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Histopathological and Pathological Features of Bacterial Infection in Agama Lizards Found Around Poultry Houses

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Abstract

Purpose: Histopathological examination of the liver of the lizard (Agama Agama) is useful for assessing propensity for ecological and environmental disease.

Materials and Methods: 100 free-roaming lizards were captured around poultry houses and histopathological examination of liver lesions after bacterial infection. Culture of liver samples revealed the presence of Staphylococcus aureus and Corynebacterium species. Macroscopically, the liver appears pale. Liver tissue blocks were fixed, stained with hematoxylin and eosin stains, and viewed on a BH2 Olympus® microscope at 200x and 400x magnification. Photomicrographs were then taken.

Findings: Microscopically, the liver parenchyma consists of normal hepatocytes arranged in chains around blood vessels and separated by narrow, clear subendothelial spaces. Histopathological examination of selected liver tissues showed that melanin-loaded melano-macrophages were distributed within the tissue. Other histopathologic features observed include in the liver tissues include; hepatocyte necrosis, severe hepatocyte vacuolization and associated melano-macrophage hypertrophy, congested blood vessels, mononuclear cell infiltration and melano-macrophage hyperplasia.

Implications to Theory, Practice and Policy: The results of this study provide insight into the histopathological picture of bacteria-infected lizards in Zaria, Nigeria, and serve as a guide for clinical manifestations and knowledge about future research on lizard livers.

Keywords: *Histopathology, Liver, Melano-Macrophages, Infection, Agama Lizard*

1.0 INTRODUCTION

Reptiles are known to be part of the subphylum Chordata, which consists of approximately 9,084 species [1]. The class is divided into four orders: Crocodilia, Rhynchocephalia, Squamata and Testudinata. Lizards belong to the order Squamata [2]. The African Rainbow Lizard (Agama agama) inhabits a wide variety of open habitats across much of Africa, including deforested forest areas [3].

The gross appearance of reptilian livers differs slightly from that of other vertebrate livers and varies between animal morphologies. Thus, it is long and thin in most snakes, but short and wide in Chelonian and most lizards [4]. The liver accounts for 3-4% of body weight in most squamate species, but generally less in chelonians and crocodilians [5]. However, their size and mass vary with season and nutritional/reproductive status [4]. The liver (in most species) arises from the endodermal gut. It usually consists of two lobes and varies in colour from dark brown to black. A gallbladder is usually present. The liver is supplied with blood via the hepatic portal vein and hepatic artery [4]. Reptile livers perform similar functions to mammals. It secretes bile for digestion, performs metabolic breakdown, and produces various metabolites. Also, Agama livers often contain large numbers of melanomacrophage centres, which can give the liver a mottled appearance [6].

Hepatopathies in reptiles are often diagnosed postmortem [7, 8]. Bacterial hepatitis in reptiles usually results from systemic bacterial infection (e.g., sepsis) and causes hepatic granulomas with multinucleated giant cells [9]. Bacterial infections often originate in the intestine and reach the liver via the portal vein [10]. Hepatomegaly, focal abscesses, and infarcts are problems resulting from hepatitis [11]. The bacteria most commonly isolated from reptiles with hepatitis are members of the Enterobacteriaceae family. More rarely, chlamydophil or mycobacterium species can be isolated from reptiles diagnosed with bacterial hepatitis [12, 13, 14].

Unravelling the microscopic changes that occur in the reptile liver after bacterial infection is a valuable resource for pathologists, clinicians and researchers [15]. Therefore, the correct diagnosis of reptilian diseases depends on it. Schaffner [16] analyzed the livers of all reptiles, but few studies have examined the livers of agama. Therefore, the aim of this study was to microscopically examine the liver of this species during bacterial infection in order to observe the extent of cell damage and reveal the host's response to infection.

2.0 MATERIALS AND METHODS

Ethics Approval

The Ahmadu Bello University Committee on Animal Care and Use (ABUCACU), Ahmadu Bello University, Zaria, Nigeria, authorized the study procedures and all animal examinations.

