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Immunohistochemical investigation of some peptides in the rudd *(Scardinius erythrophthalmus)* stomach and intestinal regions

Nurgül ŞENOL[1*], Nurevşan DENİZ[2]

[1]*Süleyman Demirel University Faculty of Health Sciences, Department of Nutrition and Dietetics, East Campus, Isparta*
[2]*Süleyman Demirel University, Faculty of Science, Department of Biology, East Campus, Isparta*

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Abstract

The current study aimed to determine the localization and density of some peptides found in the digestive tract mucosa of Scardinius erythrophthalmus (Rudd). *In accordance with the immunohistochemical examinations, it was observed that CCK immunopositive cells were found in the parts of the intestine except for the stomach. While gastrin immunoreactive (+) cells were not observed in the stomach and middle intestine region, a small amount of these cells were detected in the anterior intestine. In the posterior intestine, a very low density of gastrin immunopositive cells were detected. It was determined that glucagon immunopositive cells were found in very small amounts in the parts of the intestine except the stomach. While low density of somatostatin immunoreactive cells were observed in the stomach region, these cells were not found in the intestinal regions. In general, it was concluded in this study that the studied peptides were found in different concentrations in different parts of the intestine.*

Keywords: *Rudd, peptide, immunohistochemistry, gastrointestinal tract.*

Kızılkanat *(Scardinius erythrophthalmus)* mide ve bağırsak bölgelerindeki bazı peptitlerin immunohistokimyasal incelenmesi

Öz

Bu çalışmada Scardinius erythrophthalmus (Kızılkanat) sindirim kanalı mukozasında bulunan bazı peptitlerin yerleşim ve yoğunluklarının belirlenmesi amaçlanmıştır.

[*]Nurgül ŞENOL, ngsenol@hotmail.com, https://orcid.org/0000-0002-3090-9835
Nurevşan DENİZ, nrglsenol@gmail.com, https://orcid.org/0000-0003-0516-252X

İmmunohistokimysal incelemeler doğrultusunda CCK immunoreaktif hücrelerin midede bulunmadığı ancak ilk, orta ve son bağırsakta bulunduğu gözlendi. Mide ve orta bağırsakta gastrin immunopozitif hücreler gözlenmezken, ilk bağırsakta bu hücreler tespit edildi. Son bağırsakta gastrin immunoreaktif hücrelere az sayıda rastlandı. Glukagon immunoreaktif hücrelere midede rastlanmazken, bağırsakta az miktarda saptandı. Midede somatostatin immunopozitif hücrelerin az yoğunlukta olduğu, bağırsakta ise bulunmadığı gözlendi. Sonuç olarak peptitlerin bağırsaktaki yoğunluğunun değiştiği belirlendi.

Anahtar kelimeler: *Kızılkanat, peptit, immunohistokimya, gastrointestinal kanal.*

1. Introduction

It has been reported that there are specialized cells to secrete mucus in the lamina epithelialis layer along the digestive tract in teleost species. The density and chemical composition of mucus cells vary according to fish species and digestive tract parts. Mucus content in the lubrication of the organ, that has an important role in the protection against pathogenic microorganisms and proteolytic degeneration, in addition to the physiologically active polypeptide hormones to control the functions of different regions of the digestive tract and epithelial cells for secretion of a gland or endocrine cells between amines settled into a complex system with the ability that created it is stated [1].

Enteroendocrine cells make up about 1% of all epithelial cells in the gastrointestinal tract. Cells were detected in the gastrointestinal tract, secretory granules according to the structure of certain hormonal products and contains at least 15 different cell types, some of them of gastrin, secretin, cholecystokinin (CCK), serotonin, glucagon-like peptide, glucagon-like peptide 2, somatostatin, substance P, vasoactive intestinal peptide, gastric inhibitory polypeptide (GIP), motilin, and pancreatic polypeptide, including the peptide and amine hormones has been reported to secrete various [2]. The secretion of many endocrine cells is effective in the digestive process in fish. The secretions in the gastrointestinal tract are chemicals that regulate the structure and functions of the duct. In the gastrointestinal tract, which is considered the largest endocrine organ, these chemicals are mainly secreted by endocrine cells. Classically, chemical secretions that affect certain organs through the blood are called hormones. Recently, it has been announced that these chemicals have short peptide chains and their amino acid compositions have also been determined. Studies in fish gastrointestinal tracts have identified more than 45 gastrointestinal peptides over the past 15 years [3].

