

Research Note

**Sterilization of a Bundle of Rice Straw for Killing
Fasciola Metacercariae by Ammonia Fumigation**

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Fascioliasis caused by *Fasciola* sp. is a serious problem in Japan. This disease occurs in animals that feed on rice straw contaminated with metacercariae of liver fluke in paddy fields. Thus, inactivation of metacercariae cysted on rice straw is considered to be an important means to prevent *Fasciola* infection. In a study on this subject, Ueno *et al.* (1978) examined the survival time of metacercariae cysted on rice straw under the natural environment after harvest and reported that the majority of metacercariae had died by January or February of the following year. Under general conditions, however, farmers may find it difficult to stock a large quantity of rice straw over an extended period of time.

In recent years growing evidence has shown that ammonia treatment of rice straw is an effective technique for making the straw more palatable and digestible for ruminants (Waiss *et al.*, 1972; Tohrai *et al.*, 1978; Horton and

Stacey, 1979; Yahara *et al.*, 1981; Kamo and Nakagawasai, 1985). Moreover, from parasitologic point of view, the treatment provides an excellent disinfectant against helminthic eggs. Suzuki (1923) proved that metacercariae of *Fasciolopsis buski* were killed by immersing them in human urine for 4 days. These results suggest that ammonia may also be involved in killing metacercariae.

Therefore, an attempt was made to kill metacercariae of *Fasciola* sp. in a bundle of rice straw by ammonia treatment under laboratory conditions.

Cercariae emerged from *Lymnaea ollula* snails experimentally infected with *Fasciola* sp. in a polyethylene bag (13 × 25 cm) containing cold water and cysted zonally on the wall of the container. Sectional sheets were prepared by cutting the metacercarial zonation on the wall.

Then several pieces of sheets with encysted metacercariae were placed in 1.0 kg of rice straw (about 55% moisture). Each bundle was put into a polyethylene bag (40 × 60 cm) containing a piece of absorbent cotton on the bottom to absorb the liquid ammonia, which measured 120 ml of NH₄OH (NH₃ 25%, Wako Junyaku Co.) as 3.0% ammonia by weight (group A). As 1.5% ammonia, 60 ml of NH₄OH and an equal volume of distilled water were added to another bag containing another bundle of straw (group B). Only 120 ml of distilled water were poured into a control bag (group C). By

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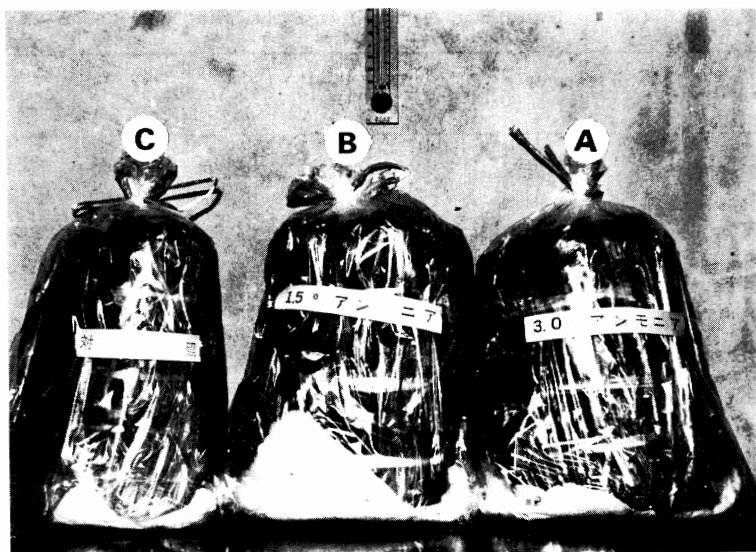


Fig. 1 Treatment of the bundles with ammonia. Bundles A and B were treated with 3.0% and 1.5% ammonia by weight, respectively. Bundle C was the control.

this method the metacercariae in each bundle were sensitized with 3.0, 1.5 and 0% of ammonia for 4 and 7 days at 24.5°C (Fig. 1).

At the termination of the treatment, ammonia concentration in the bundles was determined immediately by an ammonia detecting meter (Kitagawa Co.). At the same time, metacercariae from the three groups were observed morphologically. Twenty-eight ddY male mice, aged 5 weeks, were inoculated with 15 and 30 metacercariae from ammoniated and untreated bundles soon after the observation and sacri-

ficed 21 days later. Before autopsy, blood samples were collected from the animals of three inoculated groups. Procedure for the agar gel diffusion test with sera of mice and extract from larval flukes was the same as described previously (Yoshihara *et al.*, 1979).

