Chapter 9

Avian Cardiovascular Disease Characteristics, Causes and Genomics

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Additional information is available at the end of the chapter

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Abstract

Cardiovascular disease is common in avian species and increasing commercial economic losses and demand for healthcare in the household/smallholding veterinary sector has resulted in increased research into these disorders. This in turn has highlighted the importance of breeding, genetic testing and possibilities for future prognostic and diagnostic testing. Research into avian cardiovascular genetics has rapidly accelerated. Previously much work was undertaken in mammals with information extrapolated and transferred to birds. Birds have also been used to model cardiovascular disease and therefore knowledge has become enriched due to this endeavour. Increasingly, the avian genome is being analysed in its own right. This work is assisted by the growing number of avian genomes being published. In 2015, Nature published news on the 'Bird 10K' project, which aims to sequence 10,500 extant bird species. By 2018, the Avian Genomes Consortium had published the sequences of 45 species/34 orders. This review investigates a range of avian cardiovascular disorders in order to highlight their pathologies, epidemiology and genetics in addition to avian models of heart disease. With the availability of more reference genomes, increases in the number and magnitude of avian studies and more advanced technologies, the genetics behind avian cardiovascular disorders is being unravelled.

Keywords: avian, cardiovascular disease, genetics, pathology, epidemiology

1. Introduction to the cardiovascular system

Acute heart failure and chronic heart failure are of great concern in avian species. Heart failure represents the main cause of morbidity and mortality in broiler flocks after infectious

diseases [1]. The act of flying means that birds have a higher metabolic demand than mammals of a similar size. This means they have a higher cardiac output which is achieved by having larger hearts and pumping more blood per unit time. Additionally the cardiovascular system has become adapted in birds that dive, thus preventing inappropriate responses to submersion asphyxia [2]. Knowledge of the anatomical structure, histology and function of the avian heart are crucial in understanding how not only the healthy heart functions, but also in understanding the abnormal heart.

1.1. Structure and function of the avian heart

Similar to mammals, birds have a four-chambered heart surrounded by pericardium. The chambers effectively function as two separate pump systems to circulate blood around the body, with cardiac valves ensuring unidirectional flow of blood through the chambers and blood vessels [3]. The mammalian heart lies just into the left hand side of the thoracic cavity, whilst the avian heart lies slightly to the right of the midline [4, 5].

The heart itself it made up of multiple structural components. The cardiac muscle acts to contract rhythmically via coordination of the cardiomyocytes, potentiating movement of blood around the body [5]. The cardiac fibrous skeleton called the annulus fibrosus comprises of four connective tissue rings acting to separate the atria and the ventricles. The septum separates the heart into right and left halves, with the left side sending blood to the systemic circulation and the right side routing blood to the pulmonary circulation [6]. The septum itself contains a conduction system for the initiation and propagation of action potentials, allowing stimulation and consequent contraction of the myocardium [3, 5]. Finally, the heart is primarily supplied by two coronary arteries branching from the ascending aorta, whilst cardiac veins drain blood from the heart tissue into the right atrium, via the coronary sinus. The heart is also subdivided into atria cranially and ventricles caudally (see **Figure 1** for the histology of these structures). The atria receive the blood from the veins and then pump the blood through to the ventricles. Thinner walls are found in the atria as the blood is only pumped to the ventricles, which does not require as much muscle as that required to pump blood to the entire body [3]. Similarly, the right ventricle has a thinner wall than the left as blood is only pumped to the lungs, whereas the left ventricle pumps blood around the body [7].

1.2. Histology of the avian heart

The heart is made up of three distinct layers. The outermost layer is the epicardium, which is formed from a layer of mesothelial cells overlying adipose and connective tissue [8]. The epicardium acts as a protective layer, and contains nerves and blood vessels which supply the heart tissue [5]. The middle layer is the myocardium, which forms the greatest proportion of the heart tissue and is composed mainly from myocytes [9]. The innermost layer (endocardium) is composed of connective tissue, endothelium and smooth muscle cells and forms a protective lining over the valves and heart chambers [3].

