

A PATHOLOGICAL STUDY OF THE SALIVARY GLANDS OF RABID DOGS IN THE PHILIPPINES

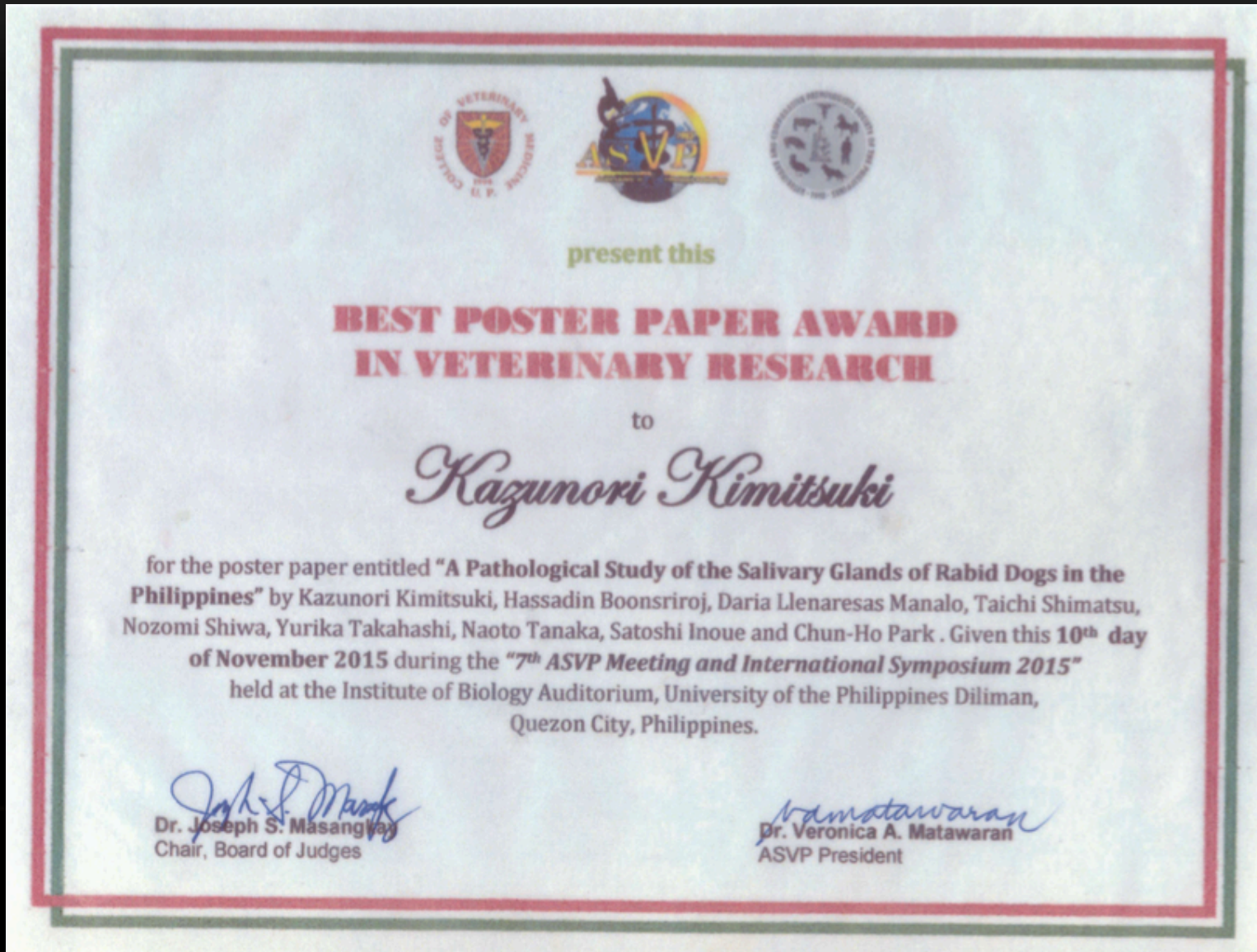
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A pathological study of the salivary glands of rabid dogs in the Philippines

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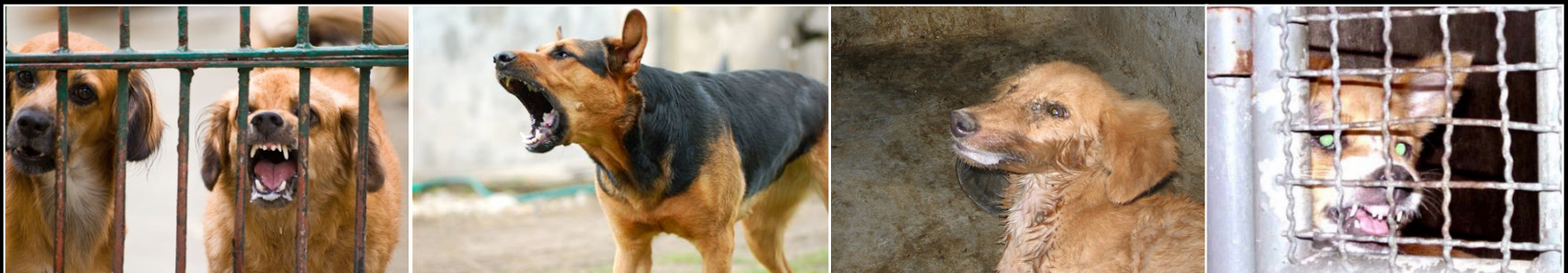
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ABSTRACT. Rabies is a zoonotic disease caused by the rabies virus. While the salivary glands are important as exit and propagation sites for the rabies virus, the mechanisms of rabies excretion remain unclear. Here, we investigated the histopathology of the salivary glands of rabid dogs and analyzed the mechanism of excretion into the oral cavity. Mandibular and parotid glands of 22 rabid dogs and three control dogs were used. Mild to moderate non-suppurative sialadenitis was observed in the mandibular glands of 19 of the 22 dogs, characterized by loss of acinar epithelium and infiltration by lymphoplasmacytic cells. Viral antigens were detected in the mucous acinar epithelium, ganglion neurons and myoepithelium. Acinar epithelium and lymphocytes were positive for anti-caspase-3 antibodies and TUNEL staining. In contrast, no notable findings were observed in the ductal epithelial cells and serous demilune. In the parotid gland, the acinar cells, myoepithelium and ductal epithelium all tested negative. These findings confirmed the path through which the rabies virus descends along the facial nerve after proliferation in the brain to reach the ganglion neurons of the mandibular gland, subsequently traveling to the acinar epithelium via the salivary gland myoepithelium. Furthermore, the observation that nerve endings passing through the myoepithelium were absent from the ductal system suggested that viral proliferation and cytotoxicity could not occur there, ensuring that secretions containing the virus are efficiently excreted into the oral cavity.

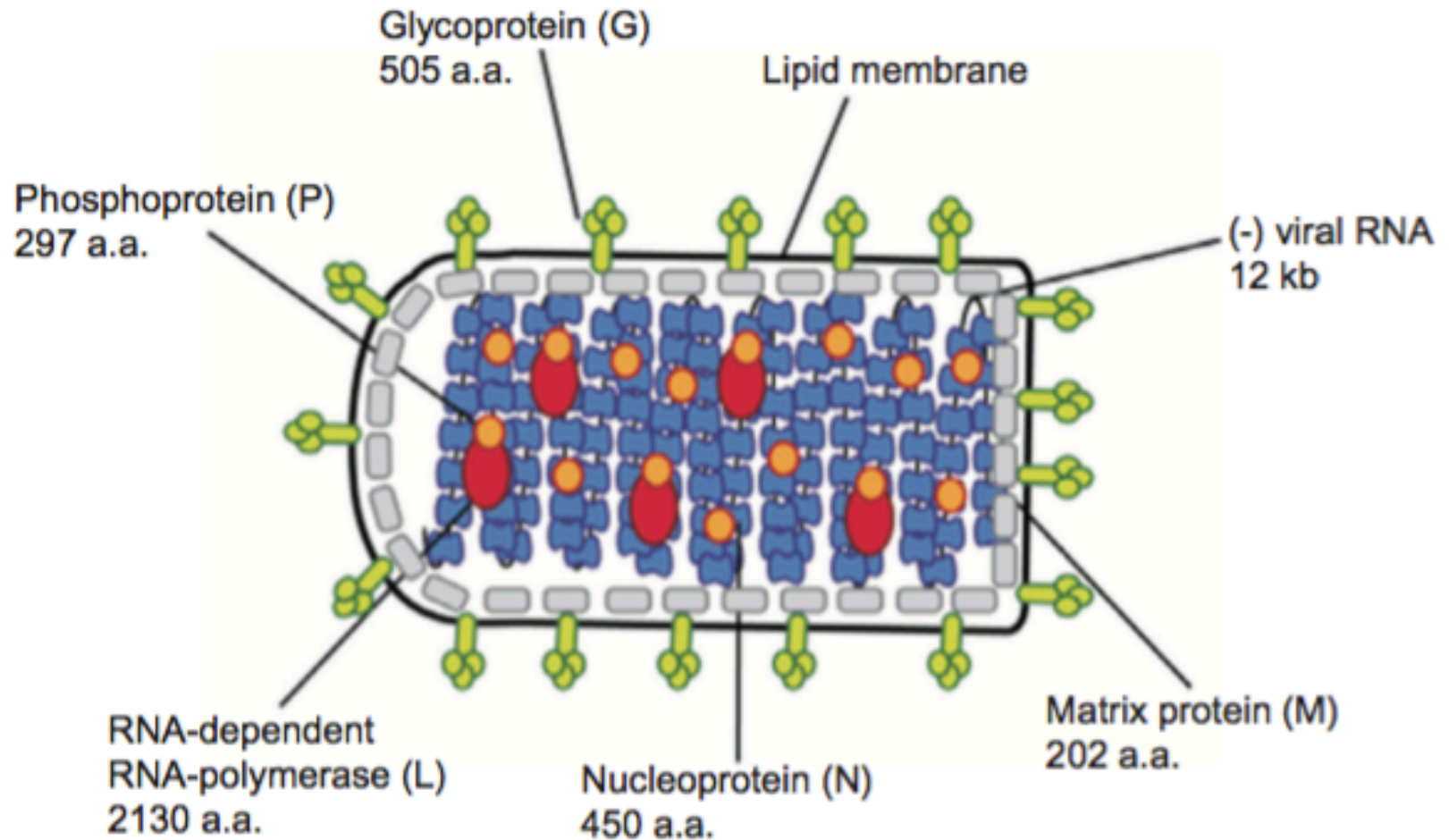
KEY WORDS: histopathology, Philippines, rabid dog, salivary gland

INTRODUCTION AND PURPOSE OF THE STUDY

- Rabies is a highly fatal zoonotic disease caused by rabies virus that affects the nervous system in humans and other mammals.
- It remains a serious global public health problem and causes more than 55,000 human deaths annually, particularly in Asia and Africa (WHO, 2013).
- In endemic countries, domestic dogs remain the major reservoir and vector for rabies virus infection and play an important role in transmission of rabies virus to humans.

