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Original Research Article

PIGEON AND FOWL HEARTS: A COMPARATIVE MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL (TROPONIN T) STUDY

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Abstract: Similar to mammals, the avian heart is a muscular pump responsible for distributing blood throughout the body. However, a larger relative heart weight to body weight (RHW/BW) was reported in birds than mammals. Furthermore, it has been revealed that smaller birds specially flying one have relatively larger hearts (relative to body mass) than larger birds (non-flying). Although this fact, there are no literatures regarding comparative descriptive variations of the heart in flying and non-flying birds that could adapt various functions, such as flying and running. Therefore, this study aimed to conduct a comparative anatomical features and morphometrical measurments between the hearts of pigeon and fowl. The present study also analyzed the immunohistochemical expression of Troponin T (TnT) as one of the major regulator of striated muscle contraction. The study revealed significant larger RHW/BW in pigeon heart. Furthermore, the hearts of both fowl and pigeon are directed caudoventrally in the cranial part of thoracoabdominal cavity, however, a more oblique position nearly parallel to the sternum was observed in pigeon`s hearts. Interestingly, the mean morphometric values (length of both cranial and caudal borders, diameter of the base, and thickness of both right and left ventricular wall and interventricular septum) of the pigeon hearts showed significant higher differences than that of fowl. Additionally, more expression of TnT was observed in pigeon hearts. Therefore, our findings suggest that such morphological and immunological varities are possibly essential factors for providing intense ventricular contraction of the pigeon`s heart to cover the high metabolic requirments for flight.

Key words: fowl; heart; pigeon; immunohistochemistry; troponin T

Introduction

The birds have an efficient cardiovascular system that permit them to adapt the metabolic demands of different physiological processes such as flight, swimming, or diving and running. It has been reported that the birds, unlike their crocodile relatives can maintain their core temperature at 37°C by the increasing the basal metabolic rate, cardiac output and blood pressure (1). The major organ involved in this system is the heart. The heart of birds, like mammals, is a muscular pump consisting of four chambers (two atria and two ventricles),

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and showed a double circulatory system with a complete separation of oxygenated and de-oxygenated blood.

Interestingly, the avian heart is significantly heavier in proportion to the body weight when compared to other vertebrates and mammales and represents 0.8-1.2% of the body weight (1). Furthermore, it was previously reported that the bird`s heart show a larger stroke volume, and higher cardiac output than do mammals of similar body mass (2,3). Additionally, a larger heart and an increase in cardiac output are required to enable the avian heart and cardiovascular system for providing enough oxygen to body tissues during flight (4). Despite the previous facts, little was so far reported concerning the hearts of birds (5-8). Furthermore, comparative morphological variations of the avian heart among flying and non flyng birds remains unclarified.

The heart is composed of contractile cardiac muscle cells (cardiomyocytes) that differs slightly from the skeletal and smooth muscle cells whereas cardiomyocyte contracts in coordination with its neighbouring cells to maintain a regular pumping rhythm. Any disturbances in such coordination leads to abnormal heart rhythms such as ventricular fibrillation (4). The contractility of cardiac muscle differs from bird to bird and from one age to another.

Troponin T is the tropomyosin-binding subunit of the troponin complex, which plays a great role in the regulation of striated muscle contraction (9). In vertebrates, three Troponin T (TnT) genes were reported; cardiac TnT (cTnT), skeletal muscle fast-twitch TnT (fTnT) and slow-twitch TnT (sTnT) (10). The cTnT is one of the regulatory proteins that control the calcium mediated interaction between actin and myosin. In avian heart, cTnT was reported to be expressed in the chicken heart (11), however, no reports about its expression among flying and non-flying birds. The present work aimed to investigate some morphological variations in pigeon and fowl hearts in relation to the presence and release of cardiac Troponin T in active flying birds rather than non-flying or resting ones.

Material and methods

Birds of study and ethics statement

This study was conducted on healthy adult pigeons and fowls (n= 16/ species) of both sexes of pure baladi breeds, obtained from commercial farms in Zagzaig, Egypt. The fowl was 3-5 months-old and 1.4±0.5 kg body weight, however, the pigeon was 6-11 months-old and 0.450±0.250 kg body weight. This experiment was carried out according to the institutional ethical committee of the Zagazig University, Egypt. The macromorphological study of this work was performed at the Anatomy and Embryology Department, Zagazig University, Egypt. The micromorphology and immunohistochemistry were done at Faculty of Veterinary Medicine, Basic Veterinary Sciences, Hokkaido University, Sapporo, Japan.

Macromorphology

Six fresh hearts from each species were dissected for further macro-morphological studies. Before removing their hearts, the anatomical positions of fowl and pigeons hearts and their relations were studied inside the thorax. Each heart was washed with normal saline and the pericardium was investigated. The pericardium was removed and the weight of the hearts was measured after cutting open in the ventricles and atria for removal of any blood residues and thrombi in its chambers. Also, the shape and surface topography of the fresh hearts were studied.