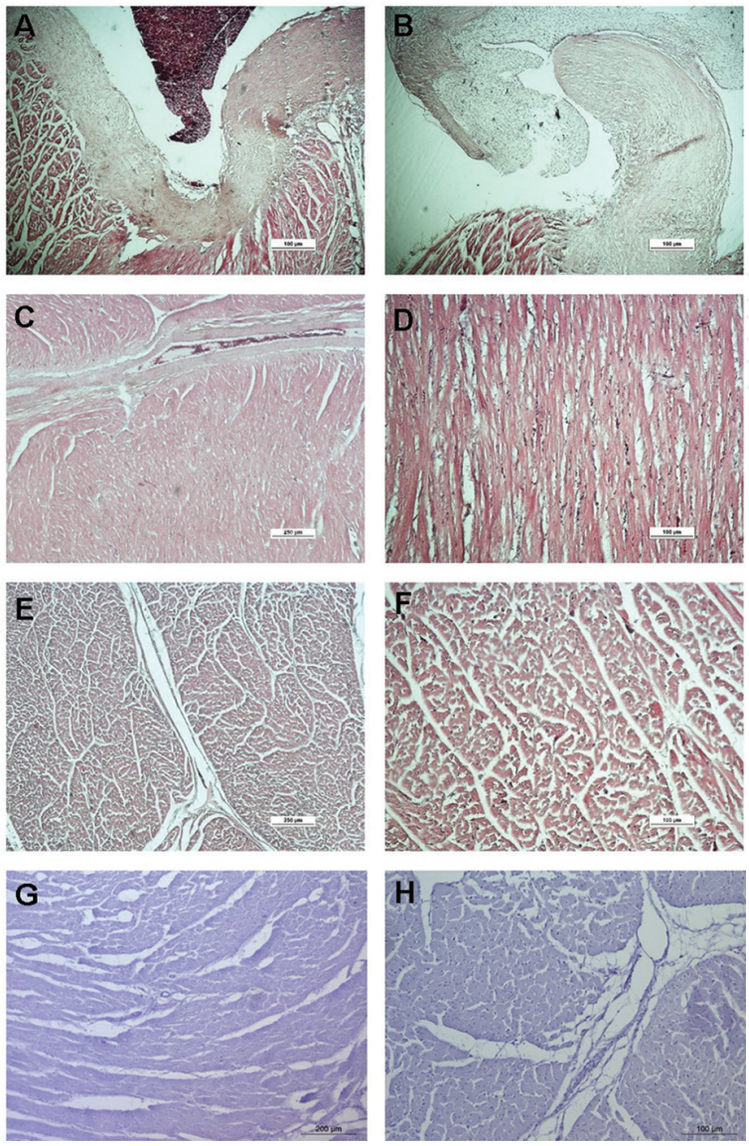

Figure 1. Chicken atrioventricular (AV) heart valve (A, B), ventricle longitudinal (C, D) and transverse (E, F) views, and the atrium (G, H). Scale bars represent 100 μm (A–C, E, G), 200 μm (H) and 250 μm (D, F). The heart histologically consists of three layers, which resemble their structure in blood vessels. The inside layer is endocardium, also covering valves. In the avian heart the right and left AV valves are in close proximity to the conduction system. The right AV valve is a single, spiral plane of myocardium, in remarkable contrast to the fibrous structure characteristic of the mammalian tricuspid valve. The vast inner part is myocardium, composed of cardiac muscle, specialised conductive tissue, valves, blood vessels and connective tissue. Cardiac muscle, the myocardium, consists of cross-striated muscle cells, cardiomyocytes, with one centrally placed nucleus. Nuclei are oval, rather pale and located centrally in the muscle cell which is 10–15 μm wide. Cardiac muscle does not contain cells equivalent to the satellite cells of skeletal muscle. Cardiac muscle cells often branch at acute angles and are connected to each other by specialisations of the cell membrane in the region of the intercalated discs. The most outer part is epicardium, consisting from mesothelial cells, submesothelial layer of connective tissue, adipose cells, nerves and vessels.

The cardiac muscle, blood vessels and endothelial lining are all derived from embryonic mesoderm, one of three primary germ layers of the early embryo [5]. The heart in turn is composed of several different cell types, all of which have a role in the function and maintenance

of the heart tissue [8]. Cardiac muscle is striated due to the arrangement of thick and thin myofilaments within the myocytes [10]. Thin filaments are composed of actin and are 6-8 nm in diameter, whilst thick filaments are composed of myosin II proteins and are around 15 nm in diameter [5, 10]. Myofilaments are organised within the sarcomere, which acts to form a single contractile unit [5]. The myocytes have central nuclei, unlike skeletal muscle fibres which have peripheral nuclei [11]. They also contain multiple mitochondria and glycogen granules, allowing for store and release of energy [5]. Cardiomyocytes are cylindrical and form a functionally continuous muscle fibre and may exhibit some branching. Unlike skeletal muscle, cardiac muscle fibres contain intercalated discs, which histologically appear as straight bands between opposing cells, but are not always visible under haematoxylin and eosin staining [5, 12]. Intercalated discs are regions containing gap junctions, which allow the transfer of ions between cardiomyocytes [13]. This enables cardiac muscle to act as a functional syncytium, by ensuring coordinated contraction of the muscle fibres [3, 12].

The endothelium is made up of simple, squamous epithelial cells known as endothelial cells, which encircle the lumen of blood vessels [14]. The cells respond to stimuli by interacting with the blood and connective tissue, and act to control functions such as permeability and blood flow [5]. Smooth muscle is derived from lateral plate mesoderm and is present in tissues throughout the body [5, 15]. Within the heart, smooth muscle is located in walls of the vasculature [16]. Smooth muscle tissue contains actin and myosin filaments similar to those seen in skeletal and cardiac muscle, however these fibres are not organised in sarcomeres and therefore do not demonstrate the same striated pattern. Smooth muscle cells are long with tapered ends and contain central nuclei. The cells are interconnected with gap junctions, enabling contraction of the muscle as a single sheet. This allows for controlled contraction or dilation of the blood vessel lumen [3, 5]. The blood vessels have the usual tunica intima (endothelial cells), media (smooth muscle cells) and externa (collagenous and elastic connective tissue) layers [5, 8].