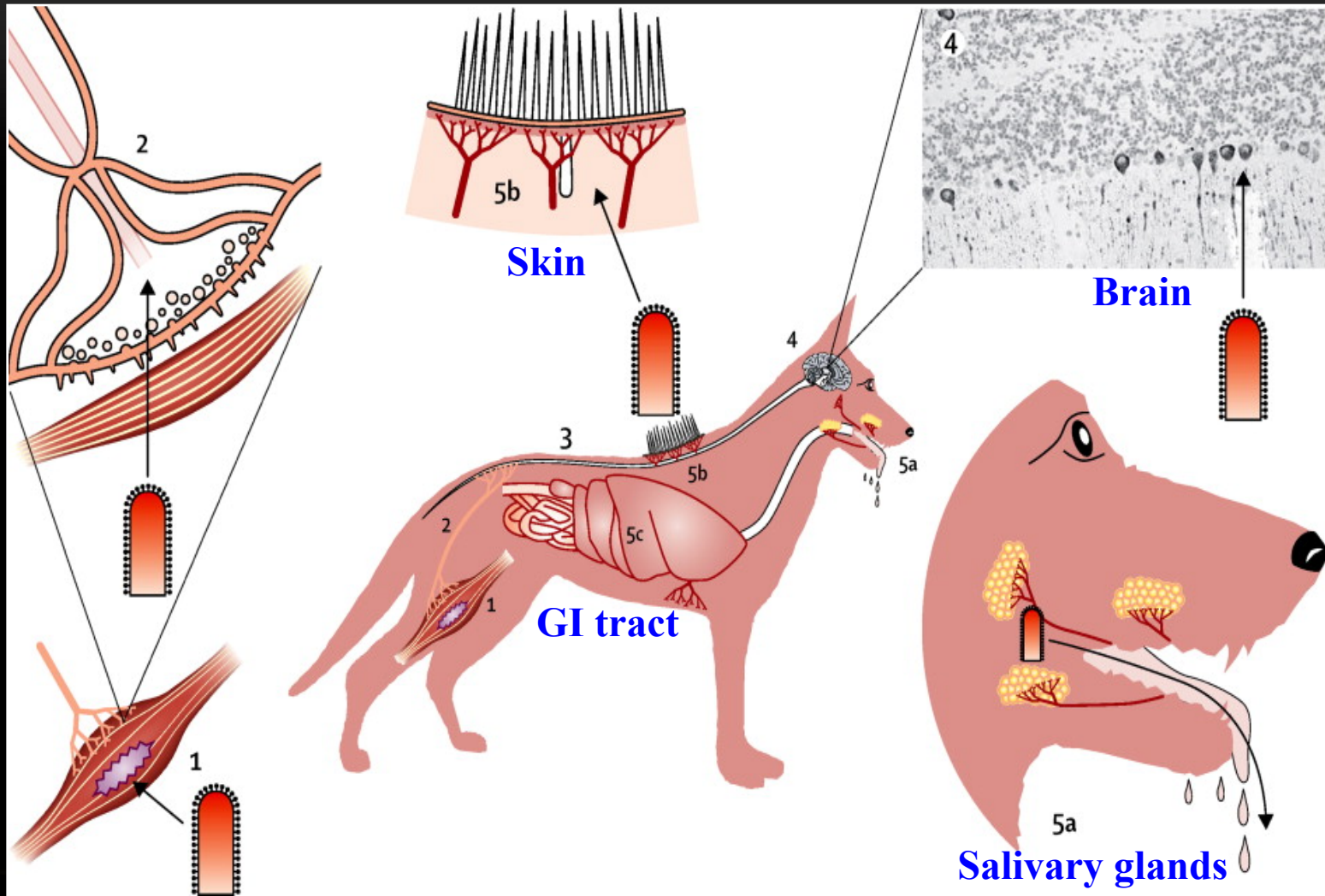


Structure of the rabies virus



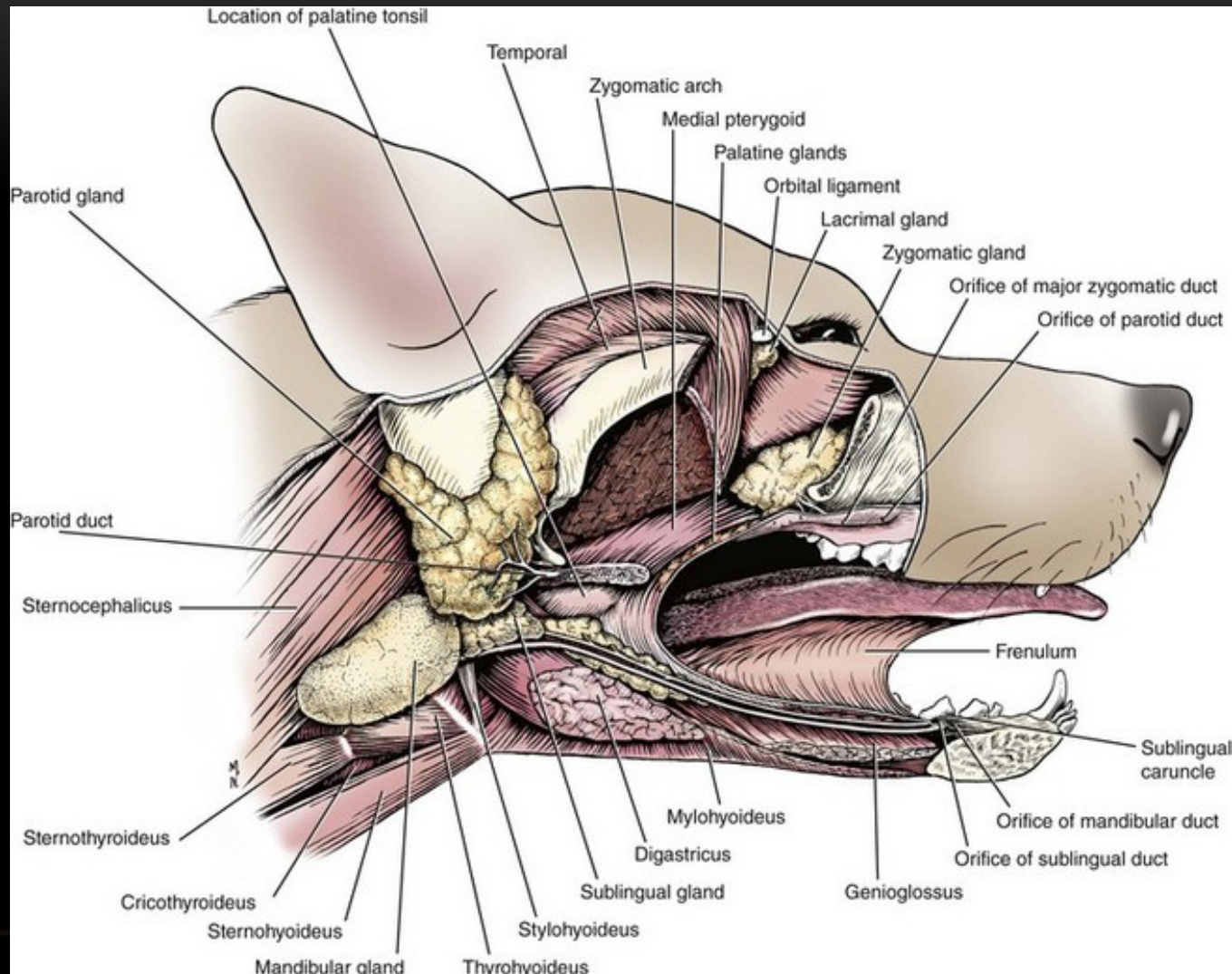
Jackson, AC. *Neuroviral*. 2014.

Pathogenesis of rabies



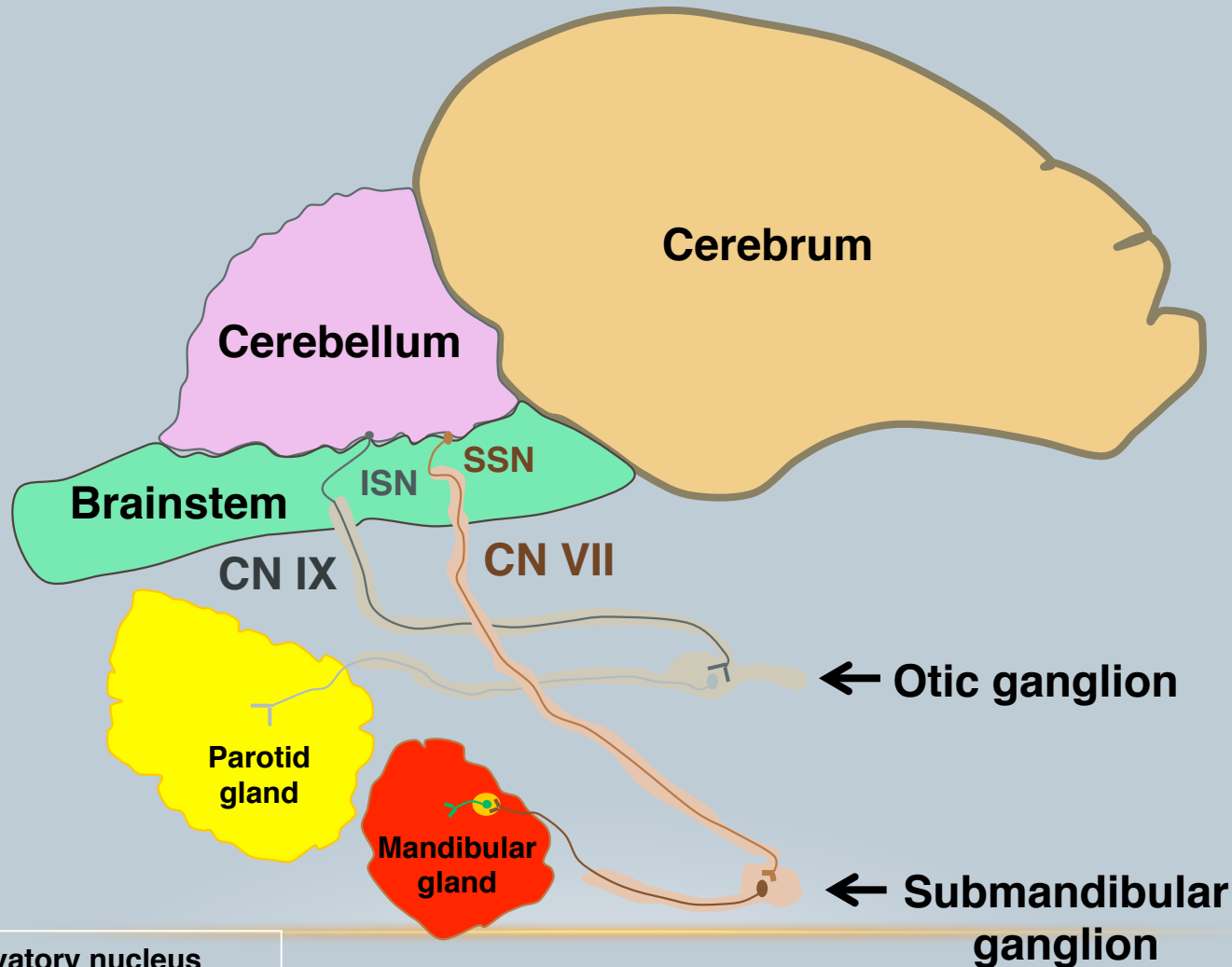
Fooks, AR. et al. *Lancet*. 2014.