In this study, it is aimed to evaluate the peptides of gastrin, glucagon, somatostatin and cholecystokinin in the digestive tract of rudd immunohistochemically.

2. Material and method

After the clove oil anesthesia of 10 adult rudd *(S. erythrophthalmus)* obtained from Lake Uluabat (Bursa, Turkiye) samples were taken from the stomach and intestines by abdominal dissection. The average length of the researched fish species is 20-25 cm and, their weight is 120-150 g.

Peroxidase anti-peroxidase (PAP) method was applied to identify cells containing CCK, gastrin, glucagon and somatostatin. Sections passed through xylol and alcohols according to this method were kept in PBS for 30 minutes under laboratory conditions to further increase rehydration after washing in water, and were taken into 0.1% trypsin with pH 7.8 at 37°C to increase antigenic labeling. This process was applied between 20-30 minutes and the sections were then kept in normal goat serum (1:100) at room temperature for 1 hour. CCK, gastrin, glucagon and somatostatin antisera were diluted 1/200 in PBS (0.01 M, pH 7.4) and sections were kept in antisera +4 ^{o}C for 12-18 hours, then washed with PBS, respectively, and kept in anti-rabbit IgG for 1 hour at room temperature. PAP (1:400) complex is applied to the sections washed again with PBS for 1 hour at room temperature, -3,3-diaminobenzidine tetrahydrochloride (DAB) (10-20 minutes) is applied for coloring, then the sections are washed with PBS, passed through alcohol and xylol and closed with entellan.

The preparations the PAP method applied were examined under the microscope, and the necessary parts photographed under the Leica DM 500 microscope.

3. Results

3.1.1. CCK immunoreactive cells

It was determined that there were CCK immunopositive cells in the parts of the intestine but these cells were not observed stomach. CCK immunoreactive cells were localized in the lamina epithelialis, lamina propria and submucosa in the intestinal sections. In the intestinal regions, it was determined that the density was higher in the middle, followed by the anterior intestine. It was observed that the density decreased in the posterior intestine. There was no significant difference in terms of localization (Figure 1, 2, 3, 4, 5, 6, 7).

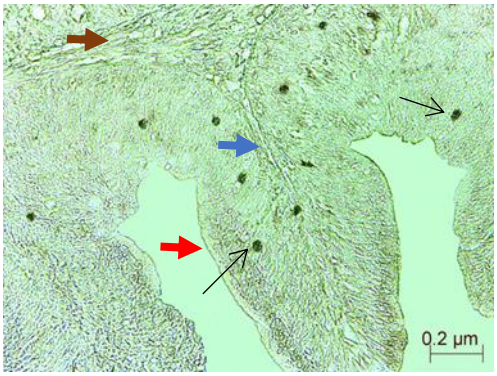

Figure 1. CCK immunopositive cells (➔) in the middle intestine, lamina epithelialis (➔), lamina propria (➔), submucosa (➔).

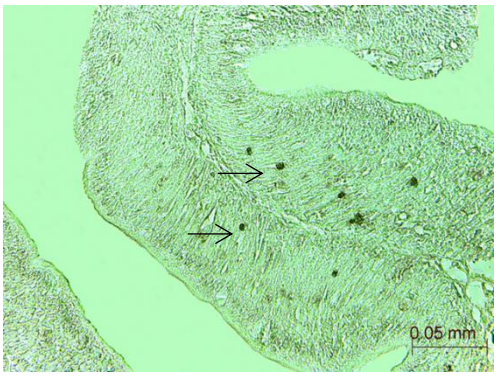

Figure 2. CCK immunopositive cells (arrows) in the middle intestine.

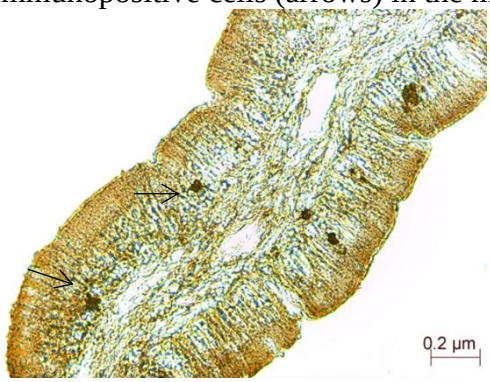

Figure 3. CCK immunopositive cells (arrows) in the anterior intestine.

