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Submitter: Uni. of QLD - UQBR Aquatics
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Submission No: 23/M391
Your ref: UQBR zebrafish pathology 2023
Date Submitted: 13 July 2023
Date of issue: 11 August 2023

Location: Otto (Conventional_cert 3112) **Species: Zebrafish**

Bacteria and Fungi	**Pos / Tested**	**Laboratory**	**Method**
Edwardsiella ictaluri	0 / 9	Cerberus	PCR
Mycobacterium chelonae	0 / 2	Cerberus	PCR
Mycobacterium fortuitum	0 / 2	Cerberus	PCR
Mycobacterium haemophilium	0 / 2	Cerberus	PCR
Mycobacterium marinum	0 / 2	Cerberus	PCR
Mycobacterium spp	0 / 11	Cerberus	PCR
Pseudomonas aeruginosa	0 / 9	Cerberus	PCR
Endoparasite	**Pos / Tested**	**Laboratory**	**Method**
Pseudocapillaria tomentosa	0 / 9	Cerberus	PCR
Pseudocapillaria tomentosa	0 / 9	Cerberus	Histo
Pseudoloma neurophila	2 / 9	Cerberus	PCR
Histopath	**Pos / Tested**	**Laboratory**	**Method**
Zebrafish Histopathology	9 / 9	Cerberus	Histo

Location: Seddon (Conventional_cert 3428) **Species: Zebrafish**

Bacteria and Fungi	**Pos / Tested**	**Laboratory**	**Method**
Edwardsiella ictaluri	0 / 9	Cerberus	PCR
Mycobacterium chelonae	0 / 2	Cerberus	PCR
Mycobacterium fortuitum	0 / 2	Cerberus	PCR
Mycobacterium haemophilium	0 / 2	Cerberus	PCR
Mycobacterium marinum	0 / 2	Cerberus	PCR
Mycobacterium spp	0 / 11	Cerberus	PCR
Pseudomonas aeruginosa	0 / 9	Cerberus	PCR
Endoparasite	**Pos / Tested**	**Laboratory**	**Method**
Pseudocapillaria tomentosa	0 / 9	Cerberus	PCR
Pseudocapillaria tomentosa	0 / 9	Cerberus	Histo
Pseudoloma neurophila	1 / 9	Cerberus	PCR
Histopath	**Pos / Tested**	**Laboratory**	**Method**
Zebrafish Histopathology	9 / 9	Cerberus	Histo

Comment:

Samples Refs. were:

Sample 01., Sample 01. PCR bead 01, Sample 02., Sample 02. PCR bead 02, Sample 03., Sample 03. PCR bead 03, Sample 04., Sample 04. PCR bead 04, Sample 05., Sample 05. PCR bead 05, Sample 06., Sample 06. PCR bead 06, Sample 07., Sample 07. PCR bead 07, Sample 08., Sample 08. PCR bead 08, Sample 09., Sample 09. PCR bead 09, Sample 10., Sample 10. PCR bead 10, Sample 11., Sample 11. PCR bead 11, Sample 12., Sample 12. PCR bead 12, Sample 13., Sample 13. PCR bead 13, Sample 14., Sample 14. PCR bead 14, Sample 15.,

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Sample 15. PCR bead 15, Sample 16., Sample 16. PCR bead 16, Sample 17., Sample 17. PCR bead 17, Sample 18., Sample 18. PCR bead 18, Sample 19. MYCO_PCR bead 01, Sample 20. MYCO_PCR bead 02, Sample 21. MYCO_PCR bead 03, Sample 22. MYCO_PCR bead 04.

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

All other tests conducted were negative.

The following samples were POSITIVE for Pseudoloma neurophila by PCR: Sample 04. PCR bead 04 (Otto (Conventional_cert 3112)), Sample 05. PCR bead 05 (Otto (Conventional_cert 3112)), Sample 10. PCR bead 10 (Seddon (Conventional_cert 3428)).

Sample 01. (Otto (Conventional_cert 3112) - Pseudocapillaria tomentosa), Sample 02. (Otto (Conventional_cert 3112) - Pseudocapillaria tomentosa), Sample 03. (Otto (Conventional_cert 3112) - Pseudocapillaria tomentosa), Sample 04. (Otto (Conventional_cert 3112) - Pseudocapillaria tomentosa), Sample 05. (Otto (Conventional_cert 3112) - Pseudocapillaria tomentosa), Sample 06. (Otto (Conventional_cert 3112) - Pseudocapillaria tomentosa), Sample 07. (Otto (Conventional_cert 3112) - Pseudocapillaria tomentosa), Sample 08. (Otto (Conventional_cert 3112) - Pseudocapillaria tomentosa), Sample 09. (Otto (Conventional_cert 3112) - Pseudocapillaria tomentosa), Sample 10. (Seddon (Conventional_cert 3428) - Pseudocapillaria tomentosa), Sample 11. (Seddon (Conventional_cert 3428) - Pseudocapillaria tomentosa), Sample 12. (Seddon (Conventional_cert 3428) - Pseudocapillaria tomentosa), Sample 13. (Seddon (Conventional_cert 3428) - Pseudocapillaria tomentosa), Sample 14. (Seddon (Conventional_cert 3428) - Pseudocapillaria tomentosa), Sample 15. (Seddon (Conventional_cert 3428) - Pseudocapillaria tomentosa), Sample 16. (Seddon (Conventional_cert 3428) - Pseudocapillaria tomentosa), Sample 17. (Seddon (Conventional_cert 3428) - Pseudocapillaria tomentosa), Sample 18. (Seddon (Conventional_cert 3428) - Pseudocapillaria tomentosa):