Anatomy of dog salivary glands



Miller's Anatomy of the dog, 2013.

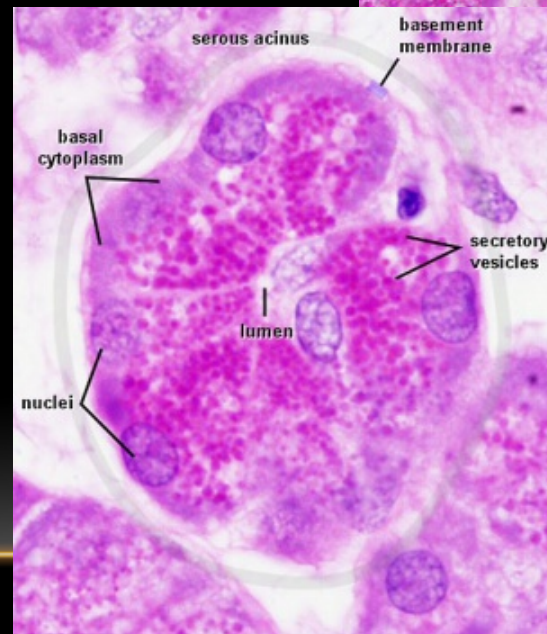
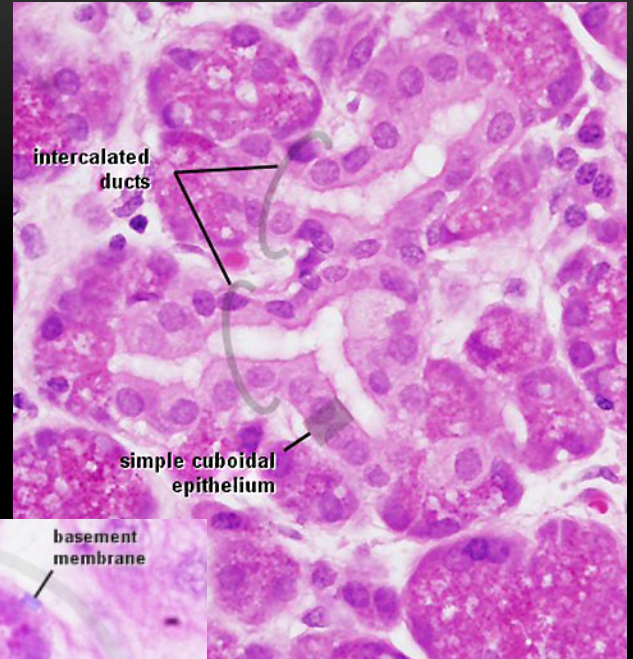
The innervation of the mandibular and parotid salivary gland in dogs



ISN: Inferior salivatory nucleus
SSN: Superior salivatory nucleus
CN VII: Facial nerve
CN IX: Glossopharyngeal nerve

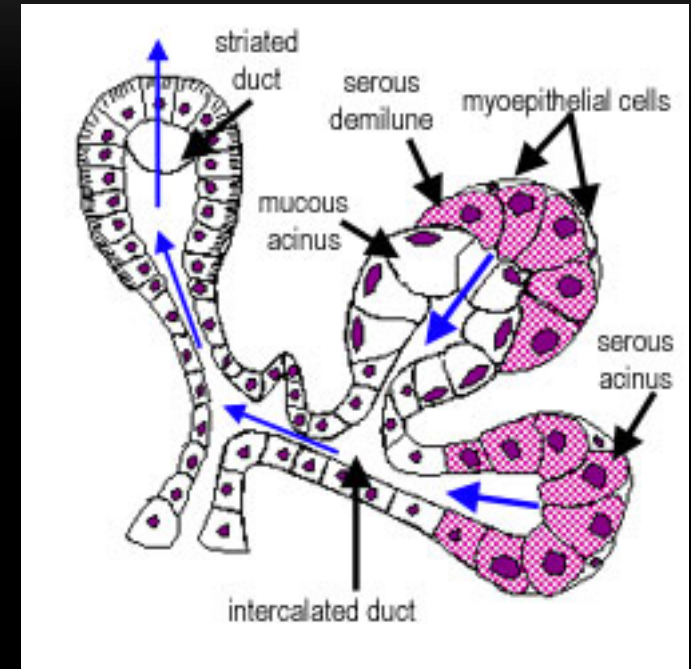
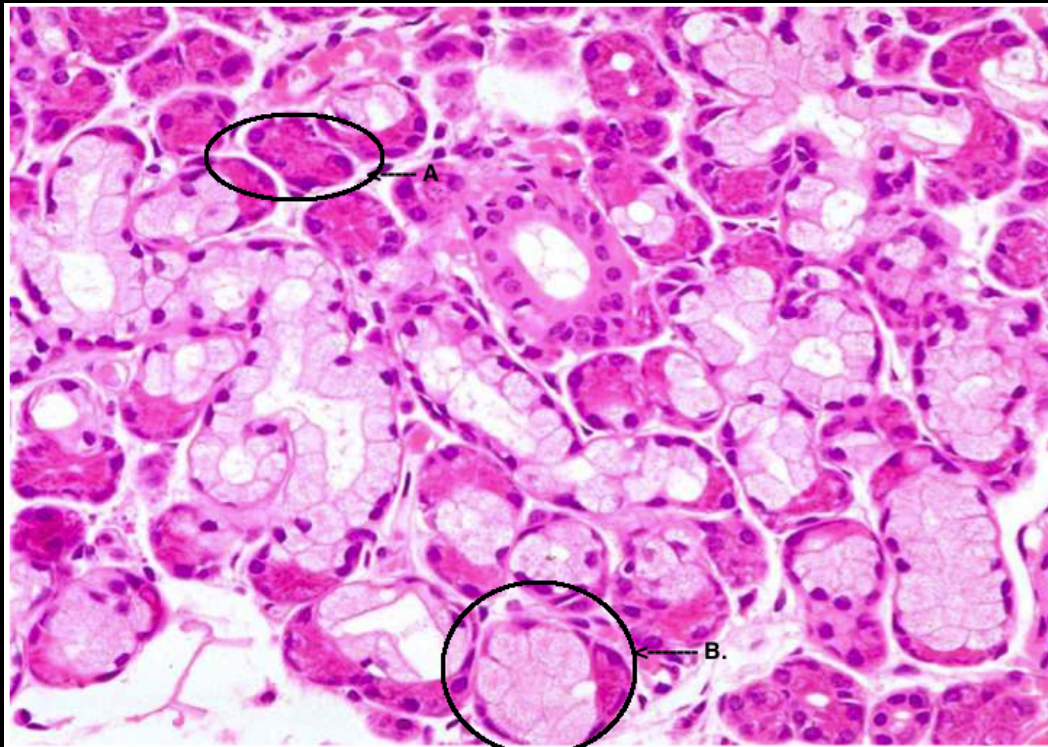
Histology of parotid salivary glands

- Serous secretory units



Histology of mandibular salivary glands

- Mixed seromucous secretory units



- The salivary glands are important as exit and propagation sites for the rabies virus.
- The dogs have the ability to excrete virus particles into the saliva up to 14 days before any clinical symptoms of rabies are apparent.
- Saliva samples can be used as alternative to brain and CSF for ante-mortem diagnosis of canine rabies.
- However, detailed pathological findings in salivary glands and the excretion mechanism remain unclear.

- The purpose of this study is to investigated the histopathological and immunohistochemical findings of the salivary glands of rabid dogs and analyzed the excretion mechanism of the virus into the oral cavity.

MATERIALS AND METHODS

Histopathological examination

- Mandibular and parotid glands of 22 rabid (4 euthanasia and 18 found dead) and 3 control dogs were used.
- Hematoxyline and eosin (HE) staining
- Special staining: Alcian blue (AB) and reticulin silver impregnation (RS)
- TUNEL assays for detection of apoptosis

- Immunohistochemistry:
 - For rabies virus antigen: anti-rabbit phosphoprotein (P)
 - For lymphocytes: anti-CD3 (T cells), CD20 (B cells) and CD79 α (plasma cells)
 - For basement membranes: anti-laminin
 - For nervous tissues: anti-neuron specific enolase (NSE) and anti-neurofilament protein (NF)
 - For myoepithelial cells: anti-alpha smooth muscle actin (α -SMA)
 - For apoptotic cells: anti-cleaved caspase-3
- Double staining: (anti-anti-P and anti- α -SMA), (anti-P and AB staining)

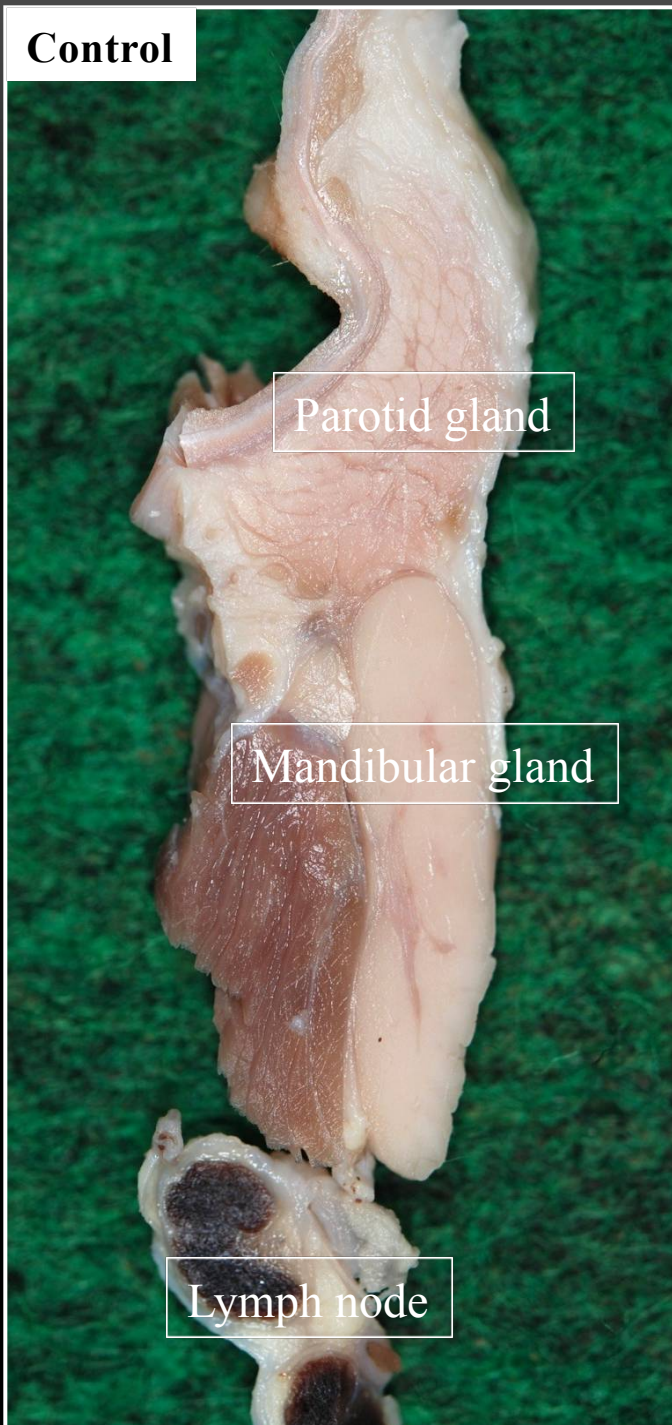
Table 1. Clinical information and laboratory findings of rabid dogs (samples obtained postmortem)

