Weissellosis

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A. Name of Disease and Etiological Agent

Weissellosis is caused by *Weissella ceti*, a gram-positive bacterium.

B. Known Geographical Range and Host Species of the Disease

1. **Geographical Range**

   Weissellosis was first described in farmed rainbow trout *Oncorhynchus mykiss* in China in 2007 (Liu, et al. 2009) and has since been identified as the causative agent of disease in outbreaks at commercial trout farms in both Brazil (Figueiredo, et al. 2012) and the southeastern United States (Welch and Good 2013). The recent identification of this disease at geographically distinct locations suggests that weissellosis is a rapidly emerging pathogen of farmed rainbow trout.

2. **Host Species**

   Weissellosis has been exclusively found in farmed rainbow trout. The susceptibility of other fish species remains unknown.

C. Epizootiology

Elevated temperature is the principal predisposing factor for weissellosis outbreaks. In North Carolina, where water temperatures vary seasonally, weissellosis has occurred exclusively during the summer months when temperatures reach 18-20°C. The disease subsides in the fall as temperatures cool, and is absent during the winter months (Welch and Good 2013). The appearance of sequential seasonal outbreaks in North Carolina since 2011 suggests that *W. ceti* has the potential to persist in areas where it has emerged, and could become an endemic disease problem. Weissellosis appears to exclusively affect the larger fish (0.25-1 kg) in a production system (Welch and Good 2013). This observation is consistent

with laboratory challenge experiments, which have demonstrated a correlation between body weight and mortality (Marancik et al. 2013). The recent identification of weissellosis on three continents suggests that this pathogen has been rapidly disseminated by some unknown mechanism(s), or that unknown environmental factor(s) are driving its independent emergence. The pathogen's route of infection and environmental reservoirs remain unknown but are the subject of ongoing research.

D. Disease Signs

1) Behavioral signs

Infected fish become lethargic and anorexic, and typically hang at the surface and congregate at the end of raceways.

Figure 1: Dead and infected fish during a weissellosis outbreak summer 2011. Photo by Tim Welch.

2) Gross and histopathologic lesions

Gross signs of disease include extensive ocular lesions (Figure 2), occasional cerebral hemorrhage (Figure 3), and dark skin coloration. The high incidence and severity of eye symptoms is non specific but appear suggestive of weissellosis and include exophthalmia, corneal and lenticular opacity, periocular and intraocular hemorrhage, and corneal rupture. Apparent blindness is believed to contribute to behavioral signs in infected and convalescent fish.

Histopathologic changes in the eye include retro-orbital inflammation advancing to granulomatous panophthalmitis with corneal ulceration, hemorrhage, and cataractous changes (Figure 4). Similar granulomatous inflammation has been found infiltrating the epicardium and superficial myocardium (Figure 5). Brain hemorrhage has not been associated with microscopic findings at this time, although vasculitis observed in sections of eye and heart suggest inflammation of blood vessels may play a role (Figure 5).

Figure 2: Adult rainbow trout displaying gross signs typical of weissellosis, including bilateral exophthalmia (upper panel), ruptured cornea and intraocular hemorrhage (lower right panel) and lenticular and corneal opacity (lower left panel). Photos by Tim Welch.