Morphometric study

Both avian and pigeon hearts were weighed and related to body mass, also a comparative gross morphometry, between fowl and pigeon hearts, were measured such as (average body weight, length of cranial and caudal border, transverse diameter, thickness of right and left ventricular wall and interventricular septum). Varnier caliper was used in current investigations.

Micromorphology

The walls of the other ten heart ventricles were opened, the blood was washed and then

the specimens were immediately fixed at 10% neutral buffered formalin and samples were taken from same areas of ventricular wall in both strains. Some of the specimens were subjected to the routine histological technique for preparations of parafine blocks. Sections of 5-7 μm thickness were obtained and stained with different histological stains such as Harris's Hematoxylin and Eosin (H&E), Periodic Acid Schiff (PAS) and Masson´s trichrome (12).

For detection of cardiac Troponin T, immunohistochemical staining of 3 μm paraffin sections with primary antibody (mouse anti-cTnT monoclonal antibody (11-13), catalog No. MA5-12960, Thermo Fisher Scientific thermofisher, Rockford, USA) at dilution of 1:1000 in 1.5% bovine serum albumin/PBS (pH 7.2). For the negative control sections, 0·01 m PBS was used instead of the primary antibody. The immunohistochemical procedures were performed according to Elewa et al. (13).

Terminology

The nomenclatures used along the course of this work were adopted using Nomina Anatomica Avium (14) and Nomina Anatomica Veterinaria (15).

Results

Macromorphology

The heart in both fowl and pigeon is situated in the cranial part of the thoracoabdominal cavity, ventral to the esophagus and tracheal bifurcation of the two lungs and just cranial to the liver (Figure 1A and B). It is directed caudoventrally, and is observed to be in a slight oblique orientation to the sternum in fowl (Figure 1C), however, a more oblique orientation, nearly parallel and resting on the sternum in pigeon (Figure 1D).

The base of the fowl heart, begins from the first intercostal space and the apex ends at the third intercostal space (Figure 1A and C). However, in pigeon, the base extends from second intercostal space and the apex reaches the sixth intercostal space, (or at the end of costal arch) (Figure 1B and D). In both fowl and pigeon, the apex is attached to the keel bone by the fibrous pericardium (sternopericardial ligaments) (Figure 1C and D). In pigeon, most of the heart is embedded between the rostral part of the right and left lobes of the liver, while, only the apex of the heart in fowl is done.

The fowl heart has an elongated cone shape with a pointed apex (Figure 1E and F). However that of pigeon, showed a flattened conical shape with a blunt apex (Figure 1G). The heart of both birds is consisted of four-chambers (two atria and two ventricles). In both studied species, the wall of the left ventricle is clearly thicker (by about five times) than the right one. The interventricular septum in both species is thicker than both right and left ventricular wall (Figure 1F and H)

Morphometric measurments:

The average heart weight in pigeon (5.02±0.07 gm) is significantly lower than that of fowl (10.97±0.22 gm), while, a higher significant difference in the average relative heart weight to body weight (RHW/BW) in pigeon (1.01±0.016) when compared to that of fowl (0.49±0.009) (Figure 2A).

The length of the cranial and caudal borders, and the transverse diameter of the fowl heart were significantly higher than that of pigeon (Figure 2B and C). These measurments were 3.21±0.05, 3.38±0.07, and 1.48±0.06 in fowl heart and were 1.7±0.02, 1.69±0.05 and 1.04±0.06 in pigeon heart, respectively. Additionally, the thickness of the right and left ventricular wall, and the interventricular septum of the fowl heart were also notably higher than that of pigeon (Figure 2B and D). These measurments were 0.59±0.04, 2.32±0.05, and 5.11±0.05 in fowl heart and were 0.36±0.02, 1.42±0.07 and 2.95±0.05 in pigeon heart, respectively.

Micromorphology

The wall of the heart in fowl and pigeon is composed of three basic layers; endocardium (tunica intima), myocardium (tunica media) and epicardium (tunica adventitia or visceral pericardium). The main thickness of the wall, particularly of the ventricle, is composed of the myocardium (Figure 3A and B). The endocardium supported by a delicate layer of collage-

