Ultrastructure of natural West Nile viral infection of raptors (*Accipiter gentilis*) found in Austria

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West Nile virus (WNV) was first identified in Africa in 1937 [1], and subsequently, has spread due to its wide vector and bird host range throughout Eastern Europe, France, Asia, Australia and America [2,3]. The host range comprises more than 317 avian species. *West Nile virus* (family: Flaviviridae, genus: *Flavivirus*) is a member of the serologically related Japanese encephalitis antigenic complex; members of that complex include *Cacipacore virus* (CPCV), *Koutango virus* (KOUV), *Japanese encephalitis virus* (JEV), *Murray Valley encephalitis virus* (MVEV), *Alfuy virus* (ALFV), *St. Louis encephalitis virus* (SLEV), *Usutu virus* (USUV), and *Yaounde virus* (YAOV) [4]. *West Nile virus* is transmitted naturally by ornithophilic mosquitoes (particularly by species of the genus *Culex spp.*) within the bird population, but some mosquitoes are also capable of transmitting the virus to mammals such as horses or humans [5]. In August 2008 a wild goshawk (*Accipiter gentilis*) showing deviant behaviour and neurological symptoms was brought to the institute for post mortem examination. The bird died a few hours later; other two goshawks and a bearded vulture (*Gypaëtus barbatus*) followed. All birds were molecular biologically and virologically tested positive for WNV lineages 2.

Besides histology including immunohistochemistry, molecular biology and virology, organ and cell culture samples of the affected birds were investigated by means of semithin sections, negative staining and ultrathin sectioning. For negative staining, infected mouse neuroblast cell culture suspension and organ suspension of ZNS-, spleen- and heart-tissue was used; for analysis of ultrathin sections, cells of infected neuroblast cell cultures (NA 42/13) and tissue of pancreas, spleen, liver and central nervous system were fixed in cold Karnovsky solution and osmium tetroxide. Ultrathin Epon-sections were finally analysed in a Zeiss 906 at 80kv.

All three birds showed widely corresponding pathomorphological findings: The organ tissue of the birds, especially the cerebellar tissue, showed severe signs of local autolysis. Formation of glial nodules and neuronal necrosis in the cerebellum and mesencephalon was evident. Neither virions nor virus-induced lesions were found in the tissue of the cerebrum. Splenic lesions were generally more subtle and consisted merely of multiple small foci of necrotic apoptotic lymphoid cells. Myocardial necrosis and fibrosis ranged from focal to widespread. Lung lesions, often around branchioles, were identified in the bearded vulture. The pathomorphological findings of all threee goshawks corresponded largely with the hungarian cases [6].

Negatively stained virions of the cell and organ suspension measured 45-55nm in diameter, were icosahedral in shape, and showed typical flaviviral morphology, that is, a dense core surrounded by a thin, diffuse outer layer (Figure 3). In ultrathin sections, flavivirus-like particles were frequently seen in the cerebellum, in pancreatic cells and in hepatocytes. In liver and pancreatic cells, the particles were found in the vacuolar system and near membranes of the endoplasmatic reticulum (Figure 2); in cells of the cerebellum, virus

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particles were usually present in cytoplasmic vacuoles (Figure 1a, 1b). At least structure of virus particles was compared with that of virions accumulated in the basal membrane of lung tissue of the bearded vulture. Flavivirus particles were also detected in the neuroblast cells of the mice cell culture (Figure 4). Replication of flaviviruses were found in invaginations, They are called vesicular packets and are assumed to originate from *trans*-Golgi-network [7].

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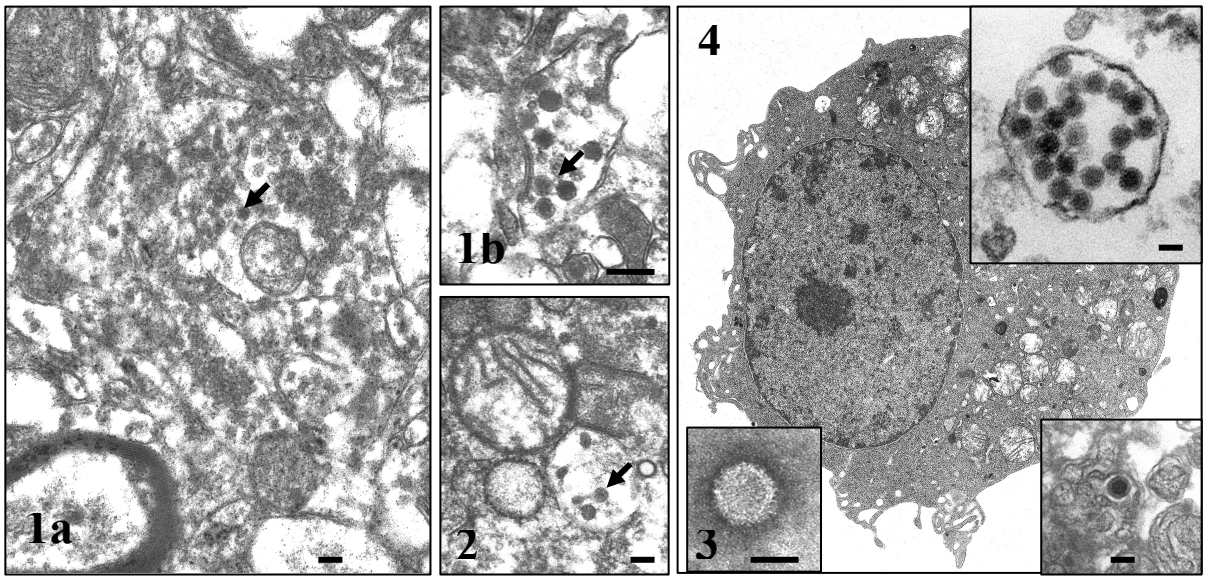

Figure 1. Goshawk. Epon section - cerebellum; nerve cell (1a) with severe signs of autolysis; virus particles in vacuoles of perikaryon and neuronal processes (1b)
Figure 2. Goshawk. Epon section - liver; virus particles in vacuolar system of hepatocytes

arrows = virions, bar = 100 nm

Figure 3. Goshawk. Negative staining - flavivirus particle from spleen suspension.
Figure 4. Infected neuroblast mouse cell. Epon section - flavivirus particles in vesicular packets (insertions)

bar = 50 nm

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