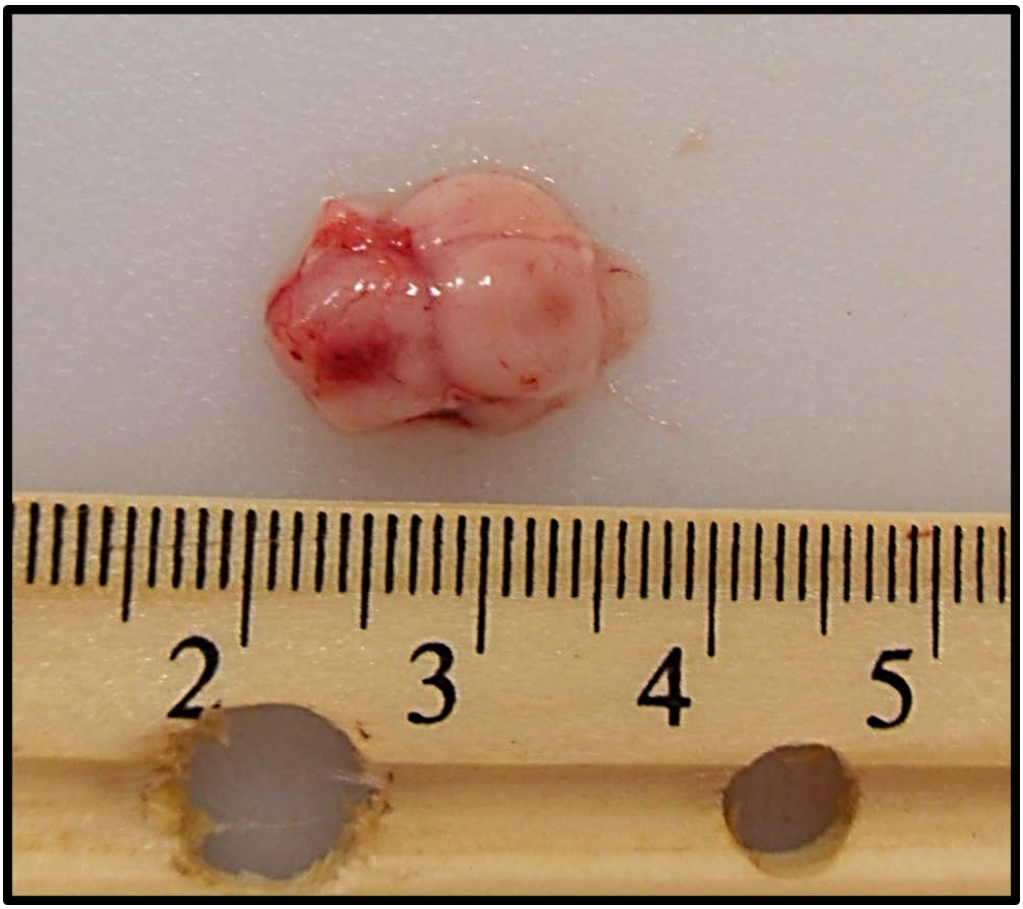

Figure 3: Brain with areas of petechial and ecchymotic hemorrhaging. Photo by Dave Marancik.

