Experimental infection of mice with *Trichobilharzia franki*, the major causative agent of swimmer's itch in Europe

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BACKGROUND

Cercarial dermatitis (CD), also known as **swimmer's itch**, is a waterborne allergic disease caused by avian schistosome larvae penetrating mammalian skin (Horák *et al*. 2015). Human CD is manifested by maculo-papulo-vesicular **skin rash** (Fig. 1) accompanied by intensive **itching** (pruritus) and **edema**. Sometimes also, a systemic reaction such as fever, cough, diarrhea, and local lymph node swelling may occur (Macháček *et al*. 2018). In Europe, CD outbreaks typically affect freshwater bodies (such as ponds and lakes) and significantly disrupt summer bathing seasons.

Trichobilharzia franki is an avian schistosome, which has been identified as a major contributor to CD cases in Europe (Fig. 2). It has a dixenic life cycle employing aquatic ***Radix auricularia*** snails as intermediate hosts and anatid birds (**mallards** or **swans**) as definitive hosts (Müller & Kimmig 1994). Mammals, including humans, are considered accidental hosts, in which *T. franki* dies in the skin. However, no data on parasite migration, pathogenicity, and interactions with the hosts are available, especially due to difficulties with the long-term maintenance of *T. franki* in the laboratory.

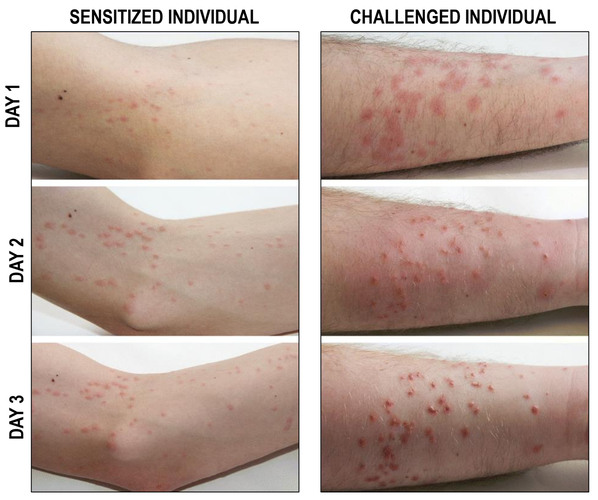

Fig. 1. Progression of cercarial dermatitis in supposedly sensitized and challenged (repeatedly exposed) individuals. The reaction in challenged one is more expressive and appears faster. Adapted from Macháček *et al*. (2018).

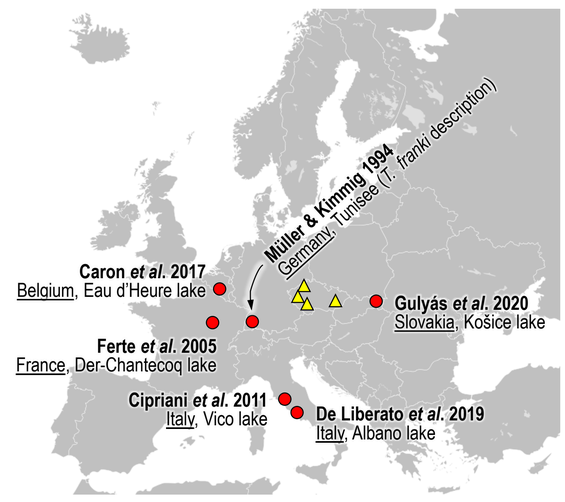

Fig. 2. European outbreaks of human cercarial dermatitis associated with *Trichobilharzia franki*: ● published reports, △ novel reports from Czechia presented in this study (see below).

STUDY DESIGN

Collection of *T. franki* isolates in the field

- Four sites with reported CD outbreaks (Czechia, July–August 2023; Fig. 3)
- Collection of *Radix auricularia* snails (Fig. 4), examination for cercariae

Determination of cercariae & infection of mice

- Morphological and molecular identification of collected *T. franki* cercariae
- Per pinnae infection of C57BL/6J female mice with 1000 cercariae (Plzeň)

Mouse harvesting & sample processing

- Mice were sacrificed and samples collected at 2 and 7 days post infection
- Clinical, histopathological, and immunological analyses

Fig. 3. Sites included in the study: **Plzeň**, Škodaland pool (49.7172092N, 13.3524572E), **Proboštov**, Proboštovský pond (50.6664067N, 13.8385653E), Dívčice, **Blatec** pond (49.1167367N, 14.3084103E) , **Lukov u Zlína**, Dolní bělovodský pond (49.2908414N, 17.7268689E).

