

Review

Heat Stress and Histopathological Changes of Vital Organs: A Novel Approach to Assess Climate Resilience in Farm Animals

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Abstract: Heat stress causes functional and metabolic alterations in different cells and tissues. There are several pathomorphological changes and biomarkers associated with heat load in adaptive and productive organs of livestock. Heat stress-induced histopathological alterations in livestock were categorized as degenerative changes (fatty degeneration, steatosis, hydropic degeneration), necrosis (pyknosis, fibrosis), circulatory disturbances (hyperemia, edema, hemorrhage, congestion, thrombosis, ischemia), growth disturbances (hyperplasia, atrophy) and focal/diffuse inflammation (vascular changes, exudation). Upon immunohistochemical analysis, the biomarkers identified in growth-related organs were *HSP70*, *HSP60*, *GABA*, *GABA_AR*, *GABA_BR*, *HSP90*, *GnRH*, *LH*, *FSH*, *m⁶A*, *Nrf2*, and *C/EBP β* . The biomarkers in the reproductive organs were *HSP70*, *Bax*, *Bcl-2*, *GABA*, *GABA_AR*, *GABA_BR*, *Caspase-3*, *HSP90*, *HSPB9*, *HSPB10*, *HSF1*, *HSP40*, *T*, *E₂*, *Cyt-C*, *CAT*, *BCL2L1*, and *VEGF*. The identified biomarkers in the immune organs were *CD3⁺ T cells*, *CD4⁺ T cells*, *CD8⁺ T cells*, *HSP70*, and *Bcl-2*. All these biomarkers could serve as reliable variables in heat stress assessment in livestock. Further, *HSP70*, *HSP90*, *HSP60*, *NPY*, *HSP27*, *Bcl-2*, *NF- κ B*, *AQP2*, *Insulin*, *CD3⁺ T cells*, *CD4⁺ T cells*, *CD172a*, *EGF*, *AQP1*, *AQP3*, *AQP4*, *AQP5*, *CRYAB*, *GHR*, *5-HT*, *CCK*, and *GLP-1* are heat stress-related biomarkers in adaptive organs that help in assessing the climate resilience of a livestock species and improving understanding about adaptive mechanisms. Among these biomarkers, *HSP70* was established to be the ideal cellular biomarker for scaling heat response in livestock. Thus, examining heat-stressed organ histopathology and identifying cellular markers by immunohistochemistry may lay the foundation for screening climate-resilient livestock breeds in the challenging climatic scenario. Further, such an approach could help in developing concepts to combat the detrimental consequences of heat stress to ensure sustainability in livestock production.

Keywords: heat stress; histopathology; *HSP70*; immunohistochemistry; livestock



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1. Introduction

Livestock plays a significant role in determining the economy of poor and marginal farmers, especially in the developing parts of the world. Climate change is one of the most emphasized factors that negatively influence livestock production. Therefore, sustaining livestock production in the changing climate scenario is paramount. Climate change associated with increased frequency of heat waves and episodes of extreme temperature damages livestock production. It is widely established that the animal exhibits normal physiological functions within the thermo-neutral zone (TNZ). At the same time, the upper and lower critical temperature of the TNZ may alter these functions [1]. High metabolic heat production coupled with rising environmental temperatures, especially in the tropical region, may severely constrain livestock production. Thus climate change-associated heat

stress can alter the economy of livestock farms. Therefore, it is very essential to identify more climate-resilient animals. Climate resilience refers to the ability of those animals that, apart from adapting to a specific environment, also produce optimally.

Heat stress may alter several biological functions, probably to change the behavior of animals to survive the stress. Of the various mechanisms exhibited by the animals to cope with heat stress, cellular and molecular changes are vital to impart the potential for the livestock to survive in extreme environmental conditions. The inability of the body to cope with increased temperatures may result in adverse pathological changes in vital organs at macroscopic and histologic levels. Thus, establishing these cellular changes could pave the way for understanding the mechanisms associated with livestock adaptation to heat stress. Establishing these changes in adaptive organs is very crucial to assessing climate resilience in livestock. The adaptive organs are those organs which govern heat dissipation mechanisms, such as adrenal gland, thyroid gland, heart, lung, skin, kidney and gastrointestinal tract (GIT). However, fewer studies have been carried out to examine heat stress-related pathologies in livestock. This warrants more efforts to understand the constraints linked to decreased productive and reproductive performance, and compromised immune functions in livestock.

This article aims to review and frame the current state of knowledge on heat stress-induced histological changes in productive and adaptive organs in livestock, and to pin down the knowledge gaps. This review also attempts to generate information on the sensitive immunohistochemistry changes associated biomarkers for heat stress. More nuanced analyses on the patterns of changes in stress markers related to heat stress are currently needed to enhance animal breeding.

2. Importance of Livestock from a Climate Change Perspective

The impact of climate change is global and is one of the defining and pressing anticipated challenges in the coming decades. The demand growth for livestock products has been escalating strikingly due to the rapid increase in global population, rising income, alterations in lifestyle and dietary shifts. Concurrently, this has paved the way to increasingly diverse livestock production to support the rising demand. On the verge of satisfying this demand, the livestock sector has significantly contributed to climate-related hazards.

It is reported that anthropogenic activities are the key drivers of the climate crisis due to global warming. The agriculture sector contributes substantially to the global greenhouse gas (GHGs) pool. The global release of methane (CH₄) from agricultural sources accounts for two-thirds of the anthropogenic CH₄ sources [2]. It is becoming clear that meat and dairy products produce the greatest environmental burden, accounting for approximately half of food-generated GHG emissions [3].

Direct GHG emissions from livestock refer to emissions produced by the animals, including enteric fermentation and manure and urine excretion [4]. The indirect emissions from the livestock sector refer to those sources such as the cultivation of feed crops used for animal feed, emissions from manure application, carbon dioxide (CO₂) emissions during the production of fertilizer for feed production and CO₂ emissions from the processing and transportation of refrigerated livestock products [5]. Other indirect emissions include net emissions from land linked to livestock, including deforestation (i.e., conversion of forest to pasture and cropland for livestock purposes), desertification (i.e., degradation of aboveground vegetation from livestock grazing), and release of carbon (C) from cultivated soils (i.e., loss of soil organic carbon (SOC) via tilling and natural processes) associated with livestock [6]. Therefore, global livestock production directly and indirectly impacts climate change, which has direct implications for the environment.

Although livestock contributes substantially to the global GHG pool, the increasing demand for livestock products makes it necessary to invest in the sector to meet the huge animal protein demand. There are reports that portray livestock as the most resilient climate sector among various sectors associated with agriculture. Further, livestock represent economic security for resource-poor farmers. Therefore, sustaining livestock production

in the changing climate scenario could play a significant role in improving the income generated for the weaker sections of the human population.

3. Significance of Heat Stress to Livestock Production

Heat stress is recognized as a severe climate shock and an important stressor that acts as a direct threat to livestock production. Heat stress results from a combination of several weather variables, including high ambient temperature, humidity, solar radiation, and wind speed, with negative impacts on animal welfare and productivity [7]. As a result, the body temperature increases, which in succession initiates compensatory and adaptive mechanisms to restore homeothermy and homeostasis.

Heat stress increases respiration and mortality, reduces fertility, modifies animal behavior and suppresses the immune and endocrine systems, thereby increasing animal susceptibility to various diseases [8]. Additionally, heat stress is of great concern as it lowers the productivity of the livestock in terms of body weight, reproductive performance, milk yield, meat quality and egg production by causing a negative influence on the feed intake of the animals [9].

High ambient temperature causes functional and metabolic alterations in different cells and tissues. Heat stress may lead to the increased production of transition metal ions (TMI), which can make electron donations to oxygen-forming superoxide or H_2O_2 , which is further reduced to a highly reactive oxygen species (ROS) causing oxidative stress [10]. Thus, the increased production and accumulation of ROS harms the immune system. Heat stress reduces the phagocytic and burst capacity of polymorphonuclear cells, which could lead to compromised immune functions in livestock. Therefore, various immune cells suffer the direct effects of heat load on functionality, and this is likely responsible for the observed impacts on disease incidence [11]. Heat stress activates the hypothalamo-pituitary adrenal (HPA) axis, resulting in increased glucocorticoid production in major livestock species, consequently decreasing the feed intake, body weight gain, relative immune organ weight and innate immunity [9,12].

