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# THE EFFECT OF EGG DOSE, AND HOST AGE AND SEX ON SUSCEPTIBILITY OF CF1 ALBINO MICE AND JIRDS *(MERIONES UNGUICULATUS)* TO PRIMARY INFECTION BY *ECHINOCOCCUS GRANULOSUS* (BATSCH, 1786)

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## SUMMARY

CF1 albino mice and jirds (*Meriones unguiculatus*) of both sexes and different ages were orally dosed with eggs of Argentine-strain *Echinococcus granulosus* (Batsch, 1786) at doses from 250 to 2800 eggs. Both host species were uniformly susceptible at the higher dose levels and some animals developed cysts from as few as 250 eggs. Weanling animals of both species were more susceptible than sexually mature individuals but no sex differences were observed in young or older animals. There were no differences in mean cyst diameters between mice with few or many cysts.

## INTRODUCTION

Laboratory animal models for primary hydatid disease have been the subject of investigation in our laboratory because the normal intermediate hosts of *Echinococcus granulosus* (Batsch, 1786), the sheep and other domestic ungulates, are inappropriate for many experimental situations for both economic and biological reasons.

Previous work demonstrated that CF1 white mice and jirds (*Meriones unguiculatus*) were highly susceptible to infection with eggs of the Argentine strain of *E. granulosus* [4] and cyst development proceeded relatively rapidly with the production of some fertile cysts as early as 195 days [5].

This paper reports the influence of egg dose and host age and sex on the susceptibility of white mice and jirds to primary hydatid cyst infection.

## MATERIALS AND METHODS

Carworth F1 (CF1) mice and *Meriones unguiculatus* were dosed orally with eggs of *Echinococcus granulosus* with an automatic syringe provided with a blunt tipped 22 gauge needle.

The eggs were obtained from worms in dogs experimentally infected with protoscolices from hydatid cysts in Argentine sheep [5] and maintained in sterile phosphate buffered saline (pH 7.2) with antibiotics [21] at 4°C, during 10-30 days before use. Several different egg batches were used but all animals in a single experiment received embryophores from the same batch.

To evaluate the effect of egg dose on host susceptibility, groups of weanling (21 days old) mice and jirds were infected with 2800, 2000, 1000, 500 or 250 eggs. In experiments dealing with host age and sex influence on susceptibility wealing and adult (older than 60 days) male and female animals of both species were used and fed with 1000 or 500 eggs. The animals were killed and necropsied 100 to 120 days after exposure. The viscera were carefully examined for the presence of hydatid cysts. After dissection of macroscopically visible cysts, the liver and lung of

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each animal were sectioned and subsequently squashed between 2 Petri dishes and observed under the dissecting microscope. Animals which died prior to sacrifice were necropsied and the infection data was incorporated with the corresponding group. Cyst maximum diameter was measured with a filar micrometer. Significant differences between groups were tested with the Chi square test for proportion of infected animals and the "t" Student's test for the number and size of cysts, taking at least 5% ($P < 0.05$) as significant level.

Percentage of eggs transformed into cysts was calculated according to:

$$\frac{\text{Mean No. of cysts per infected animal} \times 100}{\text{No. eggs administered per animal}}$$

## RESULTS

### 1) Egg dose

*CF1 mice*

The results of administration of two different egg batches at different dose levels to a total of 184 mice are summarized in Table I. The eggs used in experiment 2 were apparently more infective than those used in experiment 1, as judged by the percentage of eggs administered that developed into cysts. In both experiments the highest percentage of eggs administered which developed into cysts was observed at the lowest dose rate (250 eggs).

As the numbers of eggs administered increased there was a tendency for a higher percentage of mice to become infected and for a greater number of cysts to become established per infected animal. The increases in percentage infection with egg dose were of greater magnitude with eggs of lower infectivity (exp. 1).

