

Clinical and Histopathological Diagnosis of Acquired Visceral Melanosis (Black Liver) in Lambs from Garmian region, Kurdistan/Iraq

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Abstract:

Background: During routine post-mortem examination at the Kalar slaughterhouse in the Garmian region, Iraq, black pigmentation was observed in the livers of lambs aged 6–7 months, with occasional discoloration in the lungs, kidneys, and hearts. The affected animals showed no clinical signs prior to slaughter.

Aims: This study aimed to investigate the histopathological characteristics of the observed lesions.

Results: Tissue samples were collected and fixed in 10% neutral-buffered formalin then processed using standard histological techniques and examined microscopically. Histopathological analysis revealed a complete loss of hepatic architecture, congested central veins, coagulative necrosis, and pigment-laden Kupffer cells. Melano-macrophages and discrete pigment granules were also noted around portal areas.

Conclusions: The findings were consistent with acquired visceral melanosis (AVM), also known as “black liver.” This condition, though previously reported in other countries, is recorded here for the first time in Iraq. The etiology remains unclear, although environmental

and nutritional factors are suspected. Further studies are required to determine the origin and potential implications for animal health and meat safety.

Keyword: Black pigment, melano-macrophages, carcasses, kupffer cells, hepatocytes



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Introduction:

The liver is the largest glandular organ in animals and works to metabolize nutrients that are digested and absorbed by the digestive system (Saevan et al, 2017, and Baquerre et al, 2021). It is one of the most vital organs in the process of metabolism in mammals. Accordingly, any condition that affects the liver or causes liver failure will directly affect general body health, resulting in huge economic losses in animal production (Borai et al. 2013, and Baquerre et al, 2021) making it a global health problem (Trefts et al, 2017). This vital organ has several roles inside the body such as detoxification and elimination of endogenous wastes, processing of dietary carbohydrates, lipids, amino acids, vitamins, and synthesis of plasma proteins (John and Margaret, 2016, and Baquerre et al, 2021). It removes the toxicity of various substances from the blood supply; and thus, the liver is vulnerable to numerous pathological alterations leading to a wide range of macroscopic and microscopic pathological lesions (Saevan et al, 2017).

The liver has a unique property that allows it to regenerate itself after tissue damage, becoming indistinguishable from the original tissue within a few weeks (Baquerre et al, 2021). The occurrence and spreading of pathological lesions in the liver are affected by several factors, including season, temperature, rainfall, quality of breeding, and quality of genetic predisposition (Saevan et al, 2017). The presence of pathological lesions in the liver reflects the presence of disease in other body systems and organs, as the liver acts as a catchment for the massive absorptive part of the gut and all its occupier microorganisms (Borai et al. 2013).

Melanin was first generated by Berzelius in 1840 as any black colorings or pigmentation on an animal body (Marco et al, 2013). The source of this pigment in animals is mainly the oxidation of tyrosine (Marco et al, 2013). The most significant role of melanin is pigment formation, which is responsible for the coloration of skin (Dominika et al, 2015). The process of melanogenesis is supposed to be induced in affected organs and not absorbed from the gut (Meat and Livestock, 2024). Melanin then accumulates over time within the affected organs, turning grey and finally black, especially in liver cells (Meat and Livestock, 2024), while in kidneys, the dis-coloration was dark brown as a result of the accumulation of pigment in renal tubules (Rolf, 1960).

The term "Melanosis" is a Greek word denoting any condition characterized by abnormal dark coloration of skin or mucosa. According to its definition, it is not only the deposition of melanin, but of other dark-pigmented granules such as hemosiderin, lipofuskin, lipofuskin-like pigment or ferrum sulfate as well (Britton and Davidowitz, 2022, and Batistatou, et al, 2006). Additionally, the term is a generic term describing an abnormal grayish black or brownish black pigmentation and does not imply that the underlying pigment is specifically melanin (Chang, and Deere, 2006). Melanosis has been recognized as skin lesions in cattle, swine, and horses and in regional lymph nodes (Lyne, 1999). In some experimental cases, skin cells responded to nutritional changes and showed an increase in melanin pigmentation through topical application of agents (Scheger and Bean, 1973). Malignant melanoma was seen in esophagus of a patient (Chang and Deere, 2006). In a study conducted on mice, moderate numbers of pigmented cells have been noted in the nictations, meninges of the brain, Harderian glands, parathyroid, thymus, inner ear, and spleen (Klein-Szanto, et al, 1991).

