# The ovicidal effect of selected chemicals against eggs of *Echinococcus granulosus* *

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*Abstract*

*The ovicidal activity of various chemicals against eggs of* E. granulosus *was evaluated by measuring infectivity to $CF_1$ mice after exposing the eggs to aqueous solutions of the chemicals for 1 h at room temperature. Significant reductions in infectivity were observed in eggs treated with Lugol's iodine, 0.015% ammonium dodecylbenzenesulfonate, 70% ethanol, or 5% or 10% glutaraldehyde. No cysts developed in 10 mice that received doses of 1 000 eggs treated with 5% glutaraldehyde, and only 1 of 10 mice developed cysts after exposure to eggs treated with 10% glutaraldehyde.*

The eggs of *E. granulosus* and those of other taeniids are resistant to a large number of chemical agents. Desiccation (1) and high temperatures (2) are factors that limit egg survival in nature but screening of large numbers of chemical solutions has failed to demonstrate a compound that, when used in practical concentrations, consistently eliminates oncosphere activity and/or infectivity (3-8).

The absence of a practical ovicidal solution for decontamination of laboratory premises and instruments has dictated strict precautionary measures when working with eggs of *Echinococcus* spp. (9), and may have limited the amount of research directed towards these important zoonotic agents.

We report herein the degree of infectivity to laboratory white mice of *E. granulosus* eggs after treatment with selected chemical solutions.

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## *Materials and methods*

Gravid proglottids of *E. granulosus* were obtained from the intestines of experimentally infected dogs, and a suspension of eggs in saline solution was prepared as previously described (10). The egg suspensions were distributed in glass tubes containing 40 000 eggs per tube. After decanting the supernatants, 5 ml of an aqueous solution of each chemical was added and the tubes were placed on a slow-moving rotator (15 r/min) for 60 min at room temperature.

Two trials were carried out. In the first, eggs were treated with 2% Tween 80, sodium hypochlorite (1.5% active chlorine), 2% potassium permanganate, 10% formaldehyde solution, Lugol's iodine, 0.015% ammonium dodecylbenzenesulfonate, or 70% ethanol. In the second trial the eggs were treated with glutaraldehyde at 5 or 10%. In each trial one batch of eggs treated with 0.15 mol/l NaCl was used as the control.

After treatment, the eggs were washed twice and resuspended in saline. Aliquot volumes of 0.15 ml containing 1 000 eggs were fed by stomach tube to 21-day-old female $CF_1$ white mice. The mice were killed 100 days post-exposure and the number of cysts per animal was recorded at autopsy.

## *Results and discussion*

The infectivity of eggs following treatment with the selected chemical solutions is shown in Table 1. In the first trial, highly significant reductions in the number of infected animals as compared with the control group occurred only when the eggs were treated with Lugol's iodine, ammonium dodecylbenzenesulfonate or ethanol, with a $\chi^2$ value of 8.84 for the three combined groups versus the control; no significant differences were found in the effects of the 3 chemicals. In the second trial no cysts developed in 10 mice which received eggs treated with 5% glutaraldehyde, and 1 out of 10 mice developed 4 cysts

Table 1. Infectivity of *E. granulosus* eggs in $CF_1$ white mice after treatment with aqueous solutions of selected chemicals.

| Egg batch | Chemical and concentration used | No. infected mice/total No. mice exposed | Infected animals (%) | Eggs that developed into cysts (%) |
|---|---|---|---|---|
| 1 | control (0.15 mol/l NaCl) | 14/21 | 66.67 | 0.438 |
| | Tween 80, 2 % | 17/22 | 77.27 | 0.390 |
| | sodium hypochlorite, 1.5 % active chlorine | 19/25 | 76.00 | 0.340 |
| | potassium permanganate, 2 % | 13/18 | 72.22 | 0.244 |
| | formaldehyde solution, 10 % | 14/24 | 58.33 | 0.283 |
| | Lugol's iodine | 8/22 | 36.36 | 0.100 |
| | ammonium dodecylbenzenesulfonate, 0.015 % | 6/22 | 27.27 | 0.150 |
| | ethanol, 70° | 6/21 | 28.57 | 0.061 |
| 2 | control (0.15 mol/l NaCl) | 10/10 | 100.0 | 0.740 |
| | glutaraldehyde, 5 % | 0/10 | 0.0 | 0.0 |
| | glutaraldehyde, 10 % | 1/10 | 10.0 | 0.04 |

after receiving eggs treated with 10% glutaraldehyde. Combining both groups, a highly significant difference was obtained with respect to the control group, with a $\chi^2$ value of 25.9.

Theoretically, hydatid disease may develop if only one embryo becomes established. Parnell (6) recommended that before any compound is considered an echinococcal ovicide it must be 100% effective, otherwise it may lead to a false sense of security and neglect of precautionary measures.

The ovicidal effect of chemicals has been tested by an *in vitro* technique (4, 6, 7) and by infectivity (3, 5, 8, 10, 11). By the first method, sodium hypochlorite, potassium permanganate, 70% ethanol, Lugol's iodine, and formalin were judged to be unacceptable as ovicides. The last was also tested for infectivity in sheep by Williams (5) with negative results. Glutaraldehyde has been shown to be an effective chemosterilant against bacterial agents and can be used for this purpose even with delicate lensed instruments (12).

Although none of the chemical agents used in this study completely eliminated egg infectivity, glutaraldehyde was highly active and was completely effective at the lower concentration.

The successful inactivation of egg infectivity in mice was reported by Williams et al. (11) who used an aqueous solution (11.1 g/litre) of bunamidine hydrochloride at 37°C for 2 h.

It is recommended that further studies be carried out with the chemicals found partially successful in this study in order to determine whether ovicidal activity may be enhanced by varying exposure time, concentration or other conditions, and also to determine the effect on eggs contained within the proglottids.

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