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Features in the topography and morphology of the thyroid glands in chickens, turkeys, and ducks

P. Yonkova[1*], P. Stoyanova[2], D. Kanakov[3] and E. Vachkova[4]

[1] *Trakia University, Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, 6015 Stara Zagora, Bulgaria, orcid: 0000-0002-4396-7444*
[2] *Bulgarian Food Safety Agency, 15 Pencho Slaveykov Blvd., 1606 Sofia, Bulgaria, orcid: 0000-0001-7662-7350*
[3] *Trakia University, Department of Internal Diseases, Faculty of Veterinary Medicine, 6015 Stara Zagora, Bulgaria, orcid: 0000-0002-0506-4439*
[4] *Trakia University, Department of Pharmacology, Physiology of the Animals and Physiological Chemistry, Faculty of Veterinary Medicine, 6015 Stara Zagora, Bulgaria, orcid: 0000-0002-9291-332X*
[*] *Corresponding author:* pjon@abv.bg

Abstract

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The goal of this study was to establish some topographical and morphological peculiarities of the thyroid glands in chickens, turkeys, and ducks. On this basis, we offer practically easy access to reveal and sampling the thyroids in each of the three species of birds.

Thyroid glands of 12 broiler chickens, 12 North Caucasian turkeys, and 12 Mulard ducks were examined *in situ* after slaughter. Entire glands from 6 birds of each avian species were extirpated, fixed in formalin, and processed by standard procedures to obtain histological sections. The latter were stained with Alcian-Blue – PAS kit.

In broiler chickens and turkeys, observation and sampling of the thyroid glands were performed through cranial or ventral access. The cranial one was carried out by strongly pulling the neck in the cranial direction. The thyroids were discovered at the level of thoracic inlet in close proximity to the last thymus lobes. The right thyroid gland in chickens and turkeys formed a common glandular cluster with the right parathyroid glands. The left thyroid was entirely separated from the corresponding parathyroid glands.

Due to the significantly more caudal position of the thyroids in ducks, they were found only after the ventral opening of the thorax at the level of the tracheal bifurcation. Direct contact with the thymus and the parathyroid glands was not observed.

The thyroid colloid reacted positively after Alcian-Blue – PAS staining, and contrasted strongly against the background of the adjacent anatomical structures. In broilers and turkeys one or more thymus penetrations in the thyroid parenchyma were found. They were located subcapsularly or scattered between the thyroid follicles. Similar thymic penetrations were not detected in ducks.

The established features in the topography and morphology of the thyroid glands in chickens, turkeys, and ducks could be successfully used by veterinarians in slaughterhouses during the sampling for antithyroid agents.

Keywords: Thyroids, topography, morphology, chickens, turkeys, ducks

Introduction

The thyroid glands in birds are paired anatomical structures, between which no isthmus is observed, as in most mammals. The glands are located adjacent to the thoracic inlet, caudally to the crop, and in close proximity to the common carotid artery, jugular vein, and vagus nerve. The right thyroid gland occupies a more caudal position than the left one (Dyce et al., 1987; Baumel, 1993; Schmidt and Reavill, 2008; Onuk and Kabak, 2012; McNabb and Darras, 2015). Each thyroid is characterized by an oval shape, reddish-brown color, and average dimensions of 10 mm in length and 2 mm in width (Venzke, 1975; Dyce et al., 1987; Gadzhev, 1997; Yonkova et al., 2017; Yonkova et al., 2018; Yonkova, 2021).

Normality in the functioning of the thyroid glands in birds is a guarantee of good health status, and hence the obtaining of quality poultry products. Sometimes in practice, farmers resort to the application of thyrostatics in feed to accelerate the fattening process of animals and birds, but their meat has toxic, carcinogenic, and teratogenic properties for humans (Rosebrough et al., 2002; Serratosa et al., 2006; Kim, 2010; Boison, 2012; Manna et al., 2013).

In accordance with the requirements of European legislation, the thyroid glands obtained from birds after slaughter are used as a matrix for detecting the presence of antithyroid agents in poultry meat (European Union, 2017; Serratosa et al., 2006; Woźniak et al., 2018).

