

Submitter: Uni. of QLD - UQBR Aquatics

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Submission No: 22/M620

Your ref: 22 UQ Zebrafish
Aquaria (Otto and
Seddon)

Date Submitted: 10 November 2022

Date of issue: 17 January 2023

Location: Otto (Conventional_cert 3112)

Species: Zebrafish

Endoparasite	Pos / Tested	Laboratory	Method
Pseudocapillaria tomentosa	0 / 12	Cerberus	Histo
Histopath	Pos / Tested	Laboratory	Method
Zebrafish Histopathology	12 / 12	Cerberus	Histo
Supplementary Testing		Laboratory	Method
16S SEQ - Bacterial identificaton of pure colony	1	Cerberus	PCR

Location: Seddon (Conventional_cert 3428)

Species: Zebrafish

Endoparasite	Pos / Tested	Laboratory	Method
Pseudocapillaria tomentosa	0 / 14	Cerberus	Histo
Histopath	Pos / Tested	Laboratory	Method
Zebrafish Histopathology	14 / 14	Cerberus	Histo
Supplementary Testing		Laboratory	Method
16S SEQ - Bacterial identificaton of pure colony	1	Cerberus	PCR

Comment:

Samples Refs. were:

Sample 01 2022Sample37 -Asymptomatic Sump, Sample 02 2022Sample38 -Asymptomatic Sump, Sample 03 2022Sample39 -Asymptomatic Sump, Sample 04 2022Sample40 -Asymptomatic Sump, Sample 05 2022Sample41 -Asymptomatic Sump, Sample 06 2022Sample42 -Asymptomatic Sump, Sample 07 2022Sample43 -Asymptomatic Sump, Sample 08 2022Sample44 -Asymptomatic Sump, Sample 09 2022Sample45 -Outbox fish (fell on floor), Sample 10 2022Sample48 -Asymptomatic Sentinel, Sample 11 2022Sample49 -Asymptomatic Sentinel, Sample 12 2022Sample50 -Asymptomatic Sentinel, Sample 13 2022Sample51 -Asymptomatic Sentinel, Sample 14 2022Sample52 -Asymptomatic Sentinel, Sample 15 2022Sample53 -Asymptomatic Sentinel, Sample 16 2022Sample55 -Asymptomatic Sentinel, Sample 17 2022Sample57 -Asymptomatic Sump, Sample 18 2022Sample58 -Asymptomatic Sump, Sample 19 2022Sample59 -Asymptomatic Sump, Sample 20 2022Sample60 -Asymptomatic Sump, Sample 21 2022Sample61 -Symptomatic Sample, Sample 22 2022Sample62 -Symptomatic Sample, Sample 23 2022Sample63 -Symptomatic Sample, Sample 24 2022Sample64 -Asymptomatic Sump, Sample 25 2022Sample65 -Symptomatic Sump, Sample 26 2022Sample66 -Symptomatic Sump.

PCR/RT-PCR assays include extraction, positive and negative controls to verify the results.

Pseudocapillaria tomentosa on histology:

No evidence of Pseudocapillaria tomentosa in the sections examined of all fish.

Sample 22 2022Sample62 -Symptomatic Sample (Otto (Conventional_cert 3112) - 16S SEQ - Bacterial identificaton of pure colony), Sample 03 2022Sample39 -Asymptomatic Sump (Seddon (Conventional_cert 3428) - 16S SEQ - Bacterial identificaton of pure colony):

No Pathogens isolated by PCR.

Sample 26 2022Sample66 -Symptomatic Sump (Otto (Conventional_cert 3112) - Zebrafish Histopathology):

Addendum Report

No significant organisms were found on PCR of the Formalin-fixed Paraffin Scrolls from Samples 3A and 22A.

This does not rule out an infectious agent; the results may be false negative as a result of the long-standing

formalin fixation and/or low pathogen load as no pathogens were visible on histology in the granulomas.

Sending fresh tissue in our PCR bead tubes is preferable.

Macropathology, Morphological Summary (MD) and Aetiology if known

24 x 15ml Falcon tubes of 10% NBF:

2 x 50ml Falcon tubes of 10% NBF: labelled and containing fish.

