[Jpn. J. Parasitol., Vol. 45, No. 4, 330–332, August, 1996]

Research Note

# Vaccination of Rats with Frozen Eggs, Ethanol-fixed Eggs and Frozen Oncospheres with or without Embryophoric Blocks of *Taenia taeniaeformis*

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(Accepted August 20, 1996)

**Key words:** *Taenia taeniaeformis*; vaccination; eggs; oncospheres; embryophoric blocks; rats.

Cysticercosis and echinococcosis, caused by ingestion of eggs of taeniid cestodes, are one of the most serious parasitic zoonoses causing economic loss in the livestock and threatening human life. It is well known that mammalian hosts inoculated orally with eggs of these taeniid cestodes become completely immune to reinfection. Egg of taeniid cestodes is identical with oncosphere surrounded with embryophore which consists of embryophoric blocks. The most important immunogenic stage to induce immunity to reinfection is the oncosphere. Although highly effective recombinant vaccine against *Taenia ovis* has been produced based on the fractionated antigens of the oncosphere, it is not always easy to obtain detailed information on such vaccine candidate due to the commercial contract (Johnson *et al.*, 1989; reviewed by Lightowlers *et al.*, 1993).

*T. taeniaeformis*/rat system has been used as one of the good animal model for cysticercosis. In this system, it is known that rats vaccinated with frozen oncospheres as well as those infected with a single oncosphere of *T. taeniaeformis* became completely immune to challenge infection (Ito and Hashimoto, 1993). In the present report, we tried to evaluate the usefulness of frozen eggs and 70% ethanol fixed eggs compared with frozen oncospheres with or without embryophoric blocks using *Taenia taeniaeformis*/rat system, since if eggs are effective vaccine candidates as similar as oncospheres, they may be more useful and economic due to the simple preparation without any further processing of oncospheres without embryophores, especially in developing countries.

Frozen eggs (groups A and E), eggs fixed with 70 (v/v) % ethanol (groups B and F) and frozen oncospheres with (group H) or without embryophoric blocks (groups C and G) of *T. taeniaeformis* were used as vaccine candidates (Table). *In vitro* hatching of oncospheres was carried out according to Lightowlers *et al.* (1984) with a minor modification of the hatching solution. In the original method, eggs were resuspended with 0.5 (v/v) % sodium hypochlorite (NaClO) diluted in distilled water. We used phosphate-buffered saline (PBS, pH 7.4) for preparation of both egg suspension and 1.0% NaClO. Egg suspension in PBS was treated with the same volume of 1.0% NaClO in PBS for five–ten minutes at room temperature. There was no damage of hatched oncospheres kept in the final hatching solution (final 0.5% NaClO in PBS) even for one hr or more. Oncospheres for groups C and G were prepared using Percoll (Pharmacia, Sweden) to remove embryophoric blocks (Rajasekariah *et al.*, 1980), whereas oncospheres with embryophoric blocks for group H were just rinsed with PBS several times to remove NaClO. Eggs for groups A, B, E and F or oncospheres for groups C and G were adjusted to be

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This work was supported in part by grants from the Nissan Science Foundation and the Uehara Memorial Foundation, and a Grant-in-Aid for Scientific Research Program, Joint Research (07044243) from the Ministry of Education, Science, Sport and Culture, Japan, to A. Ito.

Table 1 Vaccine effects of frozen eggs, ethanol-fixed eggs and frozen oncospheres with or without embryophoric blocks of *Taenia taeniaeformis* in rats

| Group | No. of mice infected / No. of mice challenged | No. of metacestodes* Mean±S.D. (range) |
|---|---|---|
| *Experiment 1* | | |
| A (Frozen eggs) | 1/5 | 0.2±0.5 (0–1) |
| B (Ethanol-fixed eggs) | 4/6 | 4.7±5.6[a] (0–10) |
| C (Frozen oncospheres) | 1/6 | 0.2±0.4 (0–1) |
| D (none) | 5/5 | 40±25.6[b] (20–78) |
| *Experiment 2* | | |
| E (Frozen eggs) | 0/5 | 0 |
| F (Ethanol-fixed eggs) | 5/5 | 5.2±4.4[c] (1–12) |
| G (Frozen oncospheres) | 0/5 | 0 |
| H (Frozen oncospheres with embryophoric blocks | 0/5 | 0 |
| I (PBS only) | 5/5 | 40±11.0[d] (27–55) |

All rats for all groups other than group D were injected with the material in parenthesis emulsified with FCA at day 0. All rats were orally challenged with 200 eggs at day 30 and killed at day 60.

*All growing metacestodes in the liver were picked up with forceps and counted (Ito and Hashimoto, 1993).

[a]vs[b], [c]vs[d]: $p<0.001$ (Mann-Whitney U test).

approximately 5,000/0.1 ml of PBS for vaccination. These eggs or oncospheres were kept at –80°C for at least one month and used as frozen eggs (groups A and E) and frozen oncospheres (groups C and G), respectively. Frozen oncospheres with embryophoric blocks (group H) were prepared just before vaccination trial using frozen eggs kept at –80°C at least for one month (Negita and Ito, 1994). Thawed eggs were treated with 0.5% NaClO for a few minutes and rinsed several times with PBS to remove NaClO. Ethanol-fixed eggs, kept in 70% ethanol in PBS for at least one month at room temperature, were rinsed with PBS several times (groups B and F).

Specific pathogen-free, 5-week-old male Wistar rats were used for vaccination experiments. All group rats including group I other than group D were singly injected subcutaneously with Freund's complete adjuvant (FCA) (see Table). All rats, vaccinated subcutaneously with killed materials described above with FCA (all other than group D) or with no treatment (group D) at day 0, and challenged with approximately 200 eggs of *T. taeniaeformis*/0.2 ml of PBS at day 30, were killed at day 60 in order to count all growing metacestodes in the liver (Ito and Hashimoto, 1993). Eggs for challenge infection were prepared from gravid proglottids one day before use and stored at 4°C (Ito and Hashimoto, 1993, Takemoto *et al.*, 1995).

Table shows the vaccine effect of killed materials from two experiments in *T. taeniaeformis*/rat system. Rats injected with frozen eggs (groups A and E) or frozen oncospheres (groups C and G) or frozen oncospheres with embryophoric blocks (group H) showed complete or almost complete protection to challenge infection (>99%), whereas those injected with ethanol-fixed eggs (groups B and F) showed weaker but statistically significant protection (>86%, $p<0.001$, Mann-Whitney U test). Therefore, dead eggs kept in the freezer may be the best vaccine candidate, since we do not need any additional work to do *in vitro* hatching or isolation of hatched oncospheres without embryophoric blocks using Percoll (Rajasekariah *et al.*, 1980).

**Acknowledgements**

This paper is dedicated to Mrs. Keiko Mori, Gifu University School of Medicine, who died by a sudden heart attack on 18th July 1995, lest we forget her great help and smile.

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