One of the main differences between avian and mammalian hearts is that the right atrioventricular valve is muscular and does not contain chordae tendineae in birds. Also, the left atrioventricular valve is tricuspid in birds but bicuspid in mammals [4]. The physiology of how the heart works, however, is largely similar in mammals and birds. The avian vascular system is also similar to mammals apart from a shunt between the left and right jugular vein and an anastomosis between the femoral and ischiadic veins. In addition, when energy demand is high, blood can bypass the kidney [17]. It is also worth noting that avian erythrocytes are nucleated whereas mammalian erythrocytes are not [18].

2. Avian cardiovascular genetics

Cardiovascular disease has usually been reported to be much more common in pet birds compared to wild birds and it is thought that this is due to the longer life span of pet birds but may also be linked to exercise and diet. This review gives an overview of what has been studied and published about cardiovascular diseases in avian species and how this relates to clinical examination, management, cardiovascular genetics and cardiac development. In addition, the bird has proved to be a very informative model when undertaking cardiovascular research in

general. During development the heart is located outside of the thorax which makes it readily accessible. Therefore, avian species as research models are also discussed.

With over 10,500 bird species to choose from, prioritising the species is difficult. By 2018, 45 species/34 orders had been sequenced by the Avian Genomes Consortium under the 'Bird 10 K project [19, 20]. Although some birds such as the Red Jungle Fowl have been available since 2004, even this genome is under constant scrutiny and is updated to ensure accuracy. Since the genome was first published in 2004 there have been many improvements and complete genome builds with the latest version Gallus_gallus-5.0 being published in 2017 [21]. The arrival of next-generation sequencing presented more methods of identifying and developing what is already known, and what can be known about the genomes of each bird. In a recent publication the use of sequencing tools and bioinformatics is highlighted in relation to the ostrich genome [22]. The complexity of any genome, including the avian species is still being unravelled and the technologies used to undertake both the sequencing and bioinformatics are rapidly evolving and expanding areas. A number of innovative methods have also been used to edit the avian genome and these technologies are advancing greatly. They range from culturing and modifying primordial germ cells (PGCs) and direct in vivo transfection of PGCs, morpholino, gene targeting, TALEN, homology directed repair (HDR) and CRISP-Cas9 knockdown and knockout tools, direct injection and sperm transfection assisted gene editing (STAGE), in addition to combinations of these methods [23].

Naturally these techniques are vital in furthering our understanding of medical problems such as cardiac disorders, but they have been used for a wide range of observations. These include understanding genome evolution and ancestry, comprehending avian parasites, bacteria and viruses and is even helping towards understanding bird migration [24–27].

2.1. Difficulties in studying avian cardiovascular genetics

Despite the high levels of heart disease in many flocks of commercial birds, understanding avian cardiovascular disorders is not always easy. There is difficulty when examining a bird for cardiovascular defects. One reason for this is that birds do not have a palpable pulse so more thorough diagnostic techniques such as radiology, echocardiography, electrocardiography and post mortem investigation often need to be considered [28]. It is possible, however, to auscultate the heart but this would give little information that would help form a diagnosis [29]. Another issue is that signs of cardiovascular disease are often non-specific and could be caused by an overlying problem. Some general signs include lethargy, dyspnoea and exercise intolerance. It is also important to understand the bird's history, diet, environment and reproductive ability [28].

In many species, information relating the rate of cardiovascular disease has been investigated in both wild and captive populations and in humans. The number of papers reporting similar information is comparatively sparse in avian species. The incidence of congestive heart failure in a study of 269 Psittaciformes was as high as 9.7% [30]. Of the psittacines observed with cardiac problems, 58% had coronary heart failure considered as the cause of death, 10 with right ventricular or biventricular failure and the remaining 5 with left ventricular failure. The remaining 42% of birds affected by cardiac disease had lesions which were considered incidental or secondary [30]. Moreover, in a second study where 107 captive Psittaciformes (budgerigars (*Melopsittacus undulatus)* and Australian king parrots (*Alisterus scapularis*)) were compared,

36% had gross lesions of the heart and/or major blood vessels [31]. Interestingly 99% of the birds examined had some degree of pathological changes to blood vessels and/or the heart with 6% of the birds showed pericardial effusion, 15% showed serofibrinous coating on the pericardium and other 15% showed hypertrophy or dilatation of the ventricular myocardium [31]. It is interesting to note that these relatively long lived but captive birds have a high incidence of cardiovascular disease. The kakapo (*Strigops habroptilus*), a flightless member of the parrot family, can live for over 100 years and is kept under very close scientific and veterinary care due to the limited number alive. In addition, the kakapo is technically a wild population but benefits from extra support and care from humans due to its near extinction status, but without being fully captive. The cardiovascular system has not been specifically highlighted as a particular problem in the kakapo despite inbreeding and close monitoring. This is potentially because the data has not yet been published but it may also indicate that the data differs from other parrots, differing from the previously mentioned parrot studies. This could indicate that breed genetics play an important role when investigating cardiovascular disease in avian species.