In Australia, Norway, and the Falklands, microscopic, electronic, and biochemical studies were implemented to describe the black pigment in ovine livers. In these studies, within each particular case, similar changes were detected demonstrating the pigment is the same in each one of these cases (Meat and Livestock, 2018). The pigmented regions generally have a high concentration of melanocytes throughout the basal layer of the epi-dermis (Scheger and Bean, 1973) .

Acquired visceral melanosis, or black liver disease, affects the livers of sheep and goats. Also, other organs can be affected, resulting in black-organ disease (Meat and Livestock, 2017, Meat and Livestock, 2024). Recently, this disease has been reported in domestic animals that are kept in abattoirs in different parts of Australia (Meat and Livestock, 2024). The disease has a significant effect on animal carcasses, as it causes the condemnation of the carcasses (Meat and Livestock, 2017). The black liver condition was detected incidentally after slaughtering healthy animals (Meat and Livestock, 2018). The condition has been investigated after banning the slaughtering of diseased sheep in the Middle East (Meat and Livestock, 2018). Some cases of pigmentation (-congenital melanosis) occur in calves and occasionally in lambs and swine (John and Margaret, 2016, Meat and Livestock, 2024) .

Melanin deposits, which vary in size (about 2cm or more), are blue-black areas or black patches can be found on the liver surface (John and Margaret, 2016, Bob and Steve, 2010). The acquired melanosis represents the vast growth of non-melanin black pigment in liver cells like (e.g. kupffer and hepatocytes) of sheep. It occurs less commonly cattle after long time grazing on unimproved pastures (John and Margaret, 2016). Kupffer cells are fixed macrophages in an arrangement that

facilitates phagocytic elimination of particulates that enter the blood through the lower elementary tract (John and Margaret, 2016).

In some European countries, like Norway, the condition has been defined as hepatic lipofuscinosis (John and Margaret, 2016). In initial cases, the liver color ranges from dully gray to uniform black, and arranged in acinar pattern. It is not regularly restricted to the liver and may also be seen adjacent to the sacrum and vertebral column, and under the pleura over-lying the ribs and intercostal muscle (Bob and Steve, 2010). In severe cases, pigmentation can also be observed in the hepatic lymph nodes, lungs, and renal cortex (John and Margaret, 2016, Meat and Livestock, 2018, Bob and Steve, 2010) .

The pathogenesis and risk factors of this condition have not been properly known with limited field observations either (Meat and Livestock, 2024, Norman et al, 1996). No clinical signs can be seen in the affected animals, and the disease cannot be diagnosed while animal is alive. Following slaughtering of many black liver diseased animals, the liver appears grey to black, and presence of black grease material stuck to the knife blade. Similarly, other internal organs like the lungs, kidneys, lymph nodes, bones, tendons, and artery walls can turn black or grey. The black liver and lungs have a distinct metallic smell (Meat and Livestock, 2024, Norman et al, 1996, John et al, 2013). It may diffusely discolor the animal tissue, which may cause it to be confused in diagnosis with other diseases such as melanoma (John et al, 2013).