Any observed deviation from the shape, size, and mass of the thyroid glands in birds at slaughter raises suspicion about the administration of thyrostatics. In these cases, the histological analysis shows hyperplasia and hypertrophy of thyrocytes along with decreased or absent colloid in the follicles (Danylyk et al., 2010).

Based on the regional anatomy of the cervical and cranial thoracic region, as well as specific features of thyroid morphology in chickens, turkeys, and ducks, this study **aimed** to describe practically easy access to the thyroids in each of the three species of birds.

Materials and Methods

Bird cadavers

A total of 36 cadavers of three different bird species were dissected as follows: 12 broiler chickens at 50 days of age with an average live weight of 2.1 kg ± 0.10; 12 turkeys of the North Caucasian Bronze breed at 120 days with an average live weight of 6.0 kg ± 0.30, and 12 Mulard ducks at 90 days with an average live weight of 6 kg ± 0.20.

The birds were raised in strict compliance with each species's specific hygiene and nutrition standards and at the time of slaughter were defined as clinically healthy.

The slaughter material was provided by private licensed poultry slaughterhouses in Bulgaria as follows: broiler chickens from "Zornitsa – Commerce" Ltd., Kesarevo village, region Veliko Tarnovo; turkeys from "Eurotop", Stara Zagora, and ducks from "Premium Food Bulgaria" Ltd., Yoglav village, region Lovech.

All husbandry and experimental procedures were approved by The Animal Ethics Committee, Trakia University, Stara Zagora, Bulgaria (Approval to Project 10/2017).

In situ examination

After the slaughter, bleeding, and cleaning of the feathers, the bird carcasses designated for examination were separated from the assembly line and placed on working tables for dissection. In broiler chickens and turkeys, observation and sampling of the thyroid glands were performed through cranial or ventral access. The cranial approach was carried out after thorough cleaning of adipose tissue around the thoracic entrance and strong cranial extension of the neck. For the ventral approach, the celomic cavity was opened, and the coracoids and clavicles were released by cutting their ligaments at the shoulder joint. The sternum was lifted in the cranial direction and removed.

Thyroid glands in ducks were examined only after the ventral opening of the thorax by longitudinal incision of the sternum and pectoral muscles.

Dissections of the birds continued with removing the walls of the interclavicular air sac. The topographic position of the thyroids and their anatomical relationships with the thymus, parathyroid glands, trachea, esophagus, crop, large blood vessels, and nerves were studied *in situ*.

Thyroid sampling and Alcian-Blue – PAS staining

Thyroid samples for microscopy were obtained from 6 birds of each species (18 in total). Entire thyroid glands were fixed in a 10% aqueous solution of neutral buffered formalin (Merck KGaA, Darmstadt, Germany) for 72 hours. This was followed by washing in running water, dehydration in the ascending ethanol series, clarification in xylene, and embedding in paraffin.

With a rotor microtome Leica RM 2235 (Germany), serial histological sections with a thickness of 3-5 µm were obtained and were stained by Alcian-Blue, pH 2.5 (Cat. No. 1.01647.0500), and PAS staining kit (Cat. No. 101646) (Merck KGaA, 64271 Darmstadt, Germany). All staining procedures were performed according to the recommended product protocols (https://www.merckmillipore.com/INTL/en/product/Alcian-blue-solution-pH-2.5,MDA_CHEM-101647#anchor_PI).

The microscopic observations were performed with a Leica DM1000 LED light microscope (Switzerland), equipped with the Leica Application Suite software platform (LAS, Version 4.8.0. [Build: 154], 2003-2015, Leica Microsystems CMS GmBH).

Results and Discussion

In this study fresh carcasses of broiler chickens, North Caucasian bronze turkeys, and Mullard ducks were used. Our comparative analysis was based on the fact that these three categories of birds had reached the required slaughter age and mass at which they are used for consumption. Morphological studies were performed in parallel with sampling for antithyroid agents, which assisted the work of local veterinarians.