The Capture of Agama Lizards

100 adult specimens (male and female) of Agama agama lizards were caught inside and outside a poultry house in Zaria, Kaduna State, Nigeria. They were captured by hand or using baited sticky traps, the authors reported [17]. After collection, the samples were placed in properly ventilated containers and transported to the dissecting laboratory at Ahmadu Bello University, Department of Veterinary Pathology, Zaria, Nigeria. Lizards were euthanized and dissected. Lizard liver tissue was processed manually using the method described by Luna [18].

Liver tissue was fixed by the following steps. *Dehydration*: Samples were dehydrated in 70%, 80%, 95% and absolute ethanol for 1 hour. *Clearance*: Samples were then cleared in xylene for 24 hours. *Impregnation*: Specimens were impregnated with paraffin wax and placed in a vacuum embedding oven for 24 hours. They were then blocked with paraffin wax. The blocks were then labeled with a pencil, trimmed later, and soaked in water for 24 hours to facilitate cutting. *Sectioning*: For sectioning, the trimmed blocks containing the tissue were placed on a 5 µm thick microtome section. Sections were then placed in a tissue-floating bath of water preheated to 40°C. *Mounting*: Each glass slide was labeled with a diamond pen before being smeared with an adhesive (egg albumin).

Each section was then removed from the floating bath using a smeared glass slide and the slide was dried in an oven. *Rehydration*: The sections of the glass slide were rehydrated for 5 minutes each in xylene, absolute ethanol of 95%, 85%, and 70% concentration sequentially which is a reverse of the dehydration process. *Staining*: Each slide was rinsed in clear water, stained with hematoxylin for 5 minutes, then removed, immediately rinsed in water, soaked in ammonia for 3 seconds, and counterstained with eosin for 3 minutes. *Dehydration*: the sections on the glass slides were further dehydrated in 70%, 80%, 95%, absolute ethanol and then cleared in xylene. Finally, a synthetic mounting media (D.P.X.) was dropped on the section and a cover slip was placed on of the glass slides and allowed to dry for 24 hours.

Microscopic Examination

A total of 100 liver samples were successfully sectioned and examined. The Slides were then examined using a BH2 Olympus® light microscope. Photomicrographs were taken with a 7.1-megapixel Canon® digital camera.

3.0 FINDINGS

Gross Pathology of Lizard Liver

Livers were generally of normal size and colour in the lizards examined (Figure 1). However, the edges or the entire livers appear pale in 19 (19%) of the lizards and this is suggestive of fatty liver (Figure 2).

Figure 1: Photograph of an Apparently Normal Liver from A Lizard (Arrow)

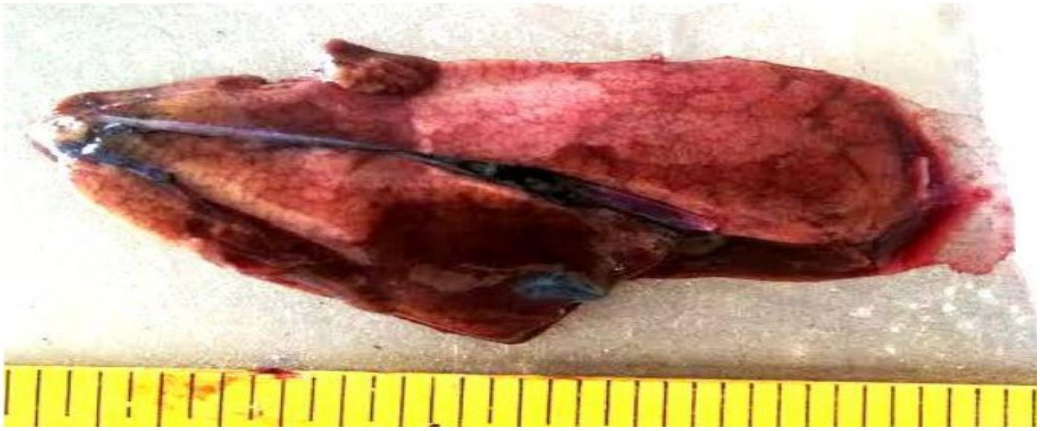

Figure 2: Photograph of a Liver from a Lizard. Note That the Tissue Has a Pale Colour Suggestive of Fatty Degeneration

