Case Definition

American Foulbrood of Honey Bees (*Paenibacillus larvae*) (Monitored)

December 2023

1. **Disease Information**
   - **1.1 General Disease and Pathogen Information:** American foulbrood (AFB) is a bacterial disease affecting brood (larval and pupal stages) of the western honey bee, *Apis mellifera*, and other *Apis* species, that is endemic in the U.S. The causative organism is *Paenibacillus larvae* (*P. larvae*), a gram-positive, spore-forming bacterium found throughout the world. *P. larvae* is passed to brood by unaffected adult bees, most commonly through spores suspended in food. When spores make their way to the bee brood gut, *P. larvae* germinates into the active, vegetative form and colonizes the gut, producing toxins and enzymes that ultimately kill the brood. AFB is considered infectious and highly contagious because the spore form of *P. larvae* is incredibly stable and hardy in the environment. Spores are resistant to heat and chemical agents and can survive for many years on scales, hive products, and other equipment. Furthermore, it takes only a few spores to cause disease in an otherwise healthy larva. Each infected larva can then produce over one billion spores. Combs of infected apiaries may show distinctive clinical signs that allow diagnosis of the disease in the field.
   - **1.2 Clinical Signs:** Brood comb of infected colonies has a mottled appearance from the mixture of healthy capped brood, uncapped cells with the remains of diseased larvae, and empty cells. Cell cappings appear moist and darkened, as well as sunken and punctured as infection develops. Dead brood are commonly older sealed larvae or young pupae whose color changes to creamy brown then dark brown to almost black with a soft consistency that becomes sticky or ropy. In some cases, the larval remains appear watery. Dead brood produce a slight to pronounced odor. The diseased brood eventually dries out to form characteristic brittle scales that adhere tightly to the lower sides of the cell. The development of a fine threadlike pupal tongue is a characteristic but rare sign of the disease. Subclinical infections are common and may require laboratory diagnosis.

2. **Laboratory Criteria**
   - **2.1 Agent Isolation and Identification:** Diagnosis of AFB is based on identification of the pathogenic agent and the presence of clinical signs in larvae. Identification techniques include microbiological characterization, and in particular the homogeneous presence of rod-shaped bacteria, polymerase chain reaction (PCR), biochemical profiling, antibody-based techniques, and microscopy. For culturing samples, five solid media are recommended: PLA (*P. larvae* agar), MYPGP agar, BHIT agar, J-agar, and Columbia sheep blood agar. Two PCR methods based on the 16S rRNA gene can be applied and used for confirmation of clinical disease and identification of bacterial

colonies after cultivation. Biochemical profiling of *P. larvae* can also be performed and is based on the catalase test, the production of acid from carbohydrates, and the hydrolysis of casein depending on the genotype. Antibody-based techniques that can be employed for identification are immunodiffusion tests and fluorescent antibody technique. Microscopic techniques used include gram staining of bacteria and carbol fuchsin staining of larval smears.

2.2 Agent Characterization: the bacterium is generally alone, without other infection.

2.3 Serology: NA.

3. **Case Classification**

3.1 Suspect Case: clinical signs, history, or epidemiology consistent with AFB in a susceptible honey bee hive.

3.2 Presumptive Positive Case: a suspect case that is positive on at least one AFB field test (lateral flow diagnostic kit, ropiness test).

3.3 Confirmed Positive Case: clinical signs and positive identification of *P. larvae* from culture and/or PCR.

4. **Reporting Criteria:** American foulbrood is a U.S. monitored disease that is reportable monthly under the APHIS National List of Reportable Animal Diseases (NLRAD).

4.1 NLRAD reporting in accordance with the NLRAD Standards for monitored diseases; and by APHIS to the World Organisation for Animal Health (WOAH).