fish
for commercial purposes. Irradiation at the maximum dose of 2200 Gy in cod and
red fish is approved by a WHO/FAO/IAEA Expert Committee recommendation. It
is also reported in Thailand that irradiation with 2000 and 5000 Gy gave the best
result for preservation of club mackerel without an off-flavour [2].

Sensory evaluation of irradiated fish may be another problem that needs to be
considered. In this study, the organoleptic test of odour and taste was performed with
a panel of 19 people. Thirteen reported a difference in odour and the majority
expressed an opinion that the irradiated fish had a stronger smell. This information
seems to be reliable; however, Koi Pla is flavoured with strong spices and it may
be difficult to distinguish between foods that have a strong smell. Additionally, some
people prefer a mild fish smell in their food, while others prefer a stronger aroma.
When the taste of the two preparations was compared, more than half the panel members said it was similar. Among those who said there was a difference, there was no clear opinion as to which dish was superior. The panelists who lived in the northeast were further questioned about the Koi Pla dish, and they all agreed that the dish made from irradiated fish would probably be acceptable and palatable to most of the people in that region of Thailand. They said that other fish dishes they prepared at home smelled even stronger than the experimental fish.

With the information that infected cyprinoids irradiated at a low dose cannot transmit the liver fluke infection, irradiation should be used as a supportive measure to control this infection. Fish can be irradiated before being put on sale in the market. The transmission of liver fluke would be controlled and people could enjoy the taste of raw fish without the risk of a parasitic infection. It could be a control technique where a source of infection is smoked fish, as in Russia. However, if this measure is to be applied to the endemic area, there will be problems of applicability, especially with Koi Pla or the popular raw fish dish in the northeast of Thailand and Laos. This dish is made from freshly caught fish from any nearby waterbed and people prefer to eat it immediately after preparation, although it can be prepared and eaten elsewhere, for instance in the rice field or at home. Because of the widespread availability of fish and the practice of immediate preparation, housewives will not find it acceptable to have to bring fish from the field and put it under an irradiation source before making Koi Pla. Besides, Koi Pla made from cold preserved fish is not acceptable to the villagers. Though it may not be practical to implement irradiation of fish for Koi Pla in Thailand, it may have a place in another common dish, Som Pla, which is made from the same kind of fish as Koi Pla. Fresh cyprinoid fish are cleaned, mixed with boiled rice and a little salt and red pepper, and are usually eaten after one or two nights of fermentation. This dish is also popular among the villagers in northeast Thailand. It was formerly prepared at home, but because of its popularity, it is now being made commercially. Recently, the fish market of Ubolratna Dam in Khon Kaen and Nong Harn Lake in Sakolnakorn made the preparation of Som Pla into a small industry and the product is being marketed throughout the country. It seems possible that irradiation of fish could be successfully implemented in this business; a small mobile irradiation plant could be constructed near the sites to irradiate the fish before it is processed and distributed.

In conclusion, irradiation of fish has been shown to inhibit the development of *O. viverrini* metacercariae and should be acceptable to the general population. The use of small mobile irradiation plants located at fish markets and fishing piers in the northeast should be tested as a control measure for liver fluke infections in Thailand.
ACKNOWLEDGEMENTS

The authors wish to thank the IAEA for financial support of this research and particularly P. Loaharanu for his suggestions and encouragement. This research could not have achieved its purpose without the co-operation and assistance received from the laboratory of Atomic Energy for Peace, Thailand. Our sincere gratitude and thanks go to the Secretary General of this organization. Thanks are also extended to the technicians at the Department of Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, who helped us conduct this study.

REFERENCES


EFFECT OF IRRADIATION USING $^{60}\text{Co}$ ON *Trichinella spiralis* LARVAE AND ON THE EGGS AND PROTOSCOLICES OF *Echinococcus granulosus*

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Pan American Zoonoses Centre

N. KAUPERT  
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Buenos Aires, Argentina

Abstract

EFFECT OF IRRADIATION USING $^{60}\text{Co}$ ON *Trichinella spiralis* LARVAE AND ON THE EGGS AND PROTOSCOLICES OF *Echinococcus granulosus*.