Histopathology of the Livers

The observed hepatocytes in the parenchyma of normal livers were well arranged in cords around the blood vessel (sinusoid), and separated from each other by narrow clear sub-endothelial space (*Disse* space). Also, melano-macrophages, laden with melanin, were widely distributed within the tissue (Figure 3). On the other hand, there were observable alterations in many of the sections examined. Necrosis of hepatocytes was seen on some of the livers, sometimes with few melano-macrophages in the area (Figure 4A). In a rare case, a dividing melano-macrophage was seen on the section of a liver (Figure 4B). There were also mild hepatocytes vacuolation associated with hypertrophied melano-macrophages (Figure 5A). Severe vacuolation of hepatocytes cytoplasm was a common finding in many of the livers (Figure 5B). Hyperplasia of melano-macrophage and multiple areas of mononuclear cellular infiltration (Figure 6A) as well as congestion of blood vessels was also a frequent observation (Figure 6B).

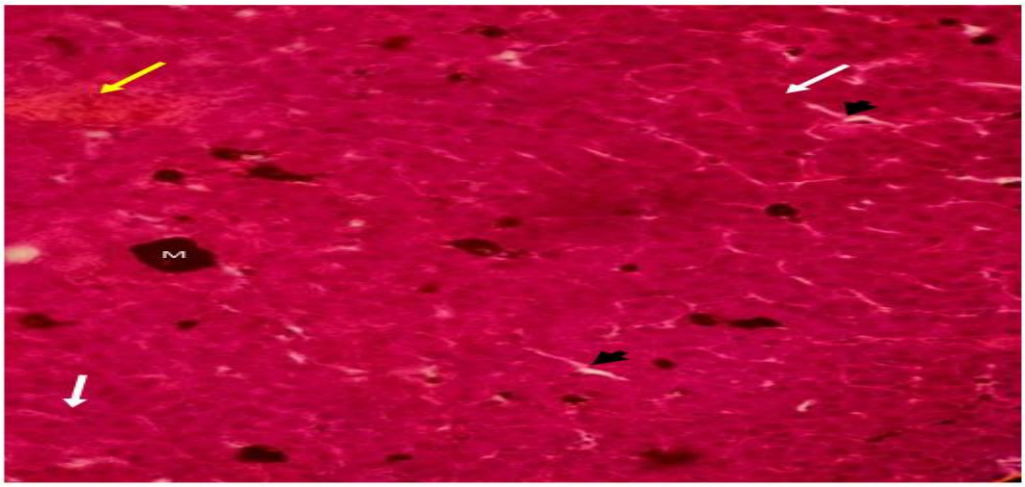

Figure 3: Photomicrograph of Liver from a Lizard. Note the Hepatocytes (White Arrows), Blood Vessel, Sinusoid (Yellow Arrow), Disse Space (Arrow Head) and Melano-Macrophage (M). H and E Stain. X400

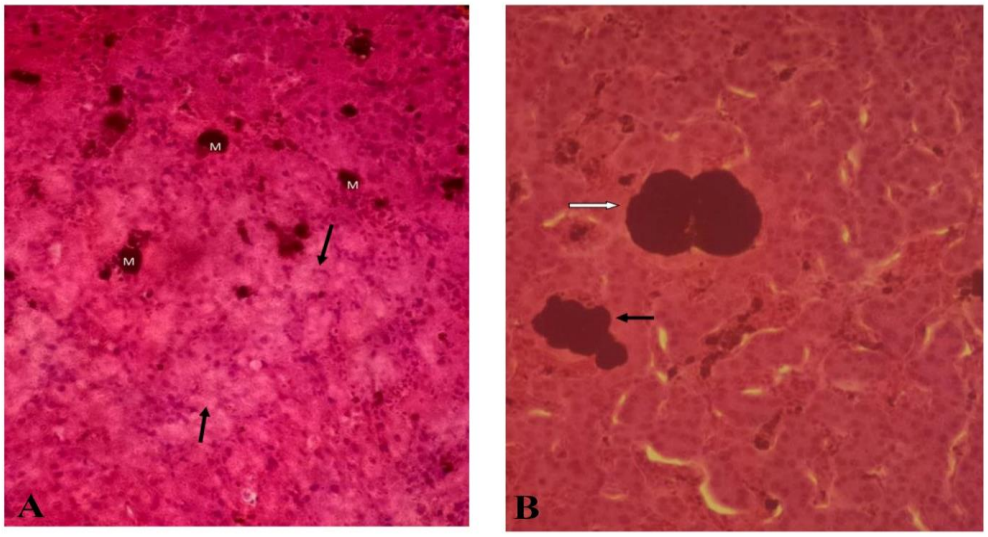

Figure 4A: Photomicrograph of Liver from a Lizard Showing Areas of Necrosis (Arrows) and Melano-Macrophages (M). H and E Stain. X400