Heat stress also results in hypofunction of the thyroid gland and affects the metabolism patterns of the animal to reduce metabolic heat production [13]. Heat stress affects cellular functions and causes impairments of the reproductive system in both sexes. In males, it reduces spermatogenic activity, while in females, it adversely impacts oogenesis, oocyte maturation, fertilization development and implantation rate [14]. Therefore, the detrimental effect of heat stress on various productive and adaptive organs negatively impacts their performance, resulting in reduced global livestock production.

In tropical and subtropical regions, it has been reported that the growth traits of farm animals—both males and females—are impaired as a result of the extreme changes in biological functions, such as disturbances in protein, water, energy and mineral metabolism. These drastic changes depress the growth traits of temperate animal breeds, at about 50% during the summer season when introduced to a tropical or sub-tropical environment due to heat-stressful conditions [15]. Consequently, heat stress, being a major environmental issue, has a detrimental impact on the growth, meat quality and productive efficiency of livestock, leading to huge economic losses [16].

The effects of heat stress on livestock growth performance are the products of the decrease in anabolic activity and the increase in tissue catabolism, mainly in fat depots and/or lean body mass, as well as decreases in thyroid hormone levels, decreased feed intake and increased tissue damage [15]. According to a study, the exposure of sheep to elevated temperatures results in a decrease in growth rate, body weight, average daily gain and total body solids [17]. Moreover, in the swine industry, the economic losses associated with heat stress are mainly explained by reduced and inconsistent growth, decreased feed efficiency, decreased carcass quality (increased lipid deposition and decreased protein accretion) and so on [18].

The heat stress-associated economic losses in the livestock sector include poor growth performance, carcass quality degradation and increased animal welfare issues. A study

in heat-stressed goats assessed the meat quality variables of color, drip loss, cooking loss, water holding capacity, shear force, muscle fiber diameter, muscle pH, sarcoplasmic protein, collagen solubility and tenderness in *Longissimus thoracis et lumborum* and *Semimembranosus* muscles, reflecting that meat quality variables were compromised due to heat stress [19]. Extremes in summertime temperatures impose negative effects on animal growth performance, alter carcass composition and increase the risk of pale, soft, exudative (PSE) or PSE-like meat in pigs, poultry and beef cattle, and dark firm and dry (DFD) meat in ruminants [20]. The impacts of heat stress on meat quality among different species or breeds of animal are reported to be inconsistent, which might be attributed to alterations in the thermo-tolerance of the farm animals.

4. Importance of Studying Heat Stress-Induced Histopathological Changes of Various Organs

Thermal insult induces diverse alterations in the histology of different organs, and such histopathological studies are carried out with the aim of shedding light on the link between heat stress and observed lesions. Researchers prioritize studying the heat stress-induced changes in histology, as it offers the advantage of identifying the differences and categorizing lesions in both heat-stressed and healthy animals. In contrast to grossly affected tissues, pathological changes in histologic sections can be easily identified. In this sense, it offers an innovative approach to obtaining an efficient diagnosis. Histopathological diagnosis helps differentiate probable etiologies and establish differential changes from the field samples. This helps generate new information based on these histological changes, and is a requisite for generating advanced therapeutic policies for diagnosis based on these insights. Experimentation on the chronology of heat stress response and pathomorphological changes is necessary to gain an understanding of the mechanisms that lead to morbidity and mortality. Applications of novel biotechnological tools and the evaluation of pathomorphological changes associated with head load in livestock have to be investigated in detail, as this aids in creating new mitigation policies (genetic and managerial).

Due to the paucity of information specific to livestock, the descriptions in this review are organized according to the limited data available. They are also compared with studies in other species. This review evaluates the changes in the histology of various organs, as it is critical to enhancing our understanding of heat load-induced pathologies in major livestock production species.

5. Histopathological Changes in Productive Organs in Heat-Stressed Livestock

Heat stress is one of the limiting factors directly affecting the productive performance of livestock animals. It detrimentally influences the functioning of productive organs and thereby retards the animal's growth, reproduction and immune competence. Histologic changes in lymphoid tissues could be the basis for understanding the changes in immunologic events responsible for the deleterious effects of heat stress [21].

The pathology is believed to be quite similar across different species of mammals, and hence when deprived of livestock-specific histopathological changes, examples from other experimental animal models are cited in this review.

5.1. Growth-Related Organs

5.1.1. Hypothalamus

The hypothalamus is a vital sub-cortical center and plays a crucial role in maintaining homeostasis, such as temperature regulation, regulation of a variety of stress responses, reproductive function integration, water and salt balance, etc. [22].

According to research in brown laying hens, morphological changes in neurons of heat-stressed hypothalamus were established, and the longer the elapsed time, the more serious the pathological damage. The oxidative stress and inflammation consequently damaged the structure of neurons and nerve fibers in the hypothalamus of the hens [23].

5.1.2. Pituitary

The histological picture of the pituitary glands of chickens under heat stress showed slight hyperplasia and hypertrophy of the basophil cells, suggesting an increase in adrenocorticotropic hormone (ACTH) output under these conditions [24]. The histological examination in an experiment using heat-stressed Japanese quails revealed that the pituicytes changed in conformation and size, with reduced lipid droplets [25].

On the rat pituitary gland, acute heat stress increased the thickness of hypothalamic axons in the posterior pituitary, and the dilation of small blood vessels with hyperemia was recorded in both anterior and posterior lobes [26]. This was in agreement with another study wherein pre-pubertal male rats were exposed to hyperthermic conditions, and the results revealed hyperemic adenohypophysis, along with hydropic degeneration and focal necrosis [27].

5.1.3. Thyroid Gland

The mass and volume of the thyroid depend on animal breed, body weight, nutrition, physiological status, season, and geographical and climatic region [28]. It has been reported that the exposure of goats to high environmental temperature decreased the thyroid activity, and consequently the level of thyroid hormones in serum declined [29]. Upon observing the ultrastructure of the thyroid gland of Zebu cattle, the extreme variability of the shape and size of follicular cells in the glands of all animals was noticed, even among the same follicle. Additionally, variable colloid droplets, lysosomes and microvilli in the thyroid suggest that the gland's function is highly sensitive to several factors such as age, climate, feeding and environmental factors [30].

A study of thyroid gland histology in the summer and winter seasons in goats revealed that the parenchyma was formed from numerous inactive follicles, flattened to low cuboidal follicular cells with little cellular organelles in the summer season as opposed to the winter season. In contrast, the goats exposed to the winter season had active follicles, high columnar cells engorged with colloid droplets and cellular organelles [31]. Similarly, the histological structures of the thyroid glands of White Yorkshire piglets exposed to lower temperatures had more active follicles when compared to those exposed to a higher temperature. The low-temperature-exposed animals also showed increased epithelium with cuboidal or columnar cells. Additionally, the colloid stained uniformly in summer and lacked vacuoles, in contrast to non-uniformly stained abundant vacuoles in winter [32]. On the other hand, the thyroid gland during the summer season in a study using Buffalo tissue samples disclosed that colloid was more homogenous with a smoother periphery than in winter. In addition, follicle distension with colloid and few vacuoles were present in both seasons. Therefore, it was concluded in the study that the thyroid glands of buffaloes showed no significant differences in histological characteristics, as they are poor thermal regulators [33]. The resting (inactive) thyroid gland leads to low metabolism, exhibiting better resistance to heat stress [34]. This serves as an acclimatization and adaptive mechanism in buffaloes [33]. Furthermore, studies have recorded variations in follicular diameter during different seasons in buffaloes [35] and also in goats, wherein the diameters of thyroid follicles in the left and right thyroid lobes varied significantly according to the season [28].

It was reported in chickens that the thyroid gland apparently reduced in size when exposed to high environmental temperatures. Additionally, in the same study, histological examinations of thyroid tissue from birds revealed a decreased height of the follicular lining cells, and they appeared as flattened squamous cells [24].