The effect of irradiation using $^{60}\text{Co}$ on *Trichinella spiralis* larvae and on the eggs and protoscolices of *Echinococcus granulosus* was studied. A dose of 30 Gy had no effect on *T. spiralis* larvae and on the eggs and protoscolices of *E. granulosus*. Irradiation of free *T. spiralis* larvae from muscle in normal saline exposed to 90 Gy and higher doses produced loss of infection in mice. There was reduction in the infectivity of eggs and protoscolices of *E. granulosus* by irradiation at a dose of 120 Gy, while there was complete loss of activity at a dose of 500 Gy.

1. INTRODUCTION

Irradiation of parasitic protozoa and helminths can cause loss of infectivity, loss of pathogenicity, interruption or prevention of completion of the life cycle and/or death of the parasite. This technology can be used as a tool to control parasitic diseases transmitted by food. However, before application of irradiation in control programmes, it is necessary to define precisely the irradiation dose which would arrest the development of the parasite without altering the quality of the food. In the present work the effect of irradiation on *Trichinella spiralis* larvae and on *Echinococcus granulosus* eggs and protoscolices was studied.

2. MATERIALS AND METHODS

Larvae of *T. spiralis* obtained from the muscle of infected pigs were experimentally administered to rats and the infection was maintained in the labora-
tory. The rats were killed after two months and larvae obtained from them were irradiated at different dose levels, and subsequently given to laboratory mice in doses of 500 irradiated larvae. Controls were also used. The mice were sacrificed after one and a half months to assess the development of the infective stage.

Dogs were experimentally infected with protoscolices of *E. granulosus*. Eggs of the parasite were obtained from dogs sacrificed 90 days post infection. The eggs, as well as protoscolices, were subjected to irradiation. Normal and irradiated eggs were given orally to mice which were sacrificed 45 days post infection. Normal and irradiated protoscolices were also inoculated intraperitoneally into mice which were sacrificed after 60 days to determine the development of the cyst.

The parasitic material was suspended in 2 mL of normal saline and irradiated with gamma rays at doses of 30, 60, 90, 120, 300 and 500 Gy using Co as the source of irradiation. The dose rate used was 36.17 Gy per minute and Fricke dosimetry was used to measure the dose from 60 to 300 Gy. Irradiation facilities of the National Atomic Energy Commission of Argentina at Buenos Aires were used.

3. RESULTS

The results of the study are shown in Tables I and II. Irradiation of *T. spiralis* larvae at a dose of 30 Gy had no effect on the number of animals which became infected. There was a significant reduction in the number of animals that became infected with larvae irradiated at a dose of 60 Gy. None of the animals which received larvae of *T. spiralis* exposed to 90 Gy and above became infected.

<table>
<thead>
<tr>
<th>Co-60 irradiation dose (Gy)</th>
<th>Proportion of animals infected</th>
<th>Percentage of animals infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10/10</td>
<td>100</td>
</tr>
<tr>
<td>30</td>
<td>10/10</td>
<td>100</td>
</tr>
<tr>
<td>60</td>
<td>3/9</td>
<td>33</td>
</tr>
<tr>
<td>90</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>120</td>
<td>0/7</td>
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</tr>
<tr>
<td>300</td>
<td>0/9</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>0/8</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE I. DEVELOPMENT OF INFECTION IN MICE AFTER EXPERIMENTAL INFECTION WITH CONTROL AND IRRADIATED *Trichinella spiralis* LARVAE
TABLE II. DEVELOPMENT OF INFECTION IN MICE AFTER EXPERIMEN­TAL INFECTION WITH CONTROL AND IRRADIATED Echinococcus granulosus EGGS AND PROTOSCOLICES

<table>
<thead>
<tr>
<th>Co-60 irradiation dose (Gy)</th>
<th>Proportion of animals infected</th>
<th>Percentage of animals infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. granulosus eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9/9</td>
<td>100</td>
</tr>
<tr>
<td>30</td>
<td>10/10</td>
<td>100</td>
</tr>
<tr>
<td>60</td>
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There was no significant effect on the eggs of E. granulosus irradiated at a dose of 120 Gy. There was a significant reduction in development at a dose of 300 Gy and there was a loss of infectivity at 500 Gy. As regards E. granulosus protoscolices, there was a significant reduction at 90 to 120 Gy, and higher doses killed the protoscolices.

