Scientific Research Publishing

Open Journal of Veterinary Medicine, 2026, 16(8), 142-160
https://www.scirp.org/journal/ojvm
ISSN Online: 2165-3364
ISSN Print: 2165-3356

# Descriptive Histological Study of the Cardiovascular and Lymphatic Systems of the Cattle Egret *Bubulcus ibis ibis*

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**How to cite this paper:** Fernández-Cruz, A.A., Ramírez-Muñoz, M.G., Zepeda-Bastida, A., Jardon-Xicotencatl, S. and Ocampo-López, J. (2026) Descriptive Histological Study of the Cardiovascular and Lymphatic Systems of the Cattle Egret *Bubulcus ibis ibis*. *Open Journal of Veterinary Medicine*, **16**, 142-160.
https://doi.org/10.4236/ojvm.2026.168011

**Received:** June 24, 2026
**Accepted:** August 28, 2026
**Published:** August 31, 2026



## Abstract

*Bubulcus ibis ibis* is a migratory bird with worldwide distribution and epidemiological relevance due to its capacity as a vector of pathogens transmissible to domestic animals and humans. This study describes the histological characteristics of the cardiovascular and lymphatic systems of this species, for which available morphological information is scarce. Three adult carcasses obtained incidentally in the surroundings of the University Campus of Tulancingo, Hidalgo, México, were used. Samples of the organs of interest were processed by paraffin embedding and stained with Hematoxylin-Eosin and with the Verhoeff method for elastic fibers. The most relevant findings were the abundance of elastic fibers in the aorta and pulmonary trunk, the identification of structures of undetermined nature adjacent to the common carotid arteries, not previously described in domestic birds, and structural differences in the cloacal bursa and spleen compared to reference avian species. The results provide a morphological basis for comparative, clinical, and epidemiological studies in this species.

## Keywords

*Bubulcus ibis ibis*, Histology, Cardiovascular System, Lymphatic System

## 1. Introduction

### Cattle Egret *Bubulcus ibis ibis*

The cattle egret is the most abundant and widespread heron species worldwide, with an estimated global population of 4 to 9.85 million birds. *Bubulcus ibis ibis* belongs to the order Ciconiiformes and has a varied diet based primarily on small invertebrates; it occasionally also captures and consumes frogs, toads, fish, crayfish, small snakes, small mammals, chicks of other bird species, lizards, and earthworms [1] [2].

Reproduction occurs at different times of year depending on local climate, location, and food availability. However, the species does not breed everywhere it is present, particularly in colder regions [1] [2].

As a native species, its habitat centers on savannas and wetlands; as an exotic species, it occupies agricultural fields and pastures. At night, the cattle egret roost and sleeps colonially in trees [1] [2].

*Bubulcus ibis ibis* is considered a semi-migratory bird distributed worldwide except in Antarctica. It is regarded as beneficial for its ability to remove most ectoparasites from animals, mainly ticks. It has also been observed feeding on larvae of the house fly (*Musca domestica*) and the blowfly (*Calliphora spp.)* in fishing villages in India and at organic waste dumps, which is considered useful given that these insects are carriers of pathogens. The species likewise feeds on insects that attack various crops. Nevertheless, it also has negative impacts on the environment [3]-[5].

According to the National Commission of Natural Protected Areas (CONANP), based on data from CONABIO, there are 348 invasive exotic species in Mexico, 16 of which are birds, including the cattle egret *Bubulcus ibis ibis*. This causes ecosystem imbalance through displacement of native species or extinction of vulnerable species due to predation, competition, and habitat destruction [6].

An additional concern is that these birds act as vectors of diseases transmissible to animal species and humans, posing a public health risk [6]. Many bird species have been shown to be susceptible to infection by influenza A virus. Regarding botulism, caused by toxins produced by *Clostridium botulinum*, cattle, birds, horses, sheep, dogs, and humans are affected, with susceptibility depending on the toxin type [7]-[9].

With respect to other bacteria, *Bacillus spp., Staphylococcus spp., Listeria spp., Streptococcus spp.*, and *Salmonella spp.* have been identified, all of which can cause infections in both animals and humans [10]-[12].

Nematodes identified include *Heterakis gallinarum, Echinostoma, Capillaria spp., Toxocara spp.,* and *Ascaridia spp.*; trematodes include *Renicola spp.* and *Apharyngostrigea spp.*; and protozoa include *Eimeria spp.* and *Toxoplasma gondii*. The presence of these parasites therefore represents a risk for poultry and humans [13]-[16].

Fungal genera identified include *Mucor spp., Rhizopus spp., Penicillium spp., Paecilomyces spp., Scedosporium spp., Alternaria spp., Microsporum spp.*, and *Trichophyton spp.*, along with yeasts such as *Cryptococcus spp., Rhodotorula*

*spp.*, and *Scedosporium spp.* [4] [17].

Based on the above, the cattle egret may constitute a public health threat due to its potential for transmitting pathogens to humans, livestock, poultry, and native wildlife, noting that more detailed studies are required to determine its pathogenic capacity [10] [12] [13].

The objective of this study is to describe the histological characteristics of the cardiovascular and lymphatic systems of *Bubulcus ibis ibis*, a species with scarce morphological literature available, to provide morphological data that contribute to the morphophysiological knowledge of the species, which is fundamental for the design of assertive population control strategies of *B. ibis ibis*, in order to help prevent the risk of transmission of pathogens to other species and humans.

## 2. Materials and Methods

### 2.1. Study Subjects

Three adult cattle egret carcasses with no apparent physical injuries were obtained incidentally between January and February 2023 in the surroundings of the University Campus of Tulancingo of the Autonomous University of the State of Hidalgo, located in the Tulancingo de Bravo Valley region, in the state of Hidalgo. The specimens presented morphological characteristics compatible with *Bubulcus ibis ibis* and were fully identified by specialized photographic comparison and phylogenetic corroboration by PCR.

The animals found were refrigerated at 4˚C immediately after discovery, and the respective necropsy was performed the same day for sample collection, provided that the necropsy study did not reveal significant post-mortem changes. In this way, the three specimens for this study were selected.

### 2.2. Collection of Biological Samples

From each of the three reference individuals, the organs and structures of their cardiovascular and lymphatic systems were carefully dissected and fixed for at least 24 hours in a 3.7% phosphate-buffered aqueous formaldehyde solution for histological study [18].