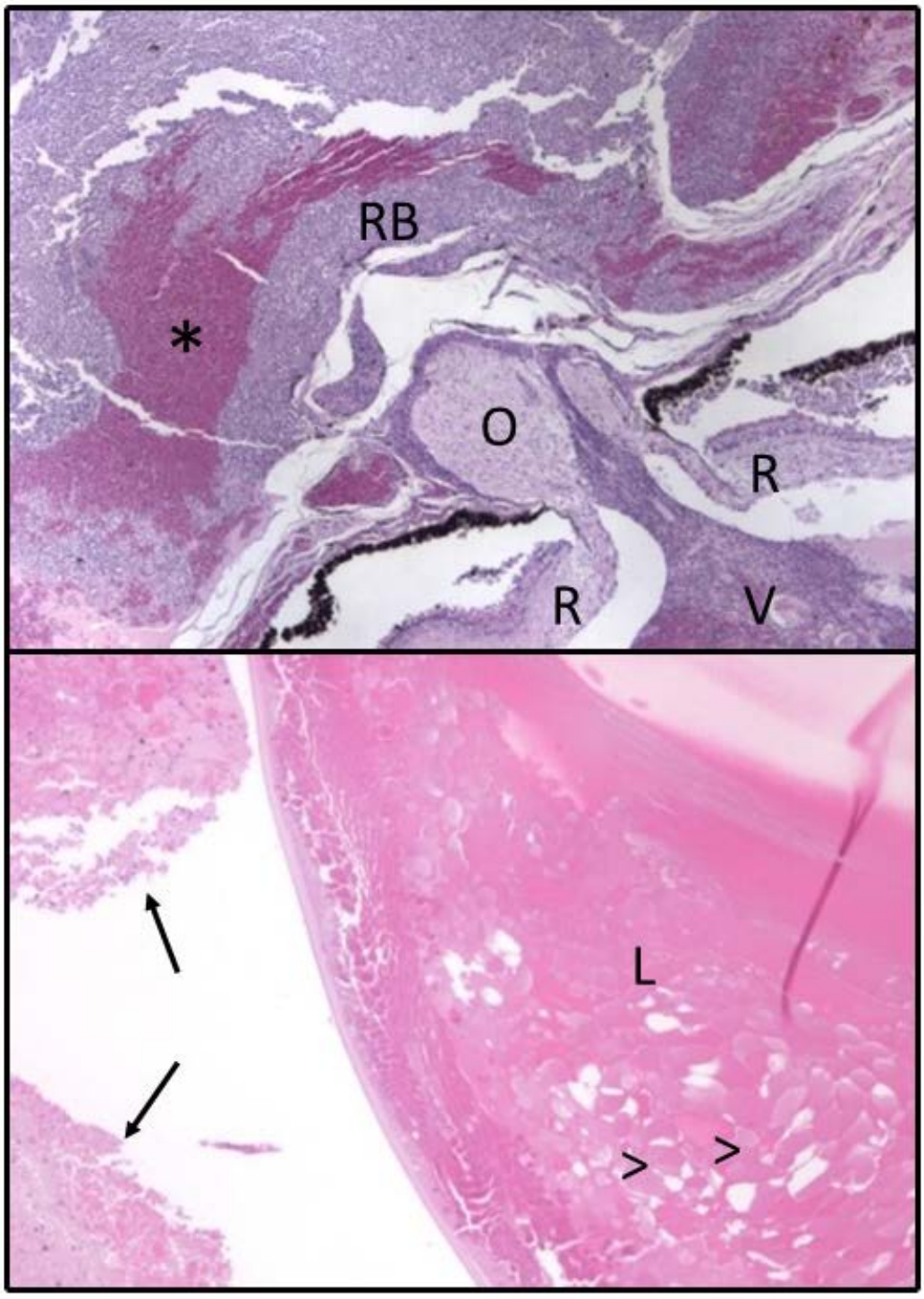

Figure 4: Ocular inflammation (top panel) with demonstrated granulomatous inflammation and hemorrhage (asterisk) effacing the retrobulbar (RB) adipose and connective tissue and extending around the optic nerve (O) and retina (R) into the vitreous of the eye. Cataractous changes (bottom panel) of the lens (L) with globular eosinophilic deposits (Morgagnian globules) (arrowheads). Within the posterior chamber of the eye is proteinacious material admixed with degenerate inflammatory cells (arrows). Photos by Dave Marancik.