4. DISCUSSION

In vivo studies on the irradiation of T. spiralis infected pork, using rat and mouse bioassay procedures, have reported that 150 Gy of gamma irradiation were sufficient to interrupt the intestinal maturation of the larvae [1]. Other in vivo studies have shown that 100 to 600 Gy of irradiation were effective in mouse bioassay and 100 to 200 Gy in rat bioassay [2]. In the present study, the T. spiralis larvae were
irradiated in vitro, and the larvae were digested free from rat muscle. Under these conditions, the radiosensitivity was lowered to a dose of 60 to 90 Gy. This variation may be due to liberation of larvae and exposure in normal saline solution as compared to muscle tissue. However, the results confirm that irradiation has a lethal effect on *T. spiralis* larvae.

In 1986, the Food Safety and Inspection Service of the United States of America added irradiation to the approved list of treatments for pork. This permits the use of a $^{60}$Co gamma radiation source to control *T. spiralis* in pork carcasses or in fresh or previously frozen cuts of pork that have not been cured, ground or heat processed [3]. However, the use of irradiation facilities in commercial operations to process infected pork on a large scale has yet to be demonstrated. Therefore, it will be necessary to make a cost–benefit analysis before its application in control programmes. Moreover, if the cost–benefit analysis is found favourable, this method could be practical in places where there are organized pig slaughtering facilities.

With respect to hydatidosis, the eggs of *E. granulosus* are present on green vegetables as a result of contamination. In hyperendemic areas where chances of infection due to contamination of green vegetables are high, irradiation of vegetables at high doses of 300 to 500 Gy can possibly prevent infection. However, before recommending this procedure, it would be necessary to see the effect of high doses on green vegetables. Moreover, this practice would need to be combined with other control methods.

The lethal effect of higher doses of irradiation of *E. granulosus* scolex can be used in the prevention of infection in dogs through the hydatid cyst present in the viscera. However, before using irradiation in practice, it would be necessary to evaluate its effect on the protoscolices contained within hydatid cysts in different infected viscera.

REFERENCES


IRRADIATION TO CONTROL INFECTIVITY OF *Toxoplasma gondii* IN MURINE BRAINS AND EDIBLE PORCINE TISSUES

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Abstract

IRRADIATION TO CONTROL INFECTIVITY OF *Toxoplasma gondii* IN MURINE BRAINS AND EDIBLE PORCINE TISSUES.

The effect of irradiation on the infectivity of *Toxoplasma gondii* tissue cysts was studied. The tissue cysts were produced in brains of mice and in edible tissues of pigs by artificial infection with oocysts of one or more different isolates of *T. gondii*. The cyst-harbouring tissues were irradiated with X rays or gamma rays at doses ranging from 0.3 to 1.0 kGy (30 to 100 krad). The source of irradiation was either a Philips X ray machine or $^{60}$Co. The results were assessed by bioassays on cats and/or mice. Some slight differences in radiosensitivity of geographically different isolates were observed. For instance, a complete inactivation of a local isolate YU TG No. 3 was achieved only after irradiation with 0.7 kGy, whereas for the same effect on the infectivity of a US isolate (ME-49) and a Chinese one (NT), irradiation with 0.4 and 0.5 kGy, respectively, was sufficient. At sublethal doses, a sharp decrease of infectivity was observed.

1. INTRODUCTION

Latent natural infection of *Toxoplasma gondii* has been demonstrated by numerous authors. Because the ingestion of raw or undercooked meat products infected with viable *T. gondii* represents one of the potential sources of toxoplasmosis in man and animals, a method of controlling the infectivity of tissue cysts without altering the main properties of edible tissues could be very useful. Dubey et al. [1]
studied the effect of irradiation on the viability of *T. gondii* in the tissues of mice and pigs and concluded that cysts in murine brains irradiated with 0.5 kGy (50 krad), and those in porcine tissues irradiated with 0.25 or 0.3 kGy (25 or 30 krad) were no longer infective to mice and cats, respectively. We have repeated some of their experiments and have extended them to different isolates.

2. MATERIAL AND METHODS

Local isolates of *T. gondii* oocysts were collected from cats fed on raw porcine diaphragms. After sporulation in 2% potassium bichromate the oocysts were stored at 4°C until used. Beside local isolates TG No. 1, TG No. 2 and TG No. 3, the US ME-49, and the Chinese NT, kindly provided by J.P. Dubey in Beltsville, Maryland, United States of America and Chang-Cun Song in Hangzhou, China, respectively, were studied.