5.1.4. Liver

The liver is one of the most crucial organs affected by high ambient temperature [36], and the exposure of conscious animal models to environmental heat stress increases portal venous radical content, which causes cellular hypoxic stress in the liver [37].

In the livers of heat-stressed Malabari goats, histopathological section revealed hepatocytes with fatty changes, and pronounced hepatic degenerative changes were also reported [38]. Similarly, in Osmanabadi goats, more degenerative changes were reported

in heat-stressed livers [39]. These changes are attributed to the sensitivity of hepatocytes to heat stress, and the effects were found to be more pronounced in goats irrespective of breed [38].

Similarly, in a study using chickens exposed to heat stress, histological changes in hepatic tissue were characterized by fatty and parenchymatous degeneration, the congestion of hepatic vessels, the nuclear degeneration of various types and leucocytic infiltration, particularly around the blood vessels, as well as a few degrees of focal necrosis [24]. However, in the same study listed above, no liver cirrhosis was observed. It has been shown that under chronic heat stress, necrosis from oxygen deficit develops in centrilobular areas of the hypoxic liver in broilers. In addition, the liver cells of broilers showed fatty degeneration by vacuolation with the dilation of sinusoids and necrosis with leukocyte granulation tissue, especially in the centritubular region. This fatty degeneration could be due to the accumulation of neutral lipids in the cytoplasm, and thus it serves as the diagnostic clue for liver injury [40]. Similarly, studies have reported the results of liver pathology, wherein the fatty degeneration of liver cells along with dilation of broiler's sinusoids and necrosis was observed with heterophils and lymphocytes [40,41]. Further, the histopathological analysis of the liver tissue of acute heat-stressed Kirin chickens showed the dilation of sinusoids and the central vein, increased inflammatory cell infiltration, vacuolar degeneration, congestion with mononuclear cells and few binuclear cells [42]. In the same study using another breed of chicken, mild dilation of the central vein and sinusoid with increased incidence of necrosis and hyperplasia was noted in the liver tissue. These established differences could be attributed to the differences in the thermo-tolerance between the breeds [42].

In a study in Japanese quail, it was reported that under heat stress, the livers of birds shrank, and the hepatocytes changed in conformation, size, and shape and decreased in the number of lipid droplets. Additionally, the nucleus conformation shrank, and the cytoplasm surrounding the nucleus showed a peripheral pallor and signs of necrosis with acidic composition [25]. In another study, morphological changes in quail liver were examined, and a typical hepatic macro-vesicular steatosis (fatty change) was found during exposure to heat stress. Similarly, other data presented indicated that chronic heat stress in broilers caused micro-vesicular steatosis in the liver, characterized by the presence of large fat droplets and vacuolar degeneration [43]. This abnormal status of the liver may be explained as heat stress accelerating the accumulation of fat in the liver [44]. The lipid accumulates in the hepatocytes as vacuoles and has a clear appearance with hematoxylin–eosin (H&E) staining [45]. Histopathology proved to be a more sensitive method to detect heat stress-induced liver tissue damage than the biochemical measurement of lipid peroxidation [46].

In a study using Bama miniature pigs that show high heat tolerance, no marked abnormal hepatic histology and hepatocyte ultrastructure were detected [36]. The histological picture of the liver of in utero heat-stressed bull calves contained significantly more cells than those of in utero cooled bull calves [47]. In a study using mice animal models, the livers of heat-stressed mice showed an increase in inflammatory macrophages and fibrosis [48].

5.1.5. Muscle

Muscle tissue plays a primary role in the metabolic adaptation to negative energy balance and heat stress [49]. Studies have revealed that sustained heat stress diminished muscle growth in lambs and pigs [50,51], and increased the production of reactive oxygen species in avian skeletal muscle [52].

Histopathological analyses of the leg muscle tissue of acute heat-stressed broilers in a study revealed increased fat tissue infiltration, degenerated muscle fiber and mild inflammatory cell infiltration, whereas in breast muscle tissue, the mild expansion of interstitial tissues with fibrosis and fat tissue infiltration were recorded [42]. The pectoralis muscle from experimental chicks had smaller myofibers and large fat deposition areas [53]. One of the common pathological changes in muscle is fat infiltration. Regarding increased fat and decreased protein content, altered meat quality was frequently reported in heat-stressed chicken meat [54]. Heat-stressed animals also exhibited changes in the composition of the

muscles and adipose deposition, which were only partially mediated by inflammation [55]. Indeed, fat deposition in heat-stressed animals has been shown to shift away from surface areas to internal stores to reduce the insulating effect and improve heat exchange [56].

5.1.6. Thymus

The maturation of T-cells, which are responsible for mounting an immune response to foreign substances, occurs in the thymus. Abnormal development of the thymus might seriously affect an animal's adaptive immune function [11]. The low weight of the thymus gland has been observed in laying hens under heat stress [57]. Similarly, the effect of in utero heat stress in calves revealed lower thymus weights and compromised post-natal immune functions [58]. Maternal heat stress could alter the typical development trajectory of immune cells and organs. However, the literature on immune outcomes is scarce in the Bovine model [11].

The thymus sections of heat-stressed broiler chickens were studied, and on examination, the reports displayed an unclear border between each lobe and a thinner thymic cortex. Moreover, under heat stress conditions, massive thymic cortex degradation with erythrocyte influx was observed in the thymus. Thus the atrophy of primary lymphoid tissues demonstrates the disruption of lymphocyte differentiation, proliferation, and the subsequent phase of immune abnormality in chickens bred under high ambient temperatures [59]. An experiment in broilers seeking to study the negative impacts of chronic heat stress on the thymus showed significant increases in the number of heterophils, apoptosis histiocytes and cell depletion [60]. Another study in Wenchang chicks revealed a reduced immune organ index accompanied by changes such as decreased thymic volume, reduced thymic lobule and lymphoid follicle. It reduced the medulla/cortex ratio, indicating a severe impact of heat stress on thymus development [61]. Continuous exposure of broiler chickens to heat stress-inducing temperatures caused lymphoid depletion of the thymus, a potential histologic marker for immunologic changes known to arise from heat stress [21]. The thymic tissue of the heat-stressed chickens showed severe necrosis with mononuclear inflammatory cell infiltration and loss of the normal architecture of thymic compartments [41].

5.2. Reproductive Organs

Heat stress reduces animal libido, fertility and embryonic survival [62]. As a non-specific environmental stress response, a negative effect on reproduction efficiency and growth performance in livestock and poultry has been observed [63]. High air temperature and humidity have a myriad of effects on cellular functions by the direct alteration and impairment of various tissues or organs of the reproductive system in both sexes of the animal [64].

5.2.1. Testes

In male animals, heat stress reduces sperm production and increases the number of deformed sperm by damaging testicular tissue [65]. Heat exposure triggers testicular degeneration, which is a process that causes deterioration of the testis structure, resulting in the loss of testicular function [66].

The histopathologic examination in heat-stressed lambs revealed a severe testicular degeneration, mainly in the germ line cells, without significant effects on somatic cells [67]. Similarly, another study also reported similar findings, which revealed testicular degeneration with significant germ line degeneration without any impact on somatic cells. Moreover, the vacuolation or disappearance of seminiferous tubules' epithelium lining, the formation of intertubular multi-nucleate giant cells, spermatogenic arrest at the spermatocyte stage and decreasing thickness of the germinal epithelial layer, thickened basement membranes with interstitial fibrosis and increased peritubular connective tissues were observed [68]. Lower testicular weight and severe damage to testicular microstructures due to chronic heat stress have been reported in rams [67]. Similarly, reports suggested that an increase in apoptosis in spermatozoa subsequently reduced testes weight in developing lambs [68]. In

heat-stressed mice, the histological results disclosed thin germinal epithelium with unclear boundaries, internal hemorrhage, decreased sperm count, tubular necrosis and degeneration in seminiferous tubule lumen, giving supporting evidence for the decreased testicular weight [69]. In a study, the histopathological changes in the testicular tubular compartment of heat-stressed goats comprised epithelial desquamation, clusters of Leydig cells with fatty degeneration, necrosis and apoptosis of germ cells, syncytial giant cells and macrophages, Sertoli cell vacuolization and thickening of the basal membrane [70]. This necrosis and apoptosis of germ cells leads to germinative epithelium degeneration, as reported by [71].