Cardiovascular system. Longitudinal and transverse sections of the heart were obtained from each bird. Blood vessel samples included three elastic arteries (aorta, pulmonary trunk and carotid artery) and one muscular artery (femoral). In addition, a jejunal sample was obtained to observe arterioles; the veins collected comprised the vena cava and the femoral vein, and the same jejunal sample was used to observe venules; furthermore, brain (to observe continuous capillaries), kidney (to observe fenestrated capillaries), and liver (to observe sinusoid capillaries) samples were also obtained from each individual.

Lymphatic system. Sample of mesentery was collected from each bird to observe lymphatic vessels and lymphoid tissue associated with this structure. Primary lymphoid organs (thymus and cloacal bursa) were also collected, and secondary lymphoid structures from each bird included spleen and jejunum samples to ob-

serve diffuse lymphoid tissue and grouped lymphoid nodules associated with their lamina propria, as well as lung samples to observe solitary lymphoid nodules associated with their parenchyma.

### 2.3. Paraffin Embedding Processing

After fixation, all samples were trimmed and placed in histocassettes for processing in an automated tissue processor (histokinette), Microm brand, model STP 1201, for 16 hours, to carry out washing, dehydration, clearing, and infiltration [18].

### 2.4. Paraffin Embedding

After processing, all samples were embedded in histological-grade paraffin at its melting point (57˚C) and allowed to solidify in blocks at room temperature for at least 24 hours before sectioning [18].

### 2.5. Sectioning

From each block, sections of 6 μm thickness were obtained using a manual rotary microtome, Leica brand, model RM2125RT, and placed in a tissue flotation bath, Premiere brand, model XH-1001, prepared with water and 3 g of gelatin for extension and adhesion to the slides. Preparations were placed in an oven at 60˚C for 5 minutes and then stored at room temperature for staining [18].

### 2.6. Staining and Mounting

Duplicate slides of the referred organs and structures from each bird were stained with the routine Hematoxylin and Eosin (H-E) stain or the Verhoeff method (V) for elastic fibers were used. The former allowed observation of the morphology of the studied organs and structures, while the latter revealed elastic fibers (stained black) associated with the studied tissue. Once stained, a coverslip was placed over the slide, encapsulating the tissue with synthetic resin, which was left to dry for 24 hours [18].

### 2.7. Observation of Histological Preparations, Morphological Analysis, and Image Capture

All histological preparations were examined using an Olympus bright-field compound microscope, model BX41, with 4X, 10X, 40X, and 100X objectives. Representative and selected images of each organ and structure were captured in TIFF format using ImagePro v. 4.0 software (MediaCybernetics) with an Evolution VF camera (MediaCybernetics) on a Vaio PC with 4 GB RAM and a Pentium processor.

## 3. Results

### 3.1. Histological Study

The histological results shown here are representative and uniform from the observations of the three specimens studied; if slight variations were observed be-

tween individuals in the structures observed, they are noted in the respective sections.

## 3.2. Cardiovascular System

### 3.2.1. Heart

The heart of *Bubulcus ibis ibis* presents three well-differentiated layers from inner to outer: endocardium, myocardium, and epicardium. The endocardium consists of an endothelium (simple squamous epithelium) beneath which lies a thin layer of dense irregular collagenous connective tissue, the subendothelium (Figure 1(A)). In some areas, this layer thickens to give rise to a subendocardium in which small, grouped muscle cells are located, corresponding to conducting myocytes (Figure 1(A)). Beneath these structures, elongated muscle cells with some branching constitute the main layer of the heart, the myocardium (Figure 1(B)), which forms the bulk of the organ wall. On the outer surface, a thin layer overlying the cardiac myocytes corresponds to the epicardium, consisting of a mesothelium (simple squamous epithelium) that anatomically constitutes the visceral pericardium (Figure 1(C)), beneath which lies a thin layer of dense irregular collagenous tissue corresponding to the subepicardium (Figure 1(C)).

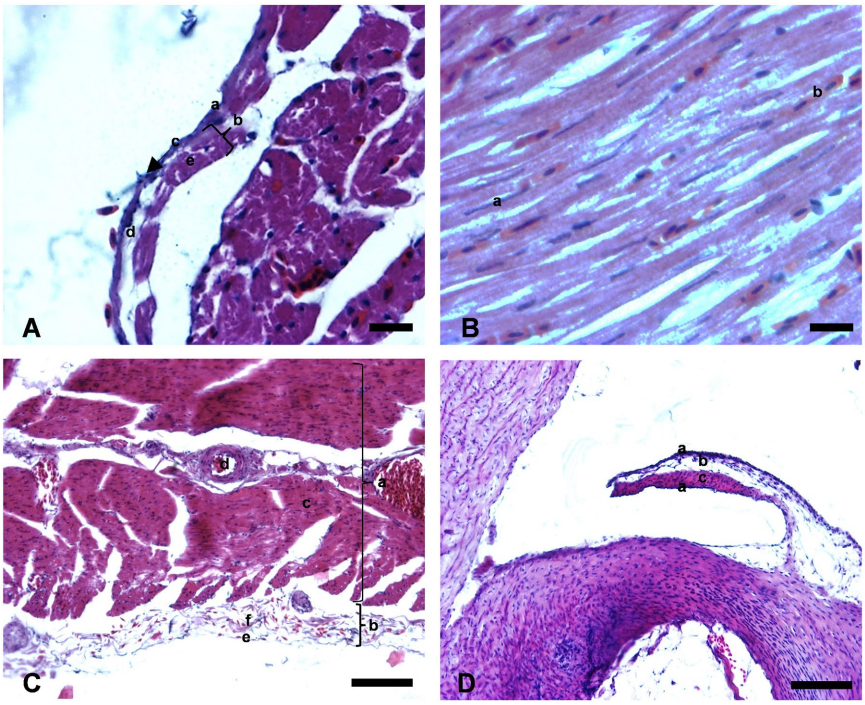


Figure 1. Heart of *Bubulcus ibis ibis*. A) Endocardium (a) and subendocardium (b) with conducting myocytes. Endothelium (c), dense irregular collagenous connective tissue forming the subendothelium (d), conducting myocytes (e). Bar: 20 µm. 400X. B) Myocardium in longitudinal section. Cardiac myocytes (a) and erythrocytes (b). Bar: 20 µm. 400X. C) Myocardium (a) and epicardium (b). Cardiac myocytes (c), blood vessel (d), mesothelium (simple squamous epithelium) (e), subepicardium (dense irregular collagenous tissue) (f). Bar: 100 µm. 100X. D) Cardiac valve. Valvular endothelium (a), dense irregular collagenous connective tissue (b), cardiac myocytes (c). Bar: 100 µm. 100X. Stain: H - E.

