【Grant-in-Aid for Transformative Research Areas（B）】

A paradigm shift achieved through the development of *in vitro* culture systems for trematodes

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Project Information	Project Number : 26B306	Project Period (FY) : 2026-2028
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Purpose and Background of the Research

●Outline of the Research

Infectious diseases caused by parasitic flatworms (trematodes and cestodes) are classified by the WHO as neglected tropical diseases (NTDs). Although the Sustainable Development Goals (SDGs) target their control by 2030, this goal remains unlikely, as the mechanisms of parasitism are still poorly understood, hindering effective control and contributing to their neglect.

A major barrier has been the assumption that large parasites cannot be cultured *in vitro*. Attempts in the 1980s failed, leaving few experimental approaches for fundamental research. Recently, we overcame this barrier through an interdisciplinary approach, incorporating expertise from planarian research. We established an *in vitro* culture system using the liver fluke (genus *Fasciola*) as a model. This achievement represents a paradigm shift in parasitology and infectious disease research (Fig. 1).

In this program, we focus on trematodes and use *in vitro* culture to elucidate parasitism. The impact is expected to extend to cestodes and parasitic nematodes, leading to new concepts that may develop into a transformative research area (A). We believe this paradigm shift will drive major advances in parasitology.

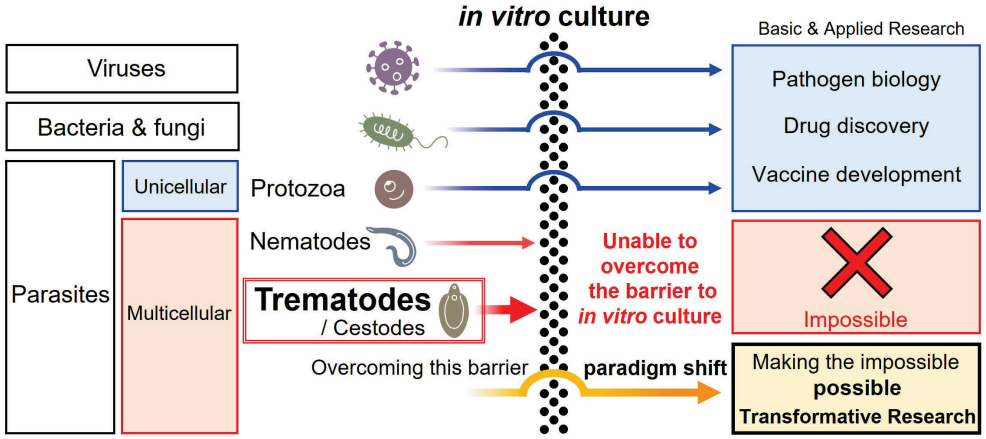

Figure 1. *In vitro* culture of pathogens & basic and applied research

●Background to the Research Program

Approximately 300 million people worldwide are affected by trematode infections, many of which are zoonotic. Fasciolosis alone causes an estimated USD 3.2 billion in annual livestock losses (Spithill, 1999). Juveniles damage the liver during migration, sometimes leading to fatal hemorrhage and severe inflammation. Despite their major impact, control measures have been largely neglected, mainly due to an insufficient research foundation arising from two key factors.

First, laboratory trematode strains require both mammalian and snail hosts, limiting the number of researchers who can work with them. The **A01 Mammalian unit** standardized maintenance protocols, enabling collaboration with the **A03 Snail and A04 Genetic modification units**.

Second, their large size (Fig. 2) led to the assumption that *in vitro* culture is infeasible. This barrier was overcome through collaboration with the **A02 Planarian unit**, revealing that a planarian sex-inducing substance (**SIS**) promotes maturation and larval development of the liver fluke *in vitro*.

This discovery led to the establishment of this research program.

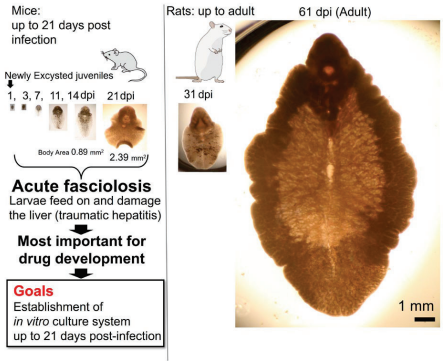

Figure 2. Liver fluke development & goals

Expected Research Achievements

●Objectives of the Research Program and Individual Projects

Projects A01–A04 will establish *in vitro* trematode culture platforms, enabling drug and vaccine target discovery and broad applications.

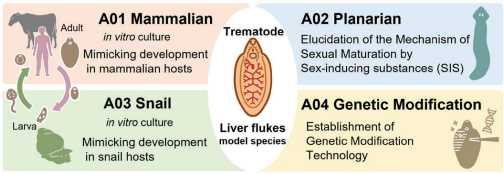

Figure 3. Project units

A01 Mammalian unit: trematode *in vitro* culture — Mimicking liver fluke development inside mammalian hosts by comprehensive analyses

SIS identified by the A02 Planarian unit enabled *in vitro* maturation, but developmental limits remain. We will identify additional factors to establish a culture system that accurately mimics development and provides a practical research platform.

A02 Planarian unit: From planarians to parasites— Molecular evolution of the SIS synthesis pathway, key factors for reproductive strategy switching

We will elucidate the biosynthetic pathway of SIS, key factors of reproductive switching in trematodes. Using planarians as a platform, we will determine its structure, identify biosynthetic genes and enzymes via transcriptomics, and achieve chemical synthesis. In collaboration with A01, we will also develop antagonists that inhibit sexualization, enabling transmission-blocking strategies.

A03 Snail unit: Artificial developmental model in snails and infection control

Asexual reproduction in snails greatly amplifies parasite numbers, making this stage key for control. We will analyze larval dynamics and identify snail-derived factors to design an *in vitro* culture system. This will enable a novel strategy to control trematode infection by blocking larval development.

A04 Genetic modification unit: Generation of genetically modified liver flukes via host-independent manipulation

Key factors underlying pathogenicity and parasitic strategies remain largely unknown due to limitations in gene-editing of obligate parasites. We will introduce genetic modifications into germline and embryonic cells, then maintain and mature parasites *in vitro* to obtain modified progeny. This platform will enable functional gene analysis and identification of genes controlling development.

Website(s)	The research program website: https://trematoda-in-vitro.vet.iwate-u.ac.jp Principal Investigator's laboratory website: https://vetparasitol.vet.iwate-u.ac.jp/