Fig. 4. *Radix auricularia*.

(1) Morphological and phylogenetic analyses of collected *T. franki* cercariae

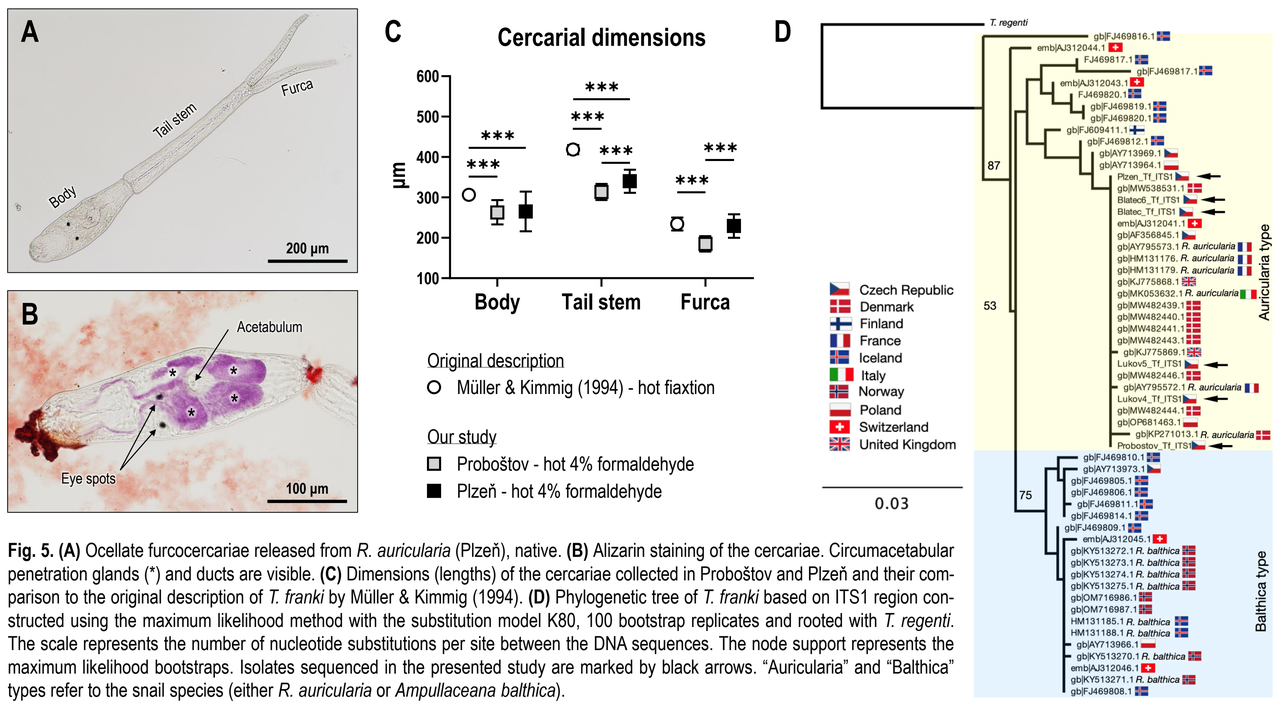

Fig. 5. (A) Ocellate furcocercariae released from *R. auricularia* (Plzeň), native. **(B)** Alizarin staining of the cercariae. Circumacetabular penetration glands (*) and ducts are visible. **(C)** Dimensions (lengths) of the cercariae collected in Proboštov and Plzeň and their comparison to the original description of *T. franki* by Müller & Kimmig (1994). **(D)** Phylogenetic tree of *T. franki* based on ITS1 region constructed using the maximum likelihood method with the substitution model K80, 100 bootstrap replicates and rooted with *T. regenti*. The scale represents the number of nucleotide substitutions per site between the DNA sequences. The node support represents the maximum likelihood bootstraps. Isolates sequenced in the presented study are marked by black arrows. "Auricularia" and "Balthica" types refer to the snail species (either *R. auricularia* or *Ampullaceana balthica*).