Naturally the other difficulty with investigating cardiovascular disease in avian species is the infrequency with which most wild populations of birds are seen by veterinary surgeons/researchers. A number of studies into cardiovascular conditions have been carried out over the years but this number declines when looking at the genetics behind each disorder. Frequently the larger cardiovascular studies are carried out on commercial flocks. These flocks hold significant economic value worldwide but breeding genetics may also be part of the cause of the high incidence rates of cardiovascular conditions observed, therefore their findings may not be as applicable to wild populations and other avian species. In addition, although commercial birds hold significant value in the food industry, the value of each bird itself can be very low, therefore making individual veterinary care difficult to justify to companies/owners.

In addition to looking at captive and wild populations of birds in order to understand cardiovascular disease, chick embryos are commonly used in research because they are relatively inexpensive in comparison to larger mammalian models and have a four-chambered heart, similar to humans. Avian species also generally have a short life-span/rapid development phases so the whole gestation can be studied in a shorter period of time than in other species [32]. Chick heart models have been used often to study the outflow tract of the developing heart for example. This review aims to look at cardiovascular disorders seen in avian species and understand some of the models of heart disease. The review highlights some of the associations of cardiac conditions with environmental factors and links and associations to genetic causes.

2.2. Congenital heart conditions

There have been reported cases of congenital cardiac defects involving a wide range of avian species. Some defects appear to be genetically linked whilst others appear to result from environmental conditions *in ovo*. There is a delicate interplay that occurs whilst the embryonic heart develops, involving both environmental, *in ovo* conditions, and genetic factors. These all have an impact on how the cardiovascular system develops. Research into human congenital cardiac defects often relies upon data obtained from chick cardiac models, where chick embryos are subjected to a varying environmental temperatures, pressures, haemodynamics and gene manipulations [33]. An example was the work on vitamin A deficiency in the

developing chick showing that it is required for specification of cardiovascular tissues and regulation of a number of key genes including GATA-4 and heart asymmetry genes [34].

Congenital heart defects within avian species have also been associated with aluminium toxicity [35]. Ventricular septal defects and ventricular myocardial defects occurred when chick embryos were dosed with aluminium. Changes in the vitelline circulation were observed in the embryos injected with aluminium, and this altered circulation has been shown to have a significant impact on the physical development of macroscopic cardiac structures [35]. External deformities including changes to cardiac surface structure, cardiac shape and changes in ventricular wall thickness were found to occur in passerine bird species exposed to environments contaminated with polychlorinated biphenyls [36]. These deformities did not result from an *in ovo* contamination, but occurred in birds born and raised in heavily contaminated environments.

Another documented cause of heart failure reported in avian species is valvular dysplasia [37]. Valvular dysplasia describes a valve which is distorted, inflexible and often fused in some way to the cardiac wall. This inhibits or completely destroys normal function of the valve, affecting blood flow through the heart chambers. Turbulent blood flow around the distorted valve can lead to further wear and damage to the valvular tissue. A documented case of this occurred in a young captive African penguin (*Spheniscus demersus*) presenting with exercise intolerance and open mouth breathing. Upon necropsy it was found that the juvenile had congenital right atrioventricular valvular dysplasia, and as a result right atrial dilatation in addition to ventricular dilation and hypertrophy [37], although no genetic basis was suggested.

Hypoxia in the chick embryo has also been linked with cardiac disease. Restricting oxygen to the chick embryo has been shown to induce left ventricle dilatation and the breakdown of cardiomyocytes. Adult chickens that have been exposed to this hypoxia have also been shown to display cardiomyopathies, most significantly left ventricular dilatation in later life and an increase in myocardial collagen. These gross changes result in impaired contractility [38]. Most studies agree that altered haemodynamics during development can lead to heart defects. Studies have been undertaken that surgically alter blood flow in an embryonic model to resemble cardiac defects seen in newborn babies. Outflow tract banding is commonly used in chick embryos around the heart outflow tract to stimulate these cardiac defects. The heart outflow tract is important because it will eventually form the intraventricular septum and semilunar valves. Therefore, changes to the heart outflow tract should help understanding of congenital defects that involve the valves and septum [39]. One study investigated how collagen content varies with and without outflow tract banding. A subdivision atlas approach was used to visualise changes in levels of collagen. This approach allows more specific spatial recognition of proteins and could be expanded on in the future to go beyond the heart outflow tract [39]. Another study used outflow tract banding to assess how haemodynamic changes effect heart tissue. It was found that the tightness of the band affected the changes seen. As tightness increased, the prevalence of cardiac defects also increased [40]. Although other studies showed that altered blood flow during development can lead to heart disease, this was the first study to assess how the degree of band tightness has an effect on the clinical presentation. Techniques such as optical pacing have also been used to increase the heart rate, thus tiring the heart, resulting in cardiac regurgitant flow [41]. The result in developing quail hearts was a number of congenital heart disease outcomes including endothelial cushion defects, valve

and septal defects and hypoplastic ventricles [41]. Naturally, altered blood flow can be due to genetics or environmental conditions, for example teratogens.

