

Experimental infection and host response of tilapia *Oreochromis niloticus* to zoonotic metacercariae *Haplorchis pumilio*

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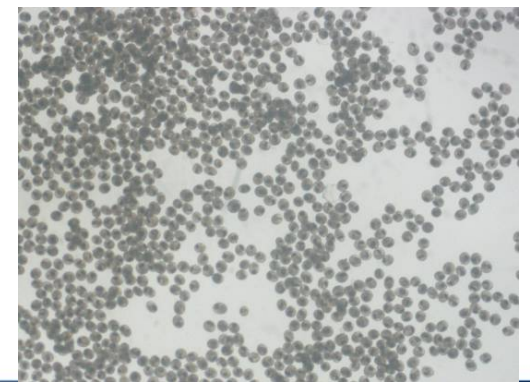
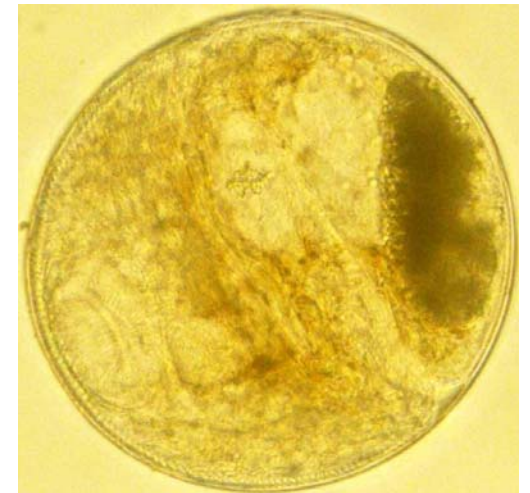
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INTRODUCTION

Fishborne Zoonotic Trematodes (FZT)

- About 40 millions of people, mainly in Asian countries such as China, Thailand, Korean and Vietnam
- Most of freshwater fish species can be served as second intermediate hosts
- Habit of eating raw, uncooked or improperly cooked fish are common in many communities
- Lack of experimental infection and interaction studies between FZT and their fish host



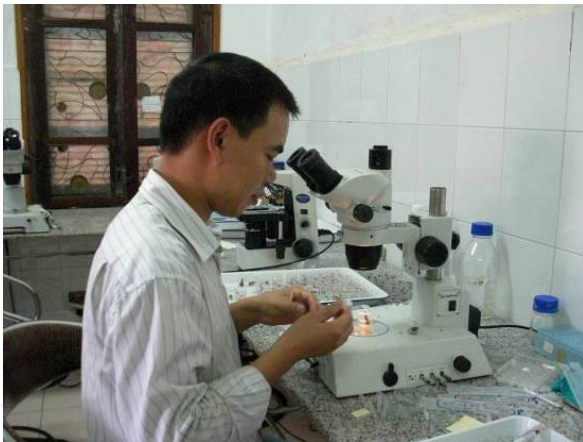
Experimental infection and host response of
tilapia *Oreochromis niloticus* to zoonotic
metacercariae *Haplorchis pumilio*

OBJECTIVES

1. To evaluate the susceptibility of tilapia to *H. pumilio* cercariae following experimental infection
 2. To determine the distribution of metacercariae in experimentally infected fish
 3. To evaluate pathological changes in situ with emphasis on cellular response induced by metacercariae infection
 4. To determine specific antibody response of tilapia against *H. pumilio* under experimental conditions
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METHOD

- Tilapia: 427 fry and 158 adult fish, free of metacercariae supplied by RIA1
- Various infected snails with *Haplorchis pumilio* cercariae collected from Nam Dinh province



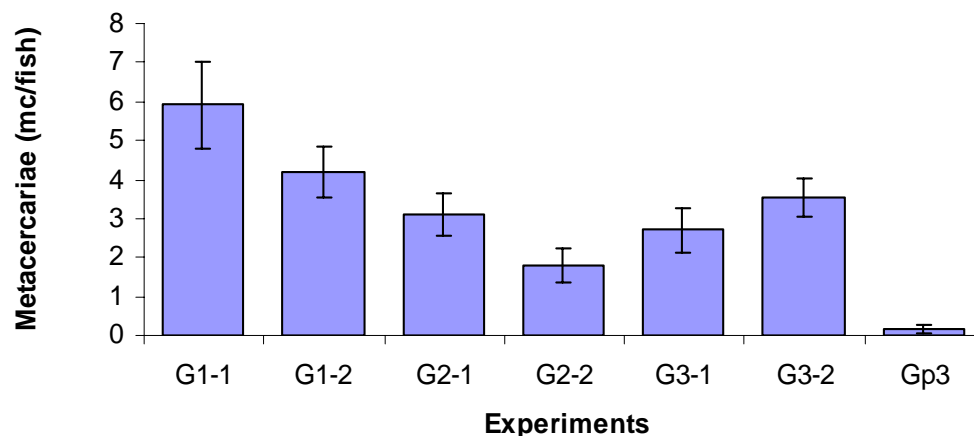
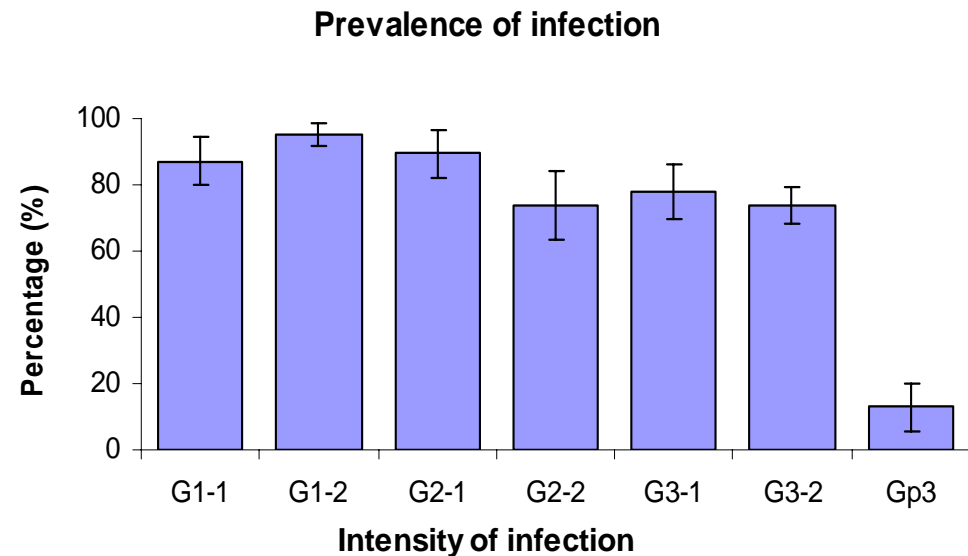
METHOD (continued...)

Factors	Group 1 Fish size (0.07±0.02 g)			Group 2 Fish size (0.11±0.03 g)			Group 3 Fish size (38.4±11.5 g)			
	G1-1	G1-2	Go1	G2-1	G2-2	Go2	G3-1	G3-2	Go3	Gp3
Number of fish	120	58	92	52	52	53	50	65	20	23
Number of snails/cercariae	34 snails	6 snails	0	3000 cer.	2500 cer.	0	25 snails	116 snails	0	500 cer.
Exposure duration (hours)	24	4.5	24	2	2	2	72	72	72	-
Water volume at challenge (ml)	500	500	500	250	250	250	2500	2700	2000	2000

METHOD (continued...)