The percentage localization of cysts according to numbers of eggs administered is shown in Table II. Regardless of dose, more than three fourths of the cysts were located in the liver or peritoneal cavity. These two sites were grouped together because it had previously been observed that many hepatic cysts become dislodged from the liver as they increase in size [5]. The second most frequent predilection site was the lungs (or pleural

TABLE I

Cyst findings in weanling female CF1 albino mice after oral exposure to *Echinococcus granulosus* eggs at different doses

| Experiment Number | Dose of eggs | Number of infected animals/ Total Number | Percentage of infected animals | Mean number of cysts per infected animal Mean ± Standard Deviation | Percentage of eggs transformed into cysts |
|---|---|---|---|---|---|
| 1 | 2000 | 20/25 | 80.0 | 6.15 ± 4.65 | 0.37 |
| | 1000 | 12/25 | 48.0 | 3.42 ± 3.20 | 0.34 |
| | 500 | 8/31 | 25.8 | 2.62 ± 1.92 | 0.52 |
| | 250 | 3/29 | 10.3 | 4.00 ± 4.36 | 1.60 |
| 2 | 2800 | 13/13 | 100 | 66.00 ± 30.03 | 2.35 |
| | 1000 | 20/21 | 95 | 23.30 ± 17.46 | 2.33 |
| | 500 | 24/25 | 96 | 7.63 ± 6.16 | 1.53 |
| | 250 | 10/15 | 66.6 | 8.10 ± 5.64 | 3.24 |

TABLE II

Percentage distribution of hydatid cysts in CF1 mice according to numbers of *E. granulosus* eggs ingested

| Experiment Number | Dose of eggs | Percentage of hepatic and peritoneal cysts | Percentage of pulmonary and pleural cysts | Percentage of cysts in other sites | Total Number of cysts |
|---|---|---|---|---|---|
| 1 | 2000 | 79.85 | 17.91 | 2.24 | 134 |
| | 1000 | 78.38 | 18.92 | 2.70 | 37 |
| | 500 | 57.14 | 42.86 | 0.00 | 14 |
| | 250 | 75.00 | 25.00 | 0.00 | 12 |
| 2 | 2800 | 91.91 | 7.46 | 0.63 | 858 |
| | 1000 | 84.40 | 11.32 | 4.28 | 468 |
| | 500 | 88.46 | 11.54 | 0.00 | 182 |
| | 250 | 79.46 | 20.54 | 0.00 | 73 |

cavity). At the higher egg doses cysts were found at other sites which included the kidney, brain, heart, spleen and subcutaneous tissues.

*Jirds*

The results of two experiments involving 114 jirds are summarized in Table III. As in mice, the percentage of jirds which became infected increased with the numbers of eggs administered, as did the mean number of cysts per infected animal (Fig. 1).

The percentage organ localization according to numbers of embryophores received is shown in Table IV. Seventy to 98 percent of the hydatid cysts were located in the lungs or pleural cavity. Hepatic cysts were only observed in the group of animals which had

TABLE III

Cysts findings in weanling jirds *Meriones unguiculatus* following oral exposure to *Echinococcus granulosus* eggs at different doses

| Experiment Number | Host characteristics | Dose of Eggs | Number of infected animals/ Total Number | Percentage of infected animals | Mean number of cysts per infected animal Mean ± Standard Deviation | Percentage of eggs developed into cysts |
|---|---|---|---|---|---|---|
| 3 | females | 2000 | 11/14 | 78.6 | 3.10 ± 1.79 | 0.16 |
| | | 1000 | 3/9 | 33.3 | 2.33 ± 1.15 | 0.23 |
| | | 500 | 2/20 | 10.0 | 2.00 ± 0.00 | 0.40 |
| | | 250 | 3/20 | 15.0 | 2.00 ± 0.00 | 0.80 |
| 4 | females and males | 2000 | 8/9 | 88.8 | 6.87 ± 3.98 | 0.34 |
| | | 1000 | 17/24 | 75.0 | 3.29 ± 1.79 | 0.33 |
| | | 500 | 5/18 | 27.7 | 1.80 ± 0.45 | 0.36 |

