Studies of Structural Stability and Inactivation by High Hidrostatic Pressure of H3N8

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The Influenza virus belongs to the *Orthomyxoviridae* family. This RNA virus presents a lipid envelope exposing two important glycoproteins, hemagglutinin(HA) and neuraminidase(NA). H3N8 is an avian Influenza virus that was originally isolated from birds, later found in horses and recently a transfer of equine influenza virus to dogs with high lethality was described. In this work, we evaluated the structural stability of this avian virus submitting the particles to high hydrostatic pressure(HHP) and other chemical (urea and guanidine hydrochloride) and physical (high and low temperatures) denaturant agents. With this aim we have used mainly spectroscopic techniques. Moreover, we investigated the particle inactivation by HHP and estimated its effects by analysis of the hemagglutination titer, neuraminidase activity, electron microscopy(EM) and infectivity assays. H3N8 seems to be a very stable particle, since the denaturant treatments (in different pHs) show small structural variations. Only when high concentration of urea and guanidine were utilized, we could observe significant changes in fluorescence emission, light scattering measurements and circular dichroism data. Small changes on the secondary structure were observed at high temperature. The use of HHP for 6 hours suppressed the NA activity, hemagglutinating and infectivity capacity. However EM shows a small population of entire particles in samples after pressurization that shows ability for replication in blind passages. Currently we are working in order to eliminate this residual infectivity found in blind passages. Our data bring more information for structural virology area of enveloped particles and reinforce the idea for application of HHP to prepare viral vaccines.

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