In heat-stressed bulls, the presence of twisted tubules of the correct round or round-oval form was observed. Additionally, a group of bulls had thickened linings, including numerous cells at different stages of differentiation. The sperm canals of some bulls were characterized by enlarged lumen, cavities in tubules with narrow spermatogenous epithelium, diluted and impaired cellular elements and a small number of sperms [72].

Buffaloes have higher performance in relation to other domestic species, demonstrating good adaptation skills [73]. In a study, the thermal factor did not result in significant changes in testicular histology in buffaloes [74]. Contrastingly, another histopathological study in buffaloes after prolonged testicular insulation revealed seminiferous tubules with the absence of vacuolized cells, numerous necrotic desquamated tubular lumen cells, and a lack of changes in interstitial cells and the basement membrane of seminiferous tubules. In the epididymis, epithelial cell vacuolization was desquamated and necrotic with pyknosis and cytoplasmic hyper eosinophilia, besides the presence of spermatozoa [73].

Testes histological evaluation in boars following scrotal insulation revealed a decrease in height of the seminiferous tubule epithelium, the presence of vacuoles in the seminiferous epithelium and a reduction in the number of primary spermatocytes and round spermatids, along with an increase in debris and remains of spermatids in the lumen of seminiferous tubules. Additionally, secondary spermatocytes were not found to be impacted [75].

The testicular structure of a heat-stressed male mice model had a disorganized germinal epithelium, with marked sloughing or obliteration of the lumen and the absence of many types of sperm cells [76]. A reduced number or loss of germ cells, such as spermatocytes and spermatids, has been reported to be due to the high sensitivity to heat in rams and adult male mice [71,77]. Heat stress disrupts the testicular tissue structure and induces oxidative stress and endoplasmic reticulum stress in chicken testes [63]. Further, histological sectioning in the same study revealed abnormalities in the distribution of spermatogenic cell layers and the absence of spermatids in the seminiferous tubules.

5.2.2. Ovary

Heat stress compromises fecundity through various mechanisms, including the induction of altered ovarian function [78]. Histological sections of goat ovaries during heat stress revealed abnormal antral follicles showing degenerating oocytes with few disorganized cumulus cells [79]. There are abundant reports about the detrimental effects of heat stress compromising oocyte quality and the alteration of follicular growth in cows [80–82] and in goats [79,83]. Another study of ovarian samples from heat-stressed buffaloes revealed a higher rate of degenerated oocytes and a low number of good-quality oocytes with a low maturation rate [84].

The histological evaluation of ovarian tissues from rabbits exposed to heat stress showed focal areas of the proliferation of fibroblast in the interstitial tissues, in addition to severe follicular degeneration characterized by oocyte lysis and enhanced follicular cell degeneration [85]. The morphology of stained follicles from ovaries of chronic heat-stressed mice revealed follicular atresia and granulosa cell apoptosis [86]. It has been found that the ovary and the oocyte operate in a unique flux between apoptosis and autophagy as a strategy to cope with thermal stress [87].

In pigs, heat stress had no impact on dominant follicle number or size [88]; however, in cows, it reduced the size of dominant follicles of the estrus cycle [89]. In laying hens exposed to short and long heat, reductions in ovarian weight and the number of large

follicles were reported [90]. On the other hand, there was no significant change in ovarian weight and size in goats exposed to heat stress [79]. Although heat stress's impact on ovarian tissues has been studied and well documented in lab animal models, information is very scarce in livestock.

5.3. Immunological Organs

The histologic changes in lymphoid tissues could be the basis for knowing the differences in immunologic events responsible for the deleterious effects of heat stress [21]. Additionally, gross changes such as significant reductions in organ weights (thymus, bursa and spleen) have also been associated with heat stress [91].

5.3.1. Lymph Node

As part of normal physiological functions, lymph nodes react to both endogenous and exogenous substances with various specific morphological and functional responses. The pathogen portal in animals is through the feco-oral route, thereby reaching the gastrointestinal tract, and therefore the mesenteric lymph node is considered to be essential for studying the immune system status in livestock [92].

Examining the mesenteric lymph nodes in Malabari goats upon exposure to a hot environment revealed changes such as a paucity of lymphocyte distribution in follicular areas and the decreased density of lymphocytes in germinal centers, reflecting compromised immune functions [92].

5.3.2. Spleen

The spleen is the largest secondary immune organ that elicits an immune response against blood-borne antigens and clears the blood of foreign entities [93]. Therefore, facilitating the understanding of histological changes in the spleen is necessary to correlate with the alteration in functionality.

The histological analysis of acute heat-stressed broiler spleens showed mild lymphoid depletion with mononuclear inflammatory cells, mild necrosis and fibrosis, mild vacuolar degeneration and hyperplasia with mononuclear cells [42]. Further, in another study, the negative impact of thermal stress caused the atrophy of the spleen in chickens [94]. In addition to the above changes, alterations in spleen weight, as well as spleen morphometry, were also noted in heat-stressed chickens [95]. Thus, spleen weight assessment in chickens might prove beneficial to identifying potential biomarkers of immune response during chronic heat stress exposure [96].

Histological changes in the heat-stressed spleens of adult Japanese quails were represented by necrosis, the degeneration and shrinkage fibrosis of lymphocytic nodules, cytoplasmic vacuolation, the infiltration and metamorphosis of lymphocytes, pyknotic nuclei, dilation of the central vein and sinusoids, edema and congested blood vessels [97].

5.3.3. Bone Marrow

The evaluation of the histological architecture in heat-challenged mice bone marrow revealed a wide dilation and thrombosis in vascular sinuses with a discontinuous endothelial cell lining. Further, ultrastructure examination revealed shrunken cells with degenerated cytoplasm, numerous apoptotic cells characterized by electron-dense pyknotic nuclei and the presence of apoptotic bodies of the nucleus [98]. These findings may be due to oxidative stress causing tissue injury through apoptosis and necrosis [99], and vascular endothelium injury during heat stress [100,101]. There is very limited literature on heat stress-associated changes in the bone marrow of farm animals, warranting more such research efforts in the future.

5.3.4. Gut-Associated Lymphoid Tissue (GALT)

The GALT is a major component of the immune system, diffusely scattered through the lamina propria and in organized lymphoid follicles such as the cecal tonsil, Peyer's patch, the bursa of fabricius and Meckel's diverticulum [102].

In a study, the cecal tonsils of heat-challenged broiler chickens showed moderate lymphoid depletion with lymphocytolysis [103]. Another study in broilers revealed that exposure to heat stress increased the presence of cell debris, vacuolation, and the degeneration of epithelial cells in the cecal tonsil [104].

In the bursa, severe lymphocytic depletion of the cortex and medulla with heterophile infiltration of the bursa was observed in heat-challenged broiler chickens [103]. Additionally many studies proved that chickens subjected to heat stress exhibited lymphoid depletion in the bursa of fabricius tissue [91,105]. In addition to the aforementioned reports, another study demonstrated the occurrence of reduced bursa weight in broilers subjected to heat stress, and a decreased number of lymphocytes in the cortex and medulla areas of the bursa [106]. This histopathology results in broilers' heat-stressed bursa manifesting intra-follicular and intra-epithelial cysts [107]. Similarly, another study in broiler chickens observed microcysts in mucosal folds, and the bursal follicles showed a small-sized cortex with slightly packed lymphocytes, medullary necrosis and hyalinization. Further, in the same study, interstitial edema with increased thickness of the inter-follicular connective tissue was also observed [41]. In heat-challenged broiler chickens, a decrease in immature B cells in bursal follicles has been reported. Further, the cell density in the medulla and cortex of bursal follicles decreased severely under heat stress [59].

6. Heat Stress-Associated Histopathological Changes in Adaptive Organs in Livestock

Livestock undergoes several morphological adaptations to cope with adverse environmental conditions. Morphological adaptation is considered crucial for the survival of animals exposed to adverse environmental conditions. In recent years, considerable progress has been made in describing the heat-induced histological changes in adaptive organs of farm animals. It is very vital to understand the significant histological changes in adaptive organs as it might help to differential the adaptive responses between normal and heat-stressed animals. This might help to differentiate between climate-sensitive and climate-resilient breeds. Therefore, understanding the histological changes in various organs linked with the adaptation process is crucial for assessing the climate resilience of livestock breeds.

