



Pathological Lesions of Complex Gill Diseases in Farmed Trout Salmon (*Oncorhynchus mykiss*) Fish in Duhok Province



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Shaaban T. Sadiq^{1*}, Alaa H. Ali Al-Hamdani² and Zanan M.A. Taha³

¹Department of Pathology and Microbiology, College of Veterinary Medicine Duhok University, Duhok, Iraq

²Department of Pathology & Poultry Diseases, College of Veterinary Medicine, Mosul University, Mosul, Iraq

³Duhok Research Center, College of Veterinary Medicine Duhok University, Duhok, Iraq

DISORDERS of the gill through the time of farming trout salmon (*Oncorhynchus mykiss*) become a major problem. The word “complex gill disease” (CGD) refers toward a variety of clinical gills diseases signs that typically occur over the course of four seasons in farms of salmon fish. The observed gross lesions and histopathological lesions are the end result of infection to pathogenic organisms. Fish pathological and normal states can be determined using histopathological examination. This study estimated the health conditions of farmed trout salmon by use the histopathological examinations as a diagnostic instrument by using the routine stain hematoxylin and eosin, and using special stain Alcian blue stain and periodic acid-fast stains samples of gills of 200 trout (*Oncorhynchus mykiss*) from salmon farms in Duhok collected and fixed. All fish have lesions on the gills. Studies using light microscopy of the treated gills revealed significant histological changes which includes hemorrhage and blood vessels congestion of the primary and secondary lamella, facultative degeneration of epithelial cells, mononuclear inflammatory cells infiltration, necrosis epithelial cells of the secondary lamella, adhesions of secondary lamella, hypertrophy of chloride cells and mucus cells, facultative degeneration of pillar and hyperplasia of chloride cells.

Keywords: Gills Lesion, Pathology, Hyperplasia, Complex gills diseases, *Oncorhynchus mykiss*.

Introduction

Currently, half of the World's fish's source for human consumption comes from aquaculture, which continued as the animal production industry with the greatest growth rate [1], though, there is currently a lack of scientific data on the health of farmed fish [2- 6] Diverse techniques for evaluating the health state of aquatic creatures may have an impact on fish welfare and significantly increase production, one of them is the histological diagnosis as instruments for disease analysis and prevention [7] . The histopathological analyses are also described as confirming and conclusive diagnoses. [8, 9] A number of chemical

and physical alterations in the body lead to histopathological alterations. [10] Combining the impacts of biotic and abiotic variables on fish health and organs functions [11- 13].

Fish tissue changes can be affected by stress of agents of environment. [14] Or affected by pathogens [15, 9] conceding the development and persistence of animals. The, histopathological investigation is a virtuous indicator of fish health condition and the significance of both lesions based on in what way it affects functions of the organs and the fish's capability to live [16]

Gill diseases in farms of salmonid fish are highly significant due to cooperated gills functions

which can cause major financial loss owing to reduced dietary conversions performances, high mortality and, where treatment is needed, the costs of treatments. [17]. Though, the disease of gills is not modifiable, Since gills are in close contact with water environments and are extremely delicate compared to the integument, they are exposed to pathogens, toxins, pollutants, and organisms occurring within farm net-pens, leading them mainly susceptible to infections and physical injury. As a result, the true extent of the problem is still unknown. Numerous pathogens have been linked with gills diseases, including parasitic infections, viruses, fungi, and bacteria [18].

It is known that infection leads to severe tissues lesion and many studies that related to infectious gills anomalies and recording lesion such as epithelia cells fusions and hyperplasia of the secondary lamellae [15, 9]. The techniques of special staining could be used for detection and diagnosis of histopathologic lesion in fish [19] So this study deal with the pathological lesion of complex gills infections of farmed trout fish (*Oncorhynchus mykiss*) at Duhok province

Material and Method

Fish sampling

Live samples of 200 trout salmon fish (*Oncorhynchus mykiss*) were collected from the seven different ponds in the two different salmon farms in Sheladiz and Amedi in Duhok province during the period from March 2021 to June 2022. The fish were killed and transported with ice to the laboratory.

Gill sampling and histological sections

The gills were excised keeping the filaments and rakers intact, rinsed in distilled water, then fixed in 10% neutral buffered formalin with monobasic and dibasic sodium phosphate at pH 6.9. For 10 days at room temperature. The samples were dehydrated in progressing in advancement of alcohol, put in xylene and fixed in paraffin wax using an automatic tissue processor and wax dispenser. Thin sections (6 µm) were cut by using a rotatory microtome.