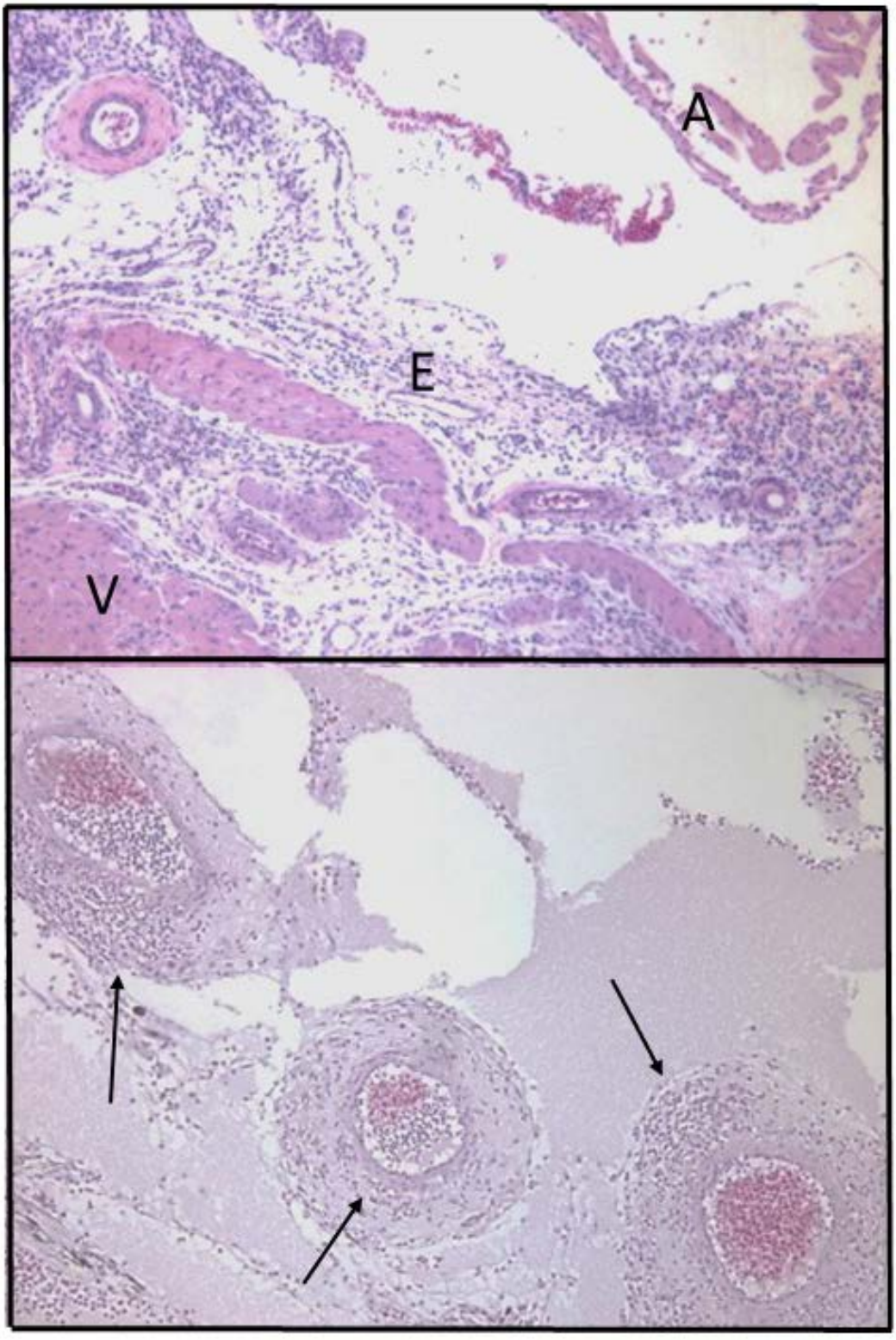

Figure 5: Myocarditis (upper panel) characterized by granulomatous inflammation expanding the epicardium (E) and infiltrating the myocardium of the ventricle (V) at the level of the bulbus arteriosus. Also present is a section of atrium (A). Vasculitis (lower panel) of

three blood vessels in the eye that are infiltrated by mixed inflammatory cells (arrows). Photos by Dave Marancik.

3) Microbiological and microscopic characteristics

W. ceti forms small (0.25 mm) white colonies displaying α-hemolytic activity on TSA agar supplemented with 5% sheep blood (TSA-Blood, Remel, USA) and does not form visible colonies when plated on any of the bacteriological media typically used for salmonid disease diagnostics (BHI, TSA or TYES). The pathogen can be isolated from multiple tissues including spleen, anterior kidney and brain. However, bacterial loads often appear highest in brain and in some cases the pathogen is exclusively found in brain. It is important to note that detectable growth and α-hemolysis (Figure 6) develop 15-18 hours after plating tissue samples onto TSA-blood and incubation at 30°C, thus making detection by culture comparatively rapid. *W. ceti* is a gram-positive rod 1.5 µm in length by 0.30 µm in diameter (Figure 7).