6.1. Adrenal Gland

Environmental stress produces structural changes in the adrenal gland [108]; therefore, testing adrenal histology could provide valuable information for assessing heat stress [109].

In broiler chickens, faint cells with blurred cellular outlines in the adrenal gland were reported [110]. The histomorphology of adrenal glands from quails under heat-stressed conditions consisted of radial cords of cells in the fasciculate zone and the foamer cytoplasm of cells in the upper part than in the lower part [108]. In the same study, it was reported that the cells in the reticular zone were smaller and darker than cells in the fasciculate zone.

Only a few studies are available describing the histological changes in the adrenal glands in farm animals during heat stress exposure. A study wherein pre-pubertal male rats were exposed to hyperthermic conditions reported mild hydropic degeneration in adrenal glands [27]. Histological investigation of the adrenal gland of heat-stressed rats revealed the existence of dark and light regions in the adrenal cortex. The cells in the light regions were filled with large lipid droplets, but those in the dark cortical regions were deprived of them. Interstitial fibrosis in all parts of the cortical zone and medulla were observed, with no mitotic figures visible in the adrenal glands of heat-stressed rats [26]. Furthermore, another study in rats revealed exciting findings, such as the prolongation of temperature stress causing adrenal gland hypertrophy. Additionally, the volumes of individual zones in the adrenal gland differed with the increase in the volume of the adrenocortical zone, while the vascular tissue remained unchanged. Therefore, it could be concluded that the increase in the proliferation rate of adrenocortical cells was an adaptive response to meet the demands of stressful conditions [111].

6.2. Lung

In heat stress, the lung is one of the most affected and injured organs, as its unique structure and impaired functions promote damages in other organs and tissues, as anoxia [112].

The principal histopathologic lesions in the lung were related to vasculatures and the massive congestion of veins and arterioles, with hemorrhages in parabronchus and alveoli in broilers. It was also reported that the lungs might also be edematous and occasionally contain focal consolidations of bronchopneumonia [40]. Similarly, in heat-stressed sheep, broncho-interstitial pneumonia with bronchiolitis obliterans was reported [113]. In this context, many studies have reported acute interstitial pneumonia affecting cattle, and this is more common during hot and dry summers [114,115].

It has been found that an increased respiratory rate followed by the stimulation of the parabrachial nuclei has caused lung edema and hemorrhage in broilers due to accelerated evaporative heat loss by convection [116]. Similarly, hyperthermia-induced changes in lung histology were examined in piglets, which disclosed excess hemorrhage and alveolar edema [117].

6.3. Heart

As a response to heat stress, heart rate and blood flow to tissues for heat exchange increase, and decreased blood flow to organs takes place. This increase in heart rate maximizes the dissipation of heat and maintains perfusion in the face of vasodilation [118]. Further, it was reported that acute congestive heart failure secondary to heat stress causes death in sheep [119].

It has been reported that sub-endocardium and sub-pericardial hemorrhages are found in the heart with congestion in heat-stressed broilers. In addition, under light microscope examination, massive myofibrillar degeneration with hemorrhage, fatty degeneration, and the vacuolation of myofibers with diffuse myocarditis in some cases were observed [40]. Another study in broilers found that chronic heat stress caused pathological organ changes, including right atrial and ventricular hypertrophy, myofibrillar degeneration, general vacuolar degeneration of myofibers and diffuse myocarditis [120].

6.4. Skin

Skin is the first defense mechanism of the immune system that responds to and resists the hostile environment of temperature, humidity and radiation [121].

The histological result of a study in heat-stressed Iraqi Buffaloes during summer demonstrated that the sweat glands were poorly developed, and lie deeply in the dermis, the secretory acinus surrounded by a few myoepithelial cells and a basal layer of epidermis loaded with abundant melanocytes. On the other hand, sebaceous glands were large, lobulated and well-developed [122].

6.5. Kidney

The kidneys play a vital role in body homeostasis. In heat-stressed sheep, gross findings have revealed bilateral, moist, pale and moderately enlarged kidneys. Additionally, histological examination revealed asymmetrically severe, acute renal tubular necrosis, tubular epithelial regeneration, neutrophil influx and cellular basophilia [113].

Generalized edema and hemorrhages, especially in renal papillae, renal tubular and subrenal capsules with an accumulation of heterophils in many inflammatory areas, have been reported in heat-stressed broilers [40]. Besides this, fatty degeneration of the renal tubular epithelia and glomeruli damage were also reported in the same study. Another study in broilers revealed edema, hemorrhage in the sub-renal capsule with glomerular damage, and the vacuolar degeneration of kidneys [120]. Heat stroke might be associated with degenerative changes in the renal tubules in mammals. Several days after heat stroke, there is often evidence of renal failure caused by the degeneration and necrosis of renal tubules [123]. Similarly, renal histopathological alterations in broiler chicken were reported,

revealing tubular necrosis, lumen dilation, the congestion of renal tubules, glomerular structural changes and lots of neutrophil granulocyte invasion in interstitial areas [124].

In a study using a mouse model, the histopathological findings of renal tissue specimens showed the degeneration of tubular epithelial cells and urinary casts [125], whereas the renal tissues from heat-stressed mice showed proximal tubular injury with brush border loss, low-grade inflammation and renal fibrosis [48].

6.6. Gastrointestinal Tract (GIT)

It has been reported that heat stress directly affects gut integrity and induces innate immune system activation, resulting in systemic inflammation in bovines [126] and laying hens [127]. The impact of heat stress in experimental goats showed significant changes in histological sections of the rumen, such as the reduction in length and thickness and the higher keratinization of rumen villi, influencing the fermentation pattern [128].

It is proven that heat stress severely affects the structure of the small intestine, including villi fracture, the shortening of the villus height, deeper crypts and mucosal epithelial cell exfoliation [129]. During heat stress, the blood flow to the intestine is reduced, leading to the ischemia and necrosis of intestinal epithelial cells [37]. Heat stress has been shown to disrupt tight junctions in the intestine, thereby increasing intestinal permeability in multiple mammalian species [130], including lactating goats [131,132], cows [126] and pigs [133]. Furthermore, it has been shown that heat stress increases the intestinal permeability of monogastric animals [134,135]. Nevertheless, it is unknown if the rumen epithelia's permeability is increased during heat stress as it is structured differently than the intestinal epithelia [136]. However, a study was carried out, and specimens of rumen papillae were microscopically examined; it was found that the corneum sloughs off in heat-stressed cows [136]. Additionally, it has also been reported in the same study that the intercellular space of the granulosum and spinosum was enlarged. Another study using rumen histological sections of goats revealed that heat stress lowered the length and thickness, while keratinization was significantly higher [128].

A study reported that heat stress in cows triggers the migration of infiltrating cells, while jejunal morphology remains unaltered. However, the histological analysis revealed clusters of infiltrating cells of an unknown cell type in mucosa and submucosa [126]. Changes in the intestinal histology of broiler chickens showed a subtle increase in the cellularity of lamina propria in the acute heat stress group, characterized by subtle, acute, multifocal lympho-plasmocytic enteritis in the jejunum [137]. Another study in broiler chickens subjected to acute heat stress revealed a reduction in ileal crypt depth. Still, no change in villus height was found, and this study concluded that due to fast intestinal mucosal re-epithelialization, there is lack of structural changes in heat-stressed chickens [138]. Similarly, chronic heat stress in pigs caused marked morphological injuries in the small intestine, such as villi tip desquamation and exposure of the lamina propria [139]. Table 1 describes the histopathological changes in heat-stressed productive and adaptive organs of livestock and other animal models.

Table 1. Heat stress-induced histopathological changes in productive and adaptive organs in livestock and other species.

Organ	Species	Histopathological Changes	Reference
Hypothalamus	Chicken	Neurons and nerve fiber structural damage	[23]
	Chicken	Basophil cell—hyperplasia and hypertrophy	[24]
Pituitary	Japanese Quail	Pituicytes—changed confirmation and size and reduced lipid droplets	[25]
	Rat	Hyperemia Hyperemia, hydropic degeneration and focal necrosis	[26] [27]

Table 1. Cont.