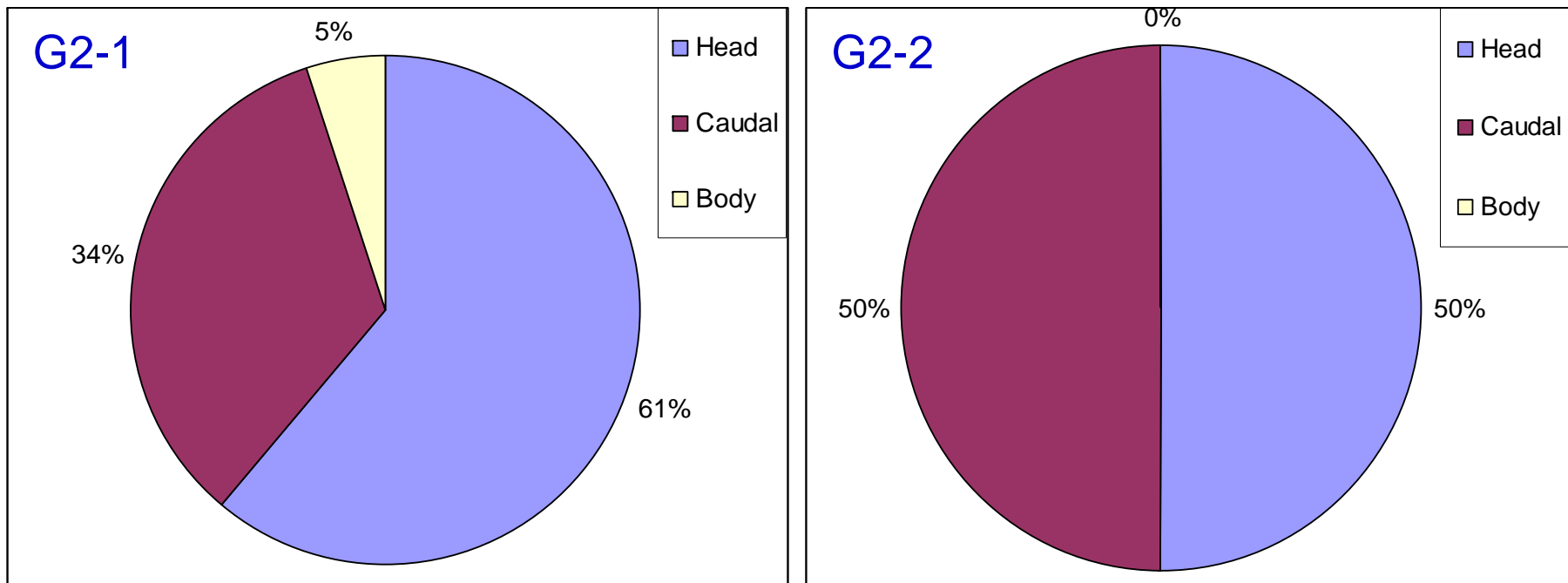
- Compression by slide (head, body and caudal parts), fish in Group 1 and 2
- Digestion by pepsin (individual fish), fish in Group 3
- Histology (buffer formalin, H&E staining), fish in Group 1 and 2
- Immunohistochemistry (Serum dilution 1:1200), fish in Group 1 and 2
- ELISA, pool sera day 1, 7, 14 and 21 of fish in Group 3

RESULT prevalence and intensity



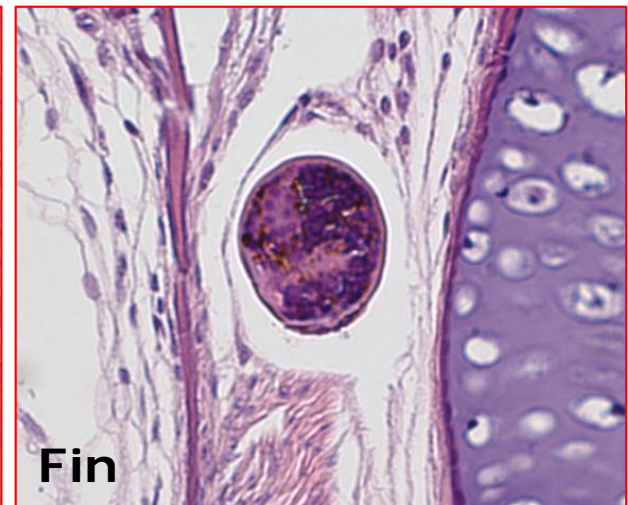
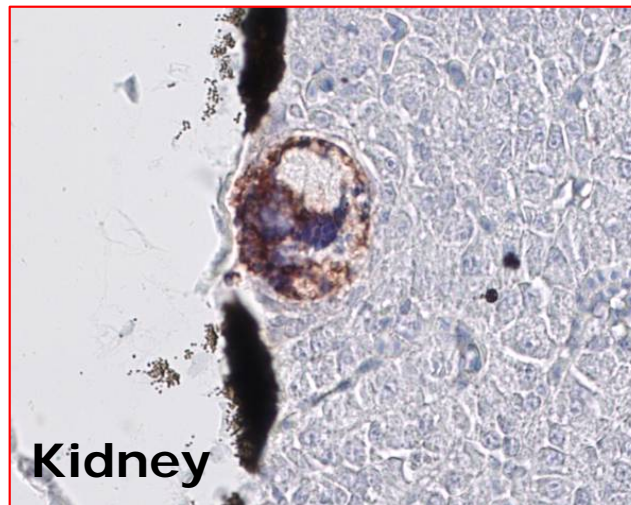
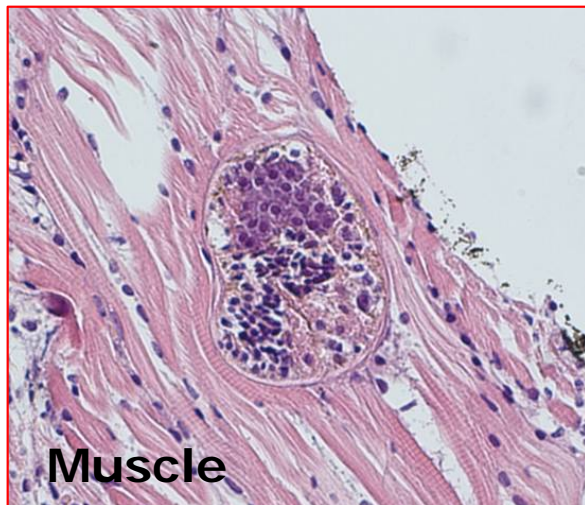
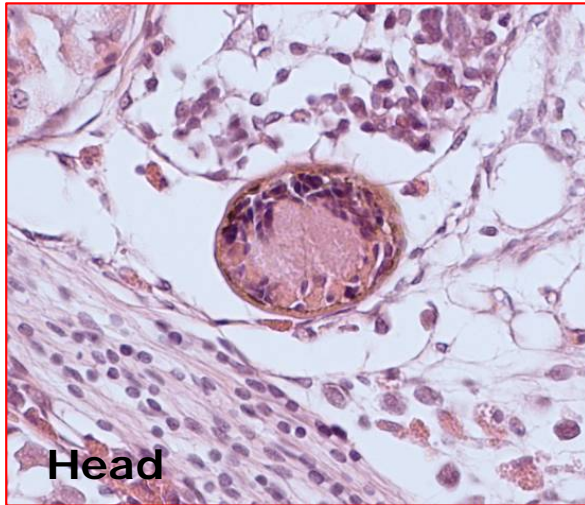
- Tilapia are susceptible to infection through the natural route while I.P. injection results in low FZT prevalence
- The dose and exposure time did not influence FZT prevalence