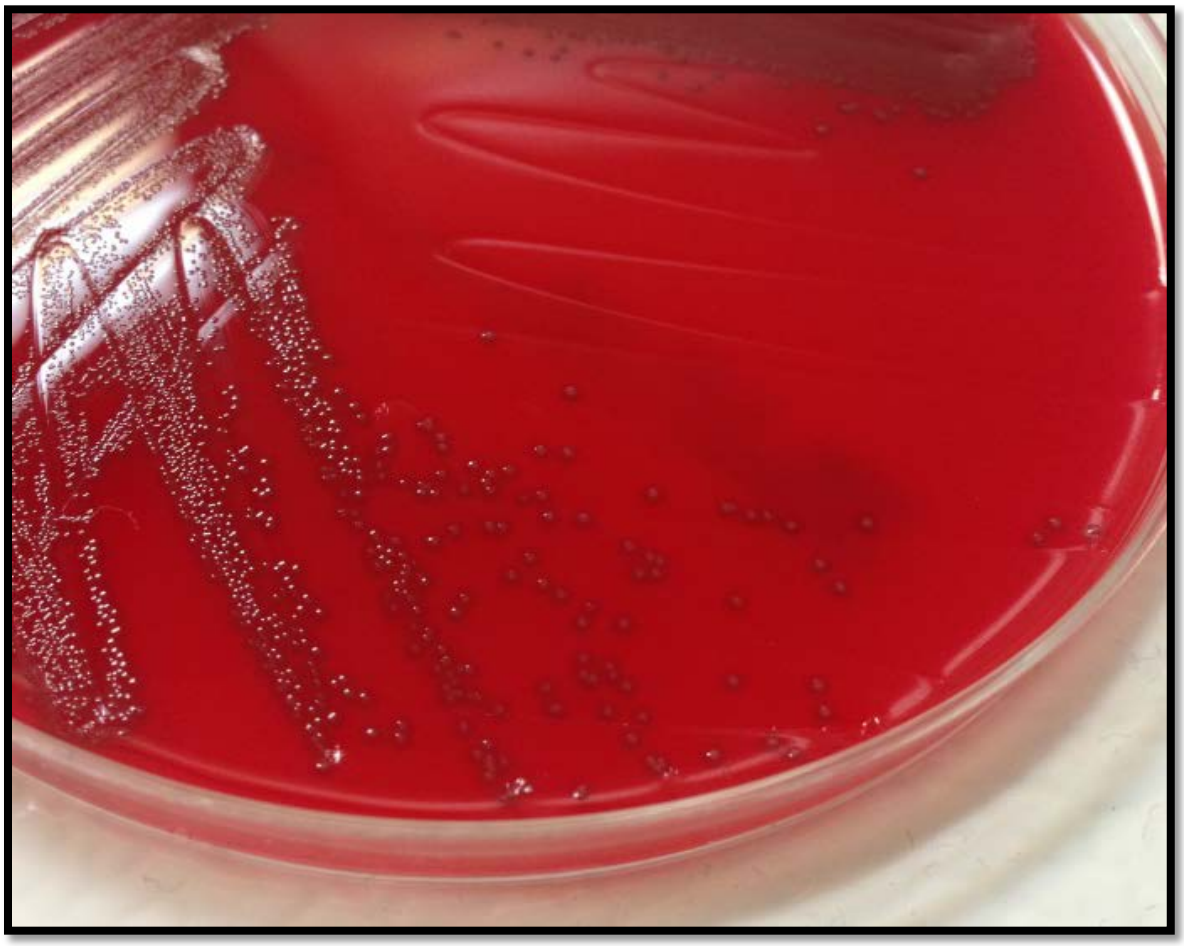

Figure 6: *W. ceti* colonies displaying α-hemolytic activity on TSA agar supplemented with 5% sheep blood after 18 hours of incubation at 30°C. Photo by Tim Welch.