No.	months	Sex	Owned	Manner of death	Vaccination	Animal conditions	Neurological symptoms	dFAT
1	48	M	Yes	Found dead	No	Free-roaming and owned	Unprovoked aggressiveness, excessive salivation	+
2	-	M	Yes	Euthanasia	Unknown	Stray	Unprovoked aggressiveness, mad biting of inanimate objects	+
3	24	F	Yes	Found dead	Yes	Free-roaming and owned	Excessive salivation, mad biting of inanimate objects, apprehensive, watchful look, paralysis	+
4	1	M	Yes	Found dead	No	Free-roaming and owned	Unknown	+
5	6	M	Yes	Found dead	No	Free-roaming and owned	Unprovoked aggressiveness, mad biting of inanimate objects	+
6	6	M	Yes	Found dead	No	Free-roaming and owned	Unknown	+
7	1	M	Yes	Found dead	No	Free-roaming and owned	Unprovoked aggressiveness	+
8	60	M	Yes	Found dead	No	Confined with household contact	Unprovoked aggressiveness, excessive salivation, apprehension, watchful look, mad biting of inanimate objects	+
9	2	F	Yes	Found dead	No	Free-roaming and owned	Unprovoked aggressiveness, mad biting of inanimate objects, aimless running	+
10	12	F	Yes	Found dead	No	Free-roaming and owned	Unknown	+
11	1	F	Yes	Found dead	No	Unknown	Unknown	+
12	36	M	Yes	Found dead	No	Free-roaming and owned	Unprovoked aggressiveness, mad biting of inanimate objects, paralysis of jaw and tongue	+
13	16	F	Unknown	Found dead	No	Free-roaming and owned	Unprovoked aggressiveness	+
14	-	-	Unknown	Euthanasia	Unknown	Stray	Unprovoked aggressiveness	+
15	2	M	Unknown	Found dead	Unknown	Free-roaming and owned	Apprehensive, watchful look, paralysis	+
16	-	-	Unknown	Found dead	No	Free-roaming and owned	Unprovoked aggressiveness	+
17	2	F	Unknown	Found dead	No	Free-roaming and owned	Unprovoked aggressiveness	+
18	8	F	Unknown	Found dead	No	Free-roaming and owned	Unprovoked aggressiveness, mad biting of inanimate objects	+
19	-	M	Unknown	Euthanasia	Unknown	Free-roaming and owned	Unprovoked aggressiveness	+
20	-	M	Unknown	Found dead	No	Free-roaming and owned	Unprovoked aggressiveness, aimless running	+
21	-	-	Unknown	Euthanasia	Unknown	Stray	Unprovoked aggressiveness	+
22	3	F	Unknown	Found dead	No	Confined with household contact	Unprovoked aggressiveness, mad biting of inanimate objects, aimless running	+

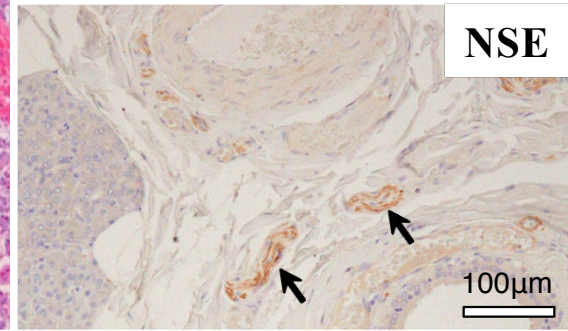
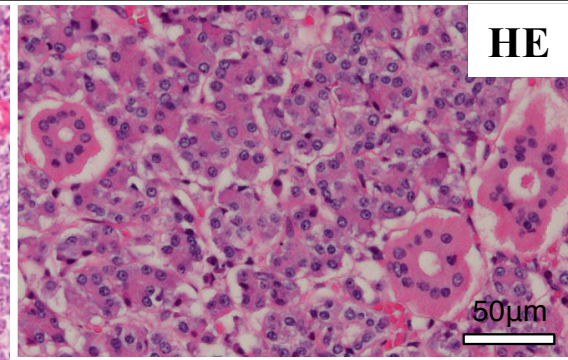
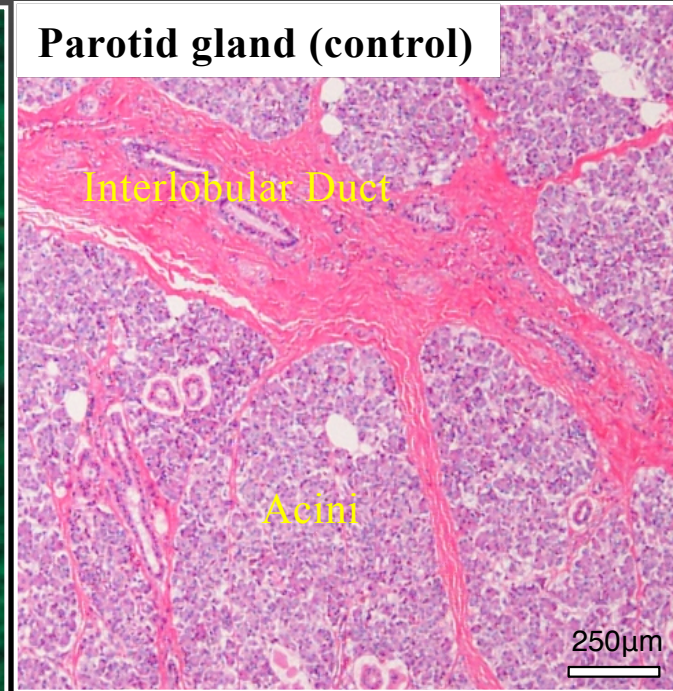
RESULTS

- Histology
 - Immunohistochemistry
 - Double staining
 - TUNEL assays
-

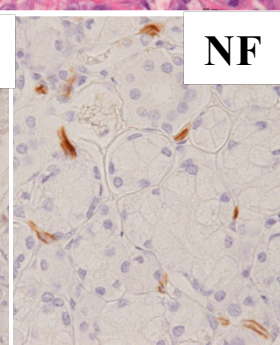
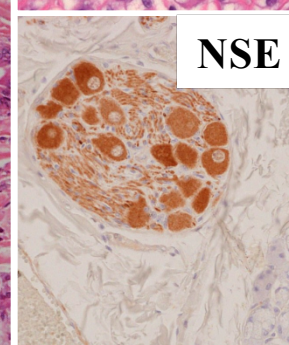
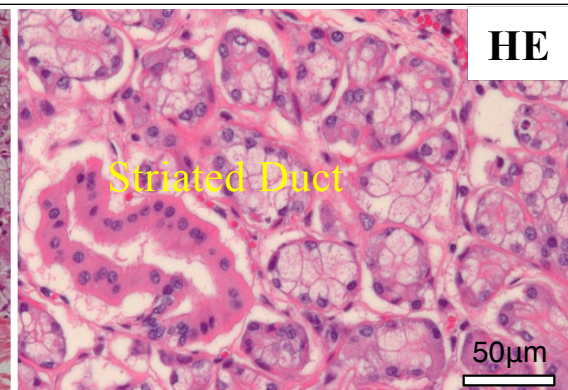
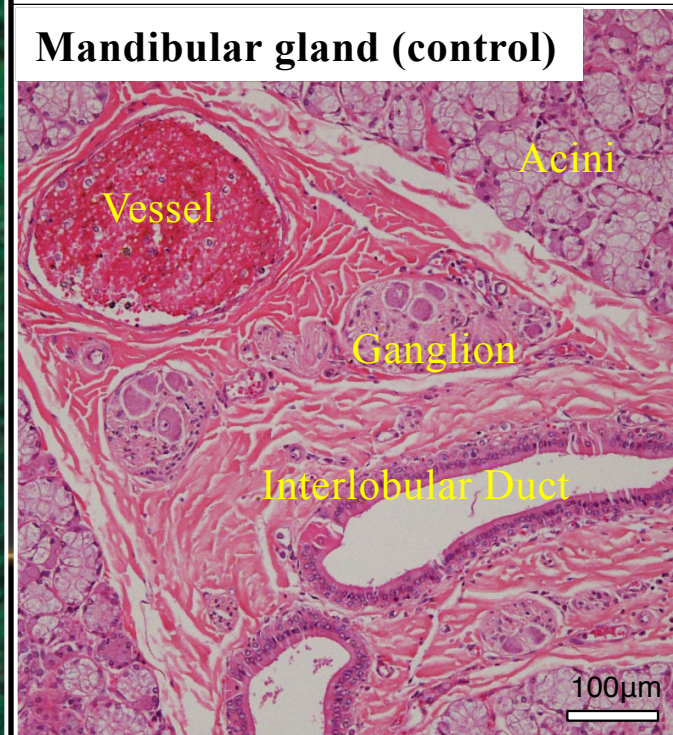
Control