RESULT distribution of metacercariae

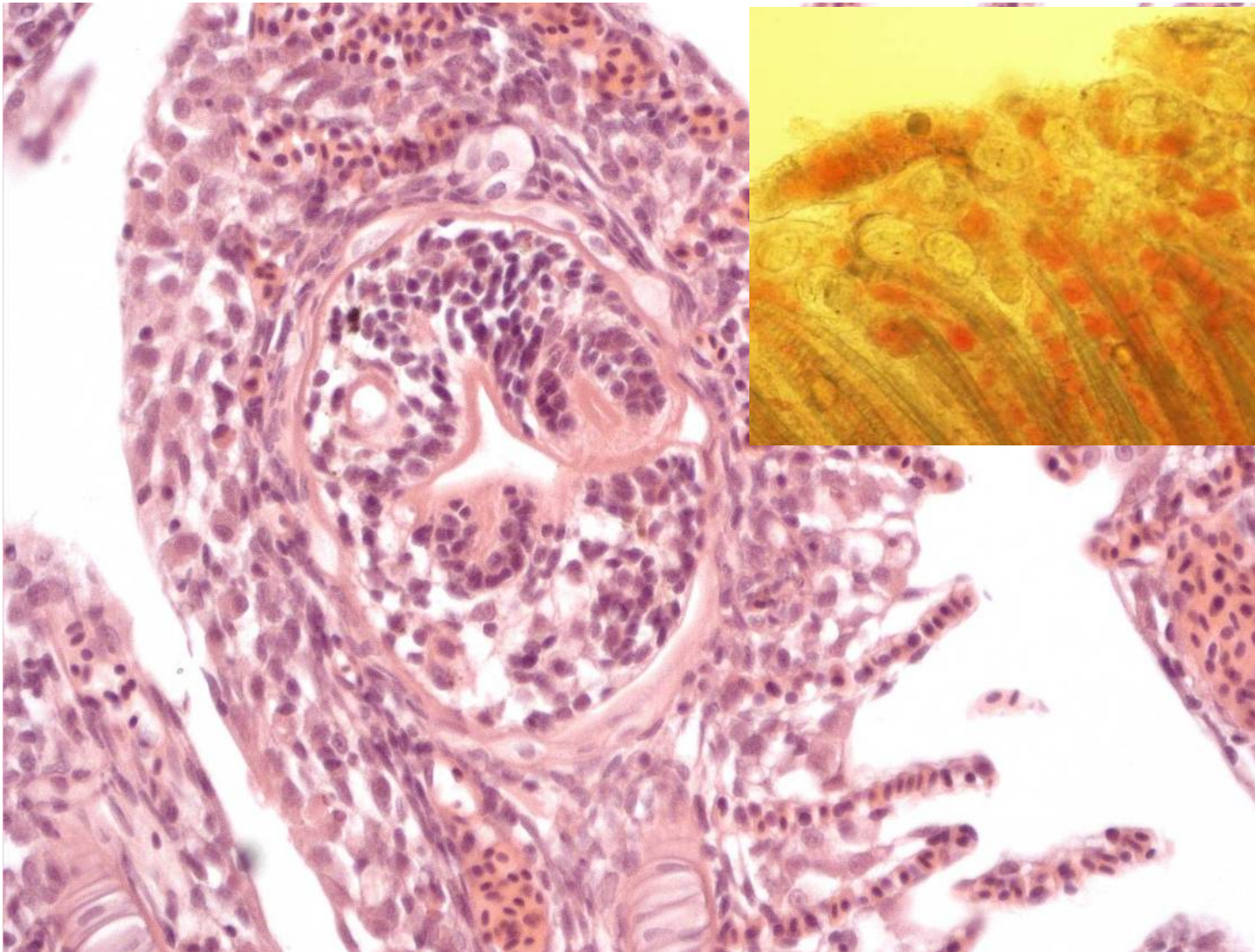


- Role of scale and mucous layer on skin in prevention of host evasion of cercariae

RESULT localization of metacercariae



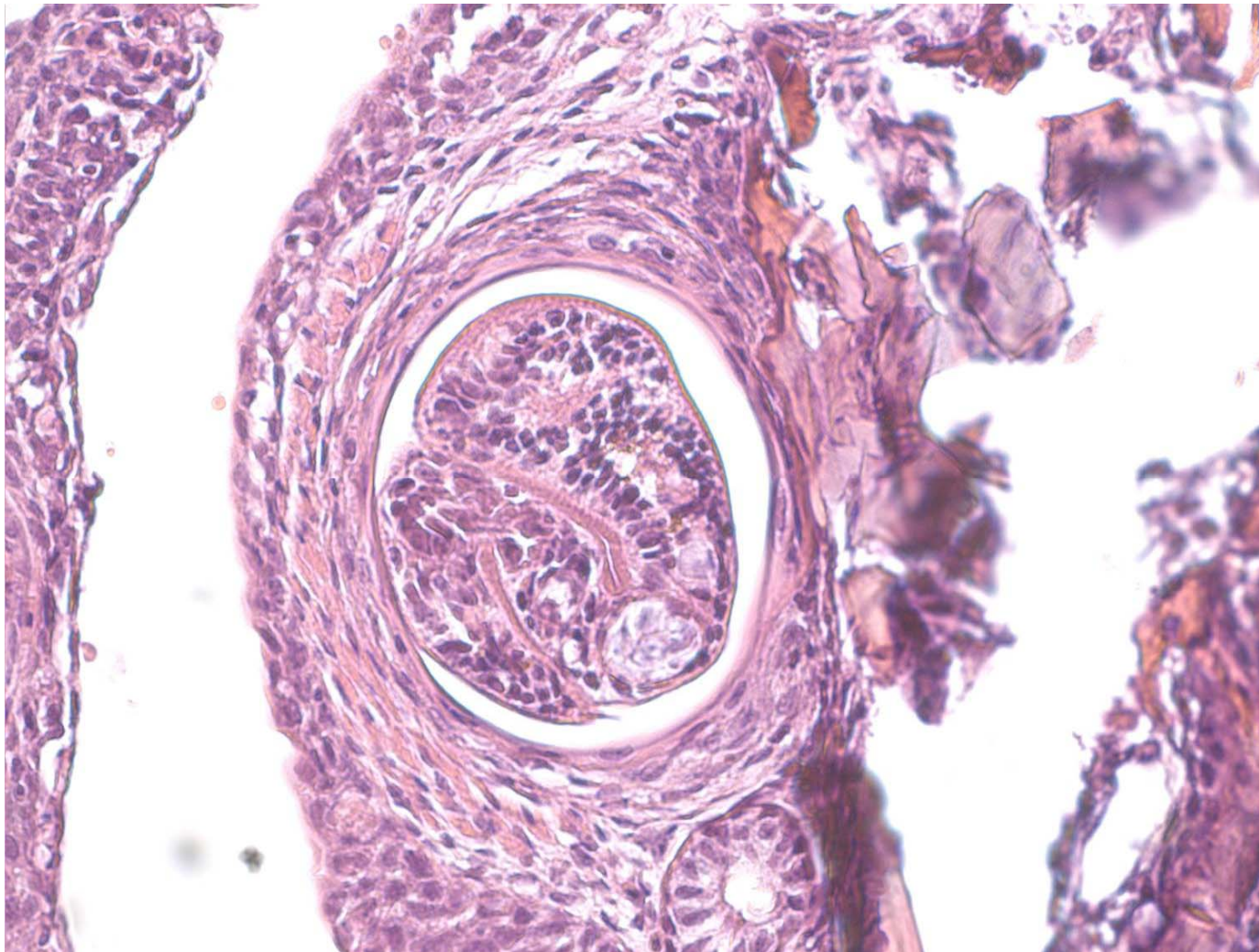
RESULT cellular response



Blood vessel
congestion,
cellular
response
(EGCs)