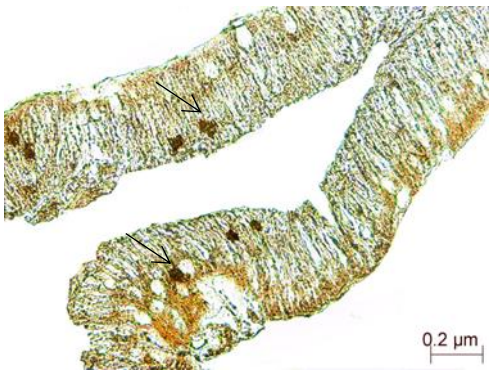

Figure 4. CCK immunopositive cells (arrows) in the anterior intestine.

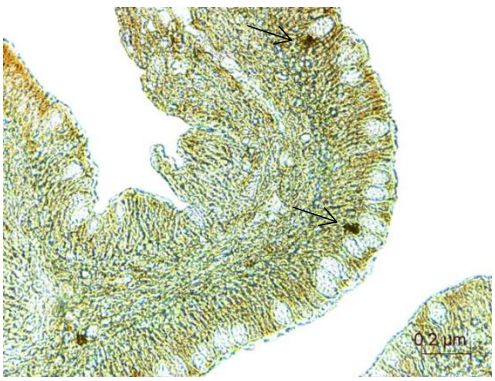

Figure 5. CCK immunopositive cells (arrows) in the posterior intestine.

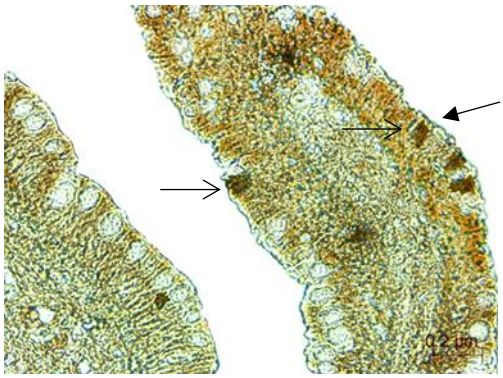

Figure 6. CCK immunopositive cells (arrows) in the posterior intestine.

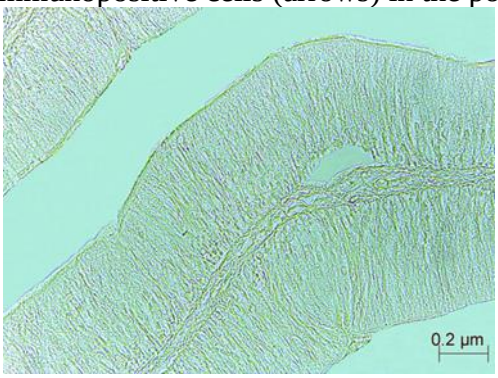

Figure 7. CCK immunonegative cells in the stomach.

3.1.2. Gastrin immunoreactive cells

Gastrin immunoreactive (+) cells were not observed in the stomach and middle intestine, while a small amount of these cells were observed anterior intestine. In the posterior intestine, very low concentration of gastrin immunopositive cells were detected (Figure 8, 9, 10, 11).

Figure 8. Gastrin immunopositive cells (arrows) in the anterior intestine.

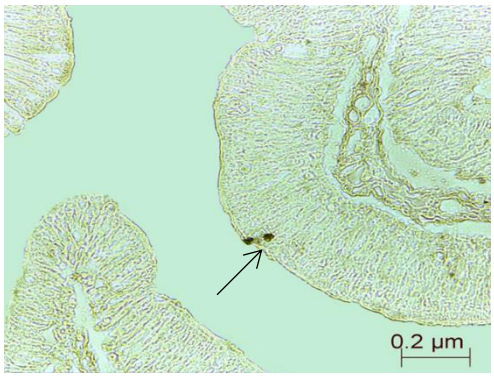

Figure 9. Gastrin immunopositive cell (arrow) in the posterior intestine.

Figure 10. Gastrin immunonegative cells (-) in the posterior intestine.