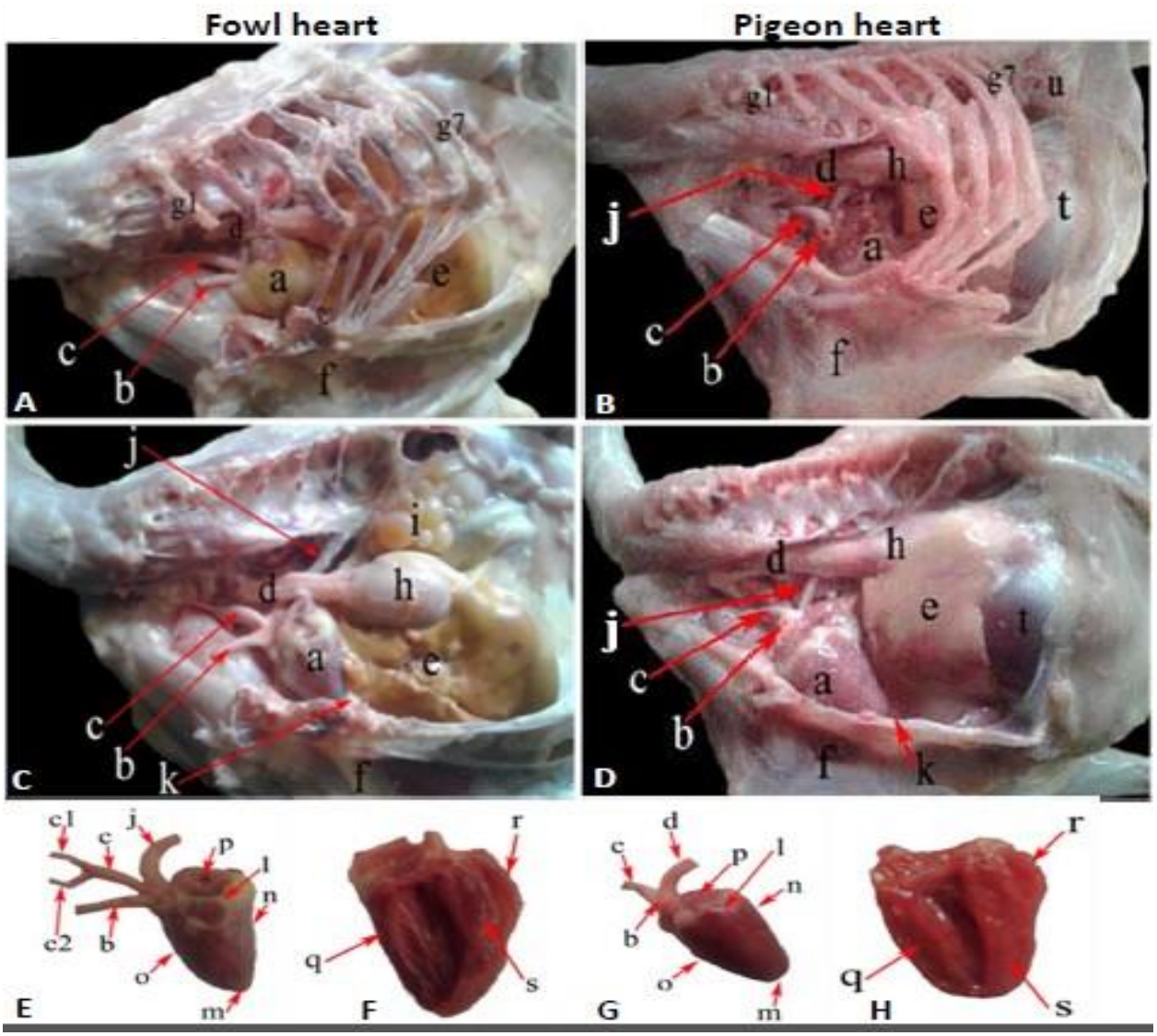

Figure 1: Photomacrographs of the fowl and pigeon hearts showing; Cor (a), Truncus pulmonalis (b), Arteria carotis communis (c), Arteria carotis communis dexter (c1) Arteria carotis communis sinister (c2) Esophagus (d), Hepar (e), Sternum (f), Costae (g1-g7), Proventriculus (h), Ovarium sinistrum (i), Aorta descendens (j) Ligamentum sternopericardiaca (K), Basis cordis (l), Apex cordis (m), Margo sinister (n), Margo dexter (o), Atrium sinistrum (p), Ventriculus sinister (q), Ventriculus dexter (r), Septum interventricular (s), Ventriculus (t), Testis (u)

nic fibres (Figure 3C), followed by a dense fibroelastic layer (subendocardial layer). The deepest layer of endocardium contains loose connective tissue and has blood vessels and nerves (Figure 3D). This latter layer is intercalated and more difficult to differentiate from the myocardial bundles in fowl, as opposed to (Figure 3E), in pigeon (Figure 3F). When compared to the fowl (Figures 3E and 4A), the deepest layer is observed to be thicker and contains numerous bundles of Purkinje fibres in the pigeon (Figure 4B). Purkinje fibres spread throughout the whole ventricular wall which are denser and thicker toward the lining side of the left ventricle when compared to the right one (Figure 4C). The myocardial fibers consist of cardiac muscle fibres running in longitudinal, circular and oblique orientations. Interestingly, the subepicardial and sub-endocardial myofibers in fowl ventricle showed longitudinal orientations (Figure 4A and C) but oblique ones were observed in pigeon (Figure 4B and D). However, the myocardial fibers in the intermediat areas revealed circular orientations in both fowl and pigeons throughout all levels (Figure 4).