Histological stains

1-Harris haematoxyllin-eosin (H&E) stain [20]

2- Alcian blue stain [21, 22]

3- Periodic acid Schiff's reagent (PAS) [23]

The sections were examined and photographed using an Olympus microscope fixed with photograph- attached cameras.

Results

The most histopathological lesions which were seen in H and E stain are the epithelial cells hyperplasia of the primary and secondary lamella, haemorrhage in the primary and secondary lamella (Fig. 1). In some sections there was congestion of the blood vessels of the secondary lamella (Fig. 2) there is facultative degeneration of epithelial cells (Fig. 3) in other sections there was increase in mucus material, climbing of dissemination epithelia's cell and mucus material between secondary lamella (Fig. 4) necrosis of the secondary filaments epithelium cell with mononuclear inflammatory cells infiltration (Fig. 5). There were also adhesions of primary lamella in some gill sections (Fig. 6).

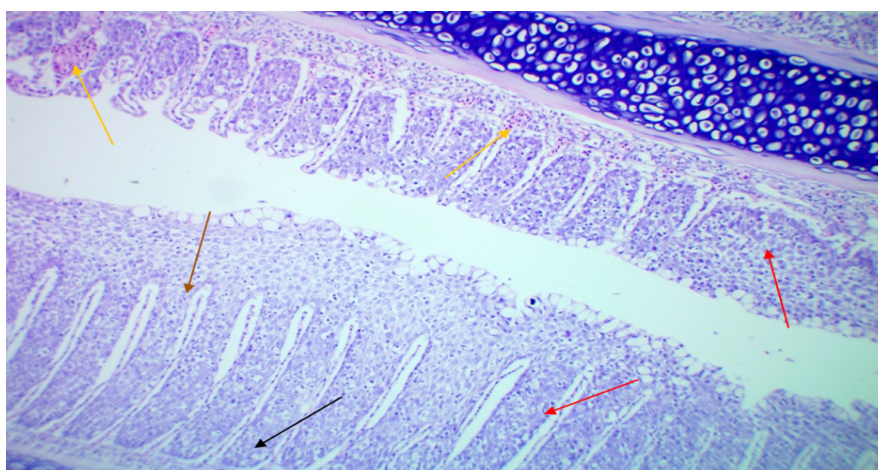


Fig.1. Hyperplasia of the epithelial cells of the primary gill filaments black arrow (—→) and secondary lamella gill filaments red arrow (—→) with haemorrhage in the primary and secondary lamella orang arrow (—→) infiltration of mononuclear inflammatory cells brown arrow (—→) H&E [40x].

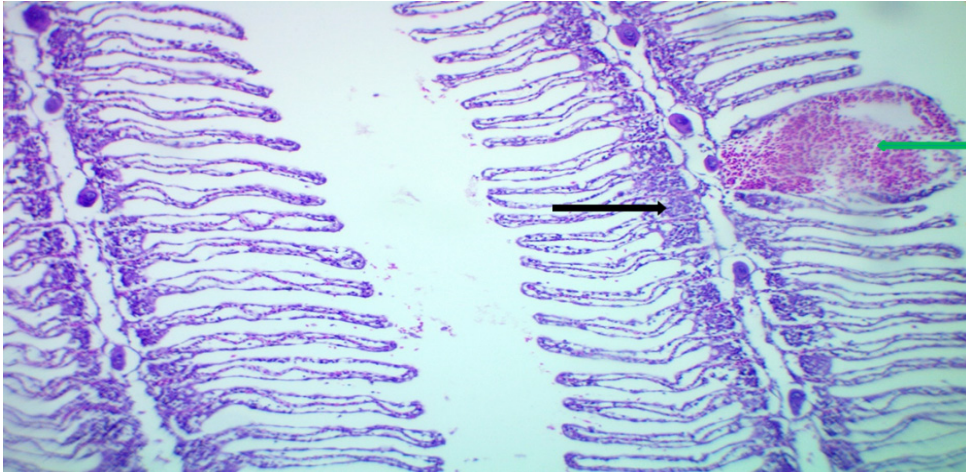


Fig.2. Hyperplasia of the epithelial cells of the primary lamellae black arrow (—→) congestion of the blood vessel of the secondary lamellae green arrow (—→) H&E [10x].