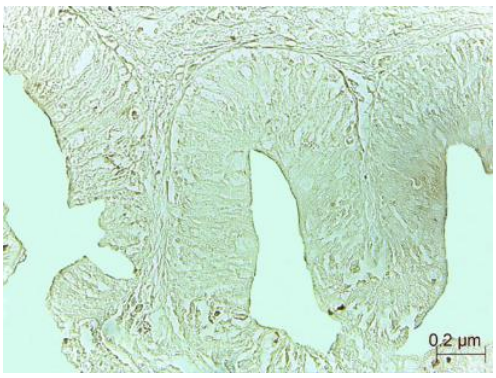

Figure 11. Gastrin immunoreactive (-) cells in the stomach.

3.1.3. Glucagon immunoreactive cells

It was determined that glucagon immunopositive cells were found in very small amounts in the parts of the intestine except the stomach. It was determined that especially the intestinal regions are located in the lamina propria (Figure 12, 13, 14, 15).

Figure 12. Glucagon immunopositive cell (arrow) cell in the anterior intestine.

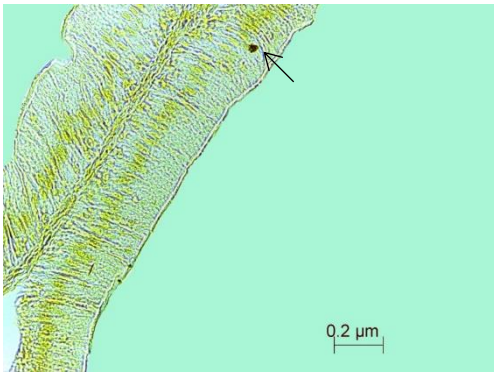

Figure 13. Glucagon immunopositive cell (arrow) cell in the middle intestine.

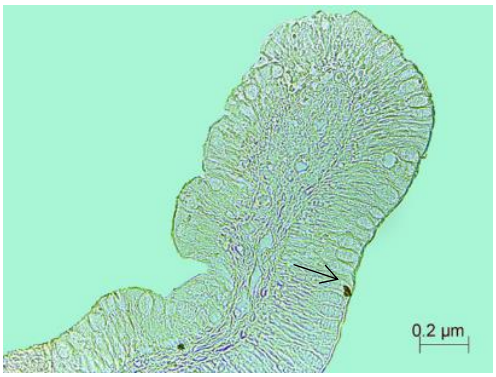

Figure 14. Glucagon immunopositive cell (arrow) in the posterior intestine.

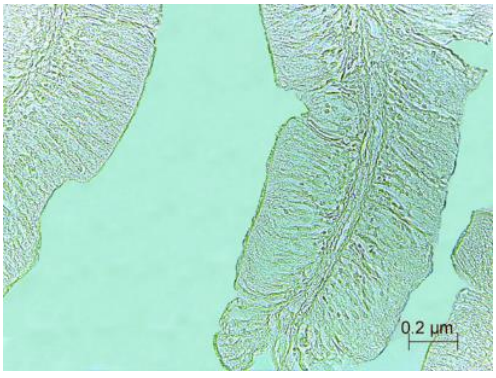

Figure 15. Glucagon immunonegative (-) cells in the stomach.

3.1.4. Somatostatin immunoreactive cells

It was observed that somatostatin immunoreactive cells were found in low density in the stomach. These cells were not found in the intestinal regions. It was determined that somatostatin immune positive cells were mostly localized in the lamina epithelialis in the stomach (Figure 16, 17, 18, 19).

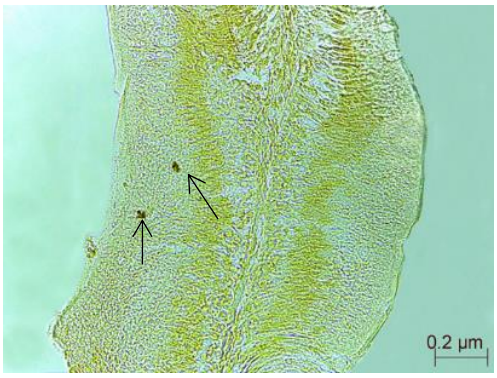

Figure 16. Somatostatin immunopositive (+) cells (arrows) in the stomach.