Organ	Species	Histopathological Changes	Reference
Thyroid gland	Buffalo	Homogenous colloid with smooth periphery	[33]
	Goat	Inactive follicles, flattened to low cuboidal follicular cells and little cell organelles	[31]
	Swine	Less active follicles, uniformly stained colloid, lack of vacuoles	[32]
	Chicken	Decreased height of follicular lining cells resembling flattened squamous cells	[24]
Liver	Cattle	Increased cells	[47]
	Goat	Fatty and degenerative changes Degenerative changes	[38] [39]
	Swine	No marked changes	[36]
	Chicken	Fatty and parenchymatous degeneration, hepatic vessel congestion, nuclear degeneration, leucocytic infiltration and focal necrosis Fatty degeneration, dilated sinusoids and necrosis in heterophils and lymphocytes Dilated sinusoids and central vein, inflammatory cell infiltration, vacuolar degeneration, congestion, necrosis and hyperplasia Microvesicular steatosis, vacuolar degeneration	[24]
			[40,41]
			[42]
			[43]
	Japanese quail	Hepatocytes—changed confirmation and size, reduced lipid droplets, cytoplasm necrosis with acidic composition	[25]
	Mice	Macrovesicular steatosis	[43]
	Mice	Increased macrophages and fibrosis	[48]
Muscle	Chicken	Leg—fat infiltration, degeneration, mild inflammatory cell infiltration Breast—expansion of interstitial tissue with fibrosis and fat infiltration Breast—fat and inflammatory cell infiltration, degenerated muscle fiber with fibrosis	[42]
		Pectoralis muscle—small myofibers and fat deposition	[53]
Thymus	Chicken	Lymphoid depletion	[21]
		Thinner thymic cortex	[59]
		Increased heterophils, apoptosis histiocytes and cell depletion	[60]
		Reduced lobule and lymphoid follicle	[61]
		Severe necrosis, mononuclear inflammatory cell infiltration	[41]
Testes	Cattle	Thickened lining, enlarged lumen, impaired cellular elements, cavities in tubules with narrow spermatogenous epithelium and less sperms	[72]
	Buffalo	No significant changes	[74]
		Necrotic desquamated seminiferous tubular lumen cells In epididymis—epithelial cell vacuolisation, desquamated and necrotic with pyknosis, presence of spermatozoa and cytoplasmic hyper eosinophilia	[73]
	Sheep	Germ line cell degeneration	[67]
		Vacuolation, intertubular multinucleate giant cells, thickened basement membrane with interstitial fibrosis and increased peri-tubular connective tissue	[68]
	Goat	Epithelial desquamation, fatty degeneration, necrosis and apoptosis, syncytial giant cells and macrophages, sertoli cell vacuolization and thickening of basement membrane	[70]
	Pig	Reduced seminiferous tubular epithelium height with vacuoles, decreased primary spermatocytes and round spermatids	[75]
	Chicken	Abnormal distribution of spermatogenic cell layers and absence of spermatids	[63]
	Mice	Disorganized epithelium and sloughing of lumen with absence of sperm cells	[76]
		Thin germinal epithelium, hemorrhage, decreased sperm count, tubular necrosis, degeneration of seminiferous tubule lumen	[69]

Table 1. Cont.

Organ	Species	Histopathological Changes	Reference
Ovary	Cattle	Reduced follicle size	[89]
	Buffalo	Degenerated oocytes	[84]
	Goat	Abnormal antral follicle, oocyte degeneration and disorganized cumulus cells	[79]
	Pig	No changes	[88]
	Chicken	Reduced number of follicles	[90]
	Mice	Follicular atresia and granulosa cell apoptosis	[86]
	Rabbit	Fibroblast proliferation in interstitial tissues and follicular cell degeneration	[85]
Lymph node	Goat	Decreased lymphocyte distribution in follicle	[92]
Spleen	Chicken	Lymphoid depletion, mononuclear inflammatory cell infiltration with hyperplasia, mild necrosis, fibrosis and vacuolar degeneration.	[76]
	Japanese quail	Necrosis, degeneration, fibrosis of lymphocytic nodules, cytoplasmic vacuolation, lymphocyte infiltration, pyknotic nuclei, dilation of central vein and sinusoids, edema and congested blood vessels	[97]
Bone marrow	Mice	Dilation and thrombosis in vascular sinuses, discontinuous endothelial lining, degenerated cytoplasm, pyknotic nuclei and numerous apoptotic cells	[98]
GALT	Chicken	Cecal tonsils—epithelial cell degeneration and vacuolation	[104]
		Lymphocytolysis and lymphoid depletion	[103]
		Bursa of fabricius—heterophil infiltration and lymphoid depletion	[103]
		Lymphoid depletion	[91,105]
		Intra-follicular and intra-epithelial cysts	[107]
Medullary necrosis, hyalinization, interstitial edema, increased thickness of inter-follicular connective tissue	[41]		
Adrenal gland	Chicken	Faint cells with blurred cellular outline	[110]
	Quail	Radial cords of cells with foamier cytoplasm, reticulate zone cells—small and dark	[108]
	Rat	Interstitial fibrosis and large lipid droplets in light region cells Hypertrophy, increased volume of adrenocortical zone	[26] [111]
Lung	Pig	Excess hemorrhage and alveolar edema	[117]
	Chicken	Congestion of veins and arterioles, edema, hemorrhages of parabronchus and alveoli	[40]
		Edema and hemorrhage	[116]
Heart	Chicken	Sub-endocardial and sub-pericardial hemorrhages, congestion, myofibrillar and fatty degeneration and vacuolation of myofibers	[40]
		Hypertrophy, myofibrillar degeneration and vacuolar degeneration of myofibers	[120]
Skin	Buffalo	Abundant melanocytes in basal layer of epidermis and large, lobulated sebaceous glands	[122]
Kidney	Sheep	Acute renal tubular necrosis, tubular epithelial degeneration, neutrophil influx and cellular basophilia.	[113]
	Chicken	Edema, hemorrhage, heterophil accumulation, glomerular damage and fatty degeneration of tubular epithelium	[40]
		Edema, hemorrhage, glomerular damage and vacuolar degeneration	[120]
		Tubular necrosis and congestion, lumen dilation and interstitial neutrophil invasion	[124]
Mice	Proximal tubular injury with brush border loss, low-grade inflammation and renal fibrosis	[48]	
		Degeneration of tubular epithelial cells and urinary casts	[125]

Table 1. Cont.

Organ	Species	Histopathological Changes	Reference
Gastro Intestinal Tract	Cattle	Sloughing off corneum in rumen papillae, enlarged intercellular space of granulosum and spinosum	[136]
		Unknown cell infiltration in mucosa and submucosa of jejunum	[126]
	Goat	Reduced length and thickness with higher keratinization of rumen villi	[128]
	Swine	Intestinal villi tip desquamation with exposure of lamina propria	[139]
	Chicken	Increased cellularity of lamina propria of jejunum Lack of structural changes due to fast intestinal mucosal re-epithelialization	[137] [138]

The direct and indirect impacts of heat stress on ruminants also impose a detrimental influence on the rumen microbiome, which in itself is a separate niche. Significant alterations in rumen microbes due to heat stress were reported by researchers in cattle [140], buffalo [141], sheep [142] and goat [143]. A transition in the dominant phylum, from *Firmicutes* to *Bacteroidetes*, was observed in caprine rumen with increasing temperature humidity index (THI) [143]. Likewise, the relative abundance of the genus *Prevotella* gradually increased with rising THI, thereby being the dominant genera. Apart from significant alteration in the production influencing bacteria, heat stress was also proved to alter the relative abundance of pathogenic bacteria such as *Erysipelotrichaceae_UCG-004* and *Treponema_2*, which increased with rising THI. Therefore, such intense association of rumen microbiota with the animals' productive performance and health status highlight the potential risks associated with heat stress in ruminants.

Figure 1 illustrates heat stress-induced histopathological changes in the productive and adaptive organs of livestock.

