

INTRODUCTION AND OBJECTIVES

- The influenza viruses are a diverse group of RNA viruses from *Orthomyxoviridae* family. Focusing on Influenza A viruses, they are primary avian viruses which can cause respiratory problems, neurological problems and in the worst cases, the death of the animal.
- Commonly apoptosis it is known as a host defence mechanism against the replication and the spread of the virus. However, studies that inhibits apoptosis in cells infected by influenza virus shows the opposite; a significant reduction of the virus replication and spread.
- The purpose of this work was to investigate the role of apoptosis caused by influenza virus A in CNS of chickens.

MATERIALS AND METHODS

Samples

The brain tissue samples were taken from the euthanized chickens in 1dpi, 2dpi and 3dpi:

- Fixed in 10% buffered formalin for 24 hours.
- Cut at three different coronal levels (figure 2) and fixed in paraffin wax.

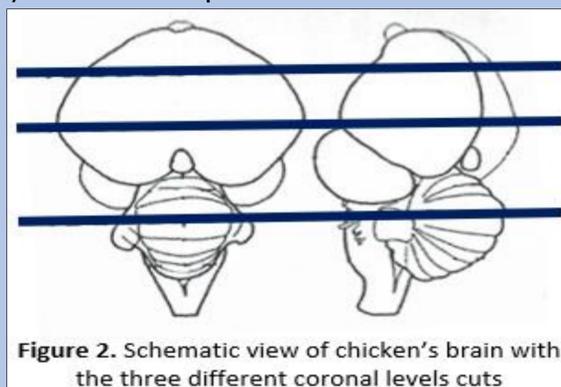
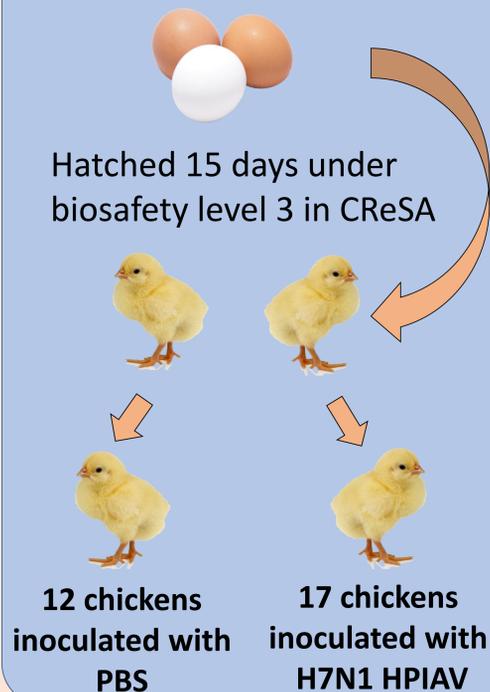


Figure 2. Schematic view of chicken's brain with the three different coronal levels cuts

Immunohistochemical studies

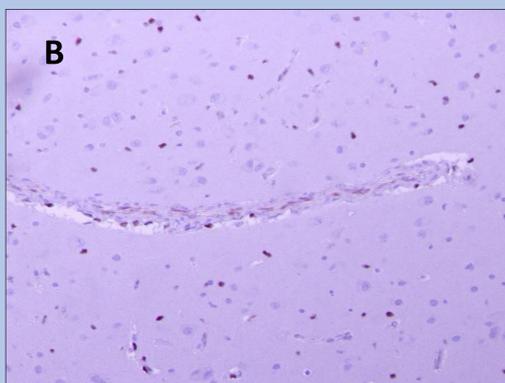
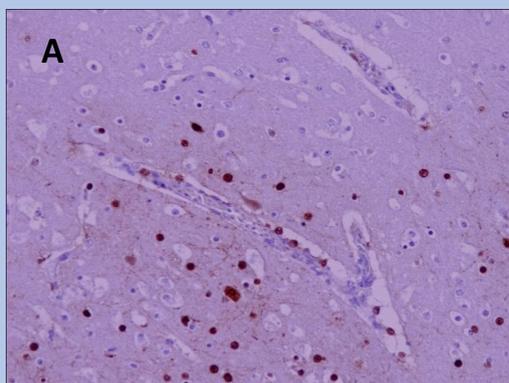
- Haematoxilyn eosin stain.
- HIC against viral antigen.
- HIC Caspase 3.



RESULTS

	IHC studies	1 dpi	2 dpi	3dpi	Not infected
Intensity	Viral antigen	-	+	++	-
	Caspase 3		+	+	+
Affected cells	Viral antigen	Not seen	- Neurons - Glial cells - Endothelial cells	- Neurons - Glial cells - Endothelial cells Nucleus: - Ependymal cells	Not seen
	Caspase 3		- Neurons - Glial cells - Endothelial cells - Ependymal cells	- Neurons - Glial cells - Endothelial cells - Ependymal cells	- Neurons - Glial cells
Distribution	Viral antigen	Not seen	Isolated/ multiple foci	Multiple foci	Not seen
	Caspase 3		Single cells	Single cells	Single cells

Chicken infected experimentally with Infuenza virus A H7N1, 3dpi, Thelencephalon. A) IHC to detect viral antigen and B) IHC Caspase 3. Positive blood vessels in both techniques.



DISCUSION

- The endothelium presented positivity in viral antigen and caspase 3. ➡ **Correlation between influenza virus and apoptosis of endothelium.**



- Influenza virus started to affect the CNS from caudal parts, like cerebellum, and continues to rostral parts.



- **Influenza A virus enters to the CNS by bloodstream.**

- Caspase 3 IHC showed an apoptosis distribution which was not correlated with the virus antigen IHC:

- **Not all the infected cells enter in to apoptosis.**

COLCLUSIONS

- ✓ Influenza virus A has an apoptotic role to reach the CNS of chickens. The virus arrives to the CNS by bloodstream and could induce apoptosis to the endothelial cells to disseminate itself through the tissue.
- ✓ More studies are needed to establish the exact mechanisms that IVA uses to cause apoptosis in CNS cells.