Figure 4B: Photomicrograph of Liver from a Lizard Showing Two Melanomacrophages, One of Normal Size (Black Arrow) and the Other in Mitosis (White Arrow). H and E Stain. X400

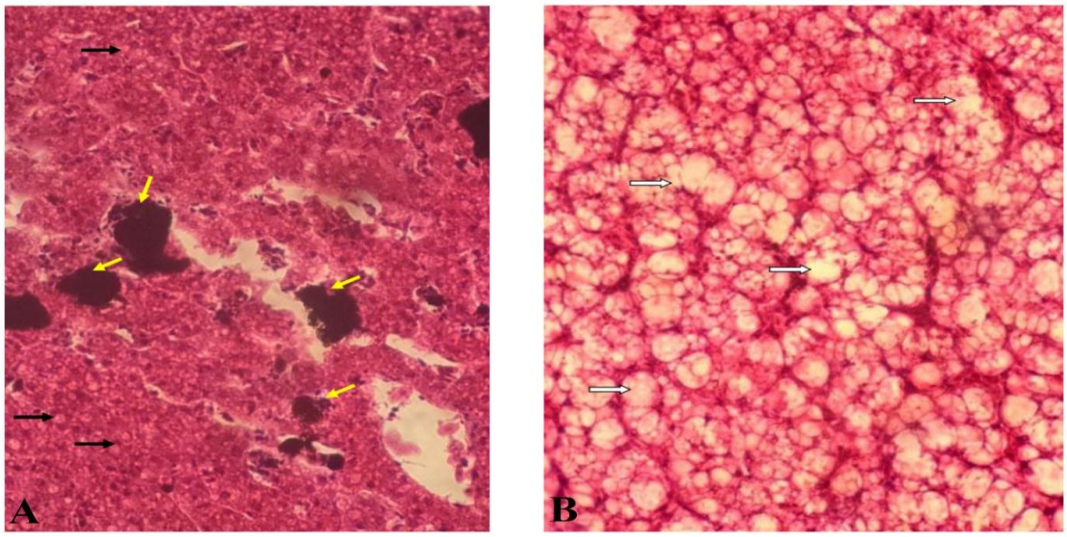

Figure 5A: Photomicrograph of Liver from a Lizard Showing Mild Vacuolation of the Hepatocyte Cytoplasm (Black Arrows) and Hyperplasia of Melano-Macrophages (Yellow Arrows). H and E Stain. X200

Figure 5B: Photomicrograph of Liver from a Lizard Showing Severe Vacuolation of Hepatocytes (Arrows). H and E Stain. X400

