

## **Diagnosis of virus belonging to the family Reoviridae in diseased pigeons, using transmission electron microscopy.**

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Pigeons (*Columbia livia*) with fatal liver disease were diagnosed by transmission electron microscopy (TEM) as laden with virus belonging to the family *Reoviridae*. In the diagnostic setting EM is valuable in the surveillance of emerging diseases and this Australian outbreak in racing and fancy pigeons was a prime exemplar of rapid EM response.

The diagnosis was made by comparison to ultrastructural libraries of viral family demarcation criteria. The family *Reoviridae* additionally has possible morphologic differentiation beyond the family, to the genus level. This is based on the presence or absence of spikes or turrets sitting on the core of the particle [1], the length of these protein spikes and their terminate appearance. In the pigeons case, by negative contrast preparation, rough spherical wheel-like particles were observed (Fig 1.) consistent with the family *Reoviridae*. Molecular characterization further classified these virions as members of the genus Rotavirus. EM and molecular sequencing are highly complementary techniques in the discovery of novel pathogens due to the absence of previously prepared virus specific reagents.

Initial grid preparations, made typically from liver stored one week post mortem before submission, also revealed large amounts of ferritin particles indicative of cellular breakdown. As the outbreak continued, fresher liver submissions and virus isolation by cell culture allowed for visually clearer EM preparations and hence more effective visualization of the outer layers of the virus particles. This was necessary to distinguish the visually similar genera - rotavirus from orthoreovirus. The absence of long spikes, less electron lucent inner ring and rounded overall outer perimeter appearance supported these particles belonging to the genus rotavirus. Thin section EM analyses were also performed on mottled areas of affected pigeon liver. Cytoplasmic inclusions of numerous electron dense particles were observed. These particles were arranged in arrays and were consistent with EM observations reported previously in a juvenile Californian pigeon [2]. Additionally, Thin Section EM was used to confirm virus isolation attempts.

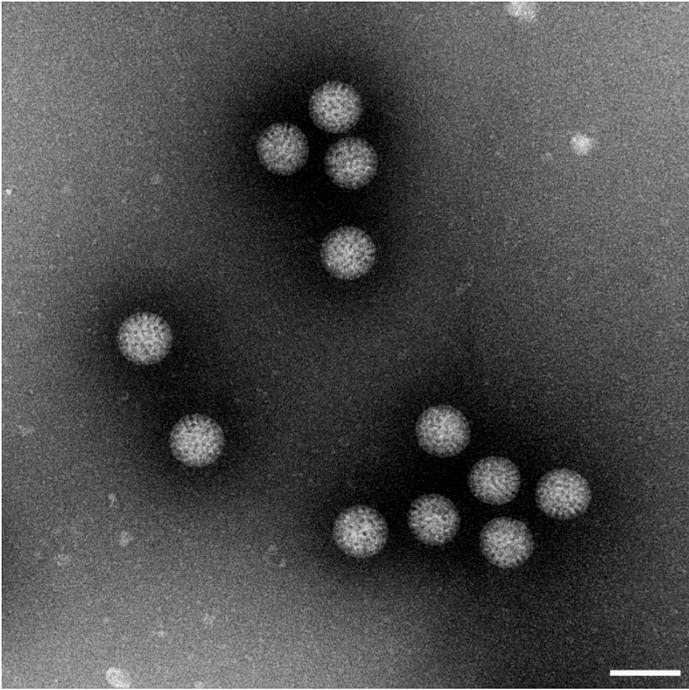


Figure 1. Negative contrast transmission electron micrograph of rotavirus particles in supernatant from fetal rhesus monkey kidney cells (MA104) inoculated with pigeon liver homogenate. Scale bar represents 100nm.

1. Goldsmith S. G. Morphologic Differentiation of Viruses beyond the Family Level. *Viruses*. 2014 Dec; 6(12):4902-4913.
2. Giannitti F., Diab S., Fish S., Woolcock P., Woods L. Hepatitis associated with Reovirus infection in a pigeon in California. Conference: 56<sup>th</sup> American Association of the Veterinary Laboratory Diagnosticians (AAVLD) and United States Animal Health Association (USAHA) Annual Meeting. October 2013.