In histological sections, black pigments are found in different parts of the affected cells such as hepatocytes and macrophages of the liver, the epithelium of the kidneys, and macrophages in the lung (John and Margaret, 2016). There is no clear evidence about the source of this pigment, but the epidemiologic features of its incidence indicate that it is a component of the diet (John and Margaret, 2016). A differential diagnosis should be taken into account while discussing this condition. Cases of copper poisoning with black kidneys, bronze-colored livers, and very dark livers in newly dead animals should be considered while discussing melanosis (Meat and Livestock, 2024, John et al, 2013). Pigments of parasitic origin are mainly related to flukes. In schistosomiasis, the liver may be grey because of the growth of black pigment in Kupffer cells (John and Margaret, 2016). Scattered black pigment on the surface of liver of animals affected by the parasite *Fascioloides magna* after postmortem examination (Karamon, et al, 2015). In studies conducted on rabbits and chickens, the histopathological lesions showed a petechial hemorrhage scattered among hepatic tissue (Abdulwahhab and Baker, 2023, Hussein and Ibrahim, 2023). In another study on rabbits, it was found that an overdose of drugs like ibuprofen caused the deposition of fat in kidneys and bleeding in other organs (Mahmood, 2024). The study aimed to

highlight this strange condition in the study area and to try to identify the reasons for its appearance, if any.

Materials and Methods:

Study Area and Animals

This study was conducted on a flock of 240 male lambs aged 6–7 months, raised for fattening in Rizgari sub-district, Garmian region, Kurdistan, Iraq. The first suspected case was observed on April 14, 2022, during routine post-mortem inspection at the Kalar slaughterhouse. Subsequently, 10 lambs were slaughtered daily, and among them, 3–4 animals consistently presented with black liver pigmentation. All animals were clinically healthy prior to slaughter and exhibited no external signs of illness .

Histological Methods

Affected livers were packaged and preserved in 10% formalin in plastic containers and sent to the veterinary anatomy and histology branch at the College of Veterinary Medicine and Surgery at Mosul University. The histological study samples were preserved with a neutral buffered formalin solution for tissue fixation and to prevent decaying and autolysis of the samples for at least 72 hours, followed by routine histological processing methods using increasing concentrations of ethyl alcohol (70%, 80%, 90%, and 100%) for dehydration, and chloroform was used as a clearing agent twice every 30 minutes. The specimens were transported to melted paraffin in two steps. Each step rests for two hours in an electron oven at 58°C. After that, the specimens were placed in molds and covered with melted paraffin wax at melting point of 58-60 °C .

The paraffin blocks were cut by a rotary microtome at 5-7 µm. Hematoxylin and eosin stains were used for general histological study and microscopic measurements (Kim et al, 2023). The histological sections were photographed, and micro-morphometric parameters were measured using the color USB 2.0 digital image camera (Scope Image 9.0- China), which was provided with image processing software.

Results:

Necropsy Findings

During the postmortem process, a sign of complete blackening of the liver appeared in several cases. Also, the presence of some black patches on the lungs, heart, and kidneys was observed, with some of these organs turning black in other cases (Figure 1).



Figure 1: A, B, C, and D: Black pigmentation of animal livers at necropsy findings.

Histopathological Findings

The pathological report confirmed that the expected diagnosis was Acquired Visceral Melanosis (AVM, black liver). Based on Hematoxylin and Eosin staining, sections of the liver with different magnifications under microscopic examination showed a loss of hepatic architecture, represented by missing the standard hepatic cord orientations. Also, some congested central veins were seen.

Another section revealed coagulative necrosis with mild infiltrations of mononuclear inflammatory cells. Kupffer cells loaded with pigment granules can be seen near the central vein. Moreover, a coagulative necrosis of the epithelial layer of bile ducts was observed, and some cells show destructive fragmentation of the nucleus (Karyorrhexis). Solitary depositions of fibrin (organized thrombus) were determined (Figure 2).