The cardiac valves consist of a number of leaflets depending on their particular structure. Each leaflet is covered by a valvular endothelium, continuous with the mural endothelium of the cardiac chambers, supported by a layer of dense irregular collagenous connective tissue and a layer of muscular tissue also covered by the endothelium (**Figure 1(D)**).

### 3.2.2. Blood Vessels

**Arteries:**

**1. Elastic: aorta and pulmonary trunk**

In *Bubulcus ibis ibis*, the aorta consists of three layers from inner to outer: tunica intima, tunica media, and tunica externa (adventitia) (**Figure 2(A)** and **Figure 2(B)**). The tunica intima consists of an endothelium (simple squamous epithelium) beneath which lies a thin layer of dense irregular collagenous connective tissue forming the subendothelium (**Figure 2(A)** and **Figure 2(B)**). The tunica media presents a stratum rich in elastic fibers arranged longitudinally relative to the vessel course, with associated collagen fibers; deeper, the characteristic circular arrangement of smooth muscle cells is observed, interspersed with elastic and collagen fibers also arranged circularly (**Figure 2(B)**). This arrangement continues to the outer surface of the vessel, where a thin collagen layer supports a mesothelium (simple squamous epithelium) that constitutes the tunica externa, in this case a serosa, given the intracoelomic location of the aorta (**Figure 2(A)**).

The pulmonary trunk shares the same structure as the aorta, consisting of three layers from inner to outer: tunica intima, tunica media, and tunica externa (**Figure 2(C)** and **Figure 2(D)**). The tunica intima is formed by an endothelium beneath which lies a thin layer of dense irregular collagenous connective tissue corresponding to the subendothelium (**Figure 2(C)** and **Figure 2(D)**). The tunica media is composed predominantly of elastic fibers arranged longitudinally with associated collagen fibers, as well as smooth muscle cells interspersed with elastic and collagen fibers (**Figure 2(D)**); this organization extends to the vessel surface, where a thin collagen layer supports a mesothelium that constitutes the tunica externa, in this case a serosa (**Figure 2(C)** and **Figure 2(D)**).

**2. Muscular: femoral artery**

The femoral artery of *Bubulcus ibis ibis* presents the following layers from inner to outer: tunica intima, tunica media, and tunica externa (adventitia) (**Figure 2(E)** and **Figure 2(F)**). The tunica intima consists of an endothelium (simple squamous epithelium) beneath which lies a thin collagen layer. The tunica media is formed by smooth myocytes arranged circularly relative to the vessel course (**Figure 2(E)** and **Figure 2(F)**). External to this layer, a zone rich in elastic fibers of varying thickness—some thick and several thin—with their characteristic undulating course, together with thick collagen fibers, forms the outer covering (**Figure 2(E)** and **Figure 2(F)**). This elastic fiber-rich region corresponds to what is routinely designated in mammals as the external elastic membrane, while the collagen layer constitutes the tunica externa, in this case the tunica adventitia (**Figure 2(E)** and **Figure 2(F)**).

