

Case Definition

[Avian Influenza \(AI\)](#)

April 2024

Addendum I: HPAI H5N1 clade 2.3.4.4b in Livestock

1. Disease Information General Disease and Pathogen Information: As of late March 2024, H5N1 clade 2.3.4.4b, genotype B3.13 per [GenoFlu](#), was detected in milk and other bovine-origin samples associated with mild illness in dairy cattle. The virus has been characterized as highly pathogenic per cleavage site analysis and bioassay testing (IVPI per WOA), and currently the B3.13 genotype is not significantly different from other genotypes affecting wild birds and poultry. Pathways of disease transmission are still being investigated and lateral transmission has been documented. The estimated herd level incubation period in dairy cattle appears variable, from approximately 12 to 21 days. The incubation period is likely multifactorial and should consider route of exposure, viral dose, production phase of the animal, and likely other factors that are still unknown at this time. The virus is found in high concentration in milk secretions of lactating cattle.

1.1 Clinical Signs: Infected cattle may be asymptomatic (subclinical) or symptomatic (clinical) and virus is predominantly found in milk and mammary tissue regardless of symptoms. Clinical signs may include a decrease in feed consumption with a simultaneous decrease in rumination and rumen motility; respiratory signs including clear nasal discharge; and subsequent acute drop in milk production. Additional clinical signs may include abnormal tacky or loose feces, lethargy, dehydration, and fever. Severely affected cattle may have thicker, concentrated, colostrum-like milk or produce no milk at all.

2. Laboratory Criteria

Milk and mammary tissue are the preferred samples from lactating animals; deep nasal swabs should be used for non-lactating animals. All non-negative samples must be forwarded to NVSL for confirmatory testing. Utility of serology for serum and milk are under investigation.

2.1 Agent Isolation and Identification: Detect presence of influenza A virus by:

2.1.1 Preferred samples from live or dead animals are tested by real-time polymerase chain reaction (PCR) assays for influenza A and those targeting H5 2.3.4.4b (subtype assays should only be used in conjunction with influenza A detection assays). Virus isolation may also be performed.

2.2 Agent Characterization: Genome sequencing methods are used to determine the amino acid sequence at the hemagglutinin cleavage site to pathotype viruses per the



WOAH Terrestrial Manual. A lineage-specific pathotyping PCR assay is available for H5 clade 2.3.4.4b at NVSL.

3. Case Definition and Reporting Criteria for Livestock

3.1 Suspect Case:

- 3.1.1** Illness compatible with H5 clade 2.3.4.4b infection; **OR**
- 3.1.2** Detection of influenza A antigen in milk using a commercially available influenza A antigen test kit (ACIA, approved by USDA) with the presence of compatible illness.

3.2 Presumptive Positive Case:

- 3.2.1** A suspect positive case as defined above with identification of influenza A H5 RNA by rRT-PCR with or without the presence of compatible illness; **OR**

3.3 Confirmed Positive Case:

- 3.3.1** Identification of HPAI H5N1 clade 2.3.4.4b NVSL by molecular assay; whole genome sequencing will be conducted to determine genotype.