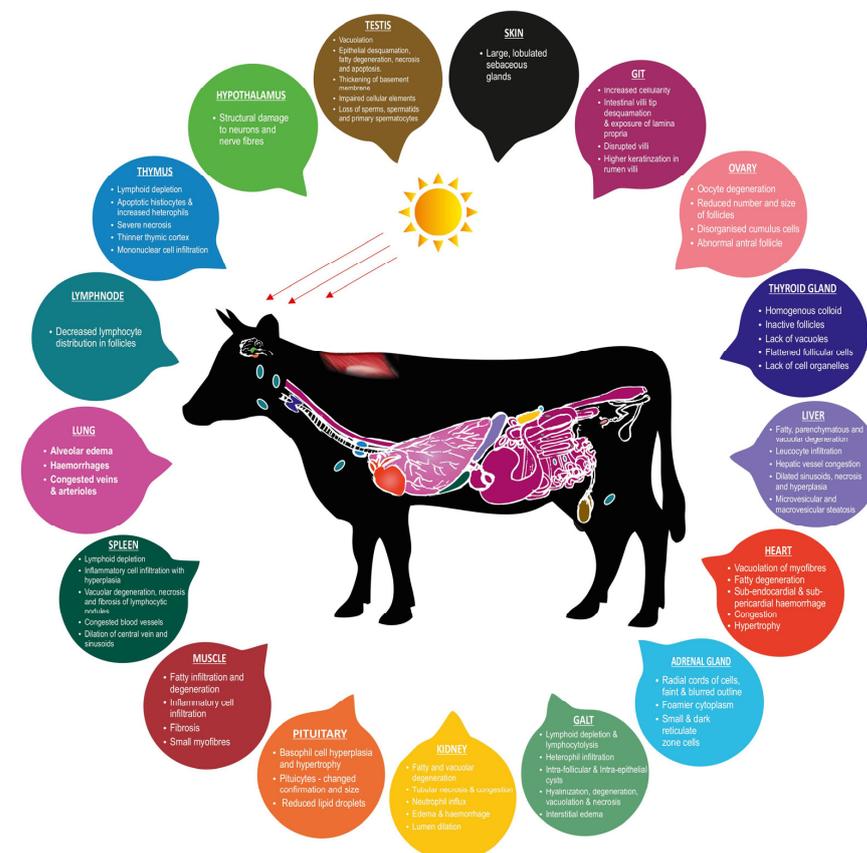


Figure 1. Heat stress-induced histopathological changes in productive and adaptive organs of livestock.

7. Immunohistochemistry (IHC) Derived Biomarkers in Adaptive and Productive Organs of Livestock

The cellular and molecular responses in livestock are fundamental, as they may lead to the identification of potential genetic biomarkers for heat stress in livestock [144]. Using molecular and physiological biomarkers for diagnosing diseases, monitoring physiological state and forecasting the risk of illness or low productive performance has become a valuable tool assisting agricultural animal production [145]. Immunohistochemistry (IHC) has been established as a solid and reliable methodology for routine diagnostic and research activities in veterinary medicine. The IHC is based on antibodies binding to a specific antigen in tissue sections [146]. The IHC technique is used in the search for cell or tissue antigens ranging from amino acids and proteins to infectious agents and specific cellular populations [147]. Various proteins, inflammation-related cytokines, blood cells and bacteria products in the bloodstream can also be used as biomarkers of heat stress [148].

It has been established that the susceptibility and tolerance to thermal load vary with individuals, breeds and species [149]. In livestock, diverse biomarkers have been identified in productive and adaptive organs as a response to heat stress due to altered cellular defense mechanisms. In growth-related organs of livestock, biomarkers, namely, the 70-kDa heat shock protein (*HSP70*), heat shock protein 60 (*HSP60*), Gamma-aminobutyric acid (*GABA*), *GABA_A* receptors (*GABA_AR*), *GABA_B* receptors (*GABA_BR*), heat shock protein 90 (*HSP90*), Gonadotropin-Releasing Hormone (*GnRH*), Luteinizing Hormone (*LH*), Follicle Stimulating Hormone (*FSH*), *N⁶-methyladenosine (m⁶A)*, Nuclear factor erythroid 2-related factor 2 (*Nrf2*), and CCAAT/enhancer binding-protein beta (*C/EBPβ*), were reported to change in response to heat stress. The expression pattern of *HSP70* was reported mainly in the hypothalamus [150], liver [150,151] and muscle [152] of chickens. In response to heat stress, the expression of *HSP60* in the liver was reported to be low in chickens [147] and high in cattle [153]. In the pituitary and hypothalamus of chickens, the expression of *GABA*, *GABA_AR*, and *GABA_BR* biomarkers was reported [154]. Another study in chickens reported that the heat load affected the expression of reproductive hormones such as *GnRH* in the hypothalamus, *LH* and *FSH* in the pituitary [155]. On the evaluation of the effect of heat stress in chicken, *HSP90* was found to be highly expressed in the liver [156]. Higher expression of *m⁶A* was reported in the hepatic tissue in sheep [157] and *Nrf2* in pigs [36] exposed to heat stress. In the pectoralis muscle of heat-stressed chicken, the *C/EBPβ* biomarker was reported to be higher [53].

In testis, the expression of *HSP70* was low in chicken [158] and cattle [159], whereas increased expression in the ovaries of pigs was reported [160] under heat-stressed conditions. Furthermore, *Bcl-2 Associated X protein (BAX)* and *B-cell lymphoma-2 (Bcl-2)* protein levels were reported to be highly expressed in sheep [161] and swine [162] testicular tissues. It has been established that testicular heating induced the apoptosis of germ cells by regulating the *BAX* and *BCL-2* protein levels [162]. Further, in heat-stressed ovaries of chicken, varied expressions of *GABA*, *GABA_AR* and *GABA_BR* biomarkers were reported [154]. Analyzing heat-challenged testicular tissue by IHC revealed higher expression patterns of *Caspase-3 protein* levels in goats [163] and pigs [164]. In chickens, the expression of *testosterone (T)* in the testis and *estrogen (E₂)* in ovaries was reported to increase at the beginning of heat exposure, followed by a decrease [155]. Other reports in heat-stressed goats revealed higher expressions of *HSPB9* and *HSPB10* [165] and *vascular endothelial growth factor (VEGF)* [163] in testicular tissues. In heat-stressed testes, higher expressions of *HSP90* has been reported in cattle [159] and cytochrome-C (*Cyt-C*) in pigs [164]. The expressions of *heat shock factor 1 (HSF1)*, *HSP40* [160] and *BCL-2 like 1 (BCL2L1)* [88] in heat-challenged pig ovaries were reported to be higher. In contrast, in cattle, catalase (*CAT*) enzyme levels were higher [166]. Moreover, microtubule-associated *protein 1 light chain 3 (LC3B)* expression levels in ovarian tissues have been reported with no difference in thermo-neutral and heat-stressed pigs [88]. In organs of the immune system, *CD3⁺ T cells*, *CD4⁺ T cells*, *CD8⁺ T cells*, *HSP70* and *Bcl-2* were reported to change in response to heat stress. In pigs' heat-stressed spleens, *CD3⁺ T cells*, *CD4⁺ T cells*, *HSP70* and *Bcl-2* showed increased expression [167]. In the gut-associated lymphoid tissues (*GALT*) of chickens exposed to heat stress, *CD3⁺ T*, *CD4⁺* and *CD8⁺ T cells* were reported [168].

they act to prevent thermal damage to proteins [179]. In general, it has been established that *HSP70* expression is regarded as a reliable biomarker for changes in body temperature [180]. Veterinary pathologists face many challenges when performing IHC because of the diversity of species studied, and there are no guarantees that antibodies will cross-react among different species [146]. However, it is necessary to adopt advanced molecular biological techniques, as they are highly beneficial in understanding the cellular- and molecular-level changes in livestock thriving in extreme environments. Table 2 describes the biomarker expression pattern in livestock in response to heat stress.

Table 2. Heat stress-associated biomarker expression pattern in livestock in response to heat stress.