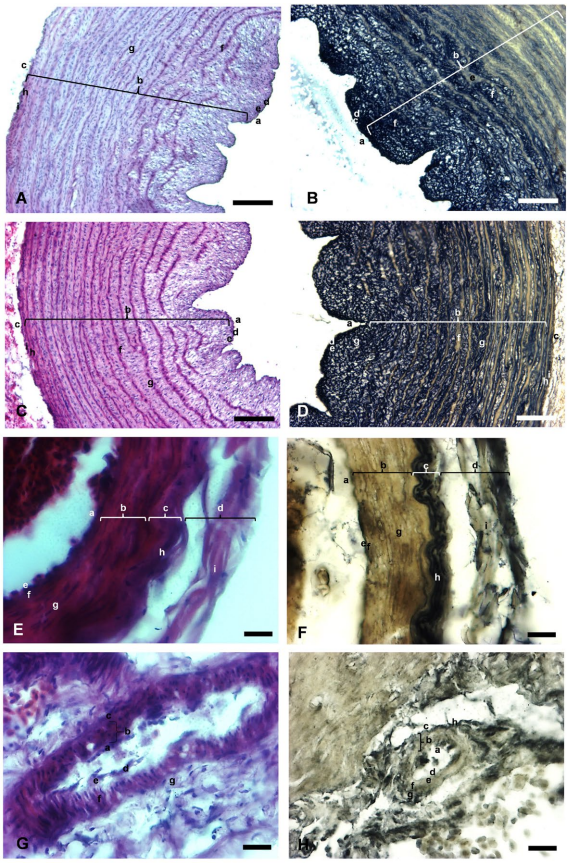

**Figure 2.** Arteries of *Bubulcus ibis ibis*. A) Aorta. All three tunicae are identified: tunica intima (a), tunica media (b), tunica externa (c). Endothelium (simple squamous epithelium) (d), subendothelium (dense irregular collagenous connective tissue) (e), smooth myocytes (f), elastic fibers (g), thin collagen layer (h), mesothelium (simple squamous epithelium) (i). Bar: 100 µm. 100X. B) Aorta. Tunica intima (a) and tunica media (b). Endothelium (simple squamous epithelium) (c), subendothelium (dense irregular collagenous connective tissue) (d), smooth myocytes (e), elastic fibers (f). Bar: 100 µm. 100X. C) Pulmonary trunk. All three tunicae are identified: tunica intima (a), tunica media (b), tunica externa (c). Endothelium (simple squamous epithelium) (d), subendothelium (dense irregular collagenous connective tissue) (e), smooth myocytes (f), elastic fibers (g), thin collagen layer (h), mesothelium (simple squamous epithelium) (i). Bar: 100 µm. 100X. D) Pulmonary trunk. Tunica intima (a), tunica media (b), tunica externa (c). Endothelium (simple squamous epithelium) (d), subendothelium (dense irregular collagenous connective tissue) (e), smooth myocytes (f), elastic fibers (g), thin collagen layer (h), mesothelium (simple squamous epithelium) (i). Bar: 100 µm. 100X. E) Muscular artery. Tunica intima (a), tunica media (b), external elastic membrane (c), tunica adventitia (d). Endothelium (simple squamous epithelium) (e), thin collagen layer (f), smooth myocytes (g), elastic fibers (h), collagen (i). Bar: 20 µm. 400X. F) Muscular artery. Tunica intima (a), tunica media (b), external elastic membrane (c), tunica adventitia (d). Endothelium (simple squamous epithelium) (e), thin collagen layer (f), smooth myocytes (g), elastic fibers (h), collagen (i). Bar: 20 µm. 400X. G) Arteriole. Tunica intima (a), tunica media (b), tunica adventitia (c). Endothelium (simple squamous epithelium) (d), thin collagen layer (e), smooth myocytes (f), collagen fibers (g). Bar: 20 µm. 400X. H) Arteriole. Tunica intima (a), tunica media (b), tunica adventitia (c). Endothelium (simple squamous epithelium) (d), thin collagen layer (e), smooth myocytes (f), collagen fibers (g), elastic fibers (h). Bar: 20 µm. 400X. Stain—H - E: A, C, E, and G. Stain—Verhoeff: B, D, F, and H.

**3. Arteriole: intestine (jejunum)**

For the description of an arteriole, blood vessels located in the periphery of the intestine (jejunum) were used as reference. Despite their small size, these vessels also present the three layers described above. The tunica intima is formed by an endothelium beneath which lies a thin collagen layer corresponding to the subendothelium (**Figure 2(G)** and **Figure 2(H)**), connecting with the tunica media, which consists of two to three layers of smooth myocytes arranged obliquely relative to the vessel axis (**Figure 2(G)** and **Figure 2(H)**); externally, a thin layer of collagen fibers constitutes the tunica adventitia (**Figure 2(G)** and **Figure 2(H)**).

## 3.3. Veins

### 3.3.1. Large: Vena Cava

In the vena cava of *Bubulcus ibis ibis*, three well-differentiated layers are identified: tunica intima, tunica media, and tunica externa, in this case a serosa (**Figure 3(A)** and **Figure 3(B)**). From inner to outer, the tunica intima consists of an endothelium beneath which lies a thin collagen layer corresponding to the subendothelium (**Figure 3(A)** and **Figure 3(B)**). The tunica media is formed by smooth myocytes arranged circularly relative to the vessel course (**Figure 3(A)** and **Figure 3(B)**). External to the tunica media, and greater in thickness, the tunica serosa is formed by thick collagen fibers (**Figure 3(A)**) and elastic fibers (**Figure 3(B)**).

### 3.3.2. Medium: Femoral Vein

The femoral vein of *Bubulcus ibis ibis* presents the same three tunicae. The tunica intima consists of an endothelium beneath which lies a thin collagen layer forming the subendothelium (**Figure 3(C)** and **Figure 3(D)**). The tunica media is formed by two to three layers of smooth myocytes arranged circularly relative to the vessel course (**Figure 3(C)** and **Figure 3(D)**). External to this, a layer approximately twice the thickness of the tunica media, formed by thick collagen fibers (**Figure 3(C)**) and elastic fibers (**Figure 3(D)**), constitutes the tunica adventitia.

### 3.3.3. Small: Small Intestine (Jejunum)

Small veins located in the periphery of the small intestine (jejunum) were used for their histological description. The vessel wall comprises the tunica intima, media, and adventitia. The tunica intima consists of an endothelium supported by a thin collagenous subendothelial layer (**Figure 3(E)** and **Figure 3(F)**). The tunica media contains two to three layers of smooth myocytes arranged obliquely relative to the vessel axis (**Figure 3(E)** and **Figure 3(F)**). The tunica adventitia consists of dispersed collagen fibers (**Figure 3(E)**) and elastic fibers (**Figure 3(F)**) and is approximately twice as thick as the tunica media.