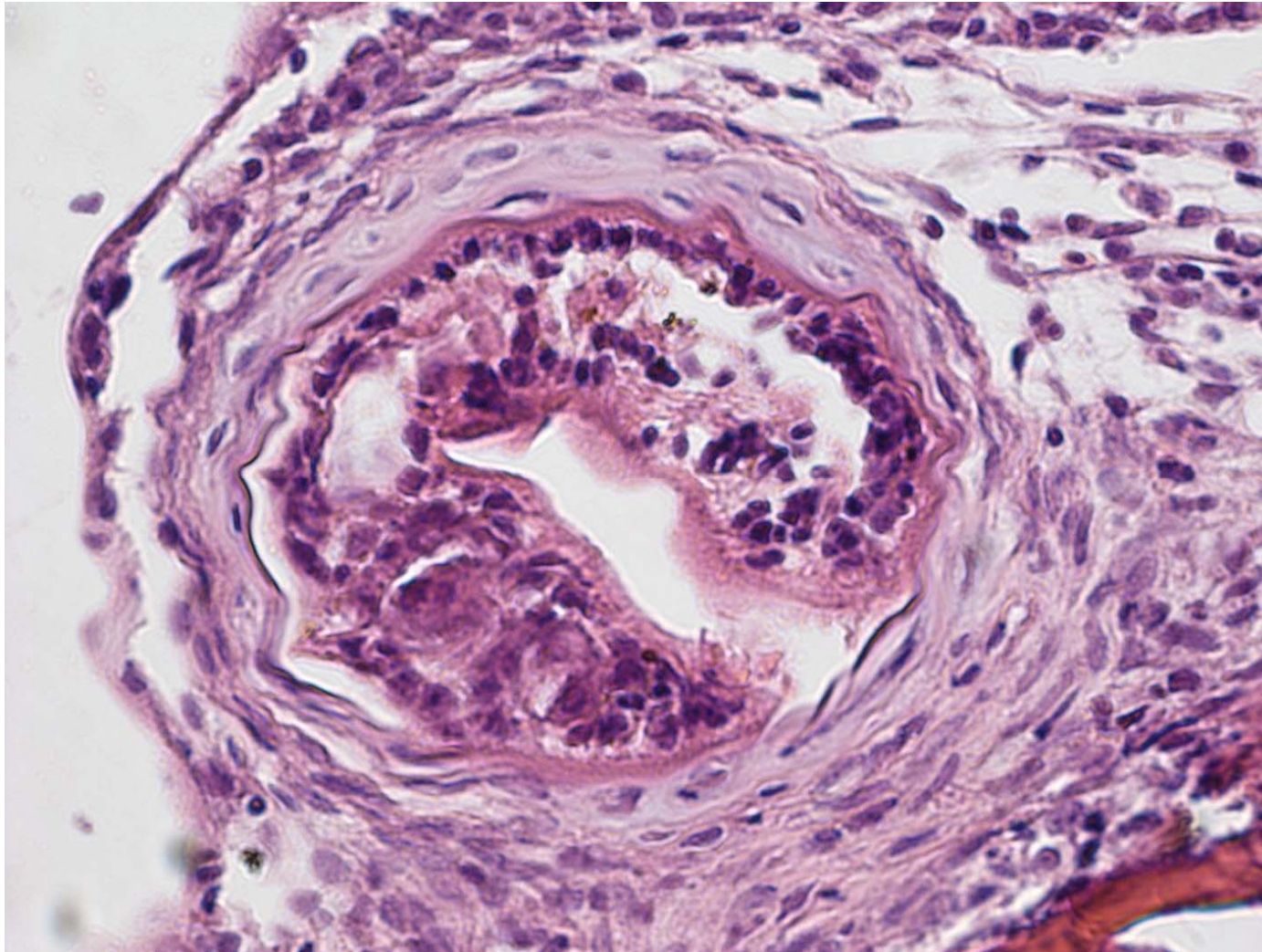
RESULT cellular response

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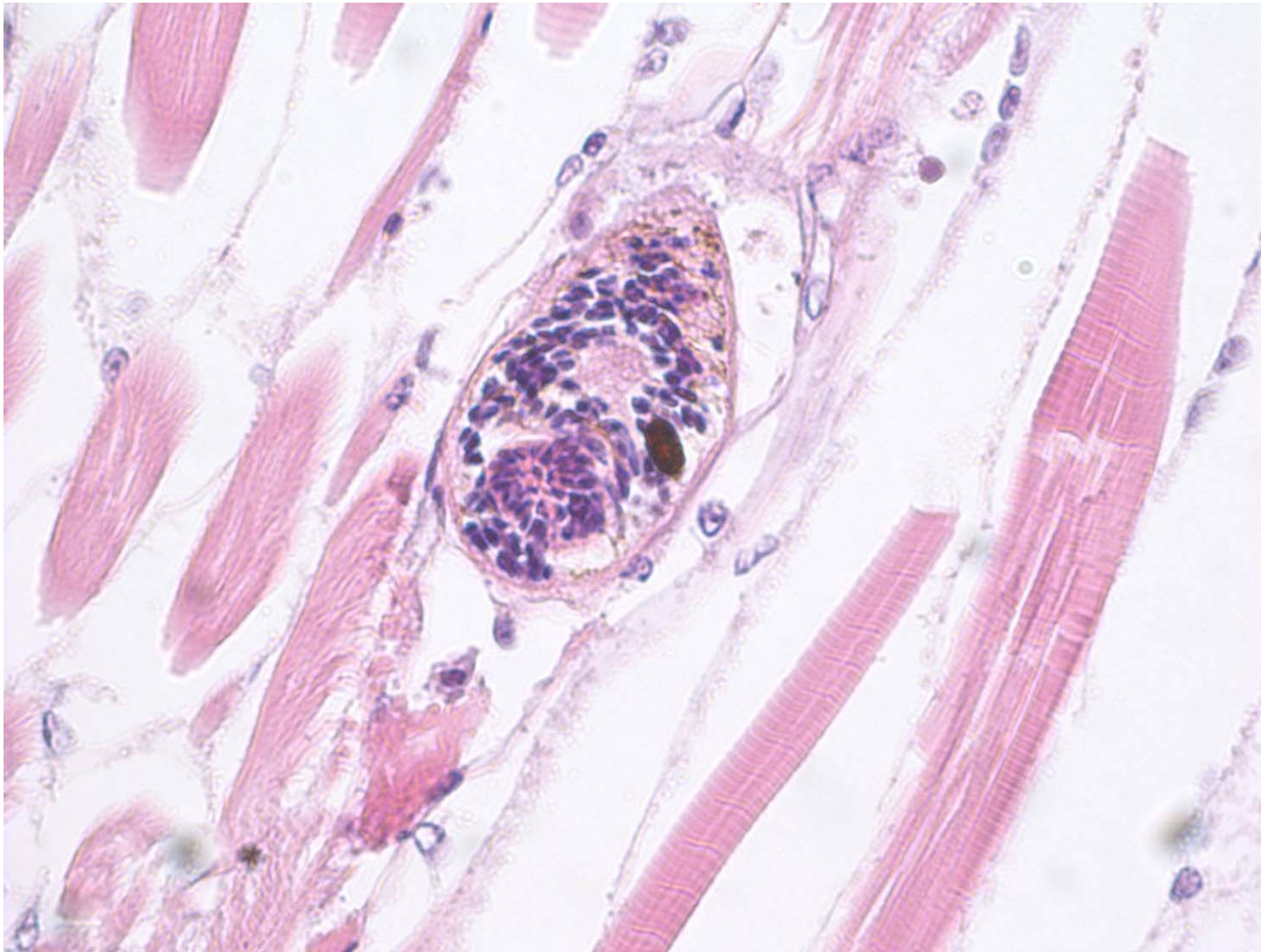
Intact
metacercariae in
the gill base
tissue

RESULT cellular response (continued...)



