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Agroterrorism: Electron Microscopy and Protecting American Livestock

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American agriculture is an enormous economic force providing more than 13% of gross domestic product. The unintentional or intentional introduction (agroterrorism) of a pathogen (fungus, bacterium, or virus) into American livestock or poultry could result in a devastating outbreak. Livestock could be lost or incapacitated disrupting that entire sector of the animal industry [1].

The Department of Homeland Security (DHS) science program at Plum Island Animal Disease Center (PIADC) is composed of three units: Targeted Advanced Development (TAD) which develops animal vaccines for the national stockpile; Disease Assessment and Forensics (DAF) which identifies disease agents and their origins; and Core Facilities (CS) which provides sequencing and microscopy support. Virus, Cellular and Molecular Imaging (VCMI) uses electron microscopy procedures such as negative staining and thin-section analyses of infected cell cultures to determine if agents submitted to Animal and Plant Health Inspection Service (APHIS) for identification are Foreign Animal Disease (FAD) agents.

Foot-and-mouth disease (FMD) is DHS's greatest concern because it affects economically critical livestock (cattle, pigs and sheep) and has the potential for its getting into wildlife populations (deer, bison, elk, etc). FMD virus (FMDV) has caused devastating outbreaks in the United Kingdom in 2001 and in Taiwan in 1997 and currently is responsible for smaller outbreaks in South America and Asia. Rapid diagnosis of FMDV, critical in controlling the spread of the disease, is complicated by the circulation of viruses that cause very similar clinical symptoms to FMDV, viruses such as vesicular stomatitis virus, vesicular exthema of swine virus, swine vesicular disease virus and ORF virus. Each of the latter viruses has a distinct EM morphology by negative staining and therefore can be readily identified in a very short time (less than one hour) from cultured cell supernatants or from vesicular fluids. A positive morphological diagnosis of FMDV, however, is not as easy it is a small (25-28 nm) virus not covered by a membrane and is in a large virus family (picornaviruses) that has the same-sized particles some of which are found in animals. In addition, FMDV is fragile and falls apart at pHs below 7.0. VCMI has developed fixation protocols for FMDV that allows for preliminary morphological identification in sections of cultured cells and tissues and is adapting protocols to permit confirmation with immunogold. Rapid processing of infected cells and tissues using microwaves will cut the time for thin section analysis from days to hours [2] and make microscopy as rapid as some antibody and nucleic acid assays without the need of reagents or primers.

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In conclusion, electron microscopy provides an invaluable first look at FADs. Confirmation of FADs especially FMDV does require antibody and nucleic acid techniques. VCMI will also present examples of using EM to characterize unknown or emerging agents of animal disease from domestic submissions.

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