(2) Clinical outcome of *T. franki* infection in mice

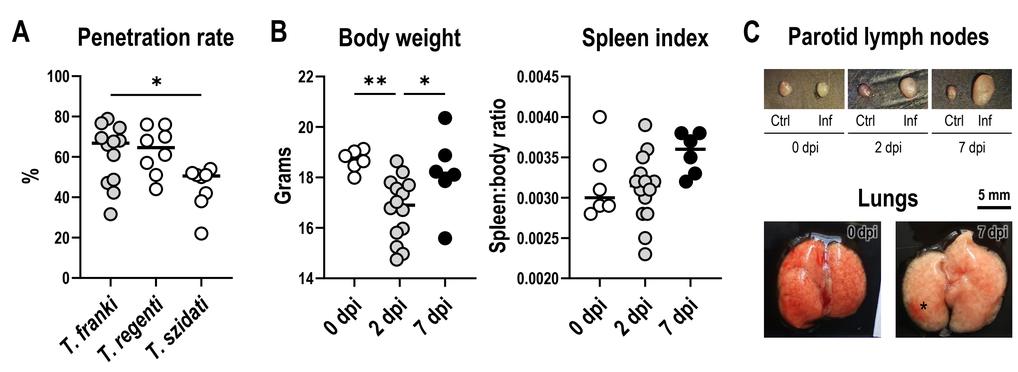

Fig. 6. (A) Penetration rate of *T. franki* cercariae after exposure to mouse pinna for 30 minutes. For comparison, similar data are shown for *T. regenti* and *T. szidati* (other avian schistosomes causing CD). **(B)** Body weight and spleen size of mice infected with *T. franki* 2 and 7 days post infection (dpi). **(C)** Enlargement of parotid lymph nodes draining the infected pinnae and a hemorrhage (*) in infected lungs.