Experimental animals were specific pathogen free (SPF) white Balb-c or grey CBA mice, and young 8–10 week old white pigs. For the production of tissue cysts mice were infected with oocysts either orally or subcutaneously, and pigs orally only. Doses of oocysts were 100 per mouse and 1000 to 10 000 per pig. Mice were killed 4–6 weeks after infection and pigs after 6, 8 or 9 weeks. Prior to irradiation murine brains were checked microscopically for the presence of *T. gondii* tissue cysts. Porcine edible tissues collected for irradiation included heart, tongue, diaphragm, gluteal muscles and brain.

For irradiation the murine brains were pooled and the porcine samples pooled and minced in a meat grinder. The source of irradiation was either a Philips 250/30 X ray machine 210 kV, 15 mA, or 60-Co at a dose rate of 0.157 kGy/min. The doses of X or gamma irradiation ranged from 0.3 to 1.0 kGy (30 to 100 krad).

The results were assessed by bioassays on cats and/or mice. The young SPF cats were fed irradiated or sham irradiated samples and their faeces periodically examined for *T. gondii* oocysts. The white Balb-c or grey CBA mice were injected subcutaneously with homogenized murine brains, or with HCl-pepsin homogenized porcine tissues, each mouse receiving 1 mL of the homogenate suspended in sterile saline and antibiotics. Each sample was fed to one or two cats and/or injected into six to twelve mice. The cat feeding test followed the principles described by Dubey and Streitel [2] and the mouse inoculation test those described by Dubey et al. [3]. The injected mice were killed 4–6 weeks after inoculation and their brains checked microscopically for tissue cysts.

3. EXPERIMENTAL SERIES AND RESULTS

After a survey of porcine and ovine diaphragms for viable toxoplasmas [4] in which three out of ten pools of porcine and four out of ten pools of ovine samples
were found positive, the effect of X irradiation on the infectivity of *T. gondii* cysts in murine brains was studied [5]. In the first experiment, performed with the isolate TG No. 1, the cyst-harbouring brains were X irradiated with 0.3 and 0.5 kGy, but neither dose was sufficient to inactivate the parasites. In the second experiment, performed with the second isolate, the infected murine brains were irradiated with 0.5 and 0.7 kGy, but no viable toxoplasmas were subsequently demonstrated. In the second year of the Research Contract, the effect of X irradiation on the infectivity of *T. gondii* cysts in the flesh of experimentally infected pigs was studied [6]. Three young pigs were orally infected with 10,000, 10,000 and 1000 oocysts, respectively, of a new local isolate (TG No. 3). One pig soon died of acute toxoplasmosis (confirmed at autopsy) and the remaining two were slaughtered after six and nine weeks, respectively. Their tissues were X irradiated with 0.3, 0.5, 0.7 and 1.0 kGy. Samples irradiated with 0.3 and 0.5 kGy regularly produced *T. gondii* oocysts in cat feeding tests and tissue cysts in mouse inoculation tests, whereas samples irradiated with 0.7 and 1.0 kGy did not. These results, compared with our previous ones [5], and especially with those of Dubey et al. [1], showed some differences in radiosensitivity of different *T. gondii* isolates. Thus, in the third year of the Research Contract the radiosensitivity of three geographically different isolates (TG No. 3, from Yugoslavia, ME-49, from the USA, and NT, from China) was compared under identical laboratory conditions, the source of irradiation being $^{60}$Co instead of a Philips X ray machine. The effect of gamma irradiation was studied on infected murine brains and the doses were 0.4, 0.5, 0.6 and 0.7 kGy. The results were assessed by bioassays on cats and/or mice. Complete inactivation of the TG No. 3, NT and ME-49 isolates was achieved with 0.7, 0.5 and 0.4 kGy, respectively [7]. At sublethal doses a sharp decrease in infectivity was observed. In the fourth and final year of the Research Contract the effect of gamma irradiation of the two more resistant isolates (TG No. 3 and NT) was studied on porcine edible tissues. For that purpose, two young pigs were orally infected with 5000 oocysts of the TG No. 3 or NT isolate and were slaughtered eight weeks later. Their minced meat and brain samples were irradiated with previously established border doses, i.e. 0.6 and 0.7 kGy for TG No. 3, and 0.5 and 0.6 kGy for NT isolate. The results were assessed by cat-feeding and/or mouse-inoculation tests. The results confirmed those obtained earlier on mice: complete inactivation of isolate TG No. 3 and isolate NT was achieved with 0.7 and 0.5 kGy, respectively. These presumably isolate-related differences in radiosensitivity should be considered in recommendations made for the control of infectivity of *T. gondii* tissue cysts by irradiation in food.

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