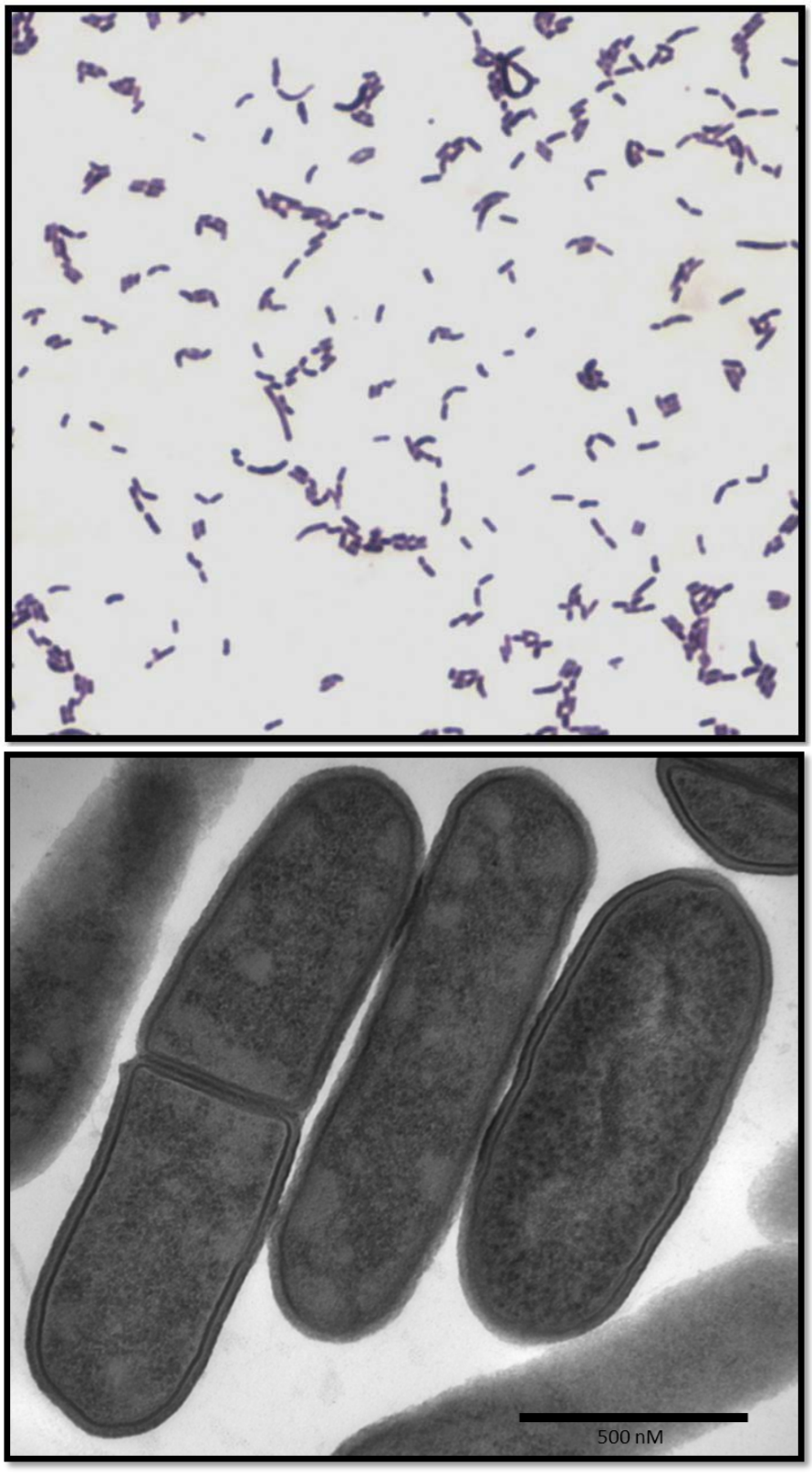

Figure 7: Light micrograph of gram stain (upper panel) and transmission electron micrograph (lower panel) of *W. ceti* cells. Light micrograph by Dave Marancik and electron micrograph by Charles Murphy.

E. Disease Diagnostic Procedures

Diagnosis is based on the observation of characteristic behavioral and gross signs and the isolation and identification of the etiological agent.

1. Presumptive Diagnosis

Moribund fish should exhibit the behavioral and gross signs of Weissellosis described in Section D. For primary isolation note that *W. ceti* is detected only when tissues are plated onto TSA blood agar and not when plated on TSA, BHI or TYES agars. Since TSA blood agar is not routinely used for salmonid diagnostics, laboratories using standard methods would not likely detect this bacterium. Additionally, brain appears to be the most reliable tissue for detection of the causative agent, and therefore when weissellosis is suspected, culture of brain onto TSA-blood agar is recommended. When plated on TSA-blood agar *W. ceti* produces visible growth and α-hemolysis within 15-18 hours when incubated at 30°C (Figure 6). The organism should be a gram-positive, catalase and oxidase negative rod 1.5 µm in length and by 0.30 µm in diameter (Figure 7). A Weissella genus-specific polymerase chain reaction assay is available to confirm genus identification (Jang, et al. 2002).

2. Confirmatory Diagnosis

Since *W. ceti*- specific PCR or serological assays have not yet been developed, confirmation of a putative *W. ceti* isolate must be accomplished by amplification and sequencing of the 16S ribosomal RNA gene. We suggest using the DNeasy Blood and Tissue kit (Qiagen) to purify total DNA from cells following the supplied protocol for the purification of DNA from gram-positive bacteria. This DNA can then be used to amplify the 16S rRNA gene as a 1465 bp fragment using the bacterial universal primers previously described (DeLong 1992). The name and sequence of the primers used are: Eubac27F AGA GTT TGA TCC TGG CTC AG and 1492R GGT TAC CTT GTT ACG ACT T. The 16S sequence should be highly related to the *W. ceti* strains found associated with recent Weissellosis outbreaks in the USA, China and Brazil. Note that the recently published draft genome sequence (Ladner et al. 2013) of the North Carolina *W. ceti* strain should facilitate the development of a species-specific PCR assay.

E. Procedures for Detecting Subclinical Infections

There are currently no methods available for detection of subclinical infection.

F. Procedures for Detecting Prior Exposure

There are currently no methods available for detection of prior exposure to *W. ceti.*

G. Procedures for Transportation and Storage of Samples

See Section 1, 1.1 General Procedures for Bacteriology.

References

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