Histopathology (HE and ZN); the following organ systems were evaluated Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, reproductive system (male), integument (including fins and tail), swim bladder.

Sample 01 2022Sample37 -Asymptomatic Sump, containing 2 fish:

1A: the larger yellow, striped fish, female

MD: focal necrogranuloma, acid-fast negative, unknown aetiology

1B: Smaller yellow striped with bent tail, male

MD: locally extensive necrotising myositis, tail; of unknown aetiology

Sample 02 2022Sample38 -Asymptomatic Sump, containing 2 fish:

2A: the larger yellow, spotted fish with long fins and tail, female

MD: microsporidiosis, encephalomyelitis and ganglioneuritis with acid-fast xenomas present, Aetiology: Pseudoloma neurophilia

2B: Smaller yellow striped fish, male

MD: locally extensive necrotising myositis, tail; of unknown aetiology

Sample 03 2022Sample39 -Asymptomatic Sump, containing 2 fish:

3A: white fish with partial tail and fins, incision into the head-body, male

MD: systemic necrogranulomatous ZN negative inflammation; paraffin scrolls will be sent for PCR

3B: yellow striped fish, female

MD: No abnormalities detected (NAD).

Sample 04 2022Sample40 -Asymptomatic Sump, containing 2 fish:

4A: Large grey, striped fish, female

MD: NAD

4B: Smaller grey spotted with slightly kinked tail

MD: microsporidiosis, encephalomyelitis and ganglioneuritis with acid-fast xenomas present, Aetiology: Pseudoloma neurophilia

Sample 05 2022Sample41 -Asymptomatic Sump, containing 2 fish:

5A: grey spotted fish

MD: This block with the tissue was unfortunately lost during histology processing.

5B: grey striped fish, male

MD: Obese fish, abundant adipose tissue. Multifocal to diffuse degenerative and necrotising myositis with no micro-organisms identified.

Sample 06 2022Sample42 -Asymptomatic Sump, containing 2 fish:

6A: grey spotted fish, male

MD: mild locally extensive necrotising myositis, tail; of unknown aetiology

6B: grey striped fish, female

MD: multifocal acute necrotic and necrogranulomatous oophoritis (EAIF)

Sample 07 2022Sample43 -Asymptomatic Sump, containing 2 fish:

7A: larger grey striped fish, female

MD: NAD

7B: smaller grey striped fish, female

MD:NAD

Sample 08 2022Sample44 -Asymptomatic Sump, containing 1 fish:

8: grey striped fish with long fins and tail, male

MD:NAD

Sample 09 2022Sample45 -Outbox fish (fell on floor), containing 1 fish:

9: grey striped fish with bent tail, female

MD: mild EAIF

Sample 10 2022Sample48 -Asymptomatic Sentinel, containing 2 fish:

10A: grey striped fish, female

MD: mild acute EAIF

10B: grey striped fish, female

MD: mild acute EAIF

Sample 11 2022Sample49 -Asymptomatic Sentinel, containing 2 fish:

11A: grey striped fish, female

MD: mild acute EAIF

11B: grey striped fish, female

MD: mild acute EAIF

Sample 12 2022Sample50 -Asymptomatic Sentinel, containing 2 fish:

12A: grey striped fish, female
MD: mild acute EAIF
12B: grey striped fish, female
MD: mild acute EAIF

Sample 13 2022Sample51 -Asymptomatic Sentinel, containing 2 fish:

13A: grey striped fish, female
MD: moderate acute EAIF
13B: grey striped fish, female
MD: NAD

Sample 14 2022Sample52 -Asymptomatic Sentinel, containing 2 fish:

14A: grey striped fish, female
MD: mild acute EAIF
14B: grey striped fish, female
MD: NAD

Sample 15 2022Sample53 -Asymptomatic Sentinel, containing 4 fish:

15A: grey striped fish, male
MD: NAD
15B: grey striped fish, female
MD: NAD
15C: grey striped fish
MD: mild multifocal necrotising myositis, tail; of unknow aetiology
15D: grey striped fish
MD: NAD

Sample 16 2022Sample55 -Asymptomatic Sentinel, containing 3 fish:

