Diagnostic electron microscopy: the final resort?

J. Mast[1]

1. CODA-CERVA, EM-unit, Groeselenberg 99, B-1180, Brussels, Belgium

jamas@var.fgov.be
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Often the failure of a series of specific diagnostic tests is required before a veterinarian or a laboratory worker decides to search for one of the few remaining electron microscopy (EM) units specialized in the diagnosis of infectious agents. The remaining sample might, but often is not, be taken and fixated properly, but expectations invariably are that, as a *deus ex machina*, this beautiful piece of machinery produces a picture of text book quality showing the long-searched-for etiological agent. Remarkably, and thanks to some weird human intervention, such whishes are not seldom granted and the scientist returns to his lab happily in the belief that in case of emergency, he always can turn to the electron microscope as a final resort.

Indeed, in this era of avian influenza, a simple negative staining allowed to visualize rota-, adeno-, pox-, reo-, paramyxo-, gumbooroo-, and herpesviruses in cultures or embryos that were clearly virus-infected, but contained no influenzavirus (Figure 1). Such results were not only obtained in chickens, but also in exotic species like partridges, pigeons and ducks, where virus-specific test are often unavailable. Also, in addition to the usual suspects, like mycoplasms and endogenous retroviruses, contaminant bovine viral diarrhea virus and simian paramyxoviruses are found in stock solutions of other viruses and even in uninfected cell lines. If no 'open-view' method, like diagnostic EM, is used such co-infections can persist unnoticed for months, influencing virus titration and seroneutralisation tests.

In certain cases, no etiological agent can be demonstrated, but interpretation of a changed cellular ultrastructure allows reorientation of the investigations. In autumn of 2003, more than 40 horses, all free ranging on meadows in the North of France and the South of Belgium suddenly died due to an atypical myopathy. Compared with control animals, affected horses demonstrated an accumulation of large intracellular lipid droplets and an open structure of the mitochondria, indicative for mycotoxin intoxication (Figure 2). This hypothesis agrees nicely with the humid climatological conditions, with the observation that only free-ranging animals were affected, and with the observation that ruminants sharing these pastures were not affected. Ruminants are less sensitive to such intoxication due to destruction of mycotoxins by the microflora of the rumen. Similarly, an increased mortality of broiler breeder chickens, was recently observed in several farms in Flanders (Belgium). EM diagnosis demonstrated no infectious agent, but ultra-thin samples showed a disturbed fat metabolism of hepatocytes. Symptoms of fatty degeneration of hepatocytes varied from advanced cell swelling, accumylation of large triglyceride globules and complete ultrastructural decay of metabolically active parenchymal hepatocytes while structural fibroblasts and erythrocytes remained intact. As a result, factors influencing fat metabolism are examined instead of chasing an illusive infectious agent. These include severe food restriction, imbalances of feed nutrients and co-enzymes, contaminating mycotoxins and chronic hypoxia due to selection for high metabolic activity.

All above-mentioned cases arrived in the EM-lab with a history of negative results in specific tests, before EM was considered, and found useful, to obtain an orientating diagnosis.

Ironically, an identical result would have been obtained, but much faster and more efficiently, if diagnostic EM would have been applied as a frontline test, rather than as a final resort.