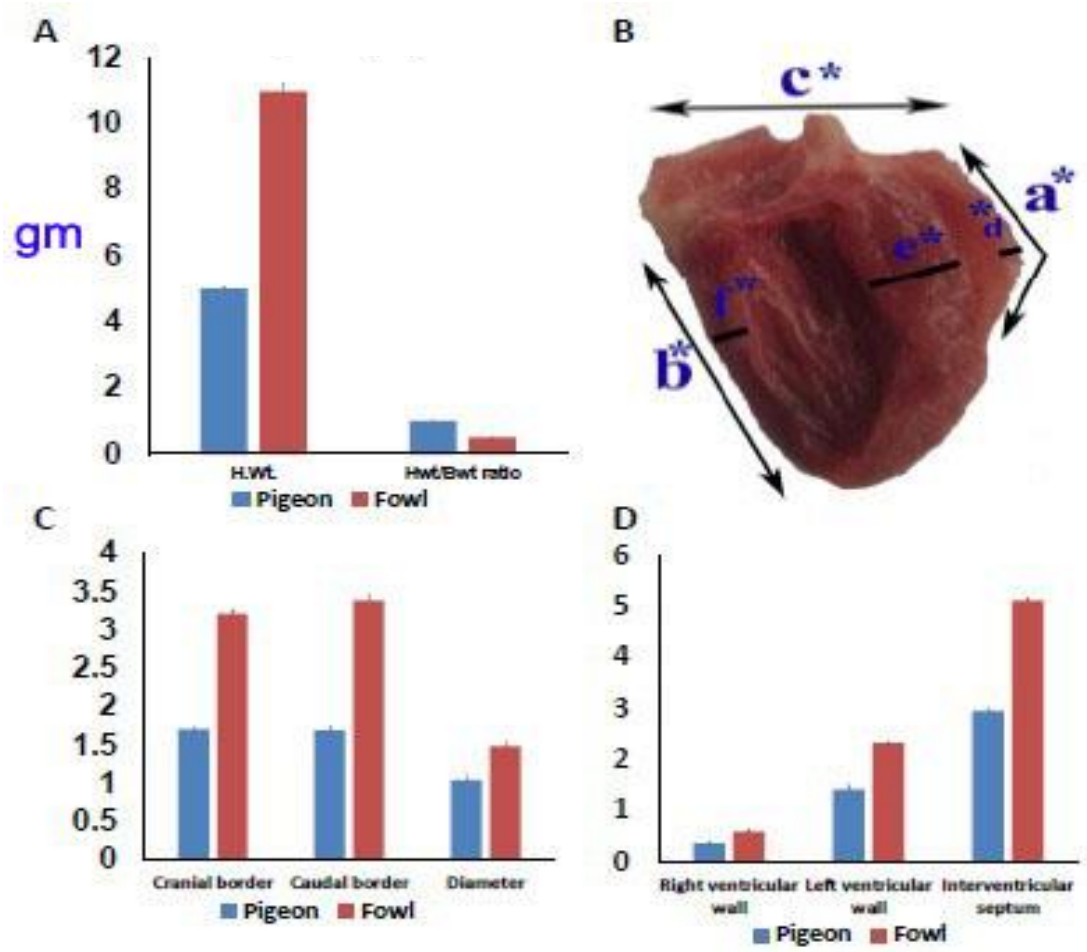

Figure 2: Morphometric measurements of fowl and pigeon hearts showing, (A) The ratio between the heart and body weight. (B) Length of cranial border (a*), Length of caudal border (b*), Diameter of heart base (c*), Thickness of right ventricles (d*), Thickness of interventricular septum (e*) and Thickness of left ventricles (f*). (C) Length of cranial and caudal borders and Diameter of heart base. (D) Thickness of right and left ventricles and thickness of interventricular septum

Masson's trichrome stained heart sections of both fowl and pigeon revealed a delicate layer of collagenic fibres surrounding the epicardium with the presence of a trivial amount of collagen fibers just around each individual myocyte, the wall of the blood vessels and Purkinje fibres (Figures 3C and D, 4E and 5A and B). The coronary blood vessels are well supplied by the myocardium present in-between its bundles, as seen most clearly in the pigeon (Figures 4E and 5A) than in the fowl (Figure 3D). H and E stained fowl heart declaired branched and symmetrically myocardial striated fibres with each other having elongated oval nuclei that run parallel with its longitudinal axis (Figure 4E and F).

Immunohistochemical staining of cTnT is located mainly in between the subendocardial layer and myocardium but it can be found in scanty amounts inside myocardial fibres in the fowl heart (Figure 5C and D). When compared to the fowl, Troponin T is more abundantly found and has a stronger reaction in the whole core of the myocardial layer of the pigeon than that of the fowl, (Figure 5E and F). The present study revealed that cardiac Troponin T found by large amounts in the heart of flying active birds, as in pigeons, and notable few amount in less active stable one as fowl. The latter indicates that there is a positive relationship between the activity of the flying birds and the amount of cardiac Troponin T in their hearts.