Encapsulation,
host cell
infiltration

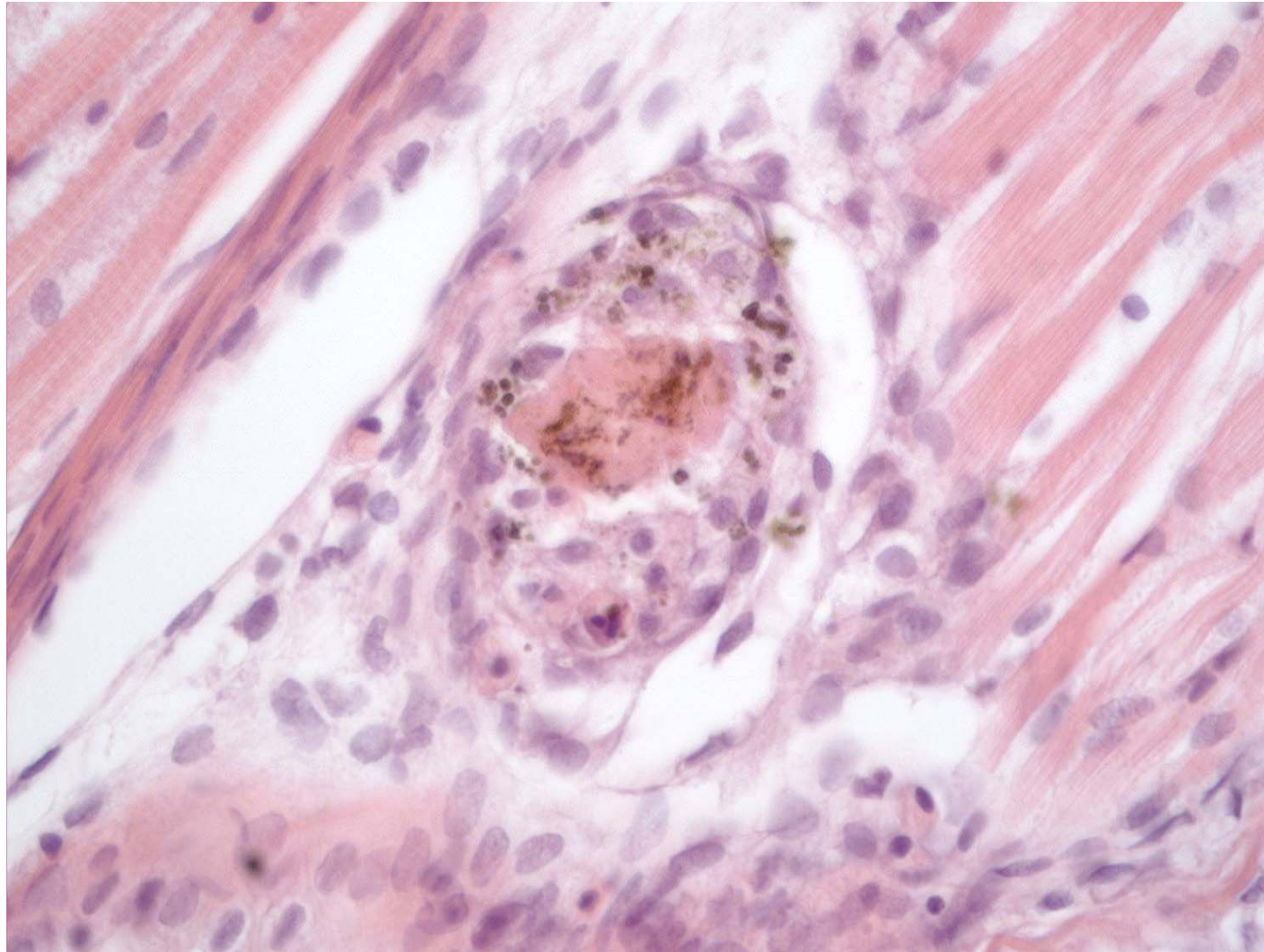
RESULT cellular response (continued...)



Muscle
degeneration,
disintegration
of muscle fibers

RESULT cellular response

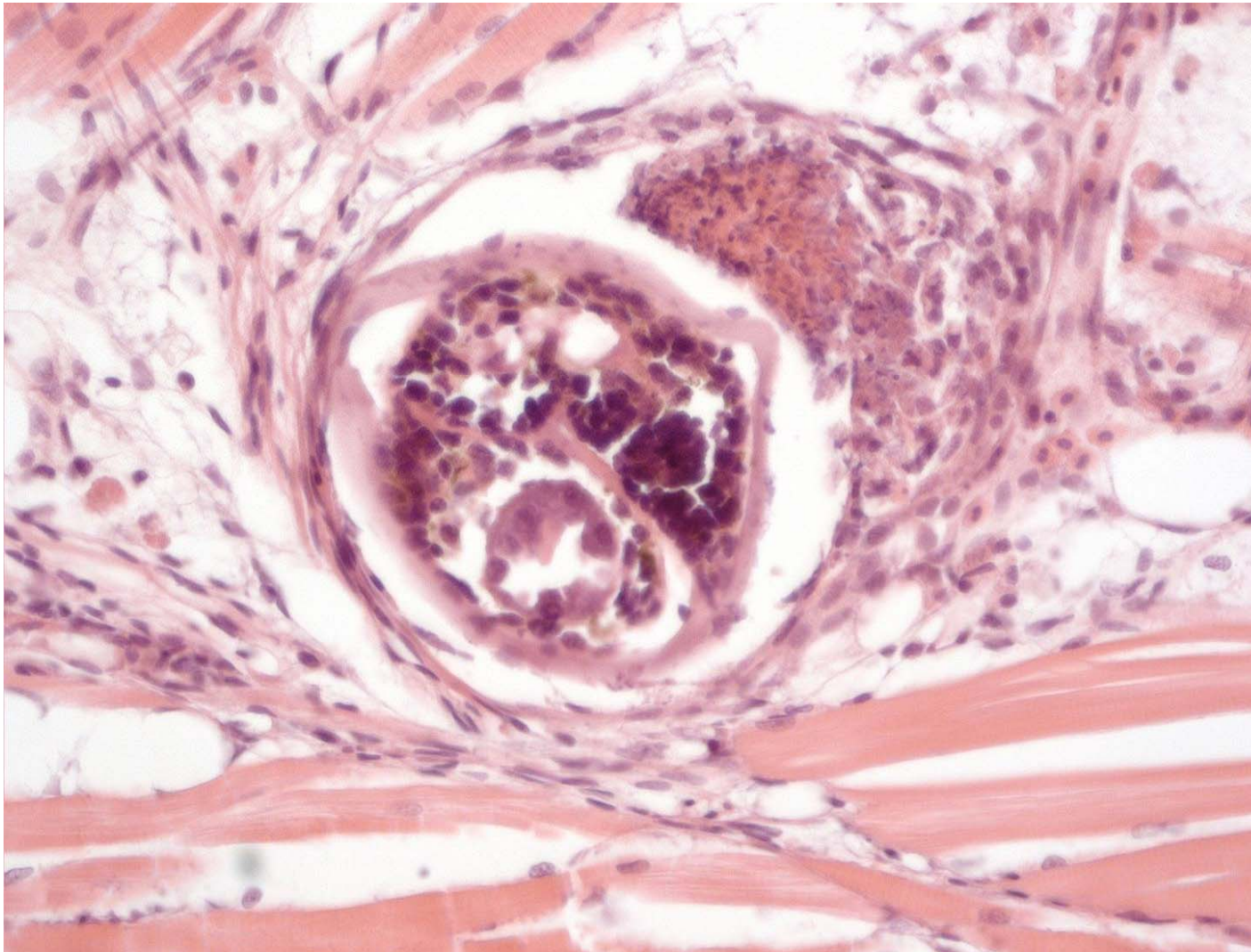
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Host cell
infiltration,
melanin-
containing cells