When the bundles were treated with ammonia for 4 days, as shown in Fig. 2, the oral and ventral suckers and excretory pores of the metacercariae in the bundles were not clear. In addition to these findings, concretions were dispersed in the metacercariae. In group A, espe-

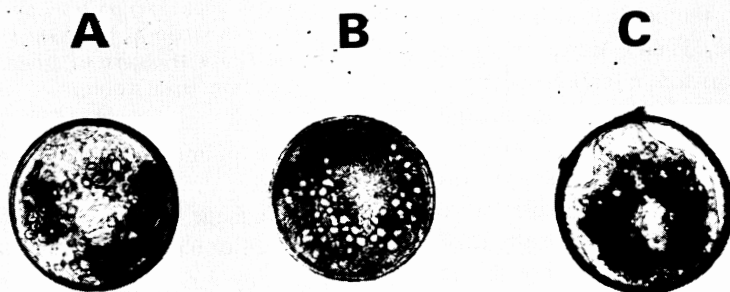


Fig. 2 Morphology of metacercariae in ammoniated and untreated bundles. A was metacercaria in the bundle treated with 3.0% ammonia and B was the same with 1.5% ammonia. C was metacercaria in the control bundle.

Table 1 Effect of ammonia treatment against *Fasciola* metacercariae in bundles of rice straw.

Group	Ammonia concentration (%)	Treatment (days)	Number of metacercariae inoculated*	Number of flukes recovered 21 days later	Ammonia in bundle (%)	AGT†
A	3.0	7	15	0, 0, 0, 0, 0 ‡	ND§	—
		4	30	0, 0, 0, 0	1.7	—
B	1.5	7	15	0, 0, 0, 0, 0	ND	—
		4	30	0, 0, 0, 0, †0	0.9	—
C	0	7	15	2, 1, 0, 1, 4	ND	+
		4	30	12, 13, 5, 7	0.1 <	+

* Metacercariae used were collected from three bundles.

† Agar gel diffusion test.

‡ At autopsy, a yellow spot, about 1 mm in diameter, was found on the surface of the liver.

§ Not done.

cially, black precipitates were observed between the degenerated concretions. In contrast with these observations, almost all the metacercariae in the bundles of the control group were normal and active.

The results of the infectivity of mice with metacercariae from the ammoniated and untreated bundles were outlined in Table 1. Macroscopically, the liver from mice fed metacercariae from the ammoniated bundles was normal, except for that of two animals, and no larval worms were found in the organ. On the contrary, presence of worms in the liver and detectable antibodies in sera of mice may prove the infectivity of untreated metacercariae in the control bag (Fig. 3).

Since the discovery of the antihelminthic effects of carbon tetrachloride and hexachloroethan on *Fasciola hepatica*, many drugs have been used widely for the control of liver fluke in cattle (Ueno *et al.*, 1964). However, cattle are never free from *Fasciola* infection as long as the animal feeds on rice straw contaminated with metacercariae.

From the result of the present study, it was clear that metacercariae in the bundles were completely killed by the treatment with 1.5% ammonia at 24.5°C for 4 days. Although the examination was carried out under laboratory conditions, it was proved that ammonia treatment was extremely effective to kill *Fasciola* metacercariae.

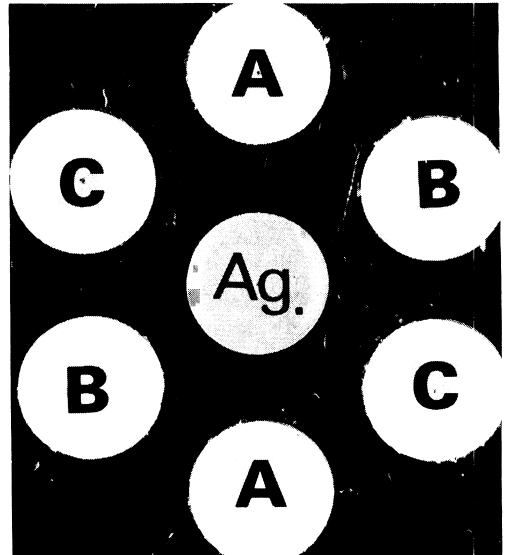


Fig. 3 Agar gel diffusion test.

A was pooled serum from mice fed metacercariae of group A, B was the same of group B and C was the same of group C. Ag was extract from larval worms.

Results of an earlier study have revealed that ammoniated products, which are used as nutritional roughage for ruminants, can be prepared by the addition of 2.5% of ammonia concentration in only 5 days if a temperature of 45°C is maintained (Tohrai *et al.*, 1978). Thus, if metacercariae in bundles of rice straw are sensitized by these conditions, there may be a significant decrease of *Fasciola* infection on a wide scale.

In parallel with the present examination, the same test is presently being carried out in the field by using rice stacks. The results will be reported in future.

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