BRIEF COMMUNICATION

EXPERIMENTAL INFECTION OF SWISS AND AKR/J MICE WITH Centrocestus formosanus (TREMATODA: HETEROPHYIDAE)

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SUMMARY

In order to better understand the biology of Centrocestus formosanus in a definitive host model, mice of Swiss and AKR/J strains were experimentally infected with 100 metacercariae of the parasite. Fourteen days post-infection, the rodents were killed and adult trematodes were recovered from the small intestine. The percentage of parasite recovery from AKR/J mice (11.4%) was significantly higher than that from Swiss mice (5.3%). Moreover, trematodes recovered from the AKR/J strain were more developed and had greater fecundity. Peculiarities concerning the mice’s immune system could explain the difference in susceptibility and in worm development seen in the present study. The data obtained confirm that mice are susceptible to infection with C. formosanus and indicate that the AKR/J strain provides a more favorable environment for parasite development.

KEYWORDS: Foodborne intestinal flukes; Trematodes; Experimental models; Heterophyids.

The use of rodents as experimental models for some human parasites has led to a better understanding of trematode biology, but important differences in particular host-parasite relationships can be observed, depending on the strains of the definitive host, especially in the case of mice. Nevertheless, there is a lack of knowledge regarding experimental definitive hosts of some human intestinal trematodes. In fact, over 70 species of foodborne flukes have already been correlated with human infection, but many of them have not been studied at length, and these include some heterophyid species.

Heterophyidae are the smallest trematodes that inhabit the intestine of birds and mammals, and the infection of human beings with these flukes has been considered an emerging parasitic disease usually acquired through the consumption of raw or undercooked fish. Heterophyes heterophyes and Metagonimus yokogawai, the two heterophyid species that most affect humans, are common in Africa, Asia and Eastern Europe. In Brazil, human heterophyiasis by Asccocotyle (Phagicola) longa has already been reported.

Additional details about the life history of the less prevalent species of heterophyids in vertebrate models are desirable. Centrocestus formosanus is an Asian species that has now been introduced to Brazil, and although it has also been identified in humans in Asia, the quantitative aspects of experimental infection of this parasite in mice are unknown. Furthermore, KIMURA et al. (2007) suggested that species of Centrocestus can be used to study human pathogenic heterophyids, since it has advantages over Metagonimus species. These authors argued that it is easier to obtain Centrocestus because of its widespread distribution and its relatively short development time in the host (three days), and in addition, rats and hamsters have been reported to be susceptible hosts.

Therefore, knowledge of the biology of the parasite in mice may be interesting not only intrinsically, but also because C. formosanus may constitute an experimental model for other parasites.

Over recent years, the inbred AKR strain of mouse and its sublineages have been acquiring greater importance for evaluating the biology of intestinal nematodes. Nevertheless, studies on trematodes using the AKR strain remain scarce and address experimental infection using the species Brachylaima cribbi, Schistosoma mansoni and Zygocotyle lunata. The permissiveness of AKR/J mice towards some of these helminths has been correlated to their immunological peculiarities such as deficiency in the complement pathway, polarization of the T helper cell (Th)-1 response, and a less substantial increase in the number of goblet cells associated with mucin deficiency in mucosa during experimental intestinal infection.

In order to better understand the biology of C. formosanus in a rodent definitive host model, the susceptibility of both Swiss and AKR/J mice...
to the parasite and the potential of these animals as experimental models were assessed.

Metacercariae of *C. formosanus* were obtained from naturally infected *Astraloheros facetus* that were caught from the Pampulha reservoir, an artificial lake located in the city of Belo Horizonte, Minas Gerais, Brazil (19°51'771"S, 43°58'542"W). The gills of these fish were removed, transferred to glass slides containing saline solution (0.85% NaCl) and analyzed under a stereomicroscope. The tissue samples containing metacercariae of the parasite were immersed in a digestion solution (0.85% NaCl in distilled water with 1% pepsin and 1% HCl, pH 2) for one hour at 37 °C. The metacercariae of *C. formosanus* were then collected and counted using a light microscope.

Swiss mice (25 ± 2 g; n = 12 females) and AKR/J mice (25 ± 2 g; n = 18 females) maintained in laboratory conditions and provided with food and water *ad libitum* were used as the experimental definitive hosts. Each animal received an oral suspension containing 100 metacercariae of *C. formosanus*. All rodents were killed by cervical dislocation, 14 days post-infection (DPI), in accordance with the local animal experimentation ethics committee (CETEA/UFMG). The small intestines had been excised and opened longitudinally using scissors in Petri dishes containing saline solution. Trematodes obtained from each rodent were killed in hot water (70 °C), fixed in 10% formalin solution, stained with acetic-alum carmine, cleared in beechwood creosote and mounted in Canada balsam.

The parasites from each strain of mice were analyzed and classified according to their sexual development (i.e. mature flukes present vitellaria and eggs in the uterus, while immature ones do not), and their intrauterine eggs were counted under a light microscope. The measurements of length and width of the body, oral sucker, acetabulum, pharynx, ovary, and testes were taken with the aid of an ocular micrometer fitted to a light microscope. The total area of the worms was measured by means of the AxioVision LE software (release 4.8.2) following image capture from a digital camera attached to a microscope (Leica DM500).

The data obtained were subjected to a Student’s t test for means, and to the χ² test or Fisher’s exact test for proportion comparisons.

Both strains of mice were susceptible to the parasite, but a significantly greater number of parasites (p = 0.006) were recovered from AKR/J mice (11.4 ± 6.2) in comparison to those from Swiss mice (5.3 ± 4.5). In the inbred strain, the worms were more developed, such that they more commonly presented vitellaria and had many more eggs in the uterus. In fact, 84.4% of flukes recovered from AKR/J mice were mature while this value in Swiss was 67.9%, with a significant difference (p = 0.007). The percentages of animals which tested positive after oral administration of metacercariae were 83.3 and 94.4%, for Swiss and AKR/J strains of mice respectively (Table 1; Fig. 1).