RESULT cellular response

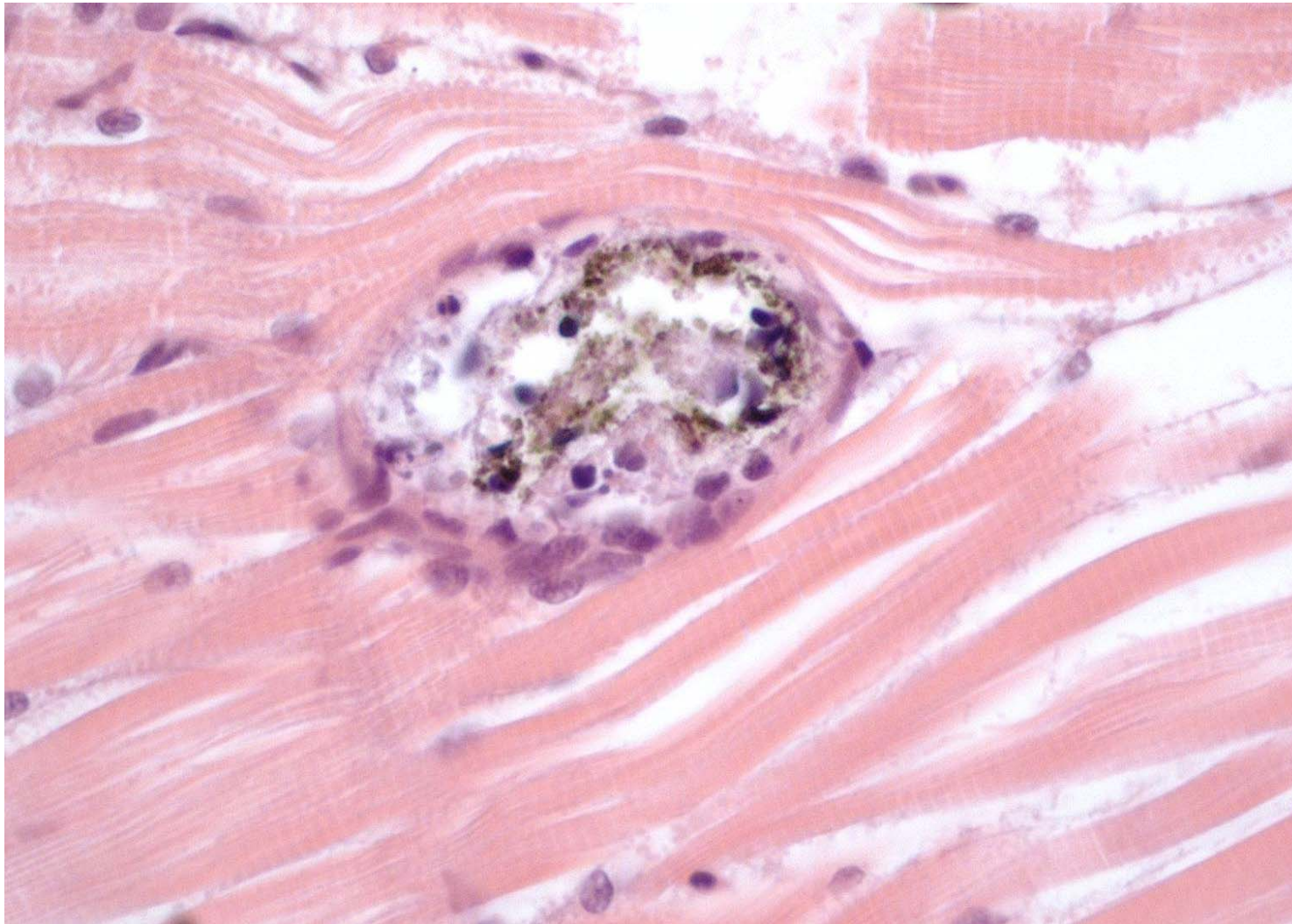
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Inflammations
and necrosis
cell debris

RESULT cellular response

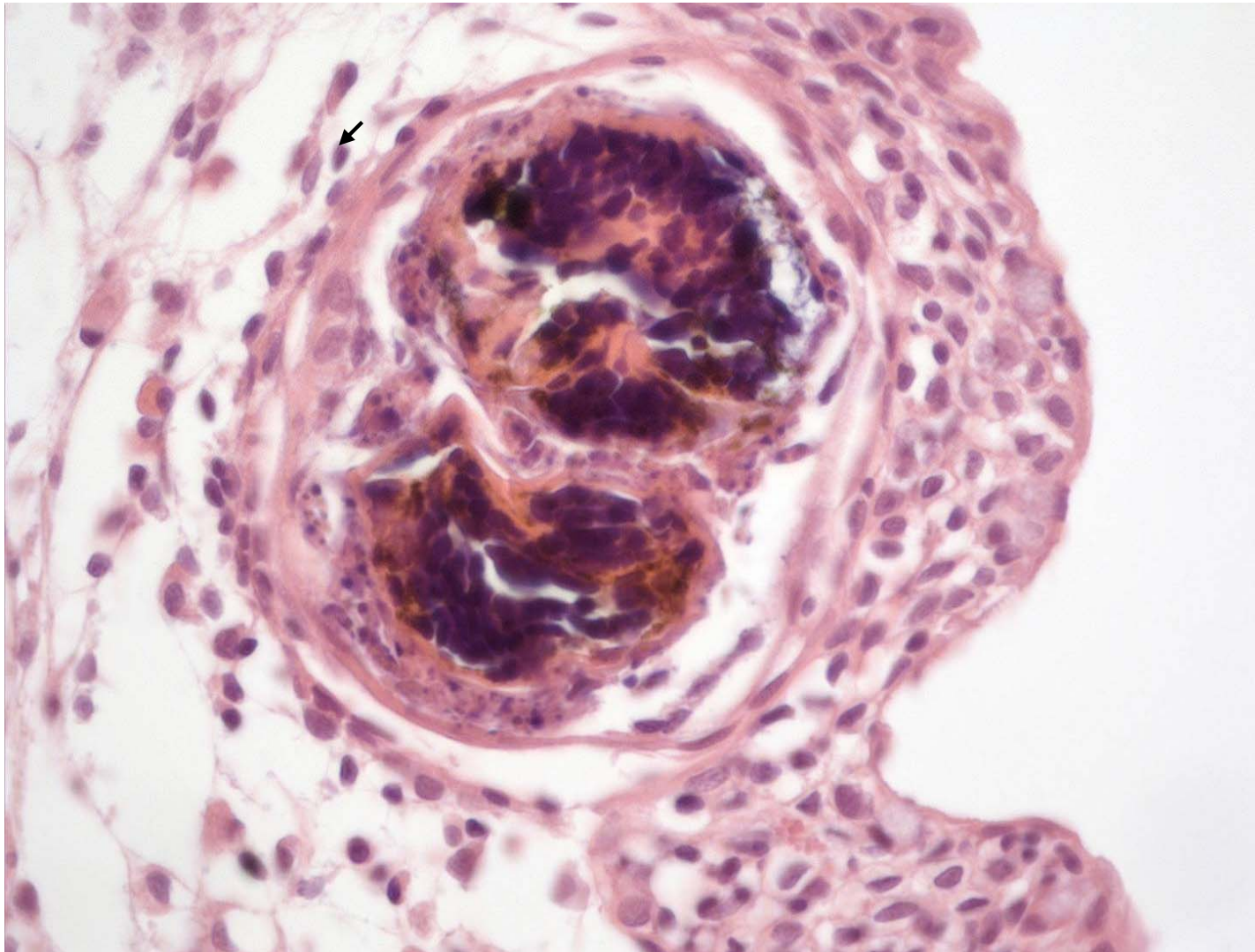
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Metacercariae
died with
necrosis
stages