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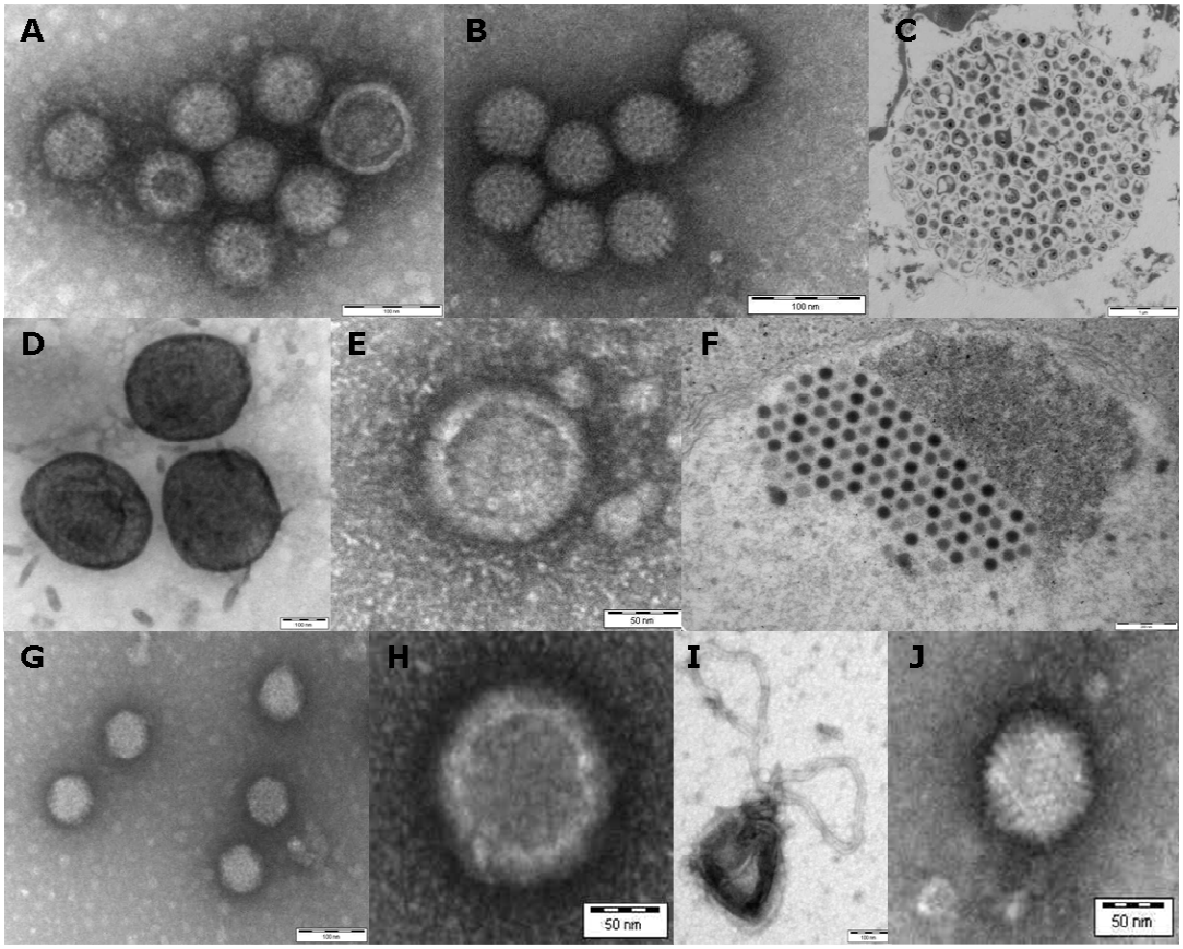

Figure 1. Differential EM-diagnosis of avain influenza and (A) partridge and (B) chicken rotavirus, (C) inclusion containing duck pest herpesvirus, (D) avipoxvirus, and (E) infectious laryngotracheitis herpesvirus, (F) chicken adenovirus, (G) chicken gumboroovirus, (H) pigeon herpesvirus, (I) paramyxovirus and (J) adenovirus.

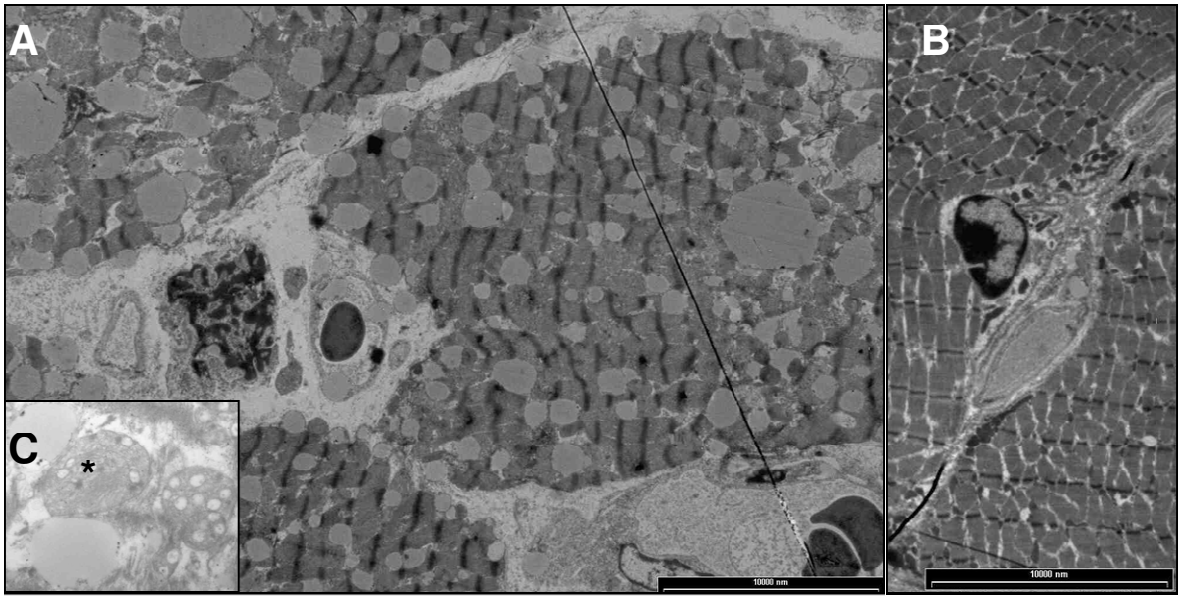

Figure 2. Representative micrographs of the cardiac muscle of (A) an adult stallion affected by acute horse myopathy and (B) of a control animal. The insert of A(C) shows mitochondria (*) at higher magnification.

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