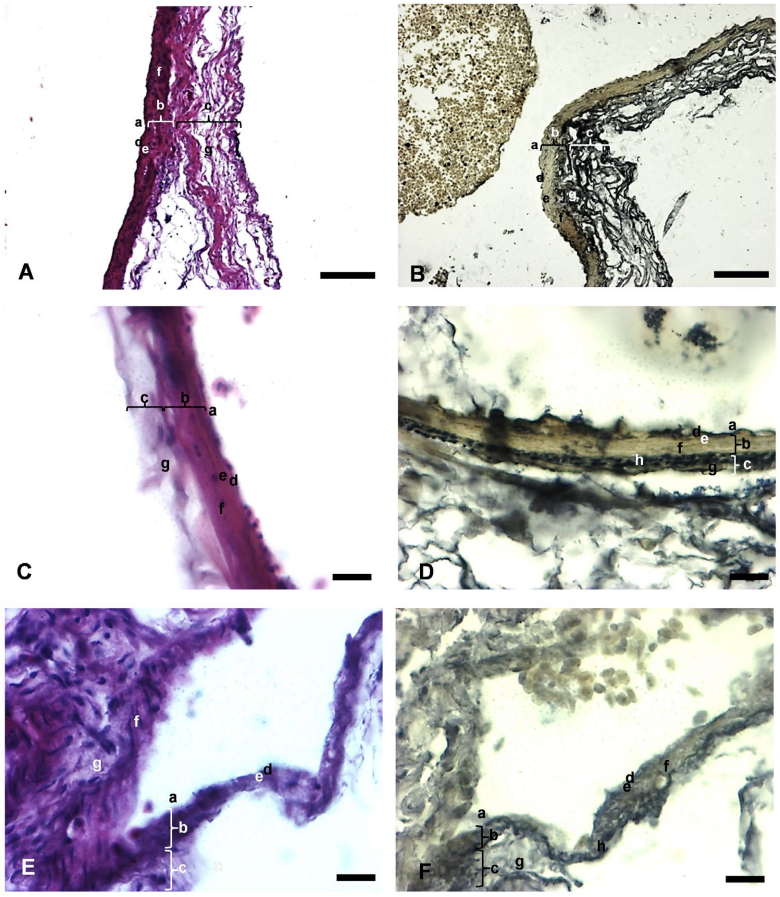


**Figure 3.** Veins of *Bubulcus ibis ibis*. A) Large vein (vena cava). All three tunicae are identified: tunica intima (a), tunica media (b), tunica externa (serosa) (c). Endothelium (d), subendothelium (thin collagen layer) (e), smooth myocytes (f), thick collagen fibers (g). Bar: 100 μm. 100X. B) Large vein (vena cava). Tunica intima (a), tunica media (b), tunica externa (serosa) (c). Endothelium (d), subendothelium (thin collagen layer) (e), smooth myocytes (f), elastic fibers (g), thick collagen fibers (h). Bar: 100 μm. 100X. C) Medium vein (femoral). All three tunicae with their corresponding structures: tunica intima (a), tunica media (b), tunica adventitia (c). Endothelium (d), subendothelium (thin collagen layer) (e), smooth myocytes (f), thick collagen fibers (g). Bar: 20 μm. 400X. D) Medium vein (femoral). Tunica intima (a), tunica media (b), tunica adventitia (c). Endothelium (d), subendothelium (thin collagen layer) (e), smooth myocytes (f), thick collagen fibers (g), elastic fibers (h). Bar: 20 μm. 400X. E) Small vein (jejunum). All three tunicae with their corresponding structures: tunica intima (a), tunica media (b), tunica adventitia (c). Endothelium (d), subendothelium (thin collagen layer) (e), smooth myocytes (f), collagen fibers (g). Bar: 20 μm. 400X. F) Small vein (jejunum). Tunica intima (a), tunica media (b), tunica adventitia (c). Endothelium (d), subendothelium (thin collagen layer) (e), smooth myocytes (f), collagen fibers (g), elastic fibers (h). Bar: 20 μm. 400X. Stain—H-E: A, C, and E. Stain—Verhoeff: B, D, and F.

## 3.4. Capillaries

### 3.4.1. Continuous: Brain

Brain tissue was used to identify and describe this capillary type, as it is a primary site of localization. These capillaries present two layers: an endothelial cell layer and a basement membrane (Figure 4(A)). The inner layer consists of endothelial

cells with a continuous arrangement, and the basement membrane is identified as the outer layer (Figure 4(A)).

#### 3.4.2. Fenestrated: Kidney

Kidney sections were used for observation of fenestrated capillaries, as the kidney is their most common site of localization. The renal glomerulus, consisting of a tuft of these capillaries, is observed (Figure 4(B)). These capillaries structurally present an endothelial cell layer, a basement membrane, and a layer formed by podocytes (Figure 4(B)).

#### 3.4.3. Sinusoidal: Liver

Liver sections were used for observation of sinusoidal capillaries, as the liver is their primary site of localization. These capillaries are identified by their sinusoidal course, irregular diameter, and the presence of erythrocytes within their lumen (Figure 4(C)). Both layers are observed: the inner endothelial cell layer and an outer thin basement membrane (Figure 4(C)).

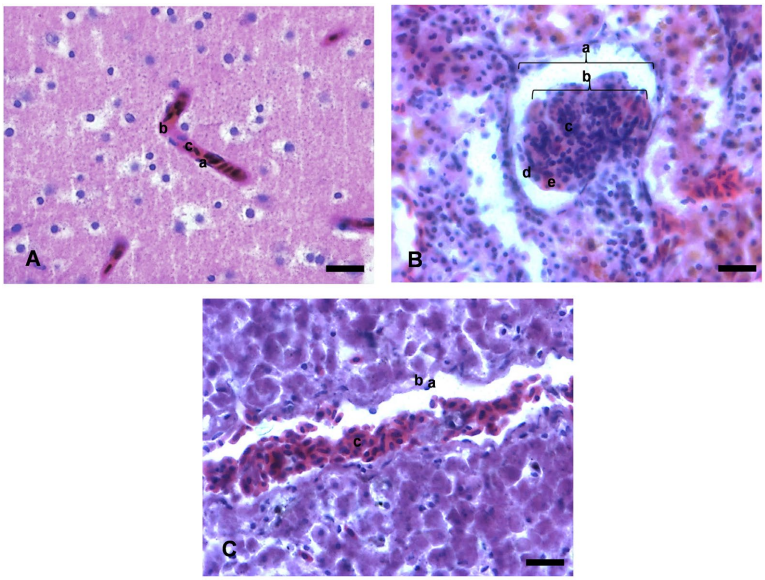


Figure 4. Capillaries of *Bubulcus ibis ibis*. **A)** Continuous capillary (brain). Endothelial cells (a), basement membrane (b), erythrocyte (c). Bar: 20 µm. 400X. **B)** Fenestrated capillary (kidney). Renal corpuscle (a), capillary tuft—glomerulus (b), endothelial cells (c), basement membrane (d), erythrocyte (e). Bar: 20 µm. 400X. **C)** Sinusoidal capillary (liver). Endothelial cells (a), basement membrane (b), erythrocyte (c). Bar: 20 µm. 400X. Stain: H - E.

### 3.5. Lymphatic System

#### Primary Lymphoid Organs and Structures

**1. Thymus**

The thymus of *Bubulcus ibis ibis* showed consistent involution with minimal variations in the three individuals studied. In general, its structure is composed mainly of adipose tissue, with some areas where it is still possible to observe scattered lymphocytic cells and loose collagenous connective tissue (Figure 5(A) and Figure 5(B)).

**2. Cloacal Bursa**

The cloacal bursa of *Bubulcus ibis ibis* presented similar histological characteristics in the three specimens studied. Externally presents a serous tunic composed of a mesothelium with associated connective tissue (Figure 5(C)). Beneath this lies a capsule composed of smooth muscle and dense irregular collagenous connective tissue, which projects thin trabeculae into the interior of the organ (Figure 5(C)). Between these, well-organized lymphocyte aggregates constitute bursal lymphoid nodules of varying size, each presenting a corona and a germinal center separated by a characteristic capillary network (Figure 5(D)). The cloacal bursa presents a lumen lined by an epithelium varying from pseudostratified columnar to simple cuboidal (Figure 5(D)). Between the epithelium and the capsule, the bursal lymphoid nodules described above collectively form cone-shaped projections directed toward the organ lumen (Figure 5(D)).