From the dissections and macroscopic observations, it was found that the topography and morphology of the thyroid glands in broiler chickens, turkeys, and ducks somewhat coincided with those described in different species of poultry, wild and ornamental birds (Venzke, 1975; Dyce et al., 1987; Gadzhev, 1997; Breit et al., 1998; Schmidt and Reavill, 2008; Onuk and Kabak, 2012; McNabb and Darras, 2015; König et al., 2016). In the descriptions of avian thyroids given by the aforementioned authors, lack information about the sequence until reaching the glands. We suggest that the thoracic inlet be used as the first anatomic landmark when applying cranial access to the thyroid glands in broilers and turkeys. Detection of the glands using this access was possible after a strong cranial drawing of the neck together with the trachea, esophagus, thymic chains, vessels, and nerves attached to it. From this point of view, the thyroid glands were observed at the level of thoracic inlet, medially to the corresponding jugular vein, dorsolaterally to the intact sternotracheal muscles, and in close proximity with the last thymic lobes (Figure 1A).

In the turkeys, the thoracic inlet was occupied by a well-developed fat depot. That is why the cranial approach was performed after the complete removal of adipose tissue (Figure 1B).

The cranial approach was easier to apply and saved time when sampling in slaughterhouses, as it was not necessary to make any incisions in the thorax. This approach was suitable for use in chickens and turkeys, but not applicable to ducks.

For more details on thyroid syntopy in chickens and turkeys, the ventral approach was used (Figure 2). This access was most suitable for observing and sampling of ducks' thyroids due to their significantly more caudal position. After the ventral opening of the thoracoabdominal cavity in the three avian species, both thyroids were found under the interclavicular air sac. In chickens and turkeys, the walls of the air sac were transparent and very fine, and their stuffing was easy. In ducks, the walls of the interclavicular air sac were observed as thick and robust membranes. Pre-cleaning of the air sac and the adipose tissue in the region facilitated the macroscopic observation of the thyroids in the birds studied.

Most of the literature's schemes and descriptions of thyroid topography are given in terms of ventral access. Gadzhev (1997), Venzke (1975), Dyce et al. (1987), Bahadir et al. (1992), Breit et al. (1998), Onuk and Kabak (2012) have used the angle between the corresponding subclavian artery and the common carotid artery as a topographic reference point for the detection of the thyroid gland. However, the current study has shown that both glands in chickens, turkeys, and ducks were located cranially from this vascular angle, in close relation with the medial surface of the respective brachial plexus and jugular vein, and laterally to the corresponding carotid artery. That is why we propose medial surface of the respective brachial plexus as an additional landmark for easy detection of thyroids in the three avian species.

The right thyroid gland in broiler chickens and North Caucasian Bronze turkeys were more caudally located than the left. Its cranial pole was lying adjacent to the last or second last lobe of the thymus chain. The right parathyroid glands (III and IV) were found tightly attached to each other and to the caudal pole of the right thyroid gland, forming a common glandular cluster. In the contact zone, the right caudal thyroid vein was easily detected. It showed a horizontal course toward the right jugular vein and served as

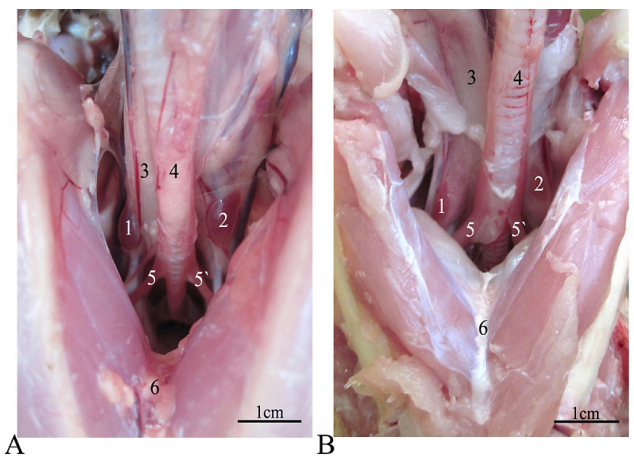

Fig. 1. Cranial view of the thoracic inlet in broiler chicken (*A*) and North Caucasian Bronze turkey (*B*).
1 – *glandula thyroidea dextra*; **2** – *glandula thyroidea sinistra*; **3** – *esophagus*; **4** - *trachea*; **5, 5′** – *m. sternotrachealis dexter et sinister*; **6** – *crista sterni*.