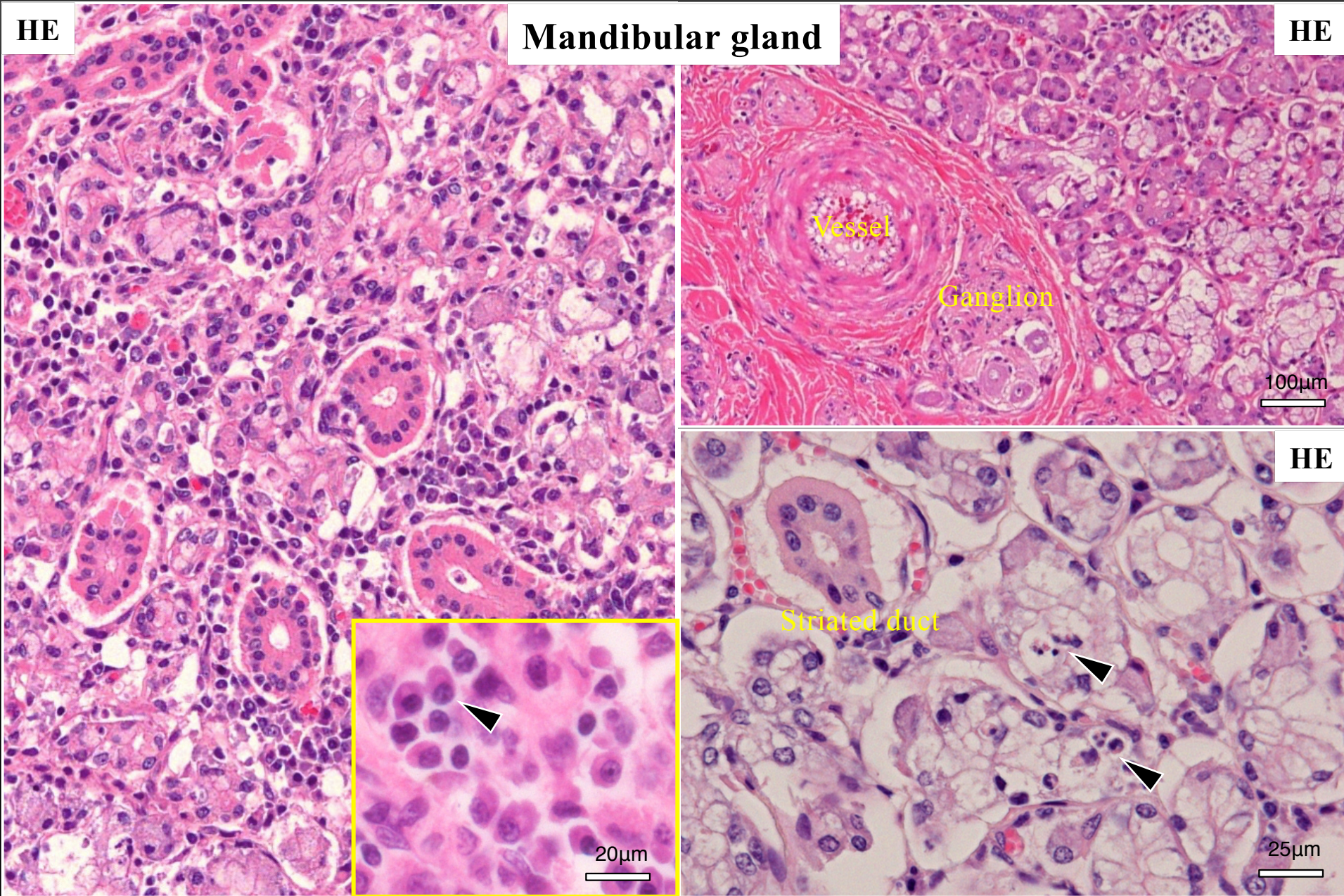
Parotid gland (control)

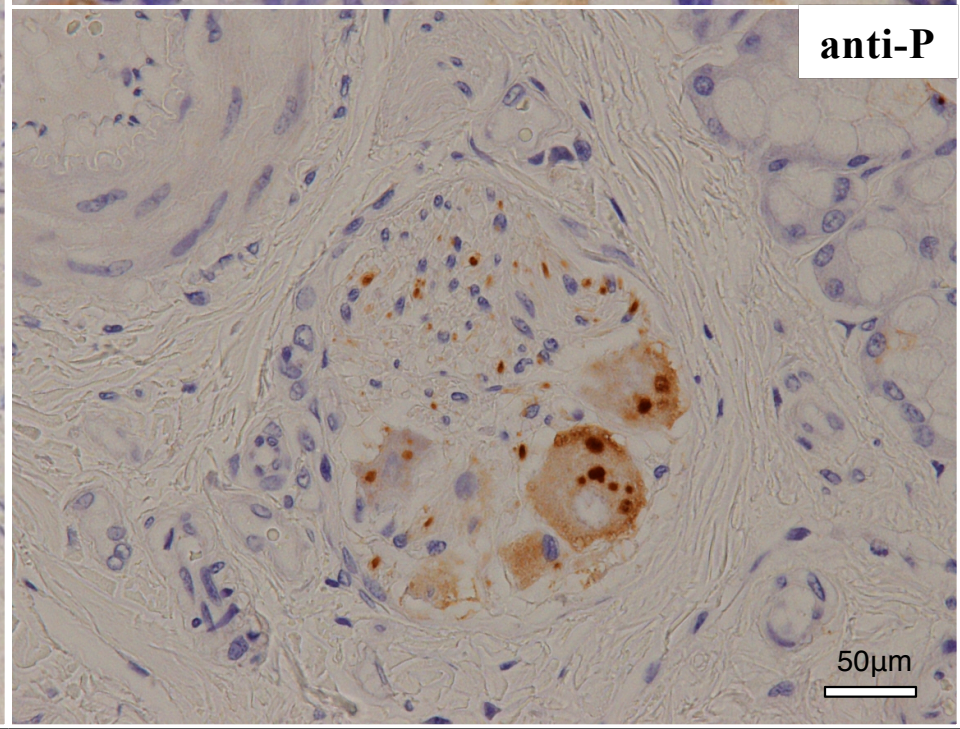
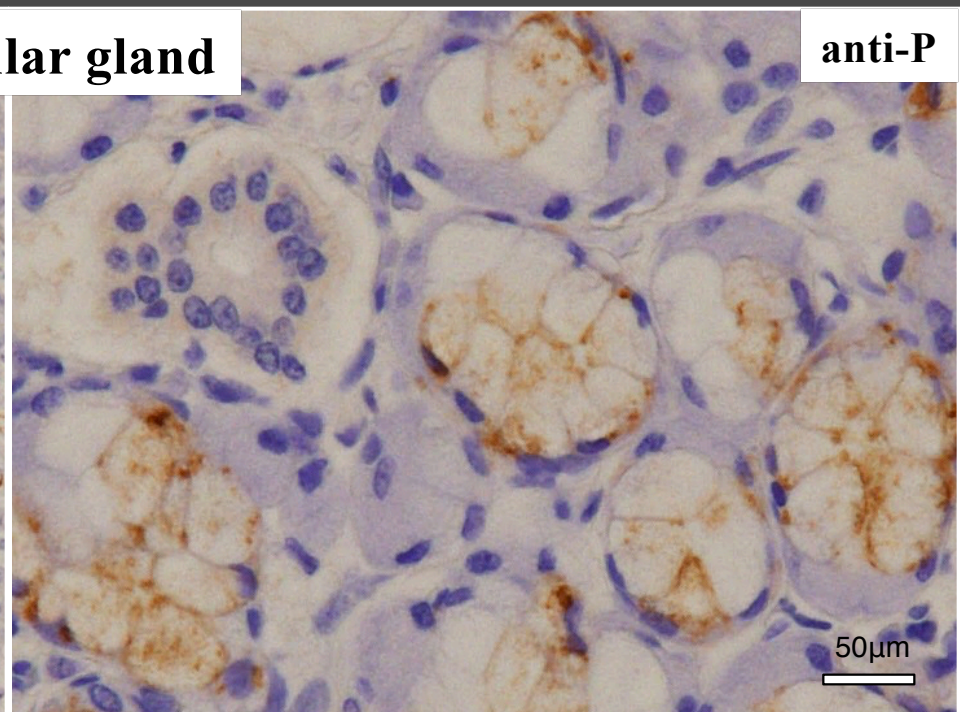
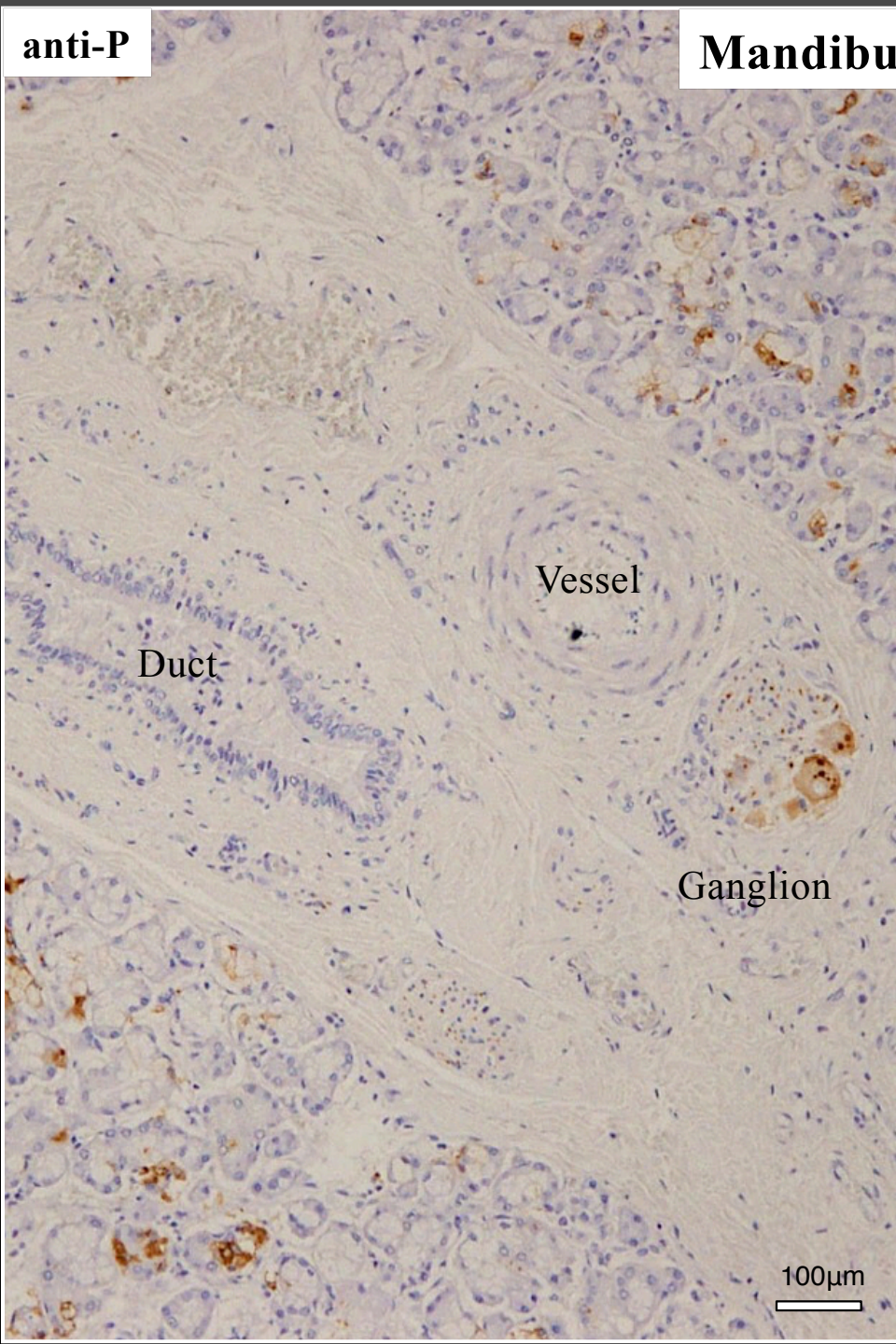


Mandibular gland (control)