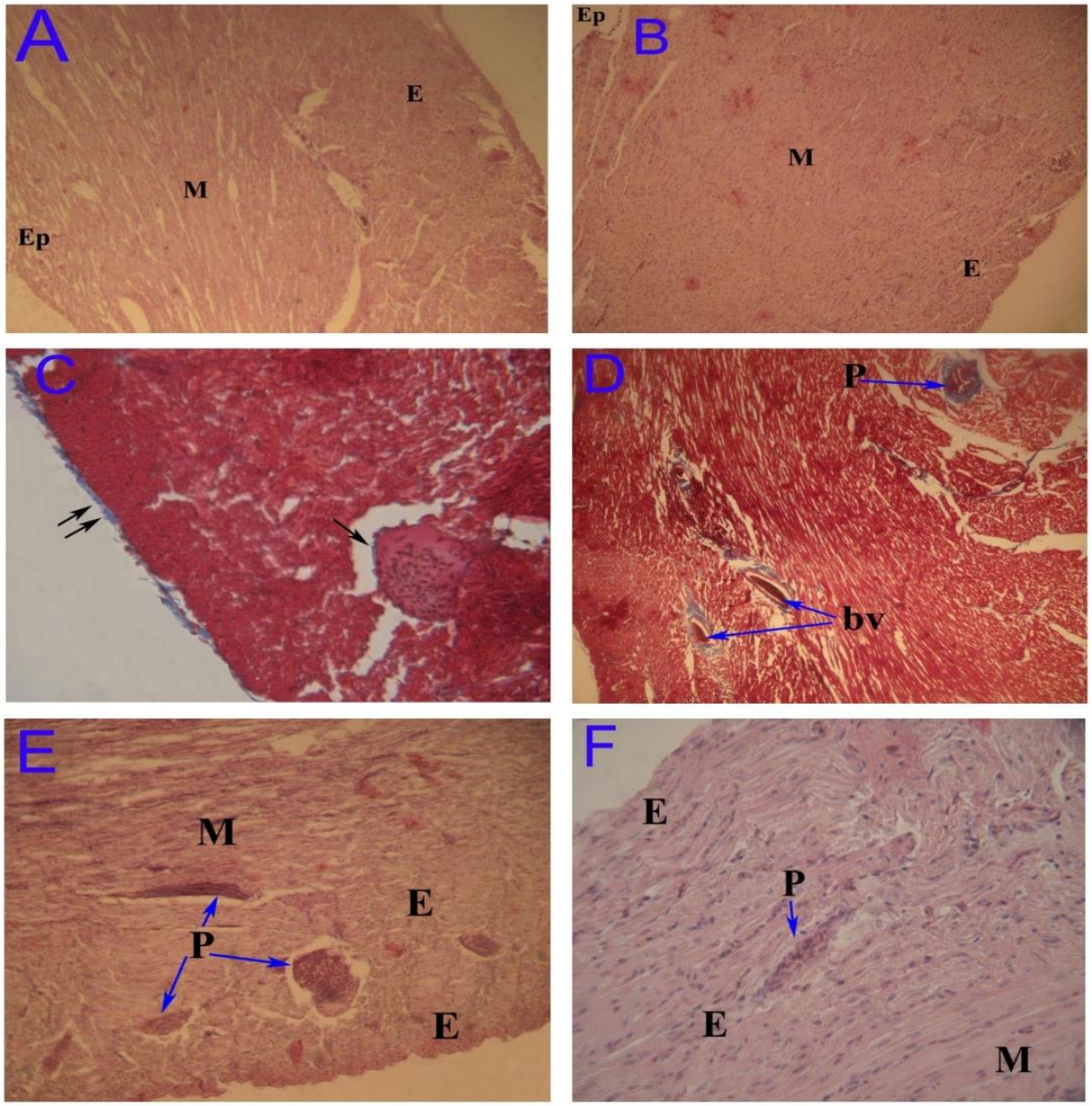

Figure 3: (A). A photomicrograph of the fowl heart (ventricular wall) showing, endocardium (E), myocardium (M) and epicardium (EP). H&E stain. X4. (B). A photomicrograph of the pigeon heart (ventricular wall) showing, endocardium (E), myocardium (M) and epicardium (EP). H&E stain. X10. (C). A photomicrograph of the fowl heart (ventricular wall) showing, a delicate layer of collagenic fibres supporting endocardium (double arrows) and mild reaction surrounding Purkinje fibres (arrow). Masson´s trichrome stain. X10. (D). A photomicrograph of the fowl heart (ventricular wall) showing, a positive reaction surrounding the wall of blood vessels (bv) and Purkinje fibers (P) in the deepest layer of endocardium. Masson´s trichrome stain. X10. (E). A photomicrograph of the fowl heart (ventricular wall) showing, endocardium (E) which intercalated and difficult to differentiate with the myocardial bundles (M). Purkinje fibers (P). H&E stain. X10. (F). A photomicrograph of the pigeon heart (ventricular wall) showing, a clear demarcation between endocardium (E) and myocardium (M). Purkinje fibres (P). H&E stain. X20