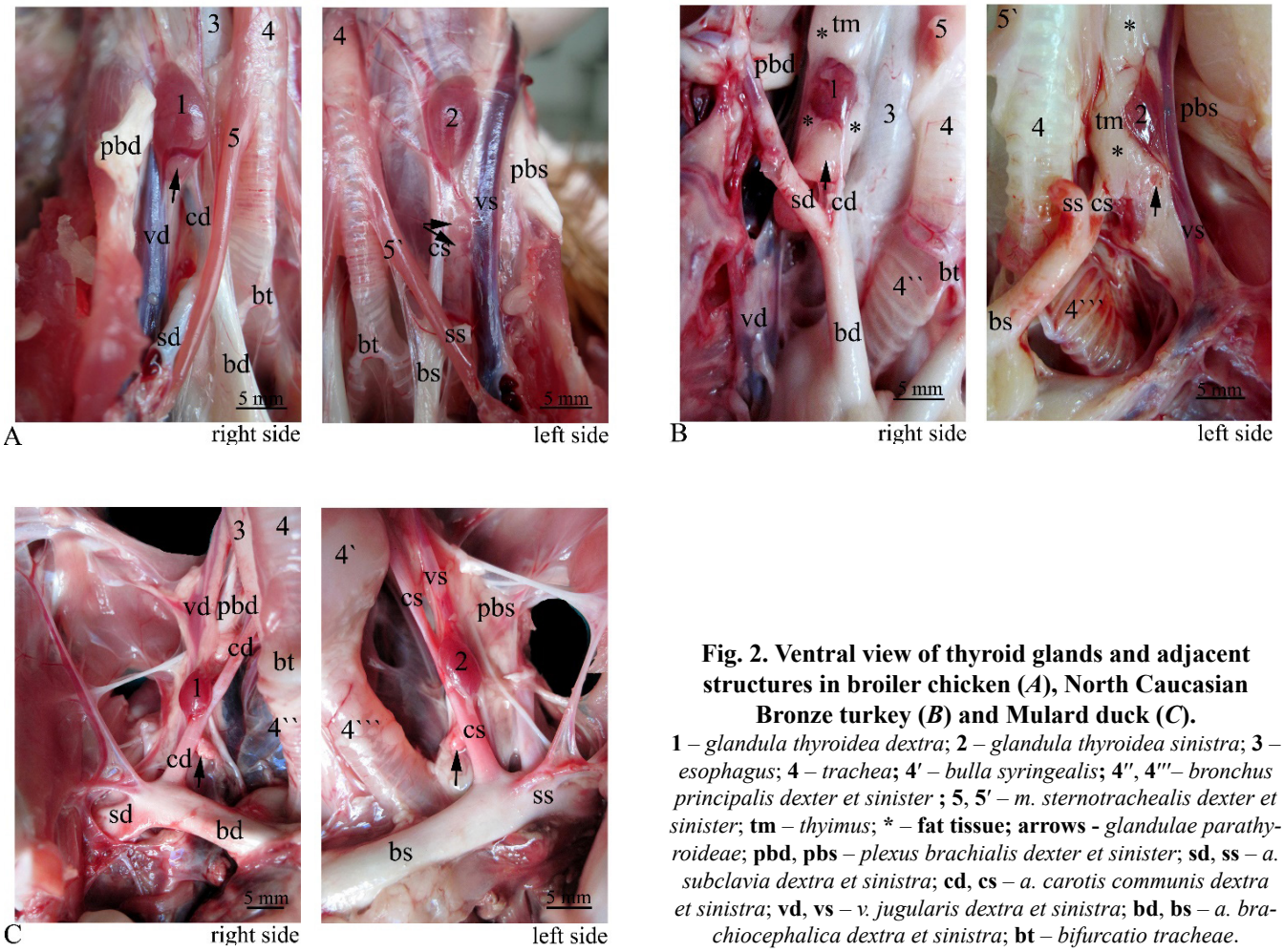

Fig. 2. Ventral view of thyroid glands and adjacent structures in broiler chicken (*A*), North Caucasian Bronze turkey (*B*) and Mulard duck (*C*).