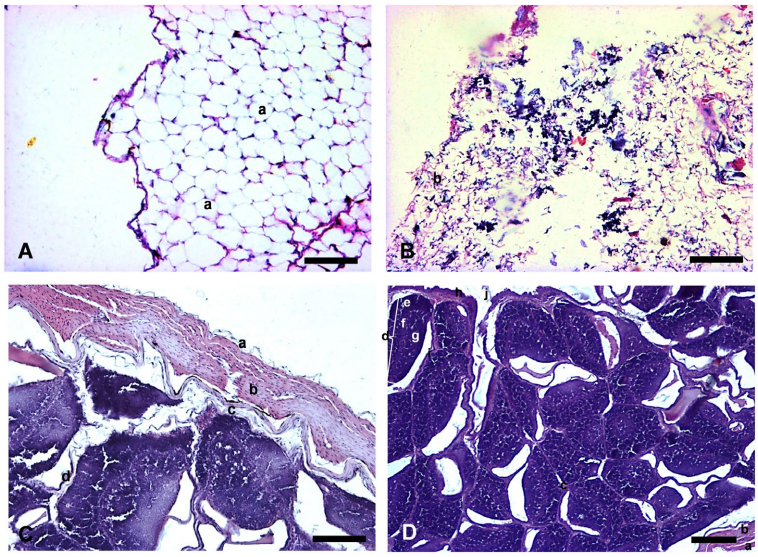


Figure 5. Primary lymphoid organs and structures of *Bubulcus ibis ibis*. A) Thymus. Involuted area showing adipose tissue (a). Bar: 100 µm. 100X. B) Thymus. Residual area with lymphocytic cells (a) and loose collagenous connective tissue (b). Bar: 100 µm. 100X. C) Cloacal bursa. Serous tunic and capsule. Mesothelium (a), smooth muscle (b), dense irregular collagenous connective tissue (c), trabeculae (d). Bar: 100 µm. 100X. D) Cloacal bursa. Cone-shaped projections directed toward organ lumen. Serous tunic (a), capsule (b), trabeculae (c), bursal lymphoid nodules (d), corona (e), capillary network (f), germinal center (g), pseudostratified columnar epithelium (h), simple cuboidal epithelium (i), lumen (j). Bar: 200 µm. 40X. Stain: H-E.

## 3.6. Secondary Lymphoid Organs and Structures

### 3.6.1. Diffuse Lymphoid Tissue

**Intestine (Jejunum):**

This type of lymphoid tissue is present in the intestine. Lymphocytic infiltration was present in the intestinal mucosa, specifically beneath the lamina propria (Figure 6(A)). This finding was consistent in the three birds studied, with virtually no variation in terms of the degree of lymphocytic infiltration of the jejunal lamina propria.

### 3.6.2. Solitary Lymphoid Nodules

**Lung:**

Solitary lymphoid nodules were found in different areas of the lung parenchyma in the three individuals studied; two representative nodules from one of the samples are shown here: the first with subpleural localization (Figure 6(B)) and the second in the pulmonary parenchyma adjacent to a pulmonary bronchus (Figure 6(C)). Lymphocytic cells are present in both, although the boundary between the germinal center and the corona cannot be precisely determined (Figure 6(B) and Figure 6(C)).

### 3.6.3. Aggregated Lymphoid Nodules

**1. Small Intestine (Jejunum): Intestinal Plaques**

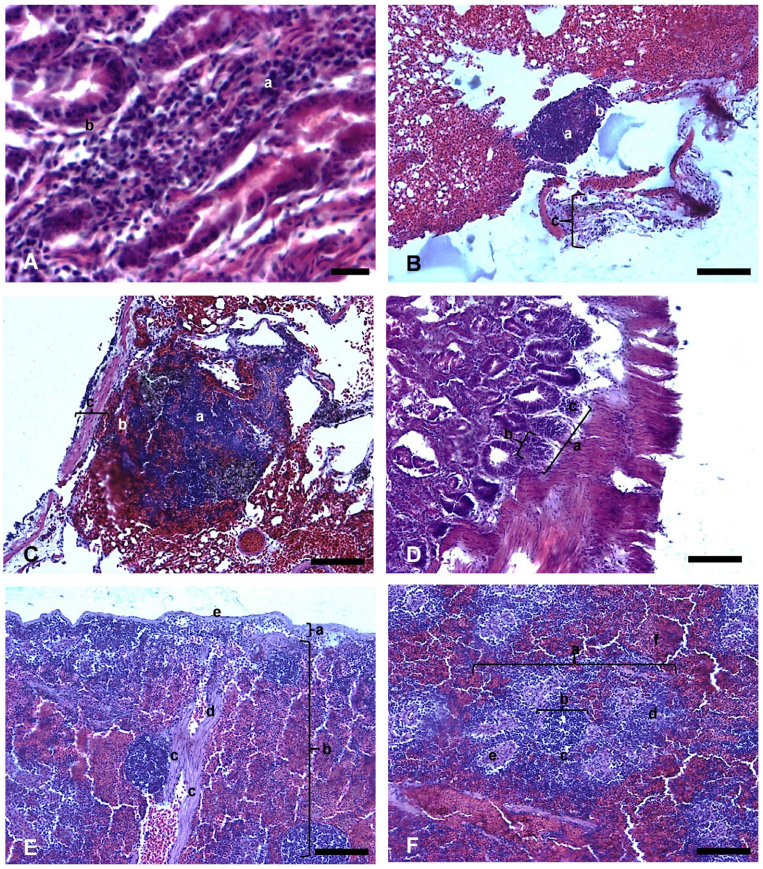


Figure 6. Secondary lymphoid organs and structures of *Bubulcus ibis ibis*. **A)** Diffuse lymphoid tissue (jejunum). Lymphocytic infiltration (a), lamina propria (b). Bar: 20 µm. 400X. **B)** Solitary lymphoid nodule (lung, subpleural location). Lymphocytic cells (a), capillaries (b), pleura (c). Bar: 100 µm. 100X. **C)** Solitary lymphoid nodule (lung, peribronchial location). Lymphocytic cells (a), capillaries (b), bronchus (c). Bar: 100 µm. 100X. **D)** Aggregated lymphoid nodules (jejunum). Intestinal plaque formed by aggregated lymphoid nodules (a). Lymphoid nodule (b), lymphocytic cells (c). Bar: 100 µm. 100X. **E)** Spleen. Capsule (a) and parenchyma (b). Trabeculae (c), dense irregular collagenous connective tissue (d), smooth muscle (e). Bar: 100 µm. 100X. **F)** Spleen. Red and white pulp. Splenic lymphoid nodules (a), germinal center (b), corona (c), lymphocytic cells (d), nodular arteriole (e), splenic cords (f). Bar: 100 µm. 100X. Stain: H - E.