There is no evidence of Pseudocapillaria tomentosa infestation in these zebrafish on histology.

Sample 01. (Otto (Conventional_cert 3112) - Zebrafish Histopathology):

Macropathology
18 formalin tubes, each containing a single zebrafish are received for histology appropriately labelled.

Sample 01
This is a pale-yellow zebrafish with no stripes.

Histopathology (HE and ZN):
Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, reproductive system (male), integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 02. (Otto (Conventional_cert 3112) - Zebrafish Histopathology):

Sample 02
This is a typical, grey-striped zebrafish.

Histopathology (HE and ZN):
Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, reproductive system (female), integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 03. (Otto (Conventional_cert 3112) - Zebrafish Histopathology):

Sample 03
This is a typical, grey-striped zebrafish.

Histopathology (HE and ZN):
Central nervous system
Within the spinal cord are multiple xenoma comprising numerous oval shaped microsporidia, approximately 5.4 × 2.7 µm. These stain pink with acid-fast ZN stain.

Musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, reproductive system (female), integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 04. (Otto (Conventional_cert 3112) - Zebrafish Histopathology):

Sample 04
This is a grey-striped zebrafish with a mild malocclusion where the lower jaw overshoots the upper jaw. The tail is curled ventrodorsally.

Reproductive system (female),
There is severe egg-associated inflammation and fibrosis (EAIF) within which there are scattered necrogranuloma.
There is no evidence of acid-fast bacterial organisms on the ZN-stained sections.

Central nervous system and musculoskeletal system
There is developmental abnormalities in both the vertebra and spinal cord at the tail.

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Gills, heart, liver, kidneys, gastrointestinal system, integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 05. (Otto (Conventional_cert 3112) - Zebrafish Histopathology):

Sample 05
This is a grey-striped zebrafish.

Reproductive system (female),
There is severe egg-associated inflammation and fibrosis (EAIF) within which there are scattered necrogranuloma.
There is no evidence of acid-fast bacterial organisms on the ZN-stained sections.

Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 06. (Otto (Conventional_cert 3112) - Zebrafish Histopathology):

Sample 06
This is a grey-striped zebrafish.

Histopathology (HE and ZN):
Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, reproductive system (male), integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 07. (Otto (Conventional_cert 3112) - Zebrafish Histopathology):

Sample 07
This is a grey-striped zebrafish.

Histopathology (HE and ZN):
Reproductive system (female),
There is severe egg-associated inflammation and fibrosis (EAIF) within which there are scattered necrogranuloma.
There is no evidence of acid-fast bacterial organisms on the ZN-stained sections.
There are no other abnormalities on the HE or ZN-stained sections.

Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 08. (Otto (Conventional_cert 3112) - Zebrafish Histopathology):

Sample 08
This is a grey-striped zebrafish.

Histopathology (HE and ZN):
Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, reproductive system (female), integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 09. (Otto (Conventional_cert 3112) - Zebrafish Histopathology):

Sample 09
This is a grey-striped zebrafish.

Histopathology (HE and ZN):
Reproductive system (female),
There is severe egg-associated inflammation and fibrosis (EAIF) within which there are scattered necrogranuloma.
There is no evidence of acid-fast bacterial organisms on the ZN-stained sections.

Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 10. (Seddon (Conventional_cert 3428) - Zebrafish Histopathology):

Sample 10
This is a grey-striped zebrafish with a mild malocclusion where the lower jaw overshoots the upper jaw.

Histopathology (HE and ZN):
Reproductive system (female)
There is mild early egg-associated inflammation and fibrosis (EAIF).

Musculoskeletal system
Focal area of skeletal muscle degeneration and necrosis in the ventral body wall beneath the liver area. No micro-organisms are present on HE or ZN stained sections.

Central nervous system, gills, heart, liver, kidneys, gastrointestinal system, integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 11. (Seddon (Conventional_cert 3428) - Zebrafish Histopathology):

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Sample 11
This is a typical, grey-striped zebrafish.

Histopathology (HE and ZN):
Central nervous system
Within the spinal cord are multiple xenoma comprising numerous oval shaped microsporidia, approximately 5.4 × 2.7 µm. These stain pink with acid-fast ZN stain.