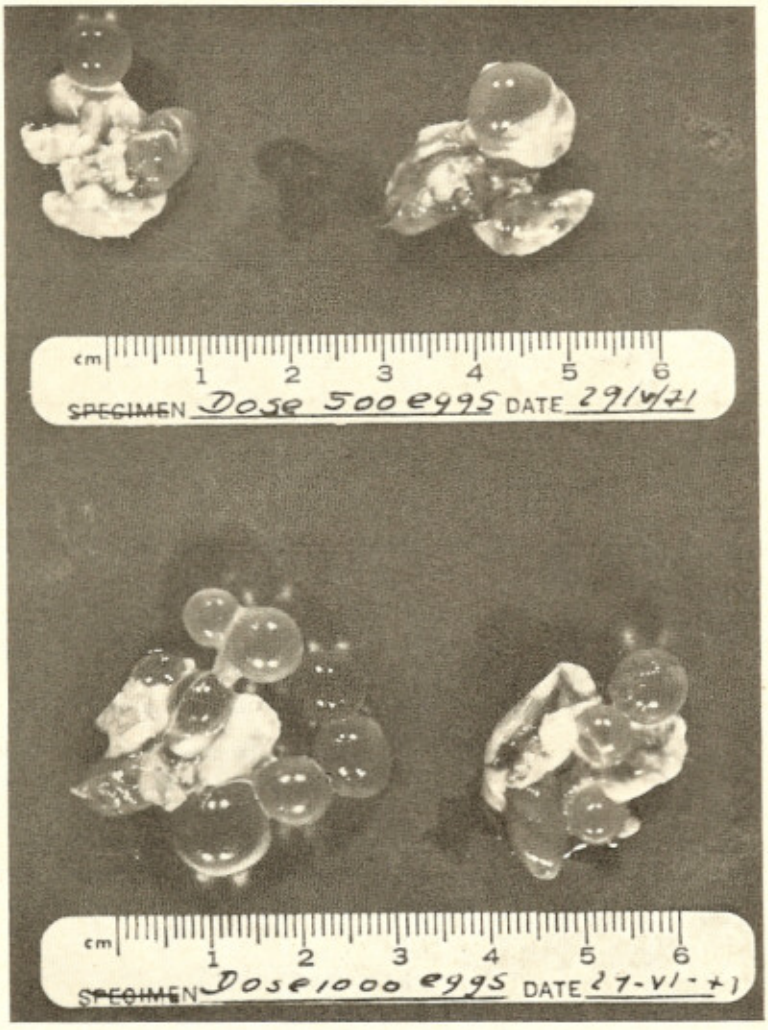


Fig. 1 — Lungs of *Meriones unguiculatus* with hydatid cysts 120 days after oral exposure to 500 and 1000 eggs of *Echinococcus granulosus*.

received 2000 eggs. Other localization sites included the kidney, spleen, heart and subcutaneous tissues.

**2) Host age**

*CF1 mice*

The results of an experiment to determine the comparative susceptibility of adult and weanling white mice following exposure to 1000 embryophores are shown in Table V. The data show that young animals of both sexes were more susceptible than adults. Although the percentage of animals which became infected with some cysts was approximately the same in young and old animals, both the numbers of cysts per infected animal and the percentage of eggs which developed into cysts were significantly greater in young animals than old ($P < 0.005$).

*Jirds*

The infection results in weanling and adult jirds following exposure to 1000 eggs are

TABLE IV

Percentage distribution of hydatid cysts in *Meriones unguiculatus* according to numbers of *Echinococcus granulosus* eggs ingested

| Dose of eggs | Percentage of hepatic and peritoneal cysts | Percentage of pulmonary and pleural cysts | Percentage of cysts in other sites | Total number of cysts |
|---|---|---|---|---|
| 2000 | 9.09 | 80.00 | 10.91 | 55 |
| 1000 | 0.00 | 98.21 | 1.79 | 56 |
| 500 | 0.00 | 70.00 | 30.00 | 10 |

TABLE V

Hydatid cysts findings in CF1 mice following oral exposure to 1000 eggs of *Echinococcus granulosus* according to host age

| Host characteristics | | Number of infected animals. Total number | Percentage of infected animals | Mean number of cysts per infected animal Mean ± Standard Deviation | Mean maximum diameter of cysts (mm) Mean ± Standard Deviation | Percentage of eggs transformed into cysts |
|---|---|---|---|---|---|---|
| Sex | Age | | | | | |
| female | weanlings | 19/20 | 95 | 23.30 ± 17.46 | 4.67 ± 2.55 | 2.30 |
| | adults | 14/14 | 100 | 7.50 ± 5.64 | 4.07 ± 3.14 | 0.75 |
| male | weanlings | 13/13 | 100 | 37.38 ± 17.58 | 5.02 ± 2.50 | 3.74 |
| | adults | 11/15 | 80 | 4.09 ± 3.50 | 3.83 ± 2.41 | 0.41 |