1 – *glandula thyroidea dextra*; **2** – *glandula thyroidea sinistra*; **3** – *esophagus*; **4** – *trachea*; **4′** – *bulla syringealis*; **4″**, **4‴**– *bronchus principalis dexter et sinister* ; **5**, **5′** – *m. sternotrachealis dexter et sinister*; **tm** – *thyimus*; ***** – **fat tissue; arrows -** *glandulae parathyroideae*; **pbd**, **pbs** – *plexus brachialis dexter et sinister*; **sd**, **ss** – *a. subclavia dextra et sinistra*; **cd**, **cs** – *a. carotis communis dextra et sinistra*; **vd**, **vs** – *v. jugularis dextra et sinistra*; **bd**, **bs** – *a. brachiocephalica dextra et sinistra*; **bt** – *bifurcatio tracheae*.

an anatomo-topographical landmark between thyroid and parathyroids (Figure 2A, B). The more caudal position occupied by the right thyroid gland in chickens and turkeys was the reason why it was released together with the liver during the mechanical removal of internal organs by vacuum in slaughterhouses. On the other hand, due to the formation of common cluster between the right thyroid and parathyroid glands, dissection during sampling was more difficult and time-consuming.

The left thyroid in chickens and turkeys was located 5-8 mm, cranially, relative to the right gland. The left gland was also in close contact with the thymus but completely separated from the corresponding parathyroid glands. Around the middle of the distance between them, the oblique course of the left caudal thyroid vein was established (Figure 2A, B). The lack of a formed cluster on the left side and the more cranial position of the left thyroid gland can be used to facilitate the preparation of samples for antithyroid agents. Similar asymmetry in glandular antimers has been found in parrots, common buzzard, rough-legged buzzard, western marsh harrier, sparrow hawk, and common kestrel (Radek & Piasecki, 2004; Radek & Piasecki, 2007).

In Mulard ducks, the two glands were positioned caudally to the thoracic inlet. As anatomical landmarks for discovering of thyroids were used tracheal bifurcation and syringeal bulla. The right thyroid gland was revealed after dorsal displacement or incision of the right sternotracheal muscle. The left thyroid was found in the angle between the left sternotracheal muscle and the syringeal bulla. Bilaterally, contact between the thyroid glands and thymus was not observed. Each parathyroid complex was situated 2-3 mm from the caudal poles of the respective thyroid gland, so the formation of the thyroid-parathyroid complexes also was not found (Figure 2C). The lack of formed glandular

clusters in ducks made sampling for antithyroid agents easier.

The thyroid glands in all studied birds differed from the surrounding structures in their oval or ovoid shape and reddish-brown to dark brown color, which was consistent with that described in many other bird species (Venzke, 1975; Dyce et al., 1987; Gadzhev, 1997; Breit et al., 1998; Schmidt and Reavill, 2008; Onuk and Kabak, 2012; McNabb and Darras, 2015; König et al., 2016).