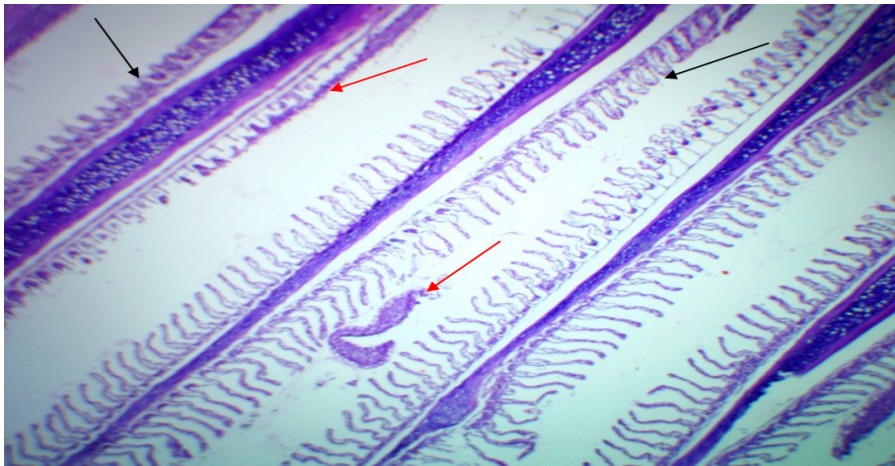


Fig. 3. Hyperplasia of epithelial cells of primary and secondary gill filaments black arrow (—→). increase in mucus discharge red arrow (—→) H&E[10x].

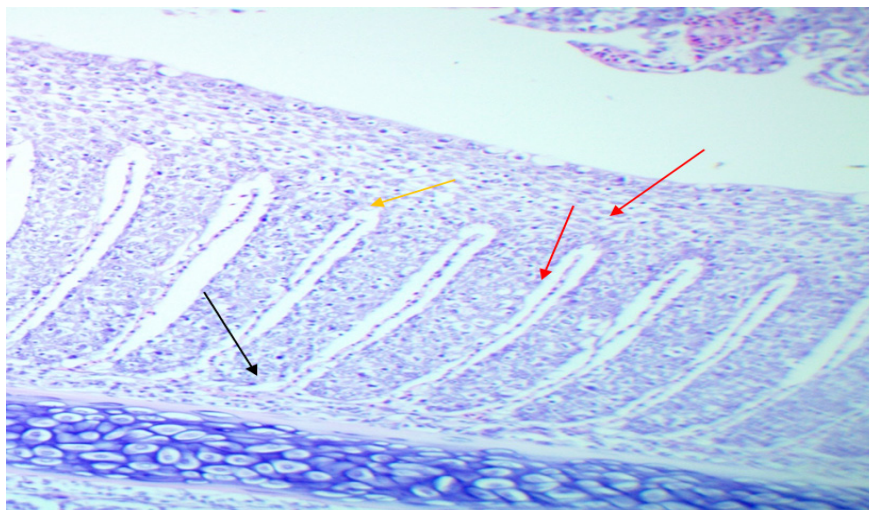


Fig. 4. Hyperplasia of the epithelial cells of primary and secondary gill filament with facultative degeneration of these cells red arrow (—→) and congestion of blood vessels of primary lamella black arrow (—→). Infiltration of monocular inflammatory cells yellow arrow (—→) H&E [40 x].

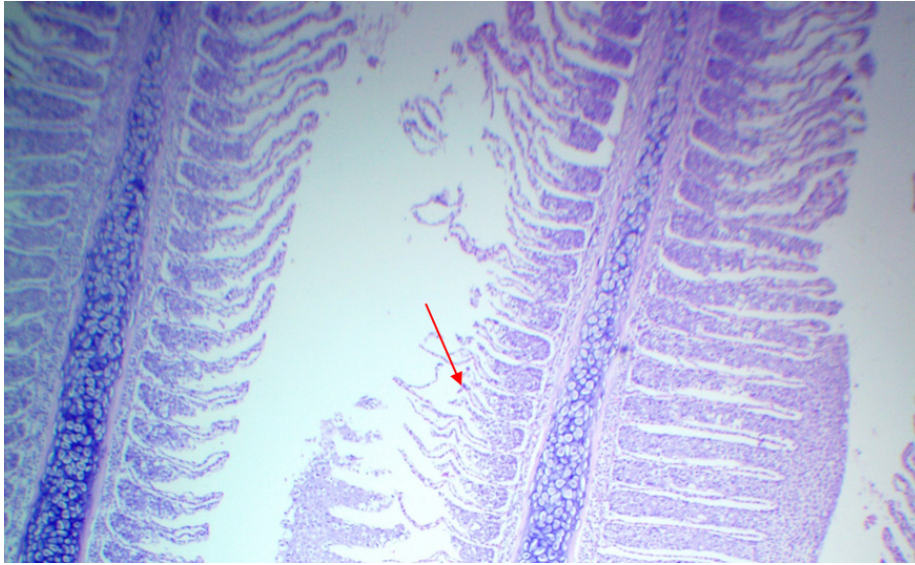


Fig. 5. Necrosis in the secondary lamella red arrow (→) H&E [10 x].