TABLE VI

Hydatid cyst findings in *Meriones unguiculatus* following oral exposure to 1000 eggs of *Echinococcus granulosus* according to host age

| Host characteristics | | Number of infected animals. Total number | Percentage of infected animals | Mean number of cysts per infected animal Mean ± Standard Deviation | Mean maximum diameter of cysts (mm) Mean ± Standard Deviation | Percentage of eggs transformed into cysts |
|---|---|---|---|---|---|---|
| Age | Sex | | | | | |
| weanlings | females and males | 17/24 | 76.6 | 3.29 ± 1.79 | 7.33 ± 0.95 | 0.33 |
| adults | females and males | 22/35 | 62.9 | 2.05 ± 0.99 | 3.76 ± 4.55 | 0.20 |

shown in Table VI. As in mice, the greater susceptibility of weanling animals was reflected in the significantly greater number of cysts per infected animal and the larger percentage of eggs which developed into cysts ($P < 0.05$).

#### 3) Host sex

*CF1 mice*

Table VII summarizes two experiments with white mice to determine the influence of host sex on susceptibility. There were no significant differences between males and females of the same age groups regarding the percentage of animals which became infected, the numbers of cysts per infected animal, cyst size and the percentage of eggs which developed into cysts.

*Jirds*

The infection results in jirds of both sexes following exposure to 500 or 1000 eggs are shown in Table VIII. There were no significant differences between sexes as measured by the percentage of animals which became infected, the numbers of cysts per infected animal, cyst size and the percentage of eggs which became cysts.

#### 4) Cyst numbers and cyst sizes

In order to determine if cyst growth was influenced by the magnitude of cyst burdens the cyst diameter data for each experiment were arranged according to the numbers of cysts found in individually infected animals (1 to 4, 5 to 14 or 15 and greater). The data for weanling mice infected at 2 dose levels in two different experiments are shown in Table IX. The mean maximum cyst diameters were consistent among animals receiving the same batch of eggs regardless of the number of cysts which developed. There were no significant differences in these or other groups (data not shown) of animals regarding cyst size and cyst burdens.

TABLE VII

Hydatid cyst findings in CF1 mice following oral exposure to eggs of *Echinococcus granulosus* according to host sex

| Host characteristics | | Dose of eggs | Number of infected animals. Total Number | Percentage of infected animals | Mean number of cysts per infected animal Mean±Standard Deviation | Mean maximum diameter of cysts (mm) Mean±Standard Deviation | Percentage of eggs developed into cysts |
|---|---|---|---|---|---|---|---|
| Age | Sex | | | | | | |
| Weanlings | females | 1000 | 12/25 | 48.0 | 3.42 ± 3.20 | 13.93 ± 7.86 | 0.34 |
| | males | 1000 | 18/44 | 40.9 | 2.65 ± 2.12 | 10.91 ± 5.01 | 0.26 |
| | females | 500 | 7/31 | 21.9 | 2.29 ± 1.79 | —— | 0.46 |
| | males | 500 | 4/22 | 18.1 | 2.00 ± 0.82 | —— | 0.40 |
| Weanlings | females | 1000 | 14/19 | 73.7 | 6.43 ± 7.11 | 4.10 ± 2.06 | 0.64 |
| | males | 1000 | 14/19 | 73.7 | 8.86 ± 8.85 | 4.38 ± 2.14 | 0.89 |
| Adults | females | 500 | 4/21 | 19.0 | 1.75 ± 0.96 | 4.44 ± 1.56 | 0.17 |
| | males | 500 | 7/23 | 30.4 | 1.71 ± 1.25 | 4.42 ± 1.95 | 0.17 |