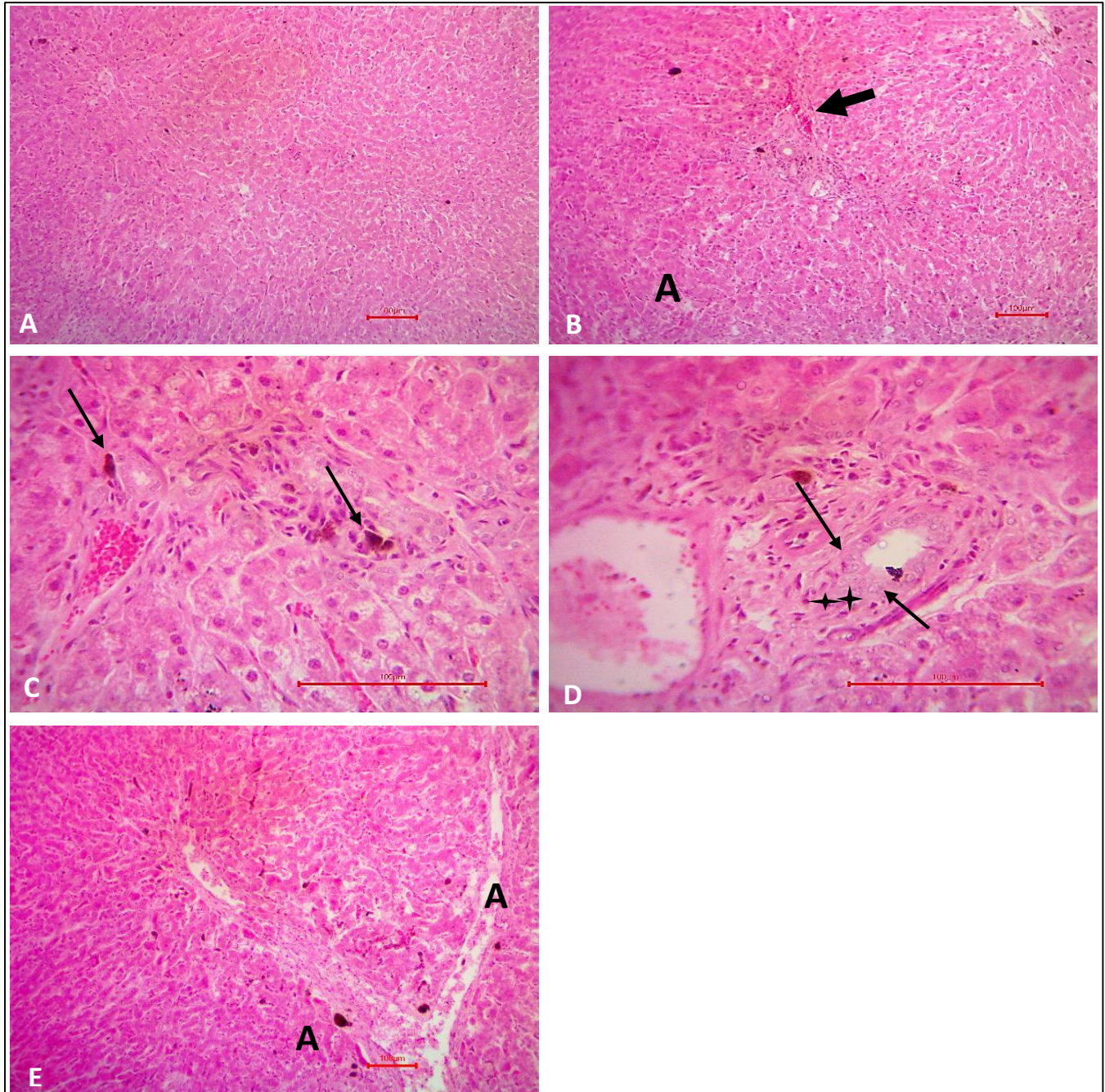


Figure 2: **A:** loss of hepatic architectures H&E 40X. **B:** Congested central veins (arrows), fissure of sinusoids (letter A) H&E (100X). **C:** Kupffer cells loaded with pigment granules can be seen near the central vein H&E 400X. **D:** Congestion of the epithelial layer of bile ducts and some cells show destructive fragmentation of the nucleus (Karyorrhexis) (stars) H&E (400X). **E:** Deposition of fibrin (organized thrombus) with pigment granules H&E (100X).

Thickening of the liver capsule and focal infiltration of inflammatory cells in the liver parenchyma with congested blood vessels was also seen. Additionally, the presence of melano-macrophages between hepatocyte plates and melanin-pigments was noticed as high concentrations of discrete granules around the portal area in different sizes and shapes. There is, however, a mass of vacuolar degeneration of hepatocytes and perivascular cuffing (Figure 3).