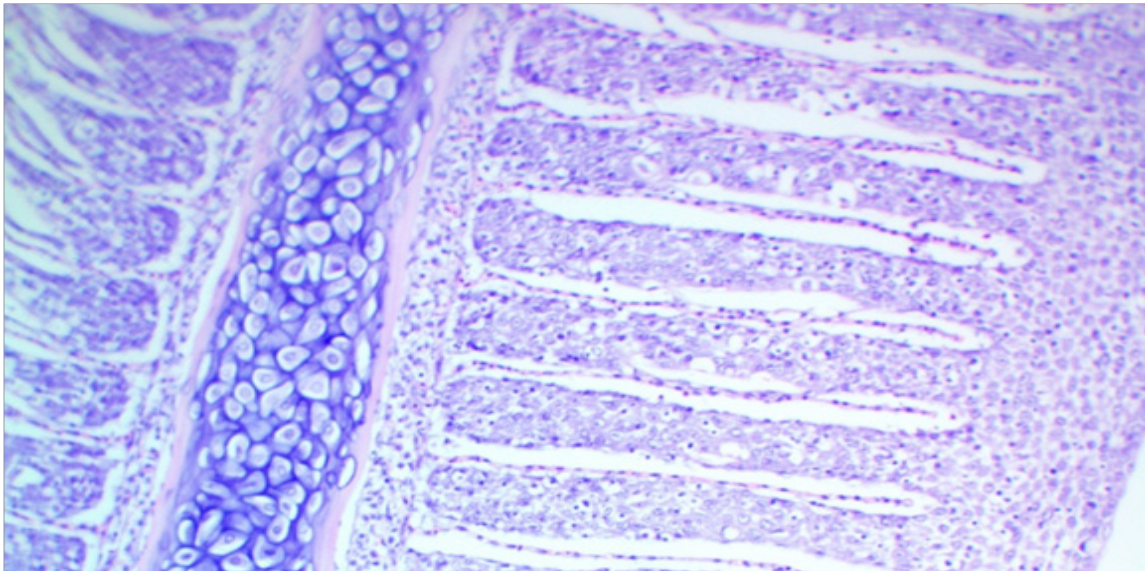


Fig. 6. Adhesion of secondary lamella .H&E [40x] .

In sections stained with Alcian blue stain there was hypertrophy of chloride cells which look as a blue cell with red nucleus and also hypertrophy of mucus cells that appear as blue cells with inapparent nucleus also vaculative degeneration of pillar cells which appear pink cells with red nucleus was seen in some section of gills (Fig.7) and other sections showed hyperplasia of mucus cells with hypertrophy of chloride cells (Fig. 8) also there was increase in number of mucus cells in other sections(Fig. 9) .

In sections that stained with PAS stain shows epithelial cells hyperplasia with facultative degeneration and hypertrophy of mucus cells which appear dark pink (magenta) and had a granular appearance, there was hypertrophy of chloride cells which appear blue with dark blue nucleus and pillar cells had blue-green nuclei and cytoplasm (Fig. 10). Other sections show adhesion of the secondary lamellae with vacuolation of epithelial cell and mucus cells hyperplasia with vacuolation of pillar cells and increase in number of chloride cells (Fig. 11)