TABLE VIII

Hydatid cyst findings in *Meriones unguiculatus* following oral exposure to eggs of *Echinococcus granulosus* according to host sex

| Host characteristics | | Dose of eggs | Number of infected animals. Total Number | Percentage of infected animals | Mean number of cysts per infected animal Mean±Standard Deviation | Mean maximum diameter of cysts (mm) Mean±Standard Deviation | Percentage eggs developed into cysts |
|---|---|---|---|---|---|---|---|
| Age | Sex | | | | | | |
| Adults | females | 1000 | 11/19 | 57.90 | 1.82 ± 0.60 | 8.81 ± 3.37 | 0.18 |
| | males | 1000 | 11/16 | 62.50 | 2.27 ± 1.72 | 7.24 ± 2.19 | 0.23 |
| Weanlings | females | 500 | 2/20 | 10.00 | 2.00 ± 0.00 | 11.25 ± 1.77(*) | 0.15 |
| | males | 500 | 6/21 | 28.60 | 1.83 ± 1.17 | 8.97 ± 2.27 | 0.18 |

(*) Only 3 cysts measured

TABLE IX

Relationship of cyst burdens and cyst sizes in weanling CF1 mice 121 days after oral exposure to eggs of *Echinococcus granulosus*

| Experiment Number | Characteristics of the host | | Dose of eggs | Numbers of cysts per animal | Mean maximum diameter of cysts (mm) | Number of cysts measured |
|---|---|---|---|---|---|---|
| | Sex | Age | | | | |
| 2 | Females | 21 days | 500 | 1 — 4 | 6.37 ± 3.08 | 20 |
| | | | | 5 — 14 | 6.20 ± 3.05 | 55 |
| | | | | ≥ 15 | 6.36 ± 2.51 | 28 |
| 6 | Males | 21 days | 1000 | 1 — 4 | 4.41 ± 1.82 | 17 |
| | | | | 5 — 14 | 4.47 ± 3.10 | 26 |
| | | | | ≥ 15 | 4.28 ± 1.81 | 67 |

## DISCUSSION

Eggs used in these experiments were obtained from worms recovered from dogs ten or eleven weeks after experimental ingestion of protoscolices. Dosage calculations were made by counting only those eggs which contained completely formed embryophores and were apparently fully mature according to the criteria of SILVERMAN [19]. Despite these precautions it was apparent that different batches of eggs varied greatly in their infectivity to white mice and jirds. This was apparent in the different proportions of administered embryophores which developed into hydatid cysts and was also reflected in the different percentages of animals which became infected at a given dose level. Part of this variation may be attributed to different periods of egg storage between removal from dogs and administration to the laboratory animals. In these experiments storage time varied from 10 to 30 days at 4°C. Using single batches of eggs we have observed changes in infectivity with storage time under these conditions (unpublished results). Morphological criteria are at best only a crude indicator of egg infectivity and studies

are presently underway to determine if percentage artificial hatching and activation can be used as an indicator of the relative infectivity of different egg batches.

The percentage of administered oncospheres which became cysts was greatest at the lowest dose level (250 eggs) but at higher dose levels there was much variation with no clear tendency for increase or decrease. The percentages of administered eggs which developed into cysts in the experiments reported here are similar to those reported previously in CF1 white mice [4, 5] and experimental infections of the common natural host, the sheep [11, 12, 16].

Some animals of both species became infected with as few as 250 eggs. The minimum infecting dose has not yet been determined but in subsequent trials 1 of 20 white mice became infected after ingesting only 50 embryophores (unpublished data). Comparison of animals which became infected and mean cyst numbers in mice and jirds which received the same egg batch (Table I, Exp. 1 and Table II, Exp. 3) revealed that the mouse was slightly more susceptible than the jird at all dose levels.

As demonstrated previously [4] cysts in mice were found predominantly in the liver whereas in jirds the predominant localization was pulmonar. In both host species, as the number of eggs administered increased cysts began to appear in other sites in addition to the primary site. In view of the recent demonstration by Heath [14] that *E. granulosus* oncospheres are likely to remain in whichever organ they encounter first it is likely that anatomic differences account for the different cyst predilection sites observed in mice and jirds. Heath proposed that the size of the oncosphere in relation to the venules and lymphatic lacteal of the intestinal villus may determine the different distribution of cysts observed in different host species. In ruminants, in which lung involvement is common, the lymphatic lacteal of the villus is relatively large and may permit the direct migration of oncospheres to the lungs. In non-ruminant animals such as the horse, pig and man, host species in which liver involvement is largely predominant, the lymphatics are smaller and, in these species, the standard migratory path may be a venous route directly to the liver [14]. Mice and jirds would appear to be excellent models to confirm this hypothesis and studies are presently underway to determine the intestinal anatomic differences which may exist.