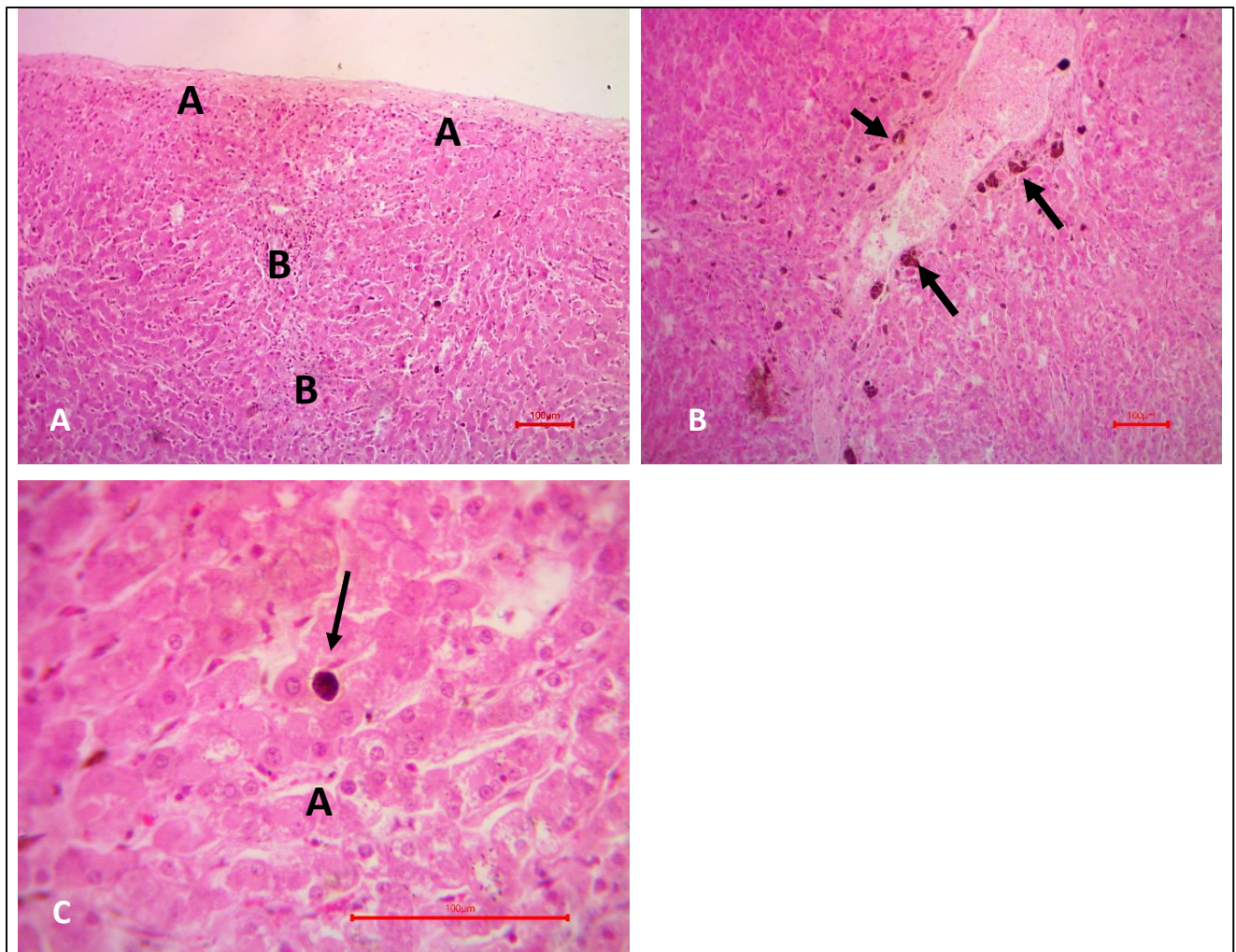


Figure 3: **A:** Thickening of the liver capsule (letter A) focal infiltration of inflammatory cells (letter B) H&E (100X). **B:** Presence of melanomacrophages and discrete granules around the portal area in different sizes and shapes H&E (100X). **C:** Mass of vacuolar degeneration of hepatocytes (A) and Kupffer cells loaded with pigment granule (arrow).