Current knowledge about the role of cardiac neural crest cells during the development of the cardiovascular system has mostly come from using quail-chick chimeras. Ablation of cardiac neural crest cells in chick embryos caused several cardiovascular defects such as abnormal artery patterning, abnormal myocardial function and an abnormal cardiac outflow tract [42]. Observing the effects of abnormal blood flow on cells will help target treatments of developmental conditions.

2.3. Ventricular septal defects

A ventricular septal defect occurs when the wall in between the left and right ventricle does not fully develop, resulting in a left to right blood shunt. Often associated with this defect is ventricular hypertrophy which later develops in order to maintain cardiac output [43]. There have been a few notable reported exotic cases, in particular a captive houbara bustard (*Chlamydotis undulata*) and Humboldt penguin. The bustard in question died at 6 months old, presenting with retarded growth; upon necropsy it was found the heart was twice the anticipated size and a ventricular septal defect was located [43]. In this case it was believed to be a genetically linked defect, supporting the theory that avian species can develop defects both as a result of *in ovo* factors and also genetic factors [43]. In the incidence of the penguin, a ventricular septal defect was detected after the Humbolt similarly presented with retarded growth. There have been reports of ventricular septal defects in two other penguins, and research suggests this defect is also the most common congenital abnormality found in caged birds [44].

Alterations in cardiac blood flow can have dramatic implications to the developing embryonic cardiac tissue. In chick embryos, studies have indicated that ligation of the vitelline vein returning blood to the chick heart, resulted in 10–72% of tested embryos sustaining a ventricular septal defect [45–47]. Left arterial ligation restricts blood entering the ventricle, therefore decreasing cardiac load on the heart. This resulted in 25% of embryos sustaining a mild ventricular septal defect [48]. Outflow tract banding results in constriction of the tract allowing blood to leave the heart, therefore causing increased cardiac pressure. This alteration resulted in 100% ventricular septal defect occurring in the embryos [48]. These studies mimic physical defects and their resulting haemodynamic changes which can occur in developing chick embryos, and how these changes impact upon the final cardiac structure. The administration of retinoic acid to chick embryos has also been associated with the development of ventricular septal defects. When a solution of retinoic acid was applied to 41 different chick embryos all at the same stage of development, 11 of these embryos later developed a ventricular septal defect [49].

2.4. Right ventricular failure

Right sided heart failure is a problem particularly relevant within populations of broiler chickens. Fast growing broiler chickens cannot meet their oxygen demand as easily as a slower growing chicken, due to the mismatch between body mass and cardiac output [50]. When put in stressful environments the proportionally small broiler heart is often unable to cope, leading to cardiac failure, seen in cases of heat stress within broiler flocks [51]. 27% of

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fast growing broiler chickens in one study were found to have arrhythmias, compared to 1% in slower growing breeds [51]. One cause of heart failure results from the upregulation of matrix metalloproteinases (MMPs) stimulated by cardiac stress, which cause collagen degradation and ultimately right ventricular dilation [50]. One 2017 study examined the impact cold temperatures had on broiler chicken hearts, and found that low temperatures activated MMP's leading to right ventricular dilation and heart failure [50]. Therefore, either extremes of temperature have been documented to induce cardiac failure in broiler species, a fact which has serious economic consequences.

The impact of diet on the risk of heart failure in broiler chickens has been much investigated, in order to find the optimum nutritional planes to maximise growth whilst reducing cardiac failure risk. It has been found that an increased salt intake resulted in right ventricular failure and mortality. The elevated salt levels resulted in an increased blood volume, leading directly to ventricular failure, and in the broiler chickens receiving the higher concentration salt solution the mortality rates reached 50% [52]. The same was not found in the slower growing white leghorn species. This research indicates that dietary control and components plays a pivotal role in maintaining the health of broiler chickens and reducing the risk of heart failure and that breed genetics may play a vital role in susceptibility. Broiler chickens are also shown to be more susceptible to blood volume expansion and consequently heart failure, potentially due to a reduced salt excretory capacity when compared with the white leghorn chickens [52]. Another study into dietary composition concluded that n-3 fatty acid supplements would act to increase the circulatory level of nitrous oxides, in turn stimulating vasodilation and therefore reducing mortality from cardiac failure [53].