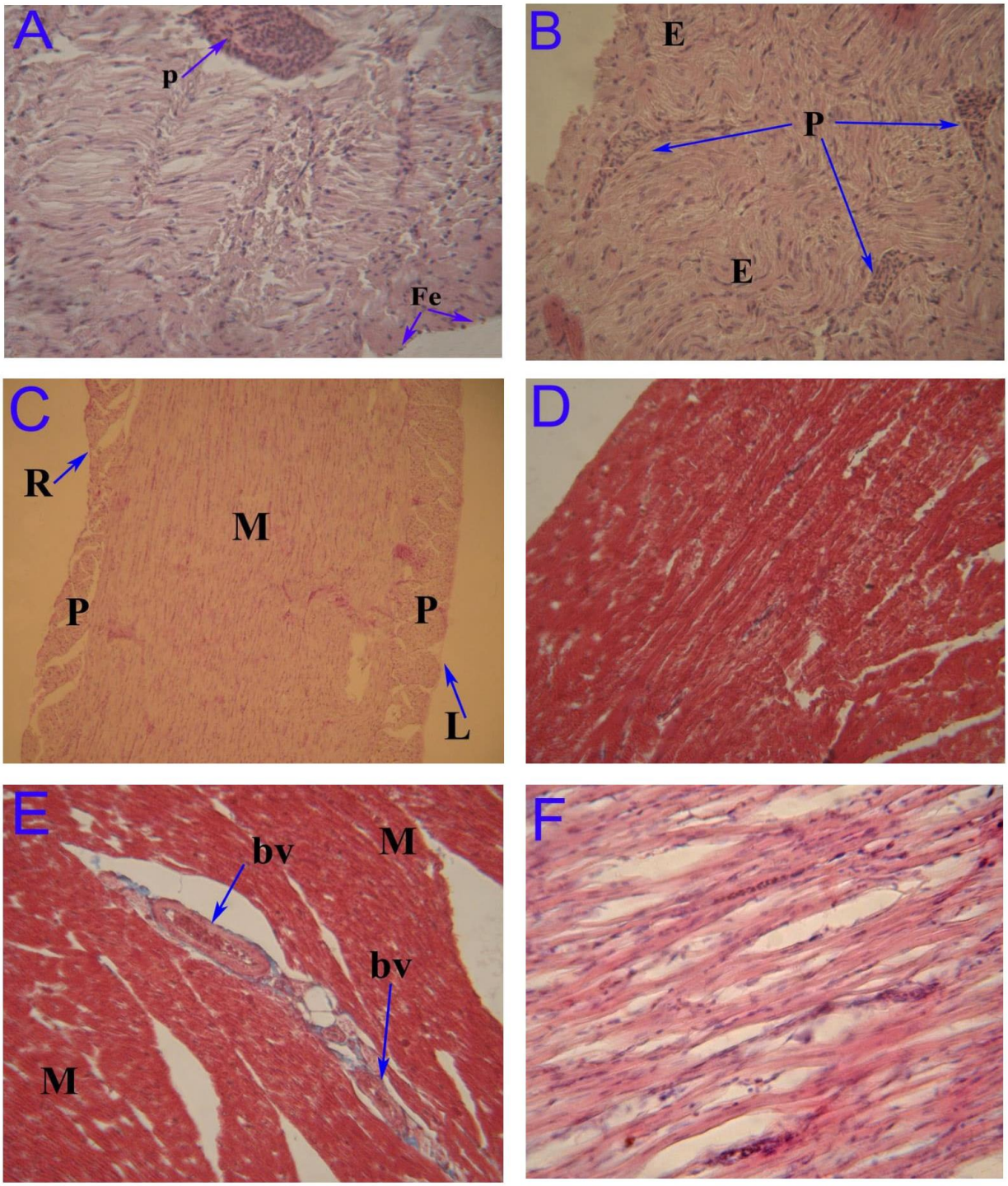

Figure 4: (A). A photomicrograph of the fowl heart (ventricular wall) showing, flattened endothelial cells (Fe) and Purkinje fibers (P). H&E stain. X20. (B). A photomicrograph of the pigeon heart (ventricular wall) showing, numerous bundles of Purkinje fibres (P) in endocardium (E). H&E stain. X20. (C). A photomicrograph of the fowl heart (interventricular septum) showing, a positive PAS reaction in the myocardial layer (M). Purkinje fibres (P), endocardium lining the left ventricle (L) and endocardium lining the right ventricle (R). PAS stain. X4. (D). A photomicrograph of the pigeon heart (ventricular wall) showing, a weak reaction between myocardial fibers. Masson's trichrome stain. X20. (E). A photomicrograph of the pigeon heart (ventricular wall) showing, numerous distributions of blood vessels having a positive trichrome reaction around its wall (bv) in myocardium (M). Masson´s trichrome stain. X40. (F). A photomicrograph of the fowl striated myocardial muscle fibers having elongated oval nuclei. H&E stain. X40