The intestine is one of the sites of localization of this lymph nodule type. Aggregated lymphoid nodules forming intestinal plaques were present in the submucosa of the intestine (Figure 6(D)). Within each nodule, lymphocytic cells are present, although the boundary between the corona and the germinal center cannot be observed with clarity (Figure 6(D)).

**2. Spleen**

The spleen of *Bubulcus ibis ibis* presents a thin capsule formed by dense irregular collagenous connective tissue and smooth muscle, with trabeculae of the same composition distributed throughout the parenchyma; both structures form part of the organ stroma (Figure 6(E)). The parenchyma contains red pulp and white pulp. In the white pulp, splenic lymphoid nodules are present; the germinal center formed by lymphocytic cells and their corona, where these cells are also present, along with nodular arterioles (Figure 6(F)). In the red pulp, splenic cords formed by large numbers of erythrocytes and lymphocytes are visible (Figure 6(F)).

### 3.6.4. Lymphatic Vessels and Associated Lymphoid Tissue

**1. Mesentery**

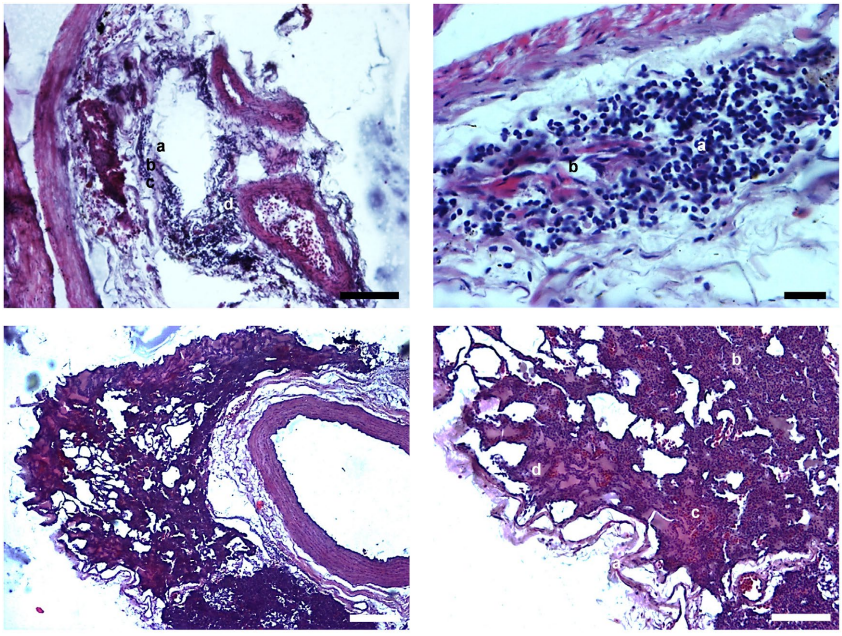


Figure 7. Associated lymphoid tissue and lymphatic vessels of *Bubulcus ibis ibis*. **A)** Mesentery. Lymphatic vessel. Tunica intima (a), tunica media (b), tunica adventitia (c), associated lymphoid tissue (d). Bar: 100 µm. 100X. **B)** Mesentery. Lymphoid tissue aggregates are associated with blood vessels. Associated lymphoid tissue (a), blood vessel (b). Bar: 20 µm. 400X. **C)** Common carotid artery. Structure of undetermined nature (a) adjacent to the blood vessel (b). Serous tunic (c), parenchyma (d). Bar: 200 µm. 40X. **D)** Common carotid artery. Parenchyma of the associated structure to it. Serous tunic (a), lymphocytic cells (b), capillaries (c), acidophilic material (d). Bar: 100 µm. 100X. Stain: H - E.

In the absence of lymph nodes, lymphocyte aggregates are visible in certain body regions. A section of structures associated with the mesentery reveals a lym-

phatic vessel presenting a thin tunica intima formed by an endothelium and dense irregular connective tissue, beneath which lie smooth muscle cells forming the tunica media; externally, a thin tunica adventitia of dense irregular collagenous connective tissue is present (**Figure 7(A)**). Associated lymphoid tissue is in the periphery of the vessel (**Figure 7(A)**). A lymphocytic tissue aggregate associated with blood vessels is also present (**Figure 7(B)**).

**2. Carotid Artery**

Associated with serosa of this blood vessel, found a very interesting structure (**Figure 7(C)**). Its parenchyma presents a homogeneous appearance of lymphocytic cells and capillaries with erythrocytes; in some areas, these cells form small cavities with acidophilic material (**Figure 7(D)**).

## 4. Discussion

Following analysis of the results, *Bubulcus ibis ibis* presents blood vessels formed by their three characteristic layers: tunica intima, tunica media, and tunica externa, except for capillaries, formed only by a basement membrane and endothelial cells. The heart presents three layers: epicardium, myocardium, and endocardium, which is consistent with descriptions in other birds [19]-[22].

Among the significant findings, a substantial amount of elastic fibers is identified in the pulmonary trunk in addition to the aorta, which may be related to structural support and the elastic recovery necessary for continuous mechanical stretching and vessel recoil due to the additional pressure generated during flight at high altitudes, considering that oxygen transfer and transport are superior in migratory birds compared to non-migratory ones [23] [24].

Birds have a cardiac output six times greater than that of mammals of comparable size, particularly during flight. Some findings suggest that the transition to high cardiac performance does not necessarily lead to greater variation in cardiac structure, indicating that heart morphology in birds is more constant and less variable than in mammals, and that this anatomical consistency may indicate evolutionary efficiency; this is consistent with the present histological study of this organ [25] [26].