RESULT cellular response

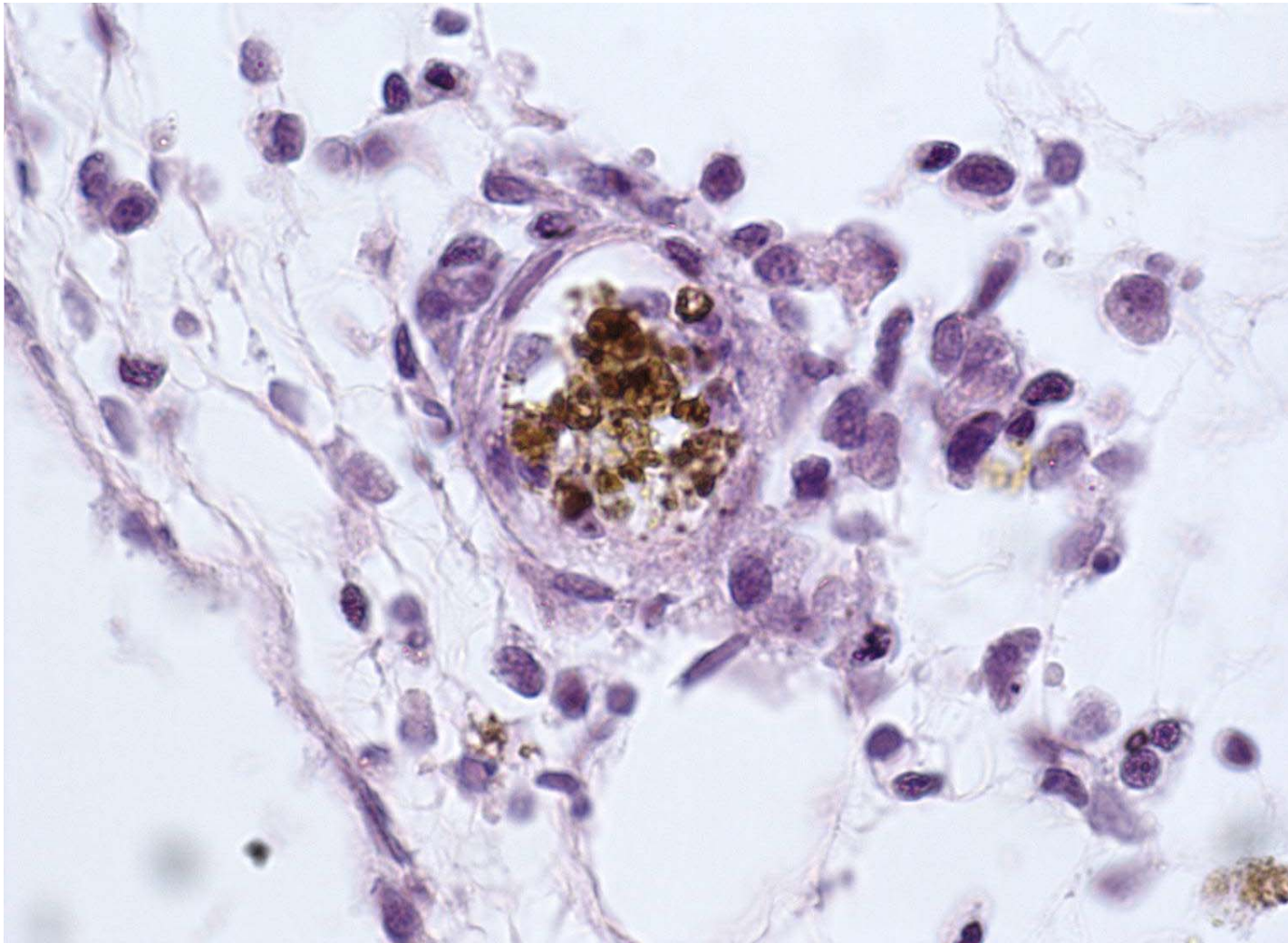
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Host cell
infiltration,
metacercariae
being in
necrosis

RESULT cellular response

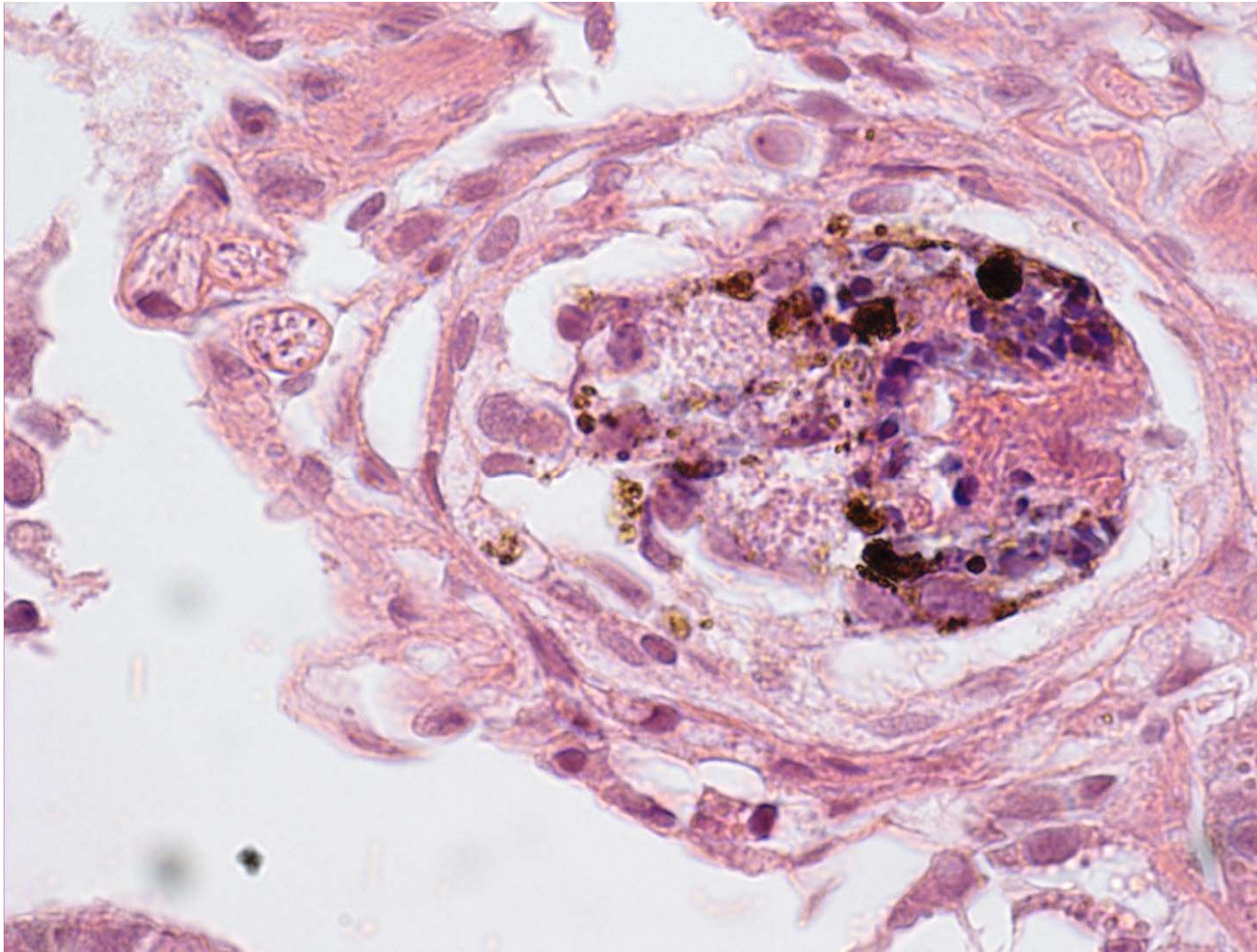
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Metacercariae
died, host cell
infiltration