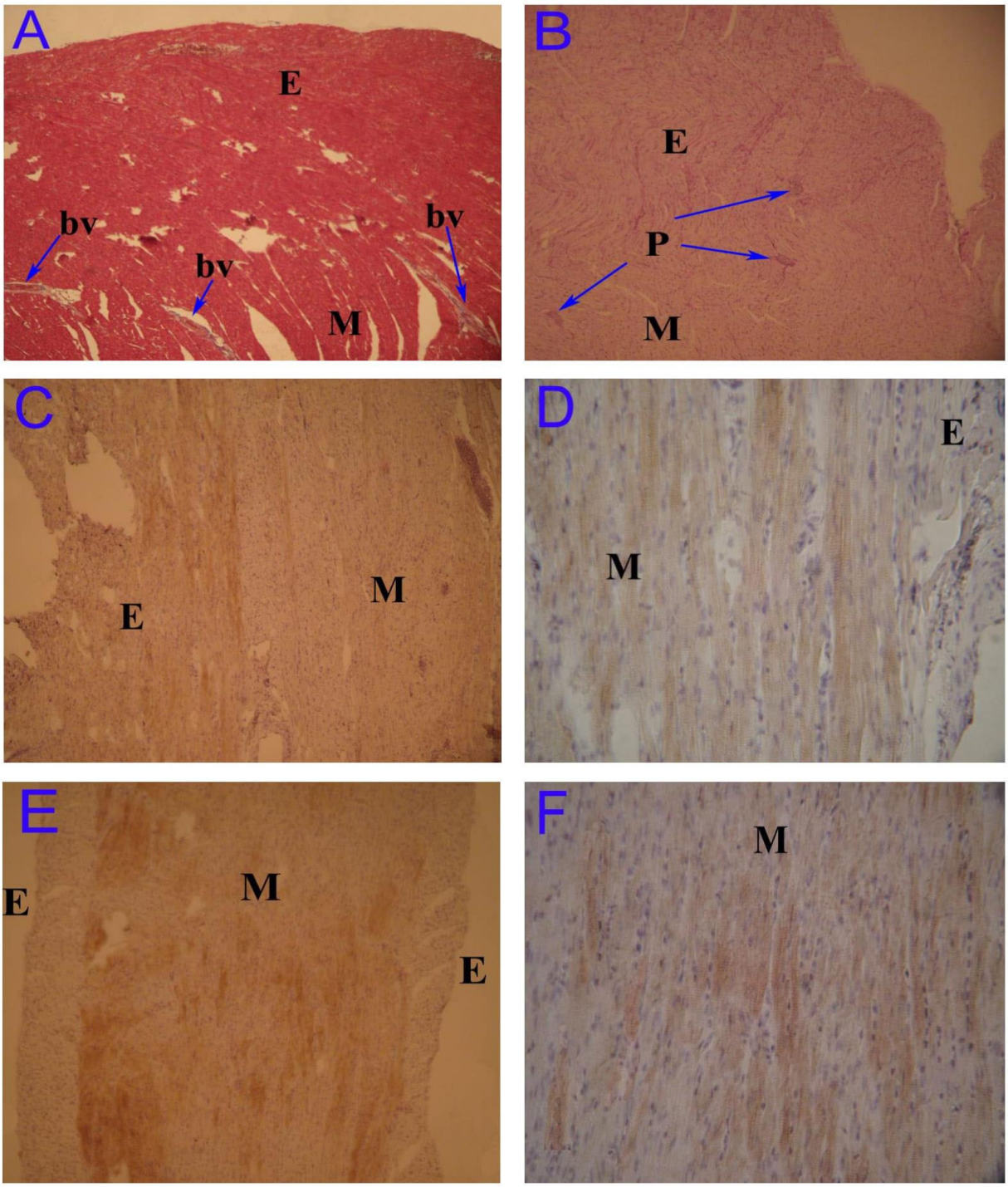

Figure 5: (A). A photomicrograph of the pigeon heart (ventricular wall) showing, numerous distributions of blood vessels (bv) in myocardium (M). Endocardium (E). Masson´s trichrome stain. X10. (B). A photomicrograph of the pigeon heart (ventricular wall) showing, distribution of Purkinje fibres (P) in endocardium (E) and myocardium (M) . PAS stain. X10. (C). A photomicrograph of the fowl heart (ventricular wall) showing, a positive troponin T reaction between the subendocardial layer (E) and myocardium (M) but scanty amount inside myocardial fibres. Immunohistochemistry. X10. (D). A photomicrograph of the fowl heart (ventricular wall) showing, a positive troponin T reaction between the subendocardial layer (E) and myocardium (M). Immunohistochemistry. X20. (E). A photomicrograph of the pigeon heart (interventricular septum) showing, a strong troponin T reaction in the whole core of myocardial layer (M). Immunohistochemistry X4. (F). A photomicrograph of the pigeon heart (ventricular wall) showing, a strong troponin T reaction in the whole core of myocardial layer. Immunohistochemistry. X20

Discussion

An efficient cardiovascular system (heart and vessels) have been reported in birds to adapt different metabolic demand including running, swimming, diving and flight. This requires that their cardiovascular system (CVS) be able to meet the demands of providing adequate delivery of oxygen to vascular beds that are taxed by extreme metabolic demands (16).