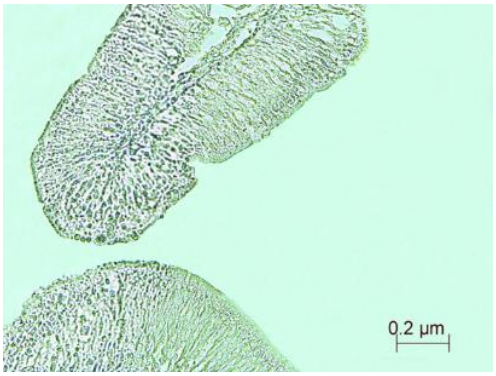

Figure 17. Somatostatin immunonegative (-) cells in the anterior intestine.

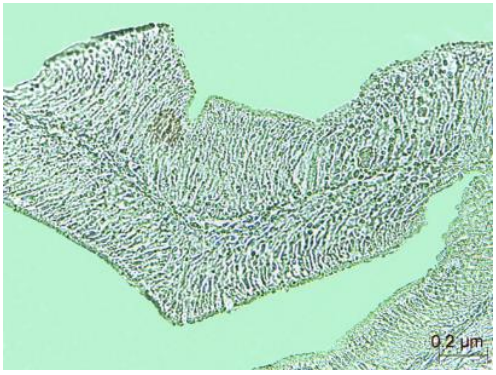

Figure 18. Somatostatin immunonegative (-) cells in the middle intestine.

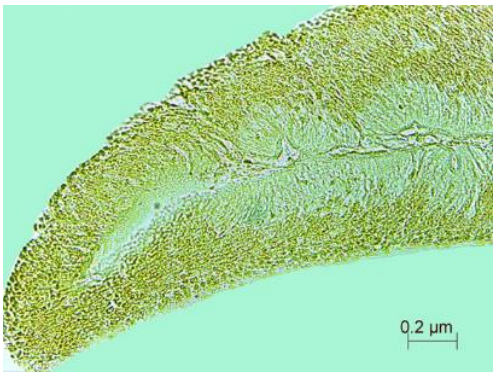

Figure 19. Somatostatin immunonegative (-) cells in the posterior intestine.

4. Discussion

The digestive system in fish consists of glands such as the pancreas and liver, which aid digestion with their secretions, and the alimentary canal. In fish, the digestive tract consists of the mouth, pharynx, oesophagus, stomach, pyloric caeca, intestines, and anus, while some fish species do not have a true stomach [4].

It was determined that ghrelin and its main receptor growth hormone secretagogue 1a, CCK, peptide YY and glucagon peptide-1 immunopositive cells were localized in the intestinal mucosa of goldfish [5]. In this study, it was determined that CCK and glucagon immunoreactive cells were found intestine but these cells were not observed the stomach.

Cardosoa et al. [6] stated that serotonin, somatostatin and gastrin immunoreactive cells are found in different densities throughout the gastrointestinal tract of *Astyanax bimaculatus* species. While somatostatin immunoreactive cells are located in the glandular and non-glandular regions of the stomach. It was determined that gastrin immunoreactive cells were located only in the glandular region of the stomach. Similarly, in this study, somatostatin cells were observed to be low in density only in the stomach, while gastrin cells were observed to be less dense in the first and last intestinal regions, differently.

In the immunohistochemical study on Korean aucha sea bass, glucagon immunoreactive cells have been observed in the small intestine and are present in low concentrations. They were scattered and oval in the epithelial mucosa. Cholecystokinin-8 (CCK-8) immunoreactive cells are localized in the small intestine and randomly distributed in the epithelial mucosa. It has been determined that somatostatin immunoreactive cells are located both in the stomach and along the gastrointestinal tract, but are more concentrated in the stomach. [7]. In this study, however, somatostatin immunoreactive cells were detected only in the stomach. On the contrary, immunoreactive cells of glucagon and CCK were detected in different densities in all parts of the intestine except the stomach region.

The presence and distribution of glucagon immunoreactive and enteroendocrine cells in *S. trutta* have been studied throughout the gastrointestinal tract in both control and infected fish species, with small amounts of glucagon-positive cells in the stomach lamina epithelialis and lumen of gastric glands. Moderate amounts of glucagon enteroendocrine cells were observed to be localized in the lamina epithelialis in the pyloric cecum and intestine [8]. In this this study, it was determined that glucagon immunopositive cells

were found in very small amounts in the parts of the intestine except the stomach. It was also determined that especially the intestinal regions are located in the lamina propria.