A clear age resistance was observed in these experiments in both mice and jirds. Although the proportion of weanling and mature animals which became infected was sometimes closely similar the mean cyst burdens and the percentage of administered eggs which became cysts were always significantly greater in weanling mice and jirds than in older animals. Cyst growth was also retarded in older jirds (Table VI) but in mice no differences were observed in cyst sizes between the younger and older animals.

Age resistance in mice has been previously shown in secondary *E. granulosus* infection [18] and primary *E. multilocularis* infection [15] and is a well known phenomenon in other host-cestode parasite relationships [6, 7, 13]. Evidence that this resistance is expressed at the intestinal level was presented by HEATH [14] who demonstrated that oncospheres of *Taenia pisiformis* appeared to make no attempt to penetrate the gut of rabbits older than 8 weeks. Some interference with the postencystment phase of cyst growth was apparent in older jirds but not in mice in our experiments. Postencystment effects may have become more apparent if the infections had been permitted to continue for longer periods, at least until the time of protoscolex development which has been observed as early as 195 days in this strain of mouse host [5]. There was no histological evidence of an increased tissue response in older jirds and, in fact, in all animals inflammatory reactions were virtually nonexistent (data not shown).

A sex influence on susceptibility to *E. granulosus* was not observed under our experimental conditions in either CF1 mice or jirds. This finding is in contrast to that observed by FRAYHA et al. [8] in secondary *E. granulosus* infection of NAMRU-3 white mice.

Sex differences in susceptibility to infections by helminths is a well-known phenomenon and has been most studied with nematodes to which male hosts are generally more susceptible (see review by SOLOMON [20]). Host-sex differences in susceptibility to larval ces-

tode infections have also been reported with *T. pisiformis* [1] and *T. taeniaformis* [2, 3, 6, 7] in which male hosts were more susceptible. In contrast, female mice were more susceptible to secondary *Taenia crassiceps* infection [9, 10]. The males of some strains of mice appeared more susceptible to *E. multilocularis* infection but this was not true with all strains [17, 22]. OHBAYASHI & SAKAMOTO [17] found that highly susceptible mouse strains did not display sex differences whereas with more resistant strains the males tended to be more susceptible. On the other hand, KAMIYA [15] reported the males of some other mouse strains to be more resistant to infection with *E. multilocularis*. It is apparent then, that sex influences on host susceptibility vary between different mouse strains and this factor may account for the fact that our CF1 mice showed no sex differences whereas FRAYHA et al. [8] who worked with NAMRU-3 albino mice found that male mice and female treated with testosterone were much more susceptible than normal females to secondary echinococcosis.

Preliminary experiments indicate that some other mouse strains are less susceptible to primary infection by *E. granulosus* (Argentine strain) (unpublished data) and we are presently testing for possible sex differences in the less resistant strains.

We are unable to find published information concerning experimental evidence for sex differences in the normal sheep host.

SCHWABE et al. [18] observed larger *E. granulosus* cysts in mice with few cysts than in those with many and suggested the possibility of a "crowding effect". Our data, in contrast, indicated that the early growth rate of cysts was not influenced by the number of cysts present.

## RESUMO

*Efeito do número de ovos, da idade e do sexo do hospedeiro, sobre a suscetibilidade de camundongos albinos da linhagem CF1 e de "jirds"* (Meriones unguiculatus) *à infecção primária pelo* Echinococcus granulosus *(Batsch,* 1786).

Ratos brancos CF1 e "jirds" (*Meriones unguiculatus*) de ambos os sexos e diferentes idades receberam, por via oral, ovos de *Echinococcus granulosus* (Batsch, 1786) de linhagem Argentina, em doses de 250 a 2.800 ovos. Ambas as espécies foram suscetíveis uniformemente às doses altas e alguns animais desenvolveram cistos com a dose mais baixa de 250 ovos. Animais recentemente desmamados, de ambas as espécies, foram mais suscetíveis que os indivíduos sexualmente maduros, porém não houve diferenças de suscetibilidade, em relação a sexo, nos animais jovens ou adultos. Não houve diferenças nos diâmetros médios dos cistos entre ratos que apresentavam poucos ou muitos cistos.

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