The altitude that chickens are raised at also has a marked effect on their risk of developing right ventricular failure. Multiple studies have shown that high altitudes can result in a disorder called pulmonary arterial hypertension. This is due to hypoxia at high altitudes which results in compensatory hypertension, and as the chickens grow larger it becomes harder for cardiac output to meet the demand. Eventually the pulmonary arterial hypertension can develop into right ventricular dilation, and eventually cardiac failure. This is the most common cause of high altitude broiler flock death [53]. Interestingly low altitude may similarly affect the heart. In a study looking at right ventricular enlargement and ascites in broiler chickens it was thought that the condition resulted from the birds being raised at low altitudes, although no conclusion was drawn as to the true cause of the conditions. Right heart failure has been associated with consumption of Furazolidone, sodium chloride and P-dioxin, however in this study none of these compounds had been ingested. Therefore further studies regarding the impact on altitude on blood volume and heart failure in broiler chickens would be of economic and scientific interest [54]. To conclude, diet, environmental temperature and altitude, exposure to toxins and genetics all play a role in the prevalence of right ventricular failure in broiler chickens, and all factors must be considered the prevention and treatment of this condition.

2.5. Atherosclerosis

Atherosclerosis is a condition that has been reported to affect a variety of avian species. It is particularly recognised within parrot species, with a study carried out in 2013 finding the prevalence of advanced atherosclerotic cases to be 6.8% within a population of 7683 parrots

[55]. The authors noted that the incidence of advanced atherosclerotic lesions was similar to that in humans aged 45–75 years old and that advancing age was an important determinant in the birds, as with humans. Interestingly, in other smaller studies, the incidence rate varies from 1.9 to 92.4% but the authors highlighted that only five psittacine genera were studied from approximately 84. Long before these recent studies in parrots, the condition was known to naturally occur in birds such as chickens [56] and up to 90% of birds from captive exotic avian orders [57] and even 100% of White Carneau pigeons studied which decreased to virtually no cases in Slow Racer pigeons [58, 59]. The White Carneau Pigeon is also used as a clinical model to study the earliest stage of the disease within human populations [60]. A genetic predisposition in this species has been investigated and is found to be linked to a single gene defect which results in the build-up of unoxidised fatty acids. A polymorphism within pro-alpha-2(1) collagen was linked to atherosclerosis giving an autosomal recessive inheritance pattern [61]. Although dietary intake will also likely play a large role in whether the birds acquire lesions [62], when White Carneau and Slow Racer pigeons were compared on a similar diet, the Slow Racer pigeons were naturally resistant to atherosclerosis [63]. More recently is has been indicated that avian species have become less favourable as a model due to the varying artery size across avian species, instead pig and mouse models have grown in favour [64].

Atherosclerosis occurs when there is accumulation of fatty lipids and cholesterol within arteries. This initial deposition can lead to fibrosis and calcification, causing occlusion and narrowing of the blood vessel [65]. The disease itself is chronic and pro-inflammatory, affecting the lining of blood vessels, and can result in strokes, arterial disease and coronary artery disease. In a case involving a male white cockatoo, atherosclerosis was found to have caused an aneurysm of the right coronary artery. It was believed that the patients diet high in fats and low in vitamin A may have led to the initial atherosclerosis, which upon post mortem was found to be widespread in vessels across the patient's body [65]. A similar case was also reported involving a female macaw, presenting with dyspnoea, upon necropsy it was found she suffered with severe atherosclerosis of the aorta and brachiocephalic arteries [66]. Prevalence of the disease within the parrot population has been shown to increase with age and is more prevalent within females [55] [67]. A comparison of observed prevalence trends with cholesterol level revealed that a systemic cholesterol increase with age is associated with an increase in the prevalence of atherosclerosis. Differences in genera, fat nutritional requirements and lipid metabolism were also found to impact upon cholesterol levels and atherosclerotic risk [67]. Male parrots in one study were found to have only 69% of the atherosclerotic risk that females have [55]. This heightened female risk has been hypothesised to be attributed to reproductive dysfunctional diseases, although further and more specific studies are required to further investigate this [55].

Atherosclerotic lesions in avian species are comparable to human lesions and are classified in a similar method to that used in human medicine. Therefore avian models have been used in the past as clinical models to study the formation, development and treatment of human atherosclerosis disease [55]. A 2015 study into the impact that cholesterol and triglycerides have on the supra aortic trunk involved examining the effect that the diet of white leghorn chickens had upon the histological appearance of their aorta. Atherosclerotic lesions were classified similarly to the system used by the American heart association [68]. High fat and high cholesterol diets increased the prevalence of severe lesions within the supra-aortic trunk,

and decreased the age at which the lesions occurred, i.e. lesions developed quicker in chickens with a higher fat diet, with severe lesions seen in chickens as young as 6 months [68]. This clinical model has been applied to increase understanding of disease development within the human population, due to the comparable nature of the disease process in avian species and humans. The same clinical model involving chickens also helped to study the effectiveness of treatment of aortic lesions with atorvastatin therapy. It was believed that this regression of the severe lesions following treatment shown in the chicken population may also be replicated within the human population [68].