As a result of the immunohistochemical study performed to examine the distribution and density of endocrine cells in the gastrointestinal tract of the zander *(Stizostedion lucioperca)*, it was determined that somatostatin-14 and bombesin immunoreactive cells were found in moderate density in the epithelium of the pylorus and Trk-B, histamine and neurotensin immunoreactive cells were absent. As for the density in the glands of the pylorus, it was determined that calcitonin gene related peptide (CGRP) and somatostatin-14 immunoreactive cells are involved [9]. Similarly, in this study, it was detected that somatostatin immunopositive cells in the stomach were predominantly localized in the lamina epithelialis.

Chena et al. [10] investigated the regional distributions of somatostatin immunoreactive cells in the stomach and small intestine sections using immunohistochemical techniques in their study. The results reported that somatostatin immunoreactive cells were found in the proventriculus, duodenum, jejunum, and ileum regions, but there was no immunoreactivity in the stomach. In this study, unlike the rudd (*Scardinius erythrophthalmus*) species, somatostatin immunoreactive cells were observed to be found in less density in the stomach. These cells were not found in the intestinal regions.

It has been reported that in *Salminus brasiliensis* detects gastrin, CCK-8, neuropeptide-Y peptides were detected in different cell types in the digestive tract, with a similar distribution as in other carnivore species. It is understood that the differences in immunoreactive cell location and density between species are generally related to the nutritional habits of the species and the anatomical and morphological features of the digestive system [11].

Dry et al. [12] demonstrated that, glucagon immunoreactive cells in *Garra rufa* were found to be few number in the expanded area after the oesophagus but increased number from the hindgut to the foregut lumen. It was determined that gastrin immunoreactive cells were found in moderate density from the oesophagus and anterior intestine, and more intensely localized in the middle and hind intestines. Somatostatin immunoreactive cells were found in high density in the oesophagus and anterior intestine, while CCK-8 positive cells were found throughout the gastrointestinal tract, but the highest density was found in the anterior intestine. In the study, CCK immunoreactive cells were detected in high density in the intestinal sections, while somatostatin immunoreactive cells were not detected in the intestinal regions.

In the study of *Alburnus tarichi* (Pearl mullet), it was observed that glucagon immunoreactive cells were rarely found in the exocrine parenchyma of the pancreas, and somatostatin immunoreactive cells were rarely localized between the epithelium and connective tissue of the exocrine ducts. It has been reported that the regional distribution, volume and density of glucagon and somatostatin immunoreactive cells in *A. tarichi* are similar to those of other teleost fish species [13]. In this study, while somatostatin immunoreactive cells were detected only in the stomach, glucagon immunoreactive cells were detected in low density in the intestinal sections except the stomach.

In the immunohistochemical study of *Chanos chanos* [14], milkfish species, it was reported that CCK immunoreactive cells were detected in the foregut and continued

towards the rectum, similar to this study. No CCK immunoreactive cells were found in the oesophagus and stomach. It has been reported that gastrin immunoreactive cells are concentrated in the foregut but decreased in the midgut, and gastrin immunoreactive cells were not detected in other digestive tract regions. In the stomach of milkfish, somatostatin immunoreactive cells are very densely distributed in the epithelium of the mucosal folds. The absence of immunoreactive cells was observed in the oesophagus and intestinal regions.

As a result, in this study, it was determined that CCK immunopositive cells were found in parts of the intestine except for the stomach. It was observed that the density in the intestinal regions was higher in the middle intestine, followed by the anterior intestine. It was determined that the density decreased in the posterior intestine. Gastrin immunoreactive (+) cells were not found in the stomach and middle intestine, while a small amount of these cells were observed in the anterior intestine. In the posterior intestine, very low concentration of gastrin immunopositive cells were detected. It was determined that glucagon immunopositive cells were found in very small amounts in the parts of the intestine except the stomach. It was observed that somatostatin immunoreactive cells were found in low density in the stomach. These cells were not found in the intestinal regions. In general, it was determined that the studied peptides were localized in different intestinal regions with different densities. However, some characteristic differences are observed in this species, which may be due to differences in the antisera tested, the methods use and/or the species investigated in the various studies.

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