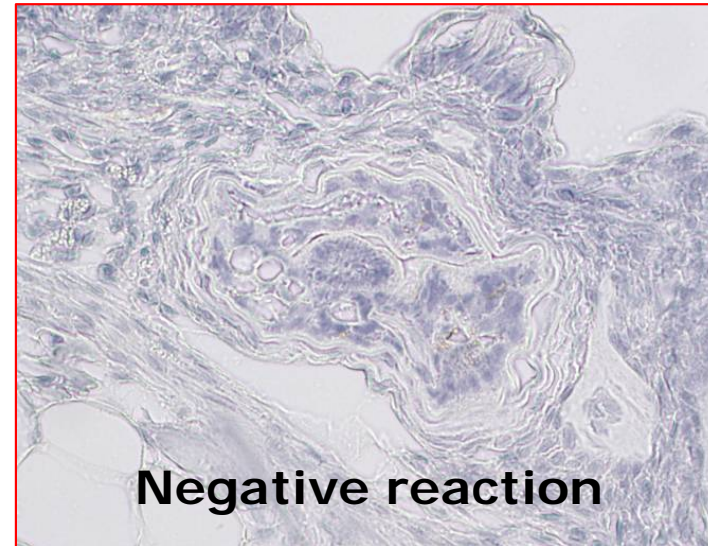
RESULT cellular response

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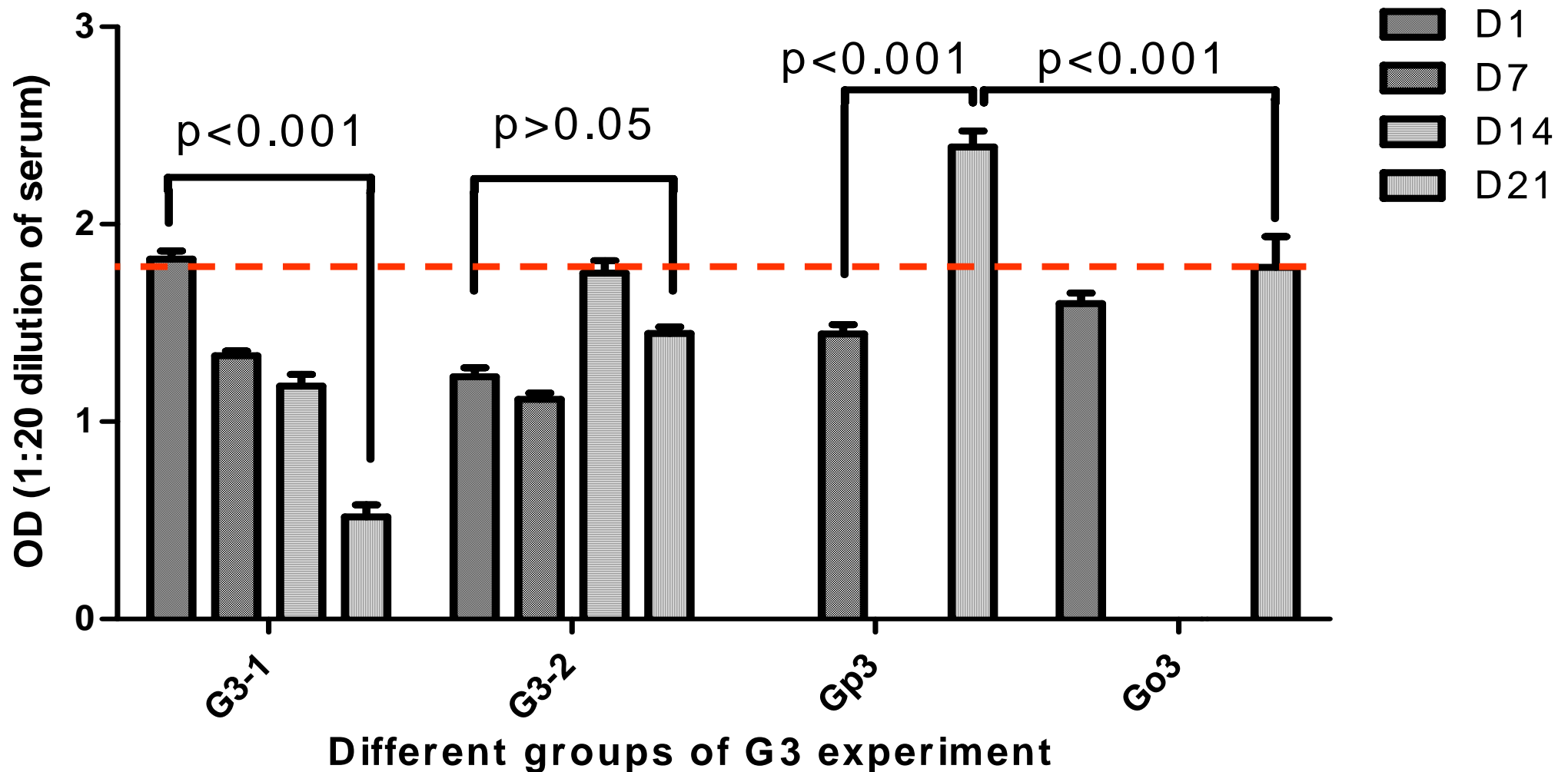


Metacercariae
died, host cell
infiltration

RESULT immunohistochemistry



RESULT antibodies response



DISCUSSION

- All fish had circulating antibodies to *H. pumilio* antigens, incl. the Go3 group
- Fish immunized with live cercariae elicited an increased antibody levels under experimental conditions while natural exposure of fish (through water) did not
- Encystment of metacercariae in natural exposure could result in an immune evasion (of metacercariae)
- Weak inflammation and low intensity of infection may not be sufficient to induce a humoral response
- Stress, high density and handling of fish may suppress circulation of antibody levels

CONCLUSION

- Tilapia *O. niloticus* is highly susceptible to infection with *H. pumilio* (74-95%) by natural exposure, but not following I.P. injection
- Highest prevalence of metacercariae in the head, followed by the caudal fin. There was less than 5% of metacercariae distributed in body region
- Metacercariae infection did in general not cause serious pathological changes in tissue. Encapsulation and degeneration/necrosis or structural changes of metacercariae found in some fish
- I.P. injection/challenge with live cercariae induced specific antibody response after boost. Natural exposure did not seem to induce humoral response

FUTURE PERSPECTIVES

- Determine correlation between the exposure time, dosage and size of fish in relation to prevalence and intensity of infection
- Assess possibility of cross-reaction between *H. pumilio* and other *Haplorchis* spp. or *Centrocestus* spp. by IHC and ELISA using polyclonal antibody
- Study cellular response to *H. pumilio* metacercariae infection in tilapia to determine infiltration of leucocytes relative to dose and exposure time post infection in fry and fingerling fish
- Study specific humoral antibodies response to *H. pumilio* following infection by improving the route of immunization and experimental condition

ACKNOWLEDGMENT

- The “Fishborne Zoonotic Parasites in Vietnam” (FIBOZOPA) project no. 91140/file no. 104.Dan.L.8.f and the Danish International Development Assistance (Danida) are gratefully acknowledged for their financial support of this study
- Further knowledge on FIBOZOPA: [http:// fibozopa2.ria1.org](http://fibozopa2.ria1.org)