16A: grey striped fish, female
MD: systemic necrogranulomatous ZN negative inflammation; paraffin scrolls will be sent for PCR
16B: grey striped fish, male
MD: Marked hepatic lipidosis with multifocal cholesterol granuloma; incidental finding
16C: grey striped fish, male
MD: NAD

Sample 17 2022Sample57 -Asymptomatic Sump, containing 2 fish:

17A: grey striped fish, male
MD: NAD
17B: grey striped fish, male
MD: NAD

Sample 18 2022Sample58 -Asymptomatic Sump, containing 2 fish:

18A: white fish, female
MD: multifocal necrogranulomatous ZN negative inflammation of the ventral body wall; paraffin scrolls will be sent for PCR
18B: grey striped fish, male
MD: multiple fibro-osseous expansions of the skeleton, gill arch, cranial bones

Sample 19 2022Sample59 -Asymptomatic Sump, containing 2 fish:

19A: yellow striped fish, male
MD: NAD
19B: grey striped fish, male
MD: NAD

Sample 20 2022Sample60 -Asymptomatic Sump, containing 3 fish:

20A: grey striped fish, male
MD: NAD
20B: grey striped fish, male
MD: NAD
20C: grey striped fingerling, 10mm long, female
MD: NAD

Sample 21 2022Sample61 -Symptomatic Sample, containing 2 fish:

21A: large ventrally opened and bulging content, grey striped fish, female
MD: locally extensive necrotising myositis, tail; of unknown aetiology
21B: grey striped fish with "snub" nose and curled tail tip, male
MD: suspected chronic healed displacement/fracture of the tail

Sample 22 2022Sample62 -Symptomatic Sample, containing 2 fish:

22A: white fish, male
MD: systemic necrogranulomatous Grams, PAS and ZN negative inflammation; paraffin scrolls will be sent for PCR
22B: white fish, female
MD: moderate, multifocal hepatic lipidosis, incidental
22C: white fish, female
MD: moderate, multifocal hepatic lipidosis, incidental

Sample 23 2022Sample63 -Symptomatic Sample, containing 2 fish:

23A: grey striped fish, male

MD: NAD

23B: grey striped fish, female

MD: NAD

Sample 24 2022Sample64 -Asymptomatic Sump, containing 1 fish:

24: Grey spotted fish with long fins and tail and a bent tail, male

MD: spindle cell tumour with invasion and replacement of the tail skeletal muscle

Sample 25 2022Sample65 -Symptomatic Sump, containing 2 fish:

25A: grey no stripes black eyed fish, female

MD: NAD

25B: grey striped fish with a stumpy, crooked tail, male

MD: NAD

Sample 26 2022 Sample66 -Symptomatic Sump. containing 2 fish:

26A: grey striped fish, female

MD: NAD

26B: grey striped fish, male

MD: NAD

Comments

P. neurophilia is an obligate, intracellular parasite which. Transmission occurs through ingestion of the mature infective spore, which can survive outside the host. Ingestion of spores probably occurs when infected fish are cannibalized and possibly transmission during spawning. It is important to remove infected fish and deceased fish as soon as possible to avoid transmission.

Egg-associated Inflammation with/without fibrosis (EAIF) has no distinct aetiology. The primary cause may reflect degeneration of eggs, which is a common occurrence in many fishes that do not spawn on a regular basis. Alternatively, EAIF may be secondary to primary infection (often mycobacterial) of the ovary. Multifocal and systemic necrogranulomatous disease is often associated with infectious agents; however, no agents could be found. No acid-fast organisms were seen on ZN-stained sections of any of the fish examined in this large group. This does not rule out Mycobacteriosis in necrogranulomatous or EAIF lesions.

There were 2 fish 18B and 24 which had tumorous lesions. The change in fish 18B were multifocal expansion of the bone buy fairly well-circumscribed, proliferations of spindle to polyhedral cells associated with lakes of osseous material. I think it is likely that this is a proliferative disorder rather than a neoplastic one. Fish 24 has a spindle cell tumour, soft tissue sarcoma which is infiltrating and replacing the skeletal muscle of the tail.



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