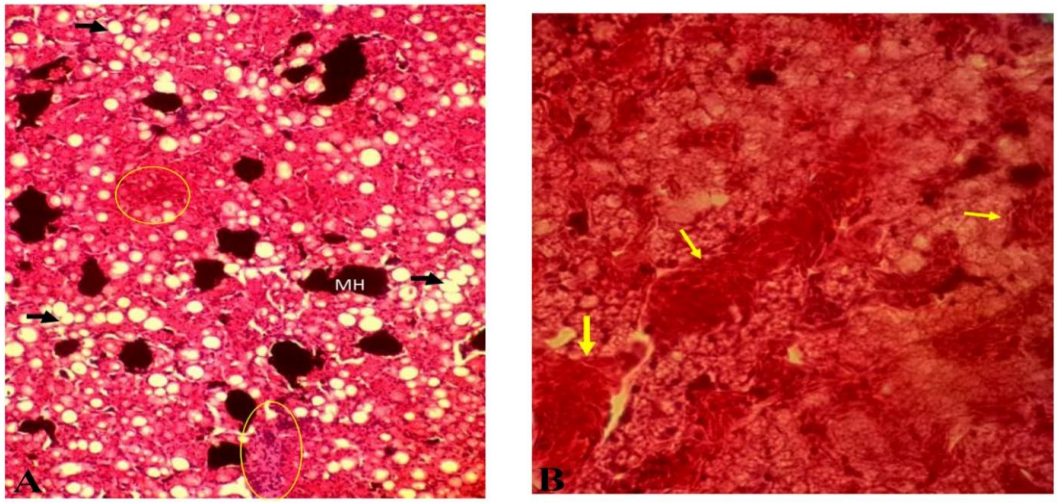

Figure 6A: Photomicrograph of Liver from a Lizard. Note the Vacuolation (Black Arrow), Melano-Macrophage Hypertrophy and Hyperplasia (MH) and Areas of Mononuclear Cell Infiltration (Circumscribed). H and E Stain. X400

Figure 6B: Photomicrograph of Liver from a Lizard Showing Congestion of the Blood Vessels (Arrows). H and E Stain. X400.

4.0 DISCCUSSION, CONCLUSION AND RECOMMENDATIONS

Although it has been established that reptiles do not have lymph nodes, accumulation of lymphoid cells are common in many tissues [19]. These lymphoid cells are called melano-macrophages or macrophage aggregates and are distinct groups of pigment-containing cells. They are also present in amphibian and some fish tissues, usually in the liver [20]. Melano-macrophages may be involved in antigen processing in a manner similar to germinal centers in mammalian lymph nodes. This is because melano-macrophages phagocytize erythrocytes, microbes, and other debris and thus need to be screened for infectious agents [21]. In mammals, persistent focal infection leads to antigenic stimulation by a reactive increase in the number of white blood cells in the area, and this reactive hyperplasia leads to enlargement of the lymph nodes (lymphadenopathy) [22]. Thus, in contrast to mammalian lymph nodes, lizard melano-macrophages are capable of stimulating an antigenic response, and the development of an enlarged melano-macrophage center (melano-macrophage hypertrophy) is a common non-specific observation in reptilian diagnostic pathology. This change is often referred to and diagnosed as melano-macrophage hyperplasia [22].

The hyperplasia of melano-macrophages in the liver observed in this study is of great immunological importance and can be explained as a defense mechanism against infectious agents. Therefore, the hypertrophy and hyperplasia of melanoma macrophages and the presence of other cellular infiltrates within the tissue observed in lizard liver in this study indicate an ongoing infection. Inflammation is characterized by mononuclear cell infiltration, primarily with leukocytes (lymphocytes and macrophages) accumulating at sites of injury to remove interfering substances. The presence of these mononuclear cells in the livers correlates with tissue necrosis, congestion, and the presence of the bacteria, Staphylococci and Corynebacterium species identified in this study.

Hepatic lipidosis is a metabolic disorder and not a single clinical disease [4]. Hepatic lipidosis may be due to glycogen or fat degeneration [23]. Factors affecting this condition have been reported to include excessive fat intake and overfeeding in crocodiles, lizards, and turtles [24]. In the current study, vacuolation may be due to lizards eating high-fat poultry diets that reptiles cannot tolerate. The apparent prevalence of hepatic lipidosis in non-breeding female reptiles is a normal occurrence. Many female reptiles undergo seasonal cycles of adipogenesis in preparation for folliculogenesis [4]. Therefore, hepatic lipidosis observed on livers of lizards in this study is more of a physiological status than a pathological feature.

On the other hand, liver necrosis, a clear pathological finding, has been reported in many liver diseases, including infections with *Aeromonas and Pseudomonas* [25], *Citrobacter freundii* and *Entamoeba invadens* [19]. This observation suggest that liver necrosis is the result of many bacterial infections. The necrosis seen in the livers of these lizards could be due to exotoxins produced by bacteria isolated from these samples. Agama lizards used in this study showed hypertrophy and hyperplasia of melano-macrophages. This indicates liver damage and/or infection as evidenced by the presence of mononuclear cell infiltrate, liver necrosis, liver congestion, and hepatic lipidosis. Therefore, the microscopic features of this study should be considered as diagnostic reference for liver pathology in agama lizards.

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