There is still no completely clear information on which vertebrates might be suitable hosts for *C. formosanus*. There are scattered reports in literature, mostly only of a qualitative nature, on infection of various hosts such as cats, rats, mice, hamsters, pigeons, chickens and ducks with this heterophyid, at different times of infection. However, rats and cats have been considered to be more favorable experimental hosts.

<table>
<thead>
<tr>
<th>Parastes</th>
<th>Swiss mice (n = 30)</th>
<th>AKR/J mice (n = 60)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body</td>
<td>L 345 ± 23</td>
<td>334 ± 29</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>W 127 ± 17</td>
<td>143 ± 18</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>A 34743 ± 4929</td>
<td>36569 ± 5596</td>
<td>NS</td>
</tr>
<tr>
<td>Oral sucker</td>
<td>L 49 ± 5</td>
<td>49 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>W 44 ± 3</td>
<td>48 ± 4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Acetabulum</td>
<td>L 40 ± 5</td>
<td>44 ± 4</td>
<td>0.0012</td>
</tr>
<tr>
<td></td>
<td>W 37 ± 4</td>
<td>39 ± 4</td>
<td>0.0136</td>
</tr>
<tr>
<td>Pharynx</td>
<td>L 34 ± 3</td>
<td>36 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>W 20 ± 2</td>
<td>21 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Ovary</td>
<td>L 37 ± 6</td>
<td>36 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>W 27 ± 3</td>
<td>26 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Testes</td>
<td>L 48 ± 7</td>
<td>48 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>W 29 ± 4</td>
<td>30 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Intrauterine eggs</td>
<td>N 5 ± 6</td>
<td>10 ± 9</td>
<td>0.0289</td>
</tr>
</tbody>
</table>

*Fig. 1 - Number of intrauterine eggs in Centrocestus formosanus recovered from Swiss (white column) and AKR/J mice (black column) at 14 days post-infection. Asterisks indicate a significant increase (p < 0.05).*

The respective mean recovery percentages of *C. formosanus* from Swiss and AKR/J strains of mice (5.3 and 11.4%, respectively) are consistent with the recovery rates obtained by CHEN (1942) using three mice that were individually infected with different numbers of metacercariae and were evaluated at different time periods after infection. In the present study, the mean recovery percentage of *C. formosanus* from mice is shown for the first time, as well as the ratio between immature and mature worms, for two different strains of mice.

The findings of the present study suggest that AKR/J mice provide a more favorable environment for parasite development. Regarding the size of the worms, those from AKR/J mice were significantly wider than
those from Swiss mice, but the length and the total area did not change. The AKR/J strain also seemed to have enabled further development of the suckers of the parasite, or perhaps they were preserved for a longer period of time. In experimental infection of hamsters with *C. formosanus*, the suckers degenerated with advancing infection, and it was suggested that these alterations could be related to the elimination of parasites from the intestine of the definitive host[^1].

In addition to the differences in the mean number of parasites, differences in morphology and sexual development were observed between the *C. formosanus* recovered from Swiss and AKR/J mice, which may indicate a phenomenon of phenotypic plasticity. It is possible that the immunological characteristics of the AKR/J strain are related to this finding. In fact, the fecundity of helminths seems to be directly linked to the host immune system and may be influenced by immunological variations correlated with strains of mice[^2,^3^], immunomodulatory drugs[^4,^5^] and experimental co-infection[^6^].

The role of intestinal goblet cells in the expulsion of the trematodes *Echinostoma trivolvis*[^7^] and *Gymnophalloides seoi*[^8^] has been demonstrated in other strains of mice, and mastocytosis and goblet cell hyperplasia with mucin activation were observed as local immune responses against *Neodiplostomum seoulense*[^9^].

Therefore, the immunological characteristics of AKR mice already mentioned[^10], and in particular the peculiarities of their goblet cells and intestinal mucins[^11], can be related to their greater susceptibility to *C. formosanus*. However, further studies on these immunopathological characteristics of the infection of AKR/J mice with this trematode are still needed.

Our data are complementary to existing information regarding the experimental model for heterophyids, therefore confirming that mice, and in particular the AKR/J strain, are susceptible and may be a promising model for studying infection with *C. formosanus*, and perhaps with other intestinal flukes. The factors directly related to higher susceptibility of mice of this inbred strain to the parasite remain unknown.

**RESUMO**

**Infeção experimental de camundongos Swiss e AKR/J por Centrocestus formosanus (Heterophyidae)**

Com o objetivo de melhor conhecer a biologia de *Centrocestus formosanus* em um modelo de hospedeiro definitivo, camundongos das linhagens Swiss e AKR/J foram experimentalmente infectados com 100 metacercárias do parasito. Quatorze dias após a infecção, os roedores foram mortos e os trematódeos adultos recuperados do intestino delgado. O percentual de recuperação do parasito de camundongos AKR/J (11,4%) foi significativamente maior que o obtido de camundongos Swiss (5,3%). Além disso, trematódeos recuperados da linhagem AKR/J apresentavam-se mais desenvolvidos e apresentaram maior fecundidade. Peculiaridades relacionadas ao sistema imunológico dos camundongos podem explicar as diferenças observadas na suscetibilidade e no desenvolvimento dos vermes no presente estudo. Os dados obtidos confirmam que camundongos são suscetíveis à infecção pelo *C. formosanus* e indicam que a linhagem AKR/J proporciona um ambiente mais favorável para o desenvolvimento do parasito.


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