Organ	Animal Model	Biomarker	Expression Pattern	References
Hypothalamus	Chicken	<i>HSP70</i>	High	[150]
		<i>GABA</i>	High	[154]
		<i>GABA_AR</i>	Low	[154]
		<i>GABA_BR</i>	Low	[154]
		<i>GnRH</i>	High to low	[155]
Pituitary	Chicken	<i>GABA</i>	High to low	[154]
		<i>GABA_AR</i>	High to low	[154]
		<i>GABA_BR</i>	High to low	[154]
		<i>LH</i>	High to low	[155]
		<i>FSH</i>	High to low	[155]
Liver	Chicken	<i>HSP70</i>	High	[150,151]
		<i>HSP70</i>	Low	[170]
		<i>HSP60</i>	Low	[147]
		<i>HSP90</i>	High	[156]
	Cattle	<i>HSP60</i>	High	[153]
Sheep	<i>m⁶A</i>	High	[157]	
Swine	<i>Nrf2</i>	High	[36]	
Muscle	Chicken	<i>C/EBPβ</i> (Pectoralis muscle)	High	[53]
		<i>HSP70</i> (Pectoral & thigh muscle)	High	[152]
Testis	Chicken	<i>GABA</i>	Low to high	[154]
		<i>GABA_AR</i>	High to low	[154]
		<i>GABA_BR</i>	High to low	[154]
		<i>T</i>	High to low	[155]
		<i>HSP70</i>	Low	[158]
	Cattle	<i>HSP90</i>	High	[159]
		<i>HSP70</i>	Low	[159]
	Goat	<i>Caspase-3</i>	High	[163]
		<i>VEGF</i>	High	[163]
		<i>HSPB9</i>	High	[165]
<i>HSPB10</i>		High	[165]	
Sheep	<i>BAX</i> (spermatocytes, desquamated cells)	High	[161]	
	<i>Bcl-2</i> (spermatids)	High	[161]	
Swine	<i>HSP70</i>	High	[181]	
	<i>BAX</i>	High	[162]	
	<i>Bcl-2</i>	High	[162,164]	
	<i>Cyt-C</i>	High	[164]	
	<i>Caspase-3</i>	High	[164]	

Table 2. Cont.

Organ	Animal Model	Biomarker	Expression Pattern	References
Ovary	Chicken	<i>GABA</i>	High to low	[154]
		<i>GABA_AR</i>	High to low	[154]
		<i>GABA_BR</i>	High to low	[154]
		<i>E₂</i>	High to low	[155]
	Cattle	<i>CAT</i>	High	[166]
	Swine	<i>HSF1</i>	High	[160]
		<i>HSP40</i>	High	[160]
		<i>HSP70</i>	High	[160]
		<i>BECN1</i>	No significant change	[88]
<i>LC3B</i>		No significant change	[88]	
	<i>BCL2L1</i>	High	[88]	
Spleen	Swine	<i>HSP70</i>	High	[167]
		<i>CD3⁺ T cells</i>	High	[167]
		<i>CD4⁺ T cells</i>	High	[167]
		<i>Bcl-2</i>	High	[167]
		<i>HSP70</i>	High	[98]
GALT	Chicken	<i>CD3⁺ T cells</i>	Low	[168]
		<i>CD4⁺ T cells</i>	Low	[168]
		<i>CD8⁺ T cells</i>	Low	[168]
Respiratory tract	Cattle	<i>HSP90</i> (Lung)	High	[159]
		<i>HSP70</i> (Lung)	Low	[159]
	Buffalo	<i>AQP1</i> (Trachea)	Low	[176]
		<i>AQP3</i> (nasal turbinate)	High	[176]
		<i>AQP4</i> (Trachea)	Low	[176]
	<i>AQP5</i> (Trachea)	Low	[176]	
Heart	Chicken	<i>HSP70</i>	Low	[169]
		<i>HSP70</i>	High	[156,171]
		<i>HSP60</i>	High	[147]
		<i>HSP90</i>	High	[182]
		<i>CRYAB</i>	Low	[169]
		<i>HSP27</i>	High to low	[175]
	Cattle	<i>HSP70</i>	High	[159]
		<i>HSP60</i>	High	[153]
GIT	Chicken	<i>NPY</i> (Proventriculus)	High	[177]
		<i>GHR</i> (Proventriculus)	High	[177]
		<i>5-HT</i> (Duodenum & Jejunum)	High	[177]
		<i>CCK</i> (Jejunum)	High	[177]
		<i>GLP-1</i> (Duodenum and Jejunum)	High	[177]
		<i>HSP60</i> (Duodenum and Jejunum)	High	[173]
		<i>HSP70</i> (Duodenum and Jejunum)	High	[173,174]
		<i>HSP90</i> (Duodenum and Jejunum)	High	[173,174]
		<i>NF-Kb</i>	High	[174]
		<i>EGF</i>	Low	[174]

Table 2. Cont.

Organ	Animal Model	Biomarker	Expression Pattern	References
	Cattle	<i>CD172a</i> (Jejunum)	High	[126]
	Swine	<i>HSP70</i> (Small intestine)	High	[167]
		<i>CD3⁺ T cells</i> (Small intestine)	High	[167]
		<i>CD4⁺ T cells</i> (Small intestine)	High	[167]
		<i>Bcl-2</i> (Small intestine)	High	[167]
		<i>Insulin</i> (Pancreas)	Low	[178]
Kidney	Chicken	<i>HSP70</i>	High	[151,172]
		<i>HSP70</i>	Low	[170]
		<i>HSP90</i>	High	[156]
		<i>AQP2</i>	High	[172]
	Cattle	<i>HSP70</i>	High	[159]
		<i>HSP90</i>	Low	[159]
		<i>HSP60</i>	High	[153]

8. Conclusions

Based on the available information in the literature, the present review highlights heat stress-induced histopathological alterations in productive and adaptive organs of livestock and the use of a panel of immunohistochemical biomarkers for diagnostic support and confirmation. To elucidate additional information, a brief account of heat stress-induced macroscopic lesions has also been catalogued. This review, citing results from different studies on heat-induced histopathological alterations in tissues, has revealed that the type and distribution of lesions varies depending on the pathologic processes encountered by the target organ. Thus, examining heat stressed-organs' histopathology and IHC remains at the epicenter of thermal stress diagnosis and assessing climate resilience in farm animals. Further, this review has given a perspective about the broad knowledge deficits in understanding heat stress-induced pathologies in livestock. These findings suggest a lack of emphasis on species-specific animal models in climate change research. It is pivotal that more work be done to assess and emphasize the impact of heat stress on productive and adaptive organs in livestock animals from a pathological perspective. This way, the livestock sector can positively address the heat stress-related economic losses incurred, and such an approach may also help the farming community to make the sector more efficient and sustainable.

9. Future Perspectives

Our findings prove that there is still a need for more research effort, as the pathogenesis of tissue injury due to heat stress needs to be better understood primarily in farm animals. In future, it is essential to evaluate distinct changes in the target organs of livestock species as a result of thermal insults to postulate accurate and relevant information to prevent pitfalls in diagnosis. The broader adoption of histopathology independently or in conjunction with immunohistochemistry could facilitate efficient diagnoses and prognoses. There is an established variation in the pathology induced by thermal insult and the ability of a livestock species to tolerate the heat load. Hence, to further our understanding, more detailed studies in livestock animal models should be undertaken to inform specific microscopic lesions. Advanced technologies provide a dynamic and powerful approach to deriving a more detailed understanding of the molecular and cellular changes in heat-stressed tissues. Therefore, the exploration of unfamiliar cellular areas by adopting new molecular tech-

niques bridges the gap in the literature. Furthermore, exploring the pathological changes at the cellular level improves the understanding of adaptive mechanisms.

Given climate breakdown, further research efforts and detailed investigations of pathologies using histopathological and immunohistochemical analysis are needed in diagnosing the irreversible detrimental effects of heat stress in livestock production. Additionally, the analysis of pathological changes serves as a reliable approach to identify potential biomarkers in assessing the impact of heat stress, and helps develop concepts to combat the detrimental consequences of heat stress in livestock. More research on molecular aspects may lay the foundation for commencing biomarkers-aided breeding programs, and assist in screening more resilient breeds in the challenging climatic scenario. These future research efforts can sustain livestock productivity and minimize the detrimental effects of heat stress on animal growth, production and reproduction.

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