(3) Histopathology of tissues affected by *T. franki* migration

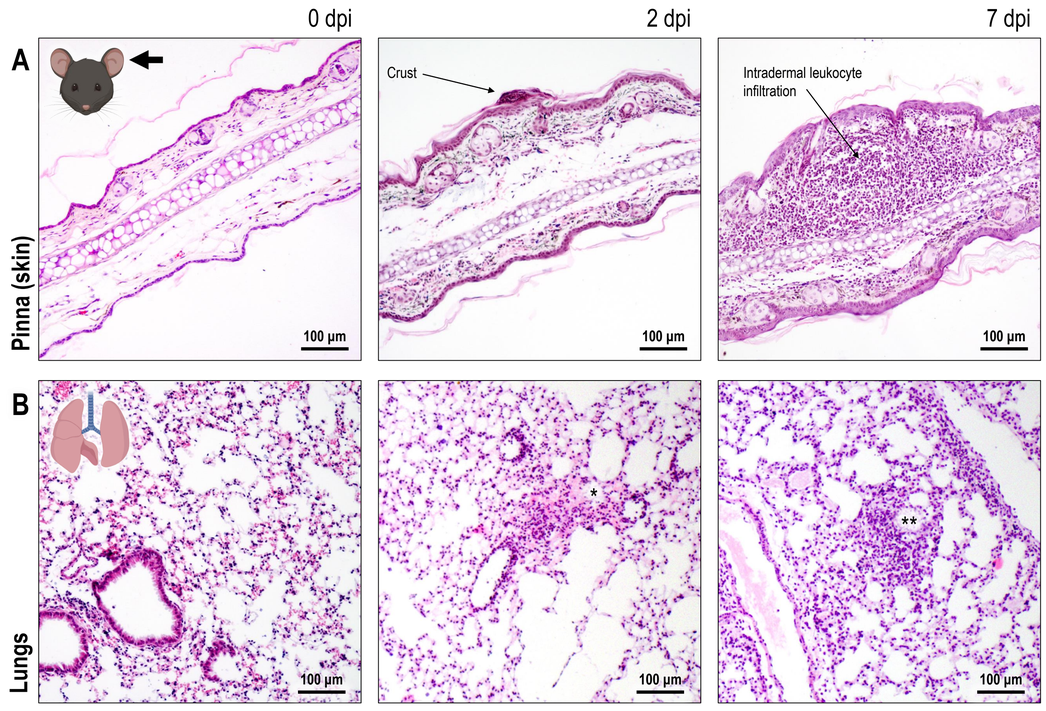

Fig. 7. (A) Penetration of mouse pinna by *T. franki* cercariae led to the formation of epidermal **crusts** with clearly defined borders at 2 days post infection (dpi). At 7 dpi, remarkable **skin thickening** was noticed along with massive leukocyte **intradermal infiltration**. **(B)** Being a visceral species, *T. franki* is expected to migrate to the lungs (similarly to human schistosomes and the avian species *T. szidati*). While migrating schistosomula were not directly detected in the lung tissue, **hemorrhages** (*) and large **eosinophil-rich lesions** (**) were noticed 2 and 7 dpi, respectively.

(4) Local and systemic host immune response against *T. franki*

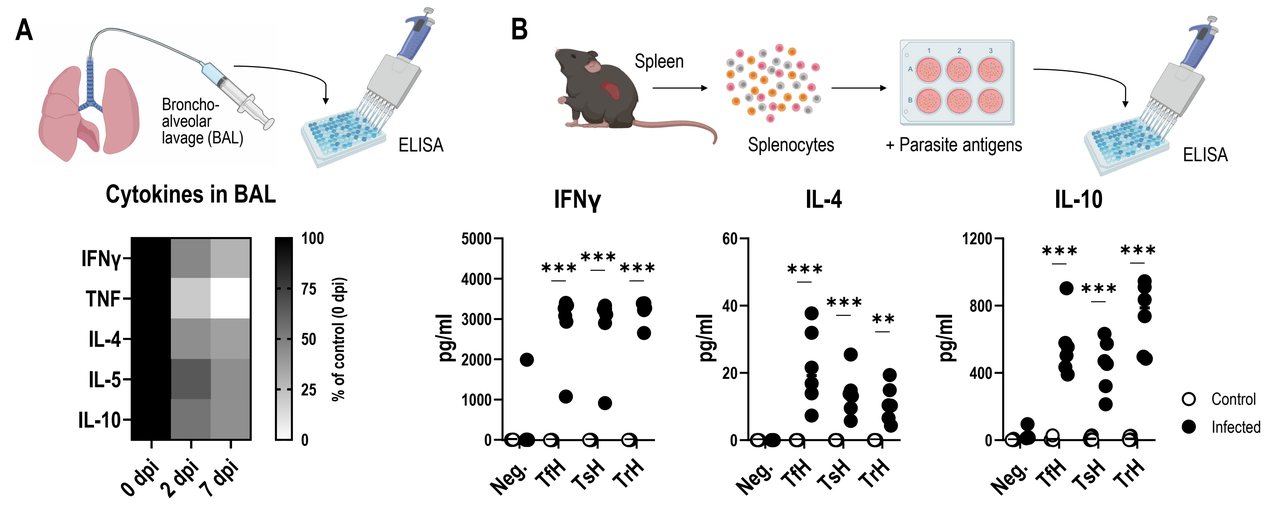

Fig. 8. (A) Bronchoalveolar lavage (BAL) was performed, and cytokines were detected in BAL fluid to assess the local immune milieu. Data are shown relatively to control (0 dpi) mice. **(B)** Splenocytes were isolated from healthy and *T. franki*-infected mice (7 dpi) and treated with the soluble fraction of *T. franki* cercarial homogenate (10 µg/ml; **TfH**). Antigen-specific cytokine production was measured after 72 hours in splenocyte supernatants. A mixture of Th1/Th2/Treg cytokines was detected, and the splenocytes also reacted to cercarial homogenates derived from other avian schistosome species (*T. regenti*, **TrH**, and *T. szidati*, **TsH**), indicating significant species cross-reactivity.

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