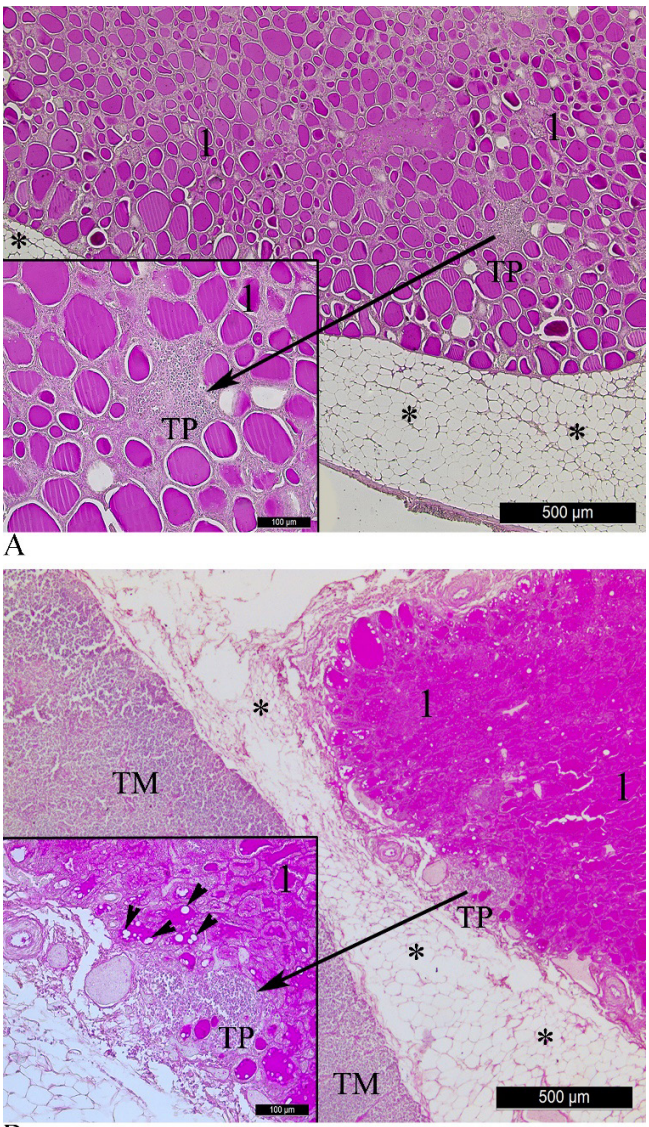

Fig. 3. Microscopic morphology of the thyroid gland in areas with thymic penetration in broiler chicken (*A*) and North Caucasian Bronze turkey (*B*). 1 – thyroid parenchyma; arrowheads – vacuoles in the colloid; TM – thymus; TP – thymic penetration; * – fat tissue. Alcian-Blue – PAS Stain. Optical magnification ×50 and ×200

Thyroid parenchyma in studied birds comprised glandular follicles with very different cross-sectional sizes. They were tightly attached to each other with oval or polygonal shape. Specific microstructural features in turkeys were numerous vacuoles in the colloid, most often located around the apical part of the epithelial cells. When stained with Alcian-Blue – PAS kit, the thyroid colloid reacted positively. Its intensely purple-red color provided a strong contrast between the thyroid gland, connective tissue, and dark blue lymphocytes in the thymus (Figure 3).

In the zones of contact between the thyroid gland and the thymus (bilaterally) and between the thyroid and parathyroid glands (right only) in chickens and turkeys, the relationship between the connective tissue capsules of adjacent glands was observed. In these areas, there were many blood vessels cut at different angles. The white adipose tissue, which filled the space between all above-mentioned structures was presented as single lobules or as a complete adipose layer of varying thickness. A very specific microstructural feature in these two bird species was the presence of one or more thymus penetrations in the thyroid parenchyma. Islets of thymus tissue were found bilaterally in glands. These formations were located subcapsularly or scattered between the thyroid follicles (Figure 3 A. B). The glandular conglomerates, as well as the thymus penetrations found in broiler chickens and turkeys, were in line with those described by Le Douarin et al. (1984), Graham et al. (2005), Grevellec and Tucker (2010), Gordon and Manley (2011) and Pandya (2020). This postnatal morphological feature expressed the common embryonic origin of the glands and has given the authors reason to talk about thymus-thyroid and thyroid-parathyroid complexes.

The absence of such conglomerates and thymic penetrations in ducks is a clear morphological sign of the different expression of regulatory genes, controlling the final position of pharyngeal derivatives in different animal species and even in individuals (Le Douarin et al., 1984; Graham et al., 2005; Grevellec and Tucker, 2010; Gordon and Manley, 2011; Pandya, 2020).

Conclusions

The established features in the topography and morphology of the thyroid gland in chickens, turkeys, and ducks could complement the anatomical literature, as well as be used for the needs of veterinary practice and food control during sampling for antithyroid agents.

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