In human medicine aneurysms are a commonly reported condition, often resulting from atherosclerosis. However there have been very few reports of this condition in avian species. An aneurysm describes an outpouching, or thin or damaged section of a vessel wall. They can result from a congenital defect, be dietary, or result from bacterial infection damage. There is one documented congenital case of it occurring in a pigeon, in this incidence the pigeon died of unrelated circumstances; however, upon necropsy was found to have a right ventricular aneurysm. This would indicate that during development there was focalised area where the heart tissue failed to correctly develop. At the time of its reporting this was the first documented congenital avian aneurysm [69]. This is interesting to note given the high levels of similarity between humans and birds in the disease otherwise.

2.6. Hypoplastic left heart syndrome

Hypoplastic left heart syndrome (HLHS) has been investigated using chick embryonic models in several studies. It is often characterised by abnormal fetal development of the left hand side of the heart and can result in a smaller/under developed left ventricle, mitral or aortic valves not forming or being small, the ascending portion of the aorta being small/underdeveloped and atrial septal defects (hole in the heart) are common in affected offspring. Left atrial ligation is used to shunt the flow of blood from the developing left ventricle to the right ventricle. This produces heart models that are phenotypically the same as hypoplastic left heart syndrome [70]. The prognosis for hypoplastic left heart syndrome in humans is poor and several operations are required. The use of stem cells to differentiate into cardiomyocytes could remove the need for operations and postnatal care but needs further investigation.

Tissue hypoxia is necessary for normal development as it stimulates signalling pathways that lead to the development of the normal myocardial architecture [71]. If the developing tissue becomes too hypoxic, however, it can lead to defects. Many studies support that hypoxia leads to an increase in fibre due to increased expression of extracellular matrix proteins [72, 73]. There is speculation, however, about the order in which the defects develop. Most literature supports that it is altered haemodynamics that lead to hypoxia that leads to fibrosis but it is possible that there are other causes of the fibrosis [73].

2.7. Cardiomyopathies

The cardiomyopathies are a complex range of cardiovascular disease and occur naturally in many species. There have been many debates on the use of several different mammals as models for cardiomyopathy but many studies are carried out in avian species. Evidence indicates

that the avian heart carried many similarities to the human heart in terms of biochemistry, physiology, function and morphology [74]. Dilated cardiomyopathy in broiler chickens has been associated with avian leukosis virus, rapid growth and pulmonary hypertension [75]. Avian leukosis virus has been implicated to cause cardiomyopathy as it is linked to 11.1% rate of dilated cardiomyopathy in infected broiler chickens in comparison to ~1.4% in non-infected broilers [75]. Turkeys have also been noted to be affected by a similar virus and had a high incidence of DCM [76]. Cardiomyocyte hypertrophy has also been noted in Japanese native fowls exposed to the virus [77] and myocarditis has also been linked to it [78]. These links in birds are important as they help to shed light on the actions of the virus. It is likely that humans and other animals are similarly affected by viruses including human immunodeficiency virus (HIV-1) and simian immunodeficiency virus (SIV) [79, 80], however more studies are required in order to draw stronger conclusions. There are many theories as to how these viruses act but some studies have shown they can up and down-regulate genes and proteins via insertional mutagenesis, inflammatory changes, physical stimulation of matrix inclusion bodies, envelope protein-induced transformation and a number of other mechanisms [75, 77, 81, 82]. Environmental factors are also a consideration. A study into the effects of polychlorinated biphenyl (PCB) 77 showed that in the tree swallow (*Tachycineta bicolor*) *in ovo* exposure to the chemical results in higher levels of cardiomyopathy and ventricular wall abnormalities [83]. Age also had an effect on normal broiler roosters in comparison to mature roosters. As the roosters aged from 7 to 42 weeks, the incidence of increased right ventricle-to-total ventricle weight ratio was significantly larger in the older population, with over 36% of roosters at 42 weeks of age within ranges believed to result in right ventricular hypertrophy [84].

Dilated cardiomyopathy in turkeys has been linked to troponin T and phospholamban (PLN) variations [85]. This represents an important finding given that within the first 4 weeks of life 2–5% of captive turkeys have been reported to have cardiomyopathy, likewise the wild turkey is also affected by this cardiovascular disorder [85, 86]. Exon 8 skipping in affected individuals has been a suggested link in cardiac troponin T and it has also been indicated that wild turkeys have the similarly low molecular weight as exon 8 is spliced out [87]. This is also notable as humans carrying specific mutations in troponin T are also affected by cardiomyopathies [88]. Over 90 mutations have been described in the troponins in humans to date which result in heart disorders including hypertrophic, dilated and restrictive cardiomyopathy in addition to left ventricular non-compaction. It has also been highlighted that despite variable regions of troponin, there are some areas which are highly conserved between species including most mammals, turkeys and chickens [88]. The areas containing the most causative mutations in humans are the T1 terminal and the C-terminal and cardiac troponin T, therefore these regions make excellent candidates for future studies, not only in turkeys but in other birds too.