Discussion

Acquired visceral melanosis is a condition or illness that infects mainly the liver and, less frequently, other internal organs, including the kidneys, heart, and lungs of sheep, goats, and cattle. It is also called black organ disease because of the accumulation of black areas within the tissue of these organs. The cause of the condition is still unknown, as no clinical signs appear on live animals but rather it can only be infected after slaughtering. The condition was recorded in abattoirs in Australia, Norway, and the Falkland Islands (Meat and Livestock, 2024).

In our study, the infected lambs did not show any clinical signs and all of them were healthy until slaughtering time. This was similar to what was reported before in many parts of the world (Meat and Livestock, 2024, Meat and Livestock, 2018). The rate of infection in slaughtered animals was about 45%, showing signs of black pigmentation in the liver. Livers showed a sign of complete blackening of the liver in more than one case. The lungs, kidneys, and, in some cases, hearts appeared slightly black too. This condition causes economic losses due to the rejection of animal carcasses at abattoirs (Meat and Livestock, 2018, Meat and Livestock, 2017). Deposits of blue-black areas of melanin were seen in calves and occasionally in lambs and swine (John and Margaret, 2016, Meat and Livestock, 2018). In a study applied to an anomaly in cattle, a black kidney condition characterized by a dark brown or almost black discoloration was observed by Rolf (1960). The study proposed that this was due to the deposition of pigment in the epithelial cells of certain portions of the renal tubules (Rolf, 1960).

Based on histological findings and pathological reports, the condition was confirmed as Acquired Visceral Melanosis (AVM, black liver). The microscopic examination showed a loss of hepatic architecture, represented by missing the standard hepatic cord orientations with congested central veins. Another section revealed coagulative necrosis with mild infiltrations of mononuclear inflammatory cells. Kupffer cells loaded with pigment granules are also seen with coagulative necrosis of the epithelial layer of bile ducts, and some cells show destructive fragmentation of the nucleus (Karyorrhexis). In another study by (John and Margaret, 2016), it was recorded that there was a huge accumulation of black pigment in Kupffer cells and hepatocytes of mature sheep and, less frequently, cattle.

In the current study, the liver parenchyma showed focal infiltration of inflammatory cells with congested blood vessels. The liver capsule was thick, with the presence of melano-macrophages between hepatocyte plates. The melanin pigments were highly concentrated with discrete granules around the portal area in different sizes and shapes. The existence of the pigment as granules in lysosomes in periportal and midzonal hepatocytes and macrophages of the liver was seen by (John and

Margaret, 2016). Is also mentioned that liver color ranges from dully gray to uniform black, and there is a prominent acinar pattern. It is not regularly limited to the liver and may also be seen adjacent to the sacrum and vertebral column and under the pleura, overlying the ribs and intercostal muscle (Bob and Steve, 2010).

The condition should be differentiated from the other similar conditions such as dark livers in animals' dead for many hours and the bronze-colored livers and black kidneys of animals dying of copper poisoning as well as pigments from parasitic origin (John and Margaret, 2016, Meat and Livestock, 2024, John et al, 2013, 22).

Conclusions:

This study reports the first confirmed cases of acquired visceral melanosis (AVM) in lambs in the Garmian region of Iraq. The diagnosis was based on consistent macroscopic and histopathological findings, including pigment accumulation within Kupffer cells and hepatocytes, vascular congestion, and hepatic architectural disruption. Although the etiology remains unclear, the observed pattern suggests a possible environmental or nutritional component. Given the economic implications of carcass condemnation and the asymptomatic nature of the condition in live animals, further investigations are required to elucidate its cause, distribution, and potential preventive measures.

Recommendations:

Based on the findings of this study, it is recommended to conduct a monthly or annual survey of random samples of sheep, goats, and cows in specific areas of Iraq to be prepared for any potential spread of this disease among animals.

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Conflict of Interest:

The authors declare no conflicts of interest.

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None.

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