Structures adjacent to the left and right common carotid arteries were identified, which have not been described in conventional bird species (ducks, geese, and chickens) [27]-[30]. Macroscopically they appear oval in orientation toward the vessel, with a brownish coloration. Microscopically, it consists of parenchyma formed by a population of lymphocytes associated with blood capillaries and acidophilic material distributed among the capillaries, all surrounded by the serosa that also envelops the carotid artery to which it is adjacent. This structure has elements compatible with those described for a hemolymphatic node, considering the presence of vascular structures with coexisting blood cells and lymphoid tissue, taking into account, however, that in this case well-defined lymphoid nodules are not observed; it should also be considered that hemolymphatic nodes are not described in any bird, being characteristic of ruminants and swine [22] [31]. Fur-

thermore, the functions described for a hemolymphatic node, such as hemocatheresis, the capture of antigens present in lymph and blood, and its participation in the activation of B and T cells in lymphoid nodules [22] [32], are by no means limited to or exclusive to mammals. Another alternative to consider would be a structure similar to the carotid sinus in mammals, but this structure is associated with the tissue architecture of the tunica media and adventitia of the carotid arteries; it is not external as in this case, and it involves nerve fibers that branch off to the glossopharyngeal cranial nerve (IX) [21] [33], which are absent in the structure described. In birds, an avian aortic bulb is described as an alternative, with histological characteristics like the carotid sinus, which performs the baroreceptor function of the latter in mammals [34] [35], but this also does not correspond morphologically to what is described in the present study. However, these observations are insufficient to establish their identity conclusively. Current knowledge indicates that the avian immune system differs from that of mammals by relying primarily on primary and secondary lymphoid organs together with diffuse mucosa-associated lymphoid tissues rather than on well-developed lymph nodes [36]-[38]. Consequently, alternative interpretations, including vascular-associated lymphoid tissue or previously undescribed lymphoid structures, should also be considered. Additional histochemical, immunohistochemical, and ultrastructural studies will be necessary to determine the composition, origin, and functional significance of these structures.

Regarding the other lymphoid organs and structures, these present the structure described in the consulted bibliography [20] [22] [39]. The spleen presents a thin capsule that projects trabeculae, which is consistent with the recent histological description of the spleen in *Bubulcus ibis ibis* reported by Abdellatif and Abdelghani-Basha [39]. Although the overall organization of the avian spleen is conserved, the thickness of the capsule and the development of the trabecular framework have been reported to vary among species, reflecting differences in splenic microarchitecture rather than in its fundamental organization [36]. Therefore, the splenic morphology observed in *B. ibis ibis* appears to represent a species-specific architectural variation while maintaining the typical histological organization of the avian spleen.

The cloacal bursa presents cone-shaped projections, suggesting a different organization of its cellular structure compared to the histological information available for other birds [20].

Although the cloacal bursa is the primary site of B-lymphocyte differentiation in birds, variations in follicular morphology and epithelial organization have been reported among avian species without affecting its essential immunological function [36]-[38].

The thymus examined in the present study corresponded to adult individuals, as evidenced by the marked involution and replacement of the parenchyma by adipose tissue. In contrast, the cloacal bursa remained structurally well preserved, indicating that it still retained its characteristic lymphoid organization. This ob-

servation is consistent with previous descriptions indicating that thymic involution may precede complete bursal regression in adult birds, although the timing and extent of involution vary among species [21] [28] [37] [38].

Regarding the remaining lymphoid structures, *B. ibis ibis* exhibited diffuse lymphoid tissue and lymphoid nodules distributed in the intestine, lung, mesentery, and in association with blood vessels. This distribution is consistent with the organization of the avian immune system, in which mucosa-associated lymphoid tissues (MALT), including gut-associated (GALT) and bronchus-associated lymphoid tissue (BALT), play a central role in immune surveillance and antigen recognition [36] [38]. As in most avian species, no true lymph nodes were identified, reinforcing the concept that birds rely primarily on specialized lymphoid organs and diffuse lymphoid tissues rather than encapsulated lymph nodes to coordinate immune responses [20] [36]-[38].

Being a migratory bird, *Bubulcus ibis ibis* has greater exposure to diverse pathogens and likewise has broad potential to function as a natural host, reservoir, and amplifying or bridge host for zoonotic agents. It is possible to infer that various mechanisms have developed to combat these external agents and ensure survival by adapting to different environments [8] [40].

The present study provides a baseline histological description of the cardiovascular and lymphatic systems of *Bubulcus ibis ibis* based on adult specimens obtained under opportunistic conditions. As a descriptive morphological investigation, its primary objective was to document normal tissue organization in a species for which histological information is scarce. Future studies incorporating specimens of different ages, broader sampling, and complementary analytical approaches will contribute to expanding the morphological characterization presented here.

## 5. Conclusions

This study provided important data contributing to avian histology and to the specific knowledge of the species, offering elements for more detailed studies on structures of interest to determine their functionality and significance, given the scarce morphological information available on migratory birds of epidemiological relevance. Knowledge of the anatomy and histology of the different systems allows the identification of tissue alterations and thus enables comparisons between normal and pathological conditions.

Although migratory birds have lower susceptibility to pathogens due to their developed immune system, they participate effectively in disease transmission; by traveling long distances they contribute to the dissemination of diseases affecting other animal species and humans, given the variety of microorganisms that have been associated with these species.

Considering their importance to the environment, preventive measures should be implemented to safeguard public health and control population growth without affecting this species or others related to it. In this regard, advances in mor-

phophysiological knowledge would undoubtedly allow for the establishment of environmentally friendly population control measures that are balanced with the species in question, considering their specific behavior and physiology.

## Ethics Statement

The authors confirm that the ethical policies of the journal have been adhered to. No ethical approval required as all specimens were adult carcasses found incidentally, and no live animals used or manipulated.

## Author Contributions

Andrea Alejandra Fernández-Cruz: Investigation, histological processing, initial data analysis, and initial writing.

María Guadalupe Ramírez-Muñoz: Investigation, histological processing, and staining procedures.

Armando Zepeda-Bastida: Investigation and supervision.

Samantha Jardon-Xicotencatl: Investigation, supervision, and writing.

Juan Ocampo-López: Conceptualization, methodology, investigation, supervision, writing, and overall coordination of the study.

All authors reviewed and approved the final version of the manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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