Avian species have also been used as models for human and non-human mammals. The Broad Breasted White turkey treated with furazolidine produced a dilated cardiomyopathy phenotype which shared many characteristic of human idiopathic dilated cardiomyopathy [74]. The white Leghorn has also been genetically altered to produce cardiomyopathy phenotypes. These have included morpholino induced knockdown of alpha myosin heavy chain causing an enlarged heart and septal defects [89]. Morpholino induced knockdown of embryonic myosin

heavy chain caused dilated cardiomyopathy, septal defects and electrical abnormalities in the developing chick heart [90]. Both proteins were also shown to be expressed from very early stages in development, when the heart is first developing *in ovo* and have also been shown to be expressed in human hearts at early developmental stages [89, 90]. It is worth noting that alpha and beta myosin mutations have also been linked to cardiomyopathies and atrial septal defects in humans [91–93], thus using the chicken models can give further insight into developmental, structural and physiological abnormalities. When looking back on the information about the structure, function and histology of the heart, it was notable that the myosins were an important feature of the avian heart, as they also are in mammalian hearts. To date no large scale study has investigated whether the disease causing mutations seen in humans and other mammals are also present in birds. In total the myosins, cardiac myosin binding protein C and troponin gene account for over 90% of the known causative mutations in human hypertrophic cardiomyopathy [94], for example. Tropomyosin I, which is associated with both actin and troponin T has also been shown to present with cardiomyopathy associated mutations in humans and more recently abnormal atrial septation, ventricular trabeculae and looping in genetic knockdown developing chickens [95]. Given the research already present in some of these genes in avian species, and their high sequence homology and expression patterns in avian cardiac tissue they present excellent candidate genes for further investigation. Given their similarities it is highly possible that further associations are present.

3. Conclusions and future directions

To conclude, a range of cardiovascular conditions have been reported that affect avian hearts. In many cases the ranges of clinical signs and treatments (where applicable) have been studied. Congenital defects, bacterial infections, dietary and environmental components have all be shown to have an impact on the presence of cardiac disease in a variety of avian species. Birds are difficult to study clinically and there are pronounced differences between wild and captive populations and differences between the species. There are also well known difficulties with using anaesthesia (often required to study or treat the birds), as the side effects of these drugs can include cardiac arrhythmias. Studying the incidence of heart disease and the genetic factors underlying the disorders has proved more problematic. Large flocks of birds produced for commercial use provide sufficient numbers of animals to study, which is usually good for genetic studies however few birds receive a post mortem in the commercial setting and most have a relatively short life. The question of inbreeding within these flocks also complicates the resulting incidence rates, and economic factors frequently affect the levels of care and veterinary interventions possible. Scientific funding is also difficult to establish given that the heart defects often present at advanced ages. Funding often relies on establishing a cost benefit, therefore would usually have to concentrate on commercial flocks rather than wild birds. The sequencing projects underway represent a big advancement towards the study of genetic disorders in avian species. They provide the much needed basic information about the avian genomes which will make it easier to study individual disorders. Appropriately powered studies using the most advanced genetic tools will highlight further causative and associated genes. Further information about the heart in general is also a key area to develop.

For example understanding the normal levels and locations of gene and protein expression during differing stages of development. Recently the transcription factor *Nkx2.5* was shown to be essential for blood vessel and cardiovascular development in the chicken, and its role has been well established in other animals, models and humans [96]. N-cadherin and retinoic A (vitamin A) are essential for appropriate cardiovascular development, without which appropriate looping, differentiation and symmetry are not achieved and chicken and quail embryos die very early in development [97]. In most animals cardiovascular disorders can affect both embryonic, young, adult and very advanced aged individuals. Vital loss of function of change of function of proteins at very specific time points can have an effect on cardiovascular health, in addition to proteins which are affected over longer time periods. Therefore understanding keys stages of development and the role that each gene plays is essential.

A large number of genetic techniques are being used in avian species in order to both understand the genome and in order to edit it. Although research into the heart is still in its infancy, there is much to be gained by continuing with the research. Many techniques are presently used in order to edit or manipulate the genome and these could be beneficial for not only animal health and welfare, but also for efficiency and economic gains. Gene editing techniques could support the food industry by enhancing meat production/egg laying traits, by inserting disease resistance and by identifying and possibly altering genetic disorder sequences. There are also possibilities for gene editing to insert characteristics into eggs (for example) to benefit human health. Naturally exploration of epigenetic modifications should also be an important factor moving forward. Studies in recent years have shown that both DNA and RNA epigenetics can have a large impact on an organism and more needs to be undertaken in the cardiovascular system in order to determine the impact in all species.

The importance of the bird as a part of the ecosystem, companion animals and as a food source mean that although they exist in great quantities, they have not been as well studied as other animals in the area of cardiovascular genetics. This is particularly true when addressing the incidence and genetic factors affecting cardiovascular disease. More research is required to quantify these diseases and to elucidate the true impact that these components have on heart development and disease.

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Conflict of interest

The authors declare no conflicts of interest.

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