Interestingly, it has been revealed that the avian have larger hearts and a greater cardiac output when compared to body mass than mammals. This cardiac output was directly proportional to the rate of O2 consumption through pumping more blood (3). Additionally, a higher O2 consumption was reported during flying than resting condition (17,18). Although these facts, the literature regarding comparative descriptive variations of the heart among flying and non- flying birds are rare. Therefore, to clarify the possible adaptations in flying birds that allows more blood to be pumped to meet the high metabolic need associated with flight, this study characterized the gross morpho-metrical variations and cTnT expresion in pigeon and fowl as a representative of among flying and non-flying birds.

In correlation with others (8,19) bird's heart has an elongated cone like shape. Moreover, the present investigation showed that some difference do exist between fowl and pigeon hearts, as can be witnessed with the pointed apex in the fowl's heart versus the blunt one of the pigeon's. Parallel to the current study other researchers stated that birds' hearts are situated in the cranial part of thoracoabdominal cavity and are embedded between the right and left lobes of the liver (8,19,20). The conical heart of fowl and pigeons are enclosed by the pericardium whose fibrous pericardium is continued ventrally to the sternum as sternopericardial ligament. This is similar to hearts of ostrich, duck, goose and turkey (21). Minor differences exist between the position of the fowl and pigeon hearts in present work and with the research conducted by other (8). The heart base lies at around against the level of the second rib, while the apex of the heart is pointed toward the sternum and situated between the fifth and sixth ribs. Concerning the weight of the heart, our results agreed with others (22) that recorded weight of chicken and pigeon heart`s is about 0.44% and 1.0% of the body weight respectively. The fowl heart accounts for 0.5 to 1.42% of the body weight, while pigeon's heart accounts for 1.1 to 1.4% of its weight (8). The results revealed that the pigeon heart has a correspondingly greater relative weight than that of the fowl's in relation to the total body weight, which is needed for basal and energy metabolism in a decent flying bird. The thickness of the left ventricular wall is about five times greater than that of the right one which is similar to findings in the hearts of fowl and turkeys (8,19), ducks (6) and ostriches (21). This proved that the left ventricle provides the power for the high pressure systemic circulation. In agreement with Hodges (23) the wall of the heart in fowl and pigeon is distinguished into three basic layers; endocardium, myocardium and epicardium. In the present study, endocardium consists of flattened endothelial cells which is supported by collagenic fibres and dense fibrelastic layer which was correlated with that described by Hodges (23) in fowl. Meanwhile, our research clarified that, the deepest layer of pigeon endocardium is thicker and contains numerous bundles of Purkinje fibres than in fowl. Notably, the myocardium structure in current investigation is closely similar to that mentioned by Hodges (23) in fowl. In this regard, the obtained result revealed that the coronary blood vessels and Purkinje fibres are shown intensively supplied the myocardium in pigeon than fowl. Cardiac troponins (cTn) are proteins that control the calcium-mediated interaction between actin and myosin, allowing contraction at the sarcomere level. Undhad et al. (24) stated that, however the concentration of the cTn can be correlated microscopic lesion and loss of immune labeling in human myocardium damage, our study confirmed that cTnT release in healthy, active flying heart of pigeon and decrease in non active (resting) one of the fowl. In the same line with Koller (25) and Anversa et al. (26), the release of cTn is possible in irreversible damage that has been

proposed either as pathognomic of human cardiac necrosis or might reflect part of a remodeling process (physiologic substrate). The present work in complete agreement with Hessel et al. (27), which demonstrated that viable cardiomyocytes release cTn as an intact protein by a stretch related mechanism. The latter author added that, metabolic inhibition of cardiomyocytes induces parallel release of intact cTnT and their degradation products, starting only after the onset of irreversible cardiomyocyt damage (28). The cTnT is less sensitive and has less utility when used as a sole marker for detecting human myocardial necrosis (29,30). In contrast to current work, the cTnT used as cardiac biomarker for the detection of myocardial injury as acute myocardial infarction and cardiac necrosis (30,31). The current work is parallel with the findings of Middleton et al. (32) who demonstrated that the elevation and release of cardiac Troponin approved a physiological not a pathological substance. It is interesting to note that, this work observed that cTnT increase in healthy myocardium of flying birds as pigeon rather than resting one as fowl.

Conclusions

In conclusion, the difference in activity (flying) between fowl and pigeon hearts affects the expression of cardiac Troponin T. Furthermore, the anatomical and histological studies of the two birds clarified some variation of the layers of their hearts. The immunohistochemical distribution of Troponin T is intensively found and has strong reaction in the whole core of myocardial layer of flying active birds as in pigeon than that of stable one as fowl. This indicates that there is a positive relationship between flying birds (pigeons) and the presence of Troponin T in their hearts.

Conflicts of interest

The authors declare no conflict of interest

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