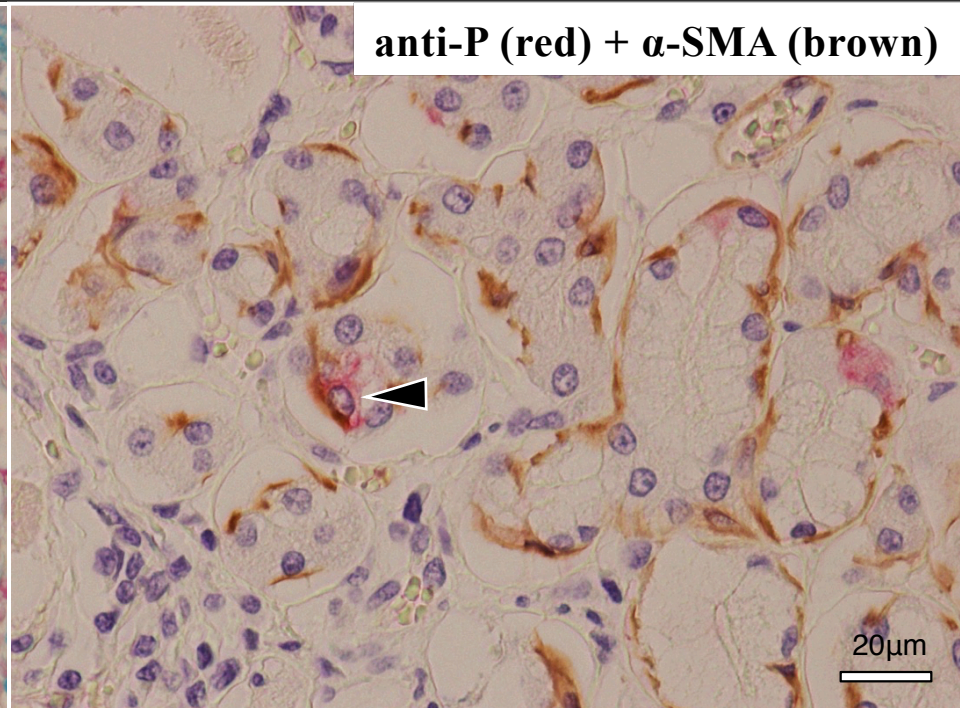
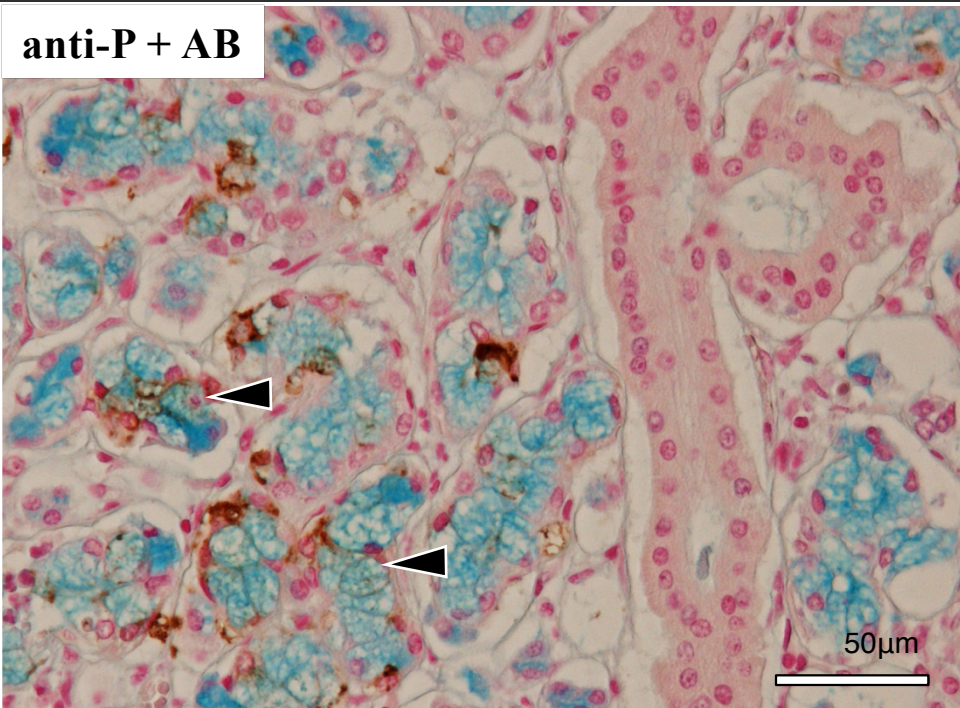


Pathological findings of the salivary gland of rabid dogs

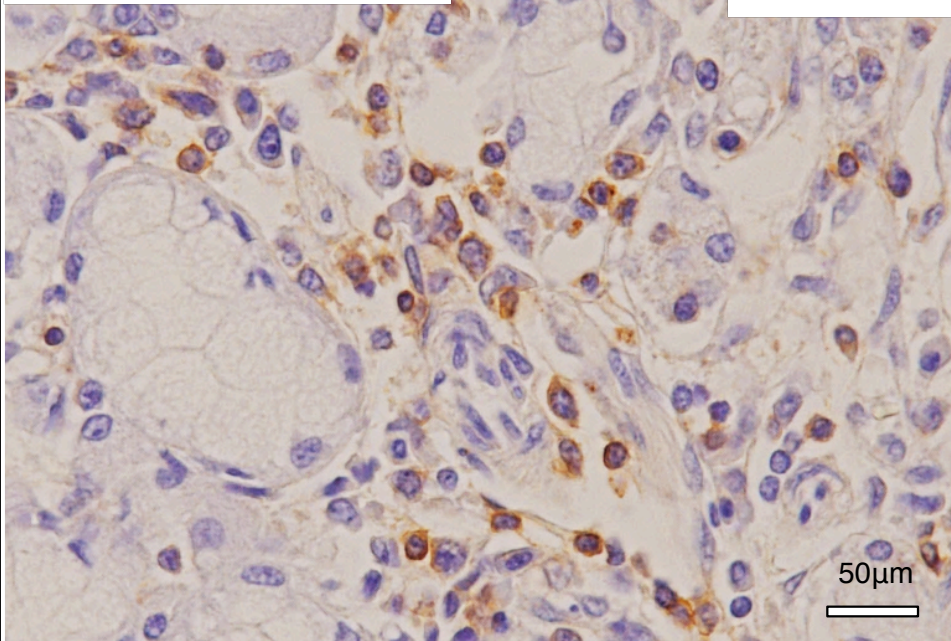




Identification of virus-infected cells by double staining

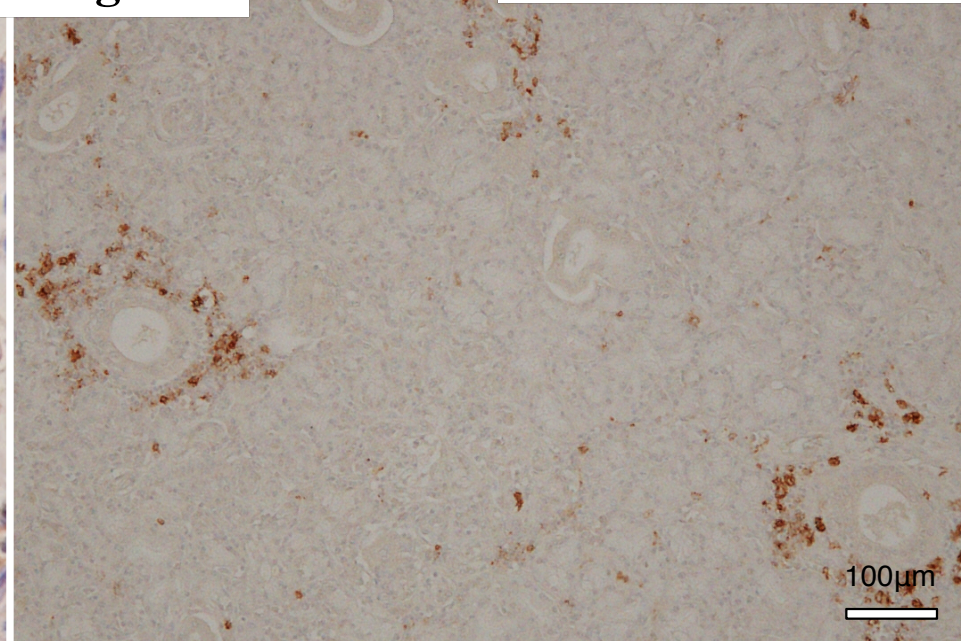


CD3 (T lymphocytes)