Musculoskeletal system
Focal area of skeletal muscle degeneration and necrosis in the ventral body wall beneath the liver area. No micro-organisms are present on HE or ZN stained sections.

Reproductive system (male)
Focal necrogranuloma, negative on ZN stained sections for bacteria.

Gills, heart, liver, kidneys, gastrointestinal system, integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 12. (Seddon (Conventional_cert 3428) - Zebrafish Histopathology):

Sample 12
This is a grey-striped zebrafish.

Histopathology (HE and ZN):
Reproductive system (female),
There is moderate egg-associated inflammation and fibrosis (EAIF) within which there are scattered necrogranuloma. There is no evidence of acid-fast bacterial organisms on the ZN-stained sections.

Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 13. (Seddon (Conventional_cert 3428) - Zebrafish Histopathology):

Sample 13
This is a grey-striped zebrafish with a mild malocclusion where the lower jaw overshoots the upper jaw.

Histopathology (HE and ZN):
Reproductive system (female),
There is moderate egg-associated inflammation and fibrosis (EAIF) within which there are scattered necrogranuloma. There is no evidence of acid-fast bacterial organisms on the ZN-stained sections.

Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 14. (Seddon (Conventional_cert 3428) - Zebrafish Histopathology):

Sample 14
This is a grey-striped zebrafish with a mild malocclusion where the lower jaw overshoots the upper jaw. The tail ends just after the anal pore and is curved dorsally.

Histopathology (HE and ZN):
Central nervous system and musculoskeletal system
There are developmental abnormalities in both the vertebrae and spinal cord at the tail.

Gills, heart, liver, kidneys, gastrointestinal system, reproductive system (male), integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 15. (Seddon (Conventional_cert 3428) - Zebrafish Histopathology):

Sample 15
This is a grey-striped zebrafish with a mild malocclusion where the lower jaw overshoots the upper jaw. The tail ends just after the anal pore and is curved dorsally.

Histopathology (HE and ZN):
Central nervous system and musculoskeletal system
There are developmental abnormalities in both the vertebrae and spinal cord at the tail.

Reproductive system (female),
There is moderate egg-associated inflammation and fibrosis (EAIF) within which there are scattered necrogranuloma. There is no evidence of acid-fast bacterial organisms on the ZN-stained sections.

Gills, heart, liver, kidneys, gastrointestinal system, integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 16. (Seddon (Conventional_cert 3428) - Zebrafish Histopathology):

Sample 16
This is a grey-striped zebrafish.

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Histopathology (HE and ZN):
Reproductive system (male)
The testis has an area of cystically dilated seminiferous tubules.

Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 17. (Seddon (Conventional_cert 3428) - Zebrafish Histopathology):

Sample 17
This is a grey-striped zebrafish.

Histopathology (HE and ZN):
Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, reproductive system (male), integument (including fins and tail), swim bladder:
No significant abnormalities.

Sample 18. (Seddon (Conventional_cert 3428) - Zebrafish Histopathology):

Sample 18
This is a grey-striped zebrafish.

Histopathology (HE and ZN):
Abdominal cavity
There is mild peritonitis with mononuclear cell infiltrate and scanty necrogranuloma. No Mycobacteria spp or other micro-organisms were identified on ZN or HE stained slides.

Central nervous system, musculoskeletal system, gills, heart, liver, kidneys, gastrointestinal system, reproductive system (male), integument (including fins and tail), swim bladder:
No significant abnormalities.

Morphological Summary
Central nervous system, 3, 11; myelitis, parasitic with multifocal xenoma; consistent with Pseudoloma neurophilia

Musculoskeletal system, 4, 10, 13, 14, 15; mandibular/maxillary anomalies
Musculoskeletal system, 4, 14, 15; vertebral malformations of the tail
Skeletal muscle, body wall, 10, 11; myositis, mild, focal necrotising

Ovary, 4, 5, 7, 9, 10, 12, 13, 15; Egg-associated inflammation and fibrosis (EAIF), mild to severe

Testis, 11; focal orchitis, necrogranulomatous, background finding
Testis, 16; seminiferous tubule dilation, background finding

Comments
The variety of developmental anomalies; jaws, vertebrae, testicular, may all reflect genetic defects; possibly inbreeding if that is a possiblility; or may be spontaneous anomalies in these fish.

P. neurophilia is an obligate intracellular parasite which results in central nervous system and skeletal muscle infection and pathology. 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, with there being the possibility of vertical transmission during spawning. It is important to remove infected fish and deceased fish as soon as possible to avoid transmission.

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. Frequently there are necrogranuloma present in the ovarian tissue and elsewhere in the body.

The occasional necrogranuloma and/or myositis in zebrafish may reflect an incidental tissue pathology. We cannot completely rule out infectious agents, but no evidence was seen on HE or ZN-stained sections. Similarly, the peritonitis in Sample 18 does not appear to be infectious or parasitic in nature.

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Peony Fung
Molecular Testing

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