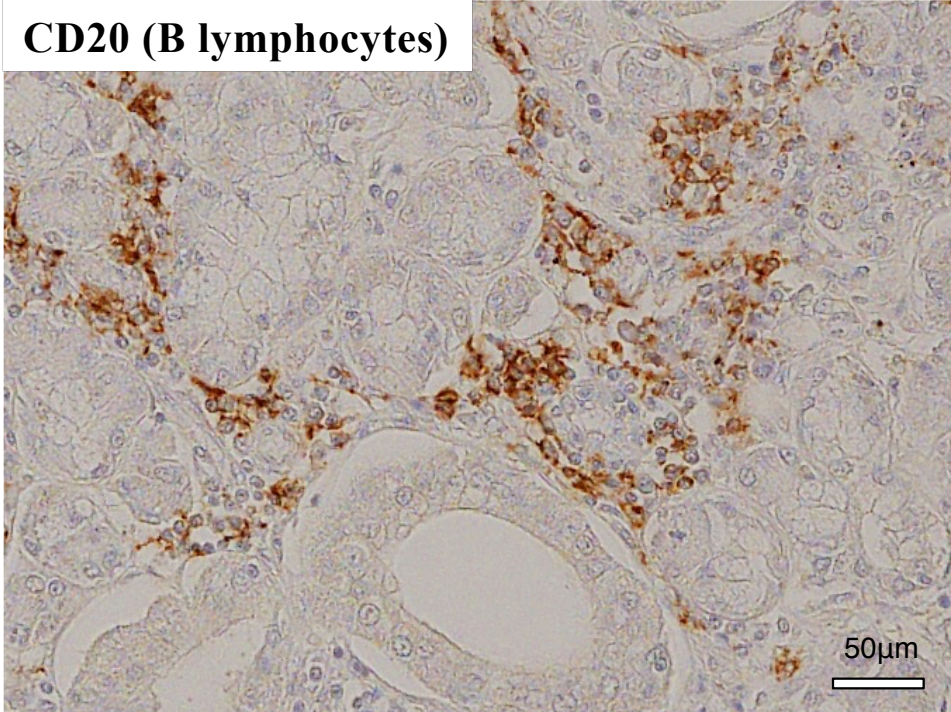


Mandibular gland

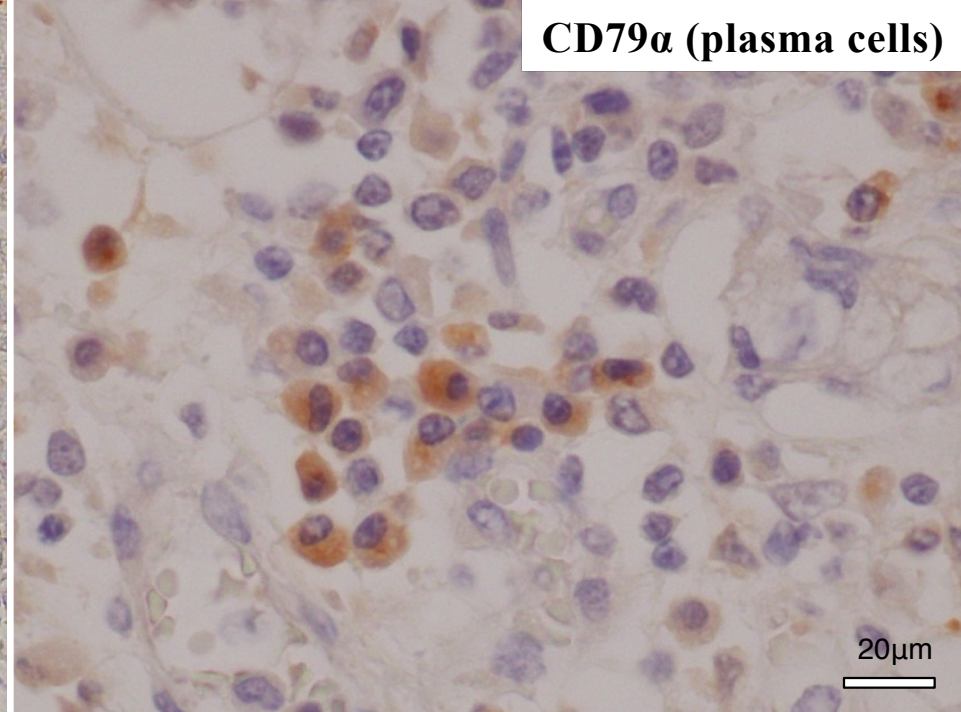
CD20 (B lymphocytes)

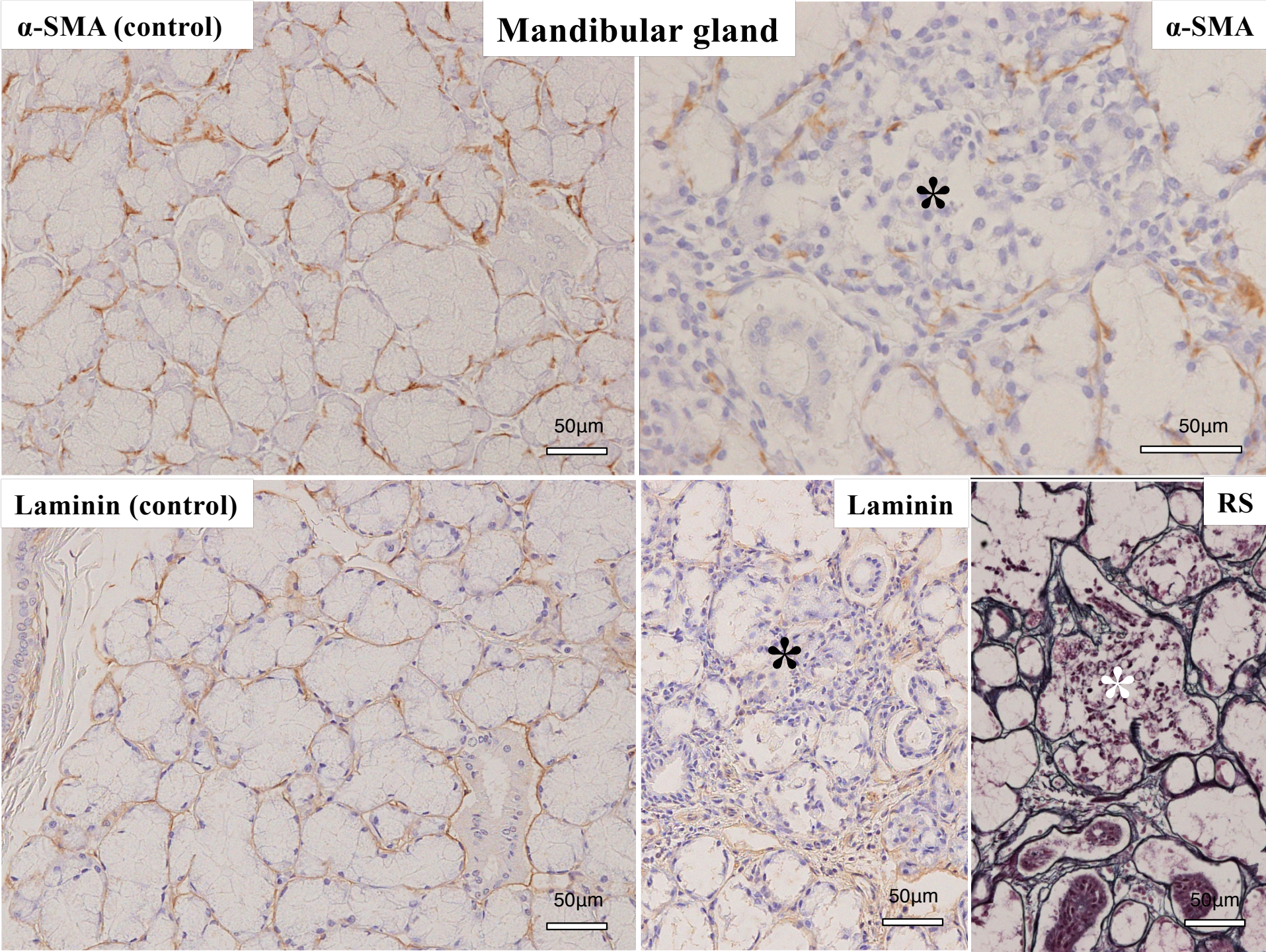


CD20 (B lymphocytes)

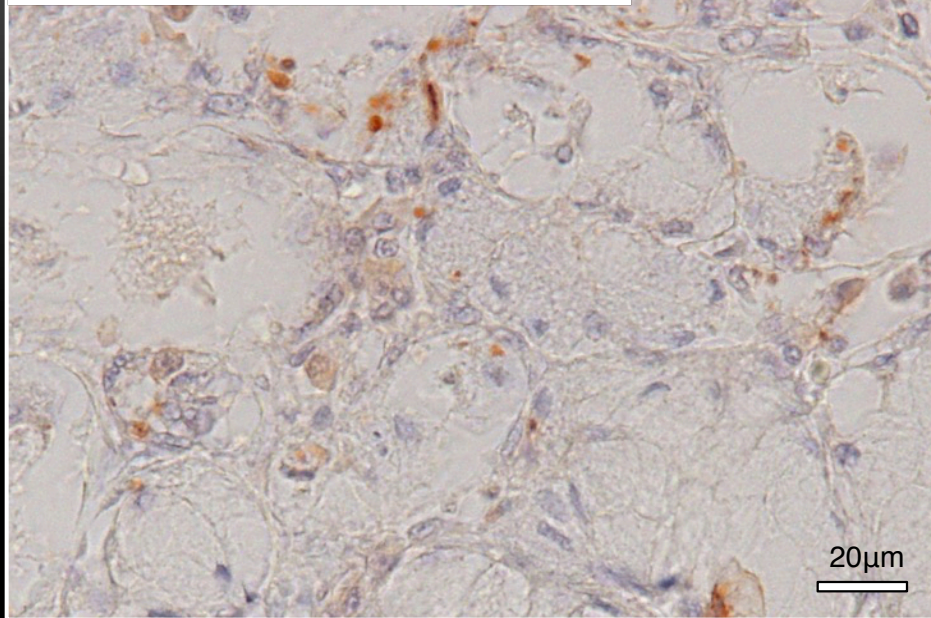


CD79α (plasma cells)

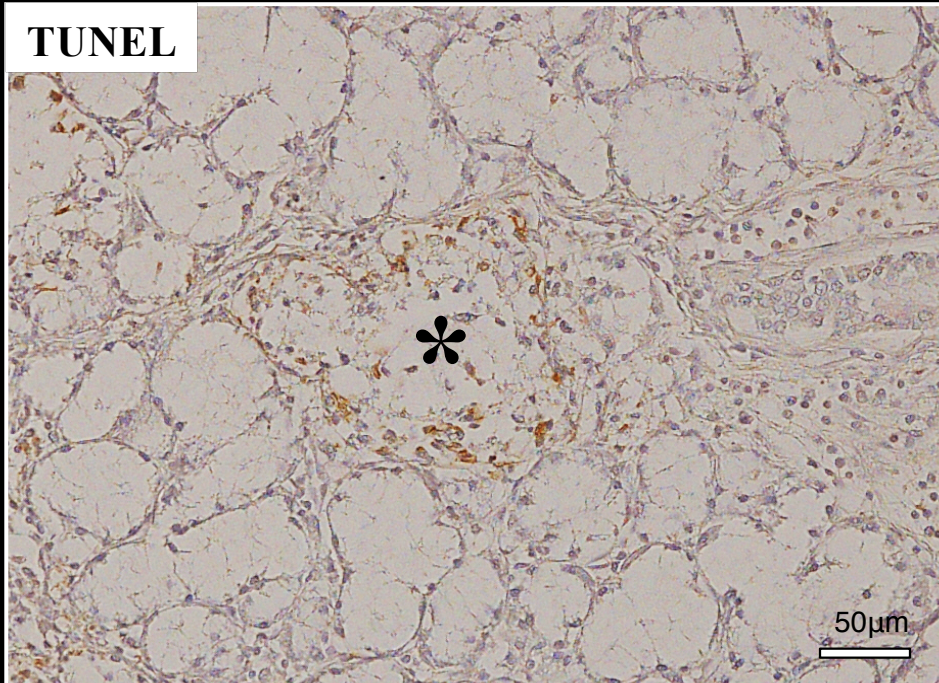




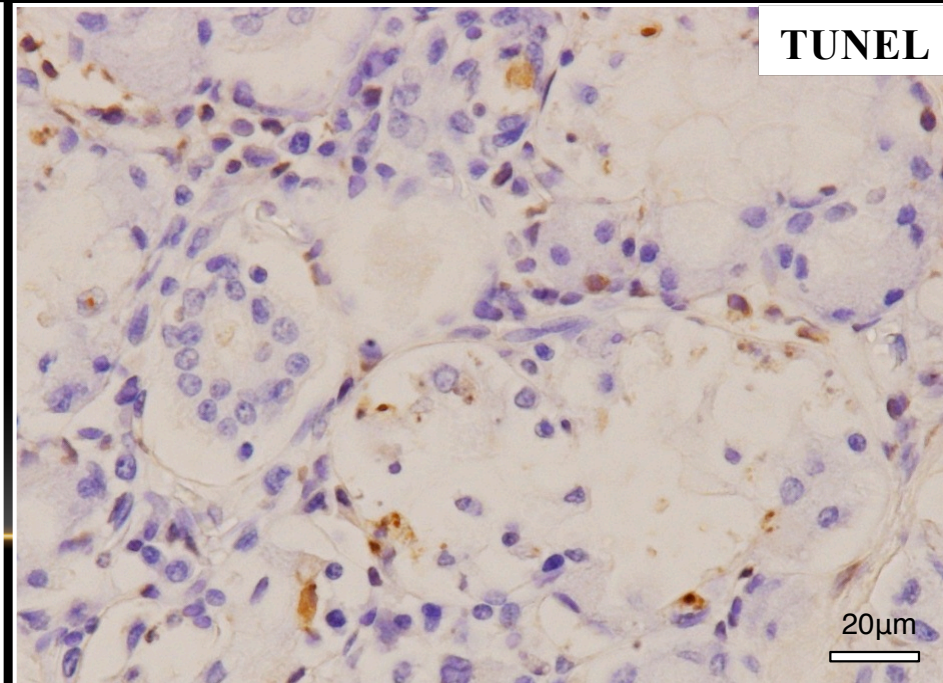
Mandibular gland, Caspase-3



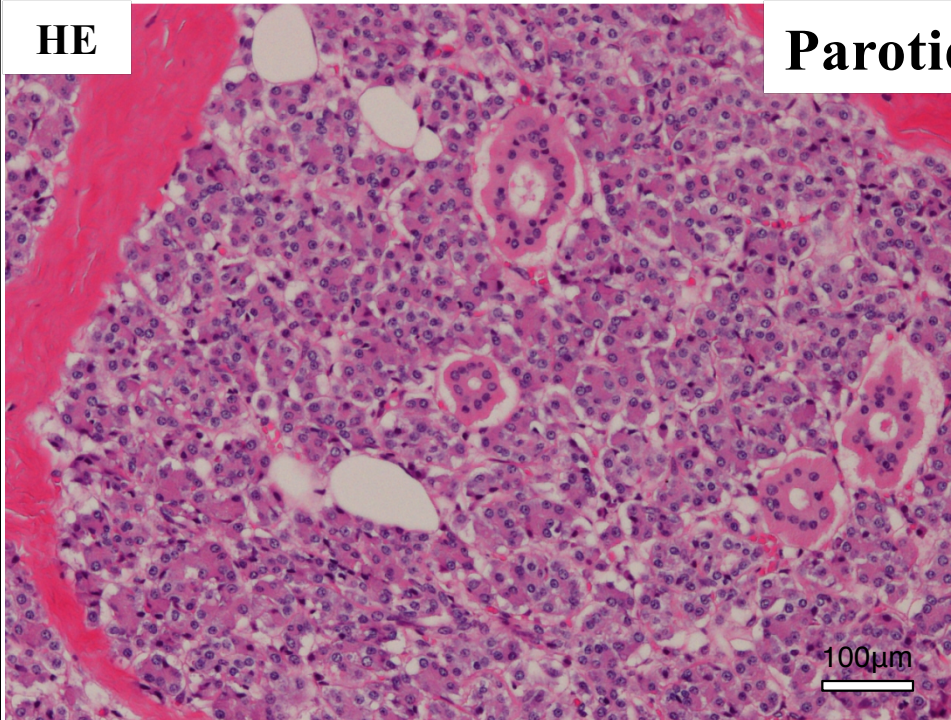
TUNEL



TUNEL

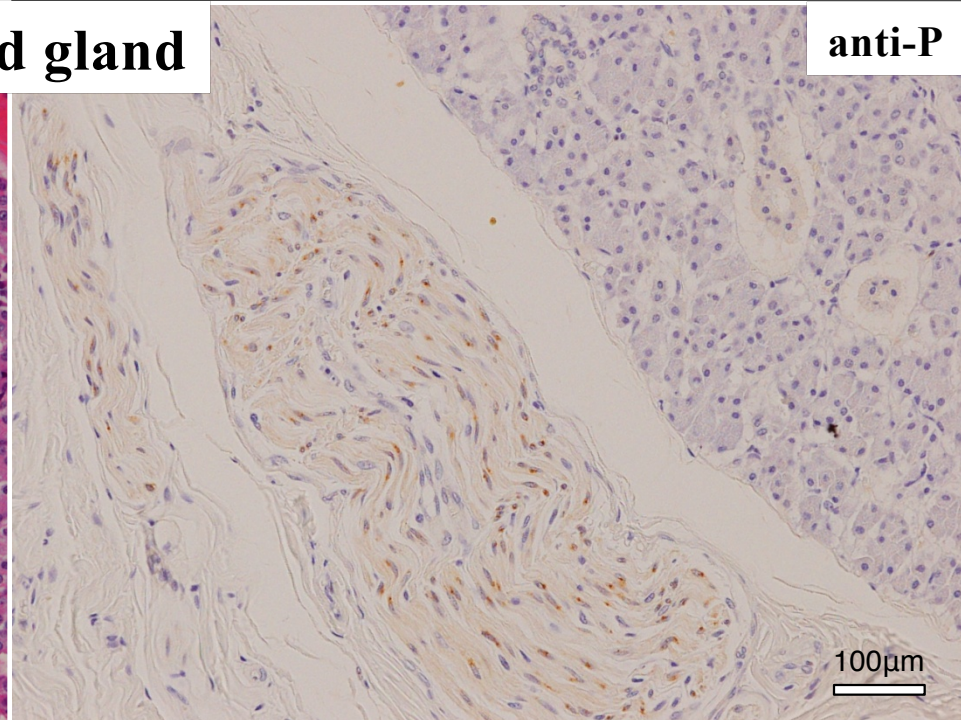


HE

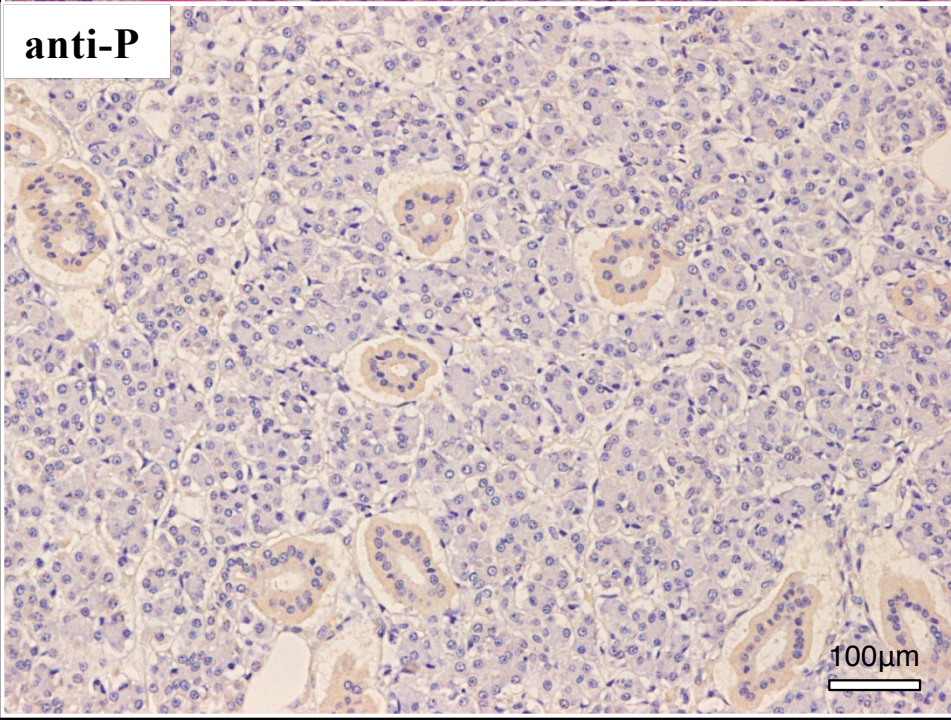


Parotid gland

anti-P



anti-P



TUNEL

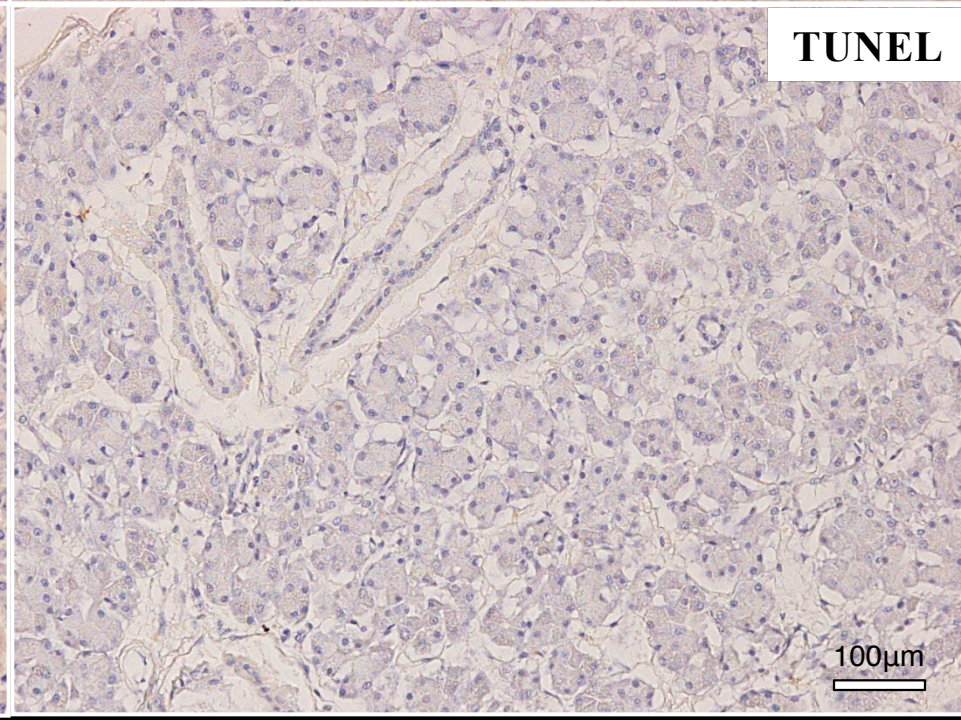


Table 2. Summary of inflammatory features and distribution of viral antigen in the mandibular glands of 22 rabid dogs

No.	Inflammatory cells	Virus antigen distribution			
		Mucous epithelium	Serous epithelium	Ductal units (striated and interlobular ducts)	Interlobular ganglion
1	Moderate	+	-	-	+
2	Mild	+	-	-	+
3	Mild	+	-	-	+
4	Moderate	+	-	-	+
5	Mild	+	-	-	+
6	Mild	+	-	-	+
7	Moderate	+	-	-	+
8	Moderate	+	-	-	+
9	Moderate	+	-	-	+
10	Mild	+	-	-	+
11	None	+	+	-	+
12	Mild	+	-	-	+
13	Moderate	+	-	-	+
14	None	+	-	-	+
15	Mild	+	-	-	+
16	Moderate	+	-	-	+
17	Mild	+	-	-	+
18	Moderate	+	-	-	+
19	Moderate	+	-	-	+
20	Mild	+	-	-	+
21	None	+	-	-	+
22	Moderate	+	-	-	+

+: Positive, -: Negative.

CONCLUSION

- These results confirmed the path through which the rabies virus descends along the facial nerve after proliferation in the brain to reach the ganglion neurons of the mandibular glands.
- Viral proliferation and cytotoxicity could not occur in duct system, ensuring that secretions containing the virus are efficiently excreted into the oral cavity.