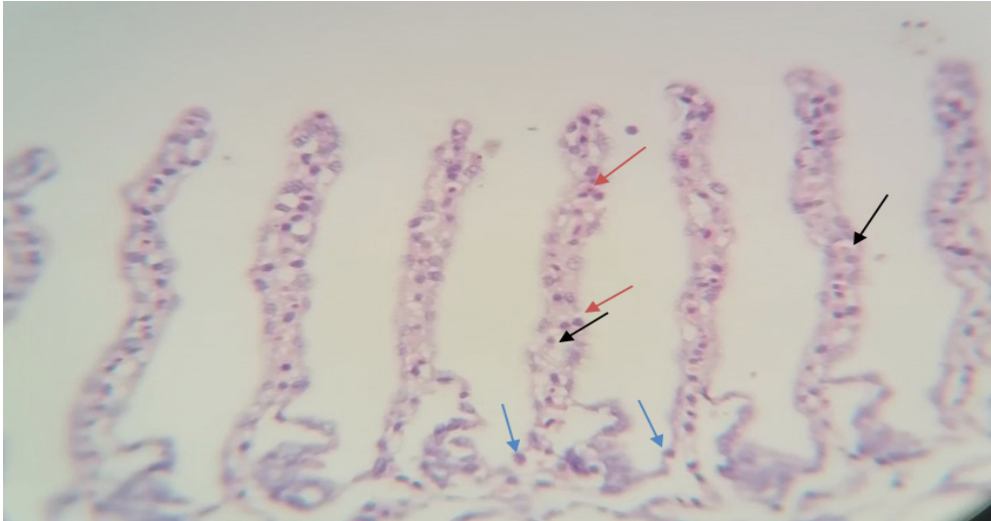


Fig. 7. Hypertrophy of chloride cells blue arrow (→) with hypertrophy of mucus cells red arrow (→) and valuation of pillar cells black arrow (→) Alcian blue[40x].

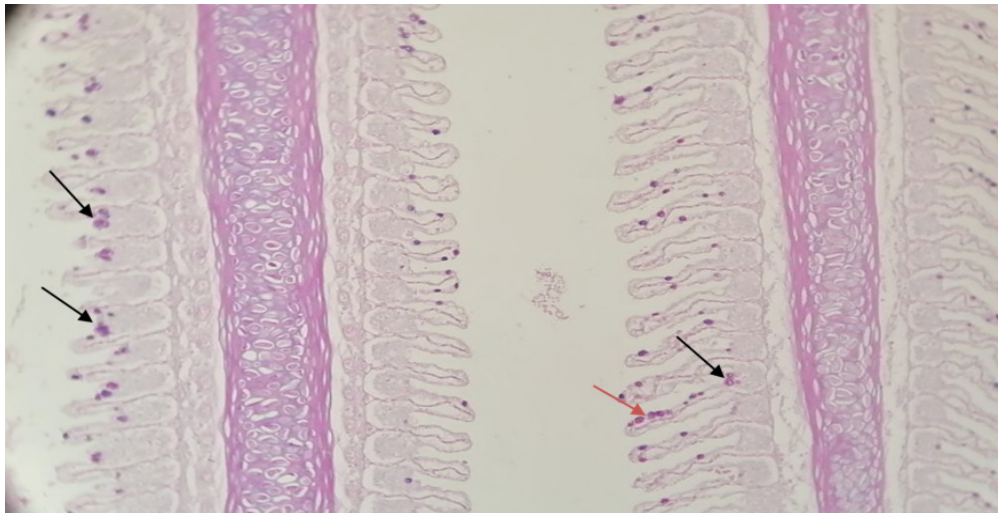


Fig. 8. Hyperplasia of mucus cells red arrow (→) with hypertrophy of chloride cells black arrow (→) Alcian blue blue [10x]

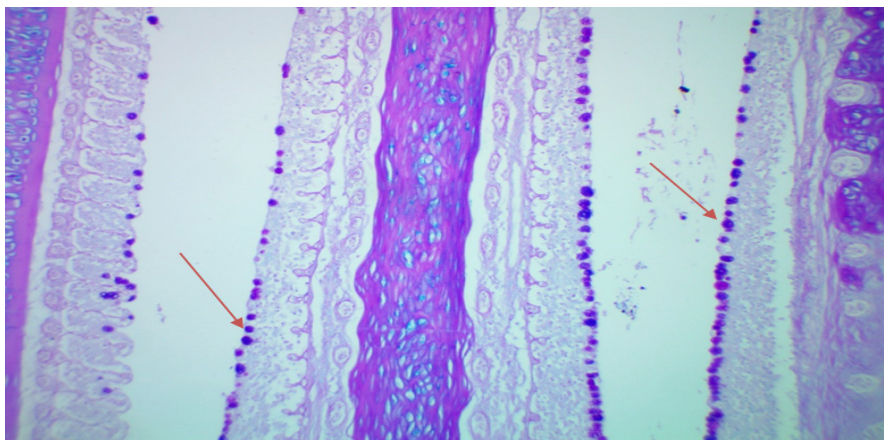


Fig. 9. Increase in number of mucus cells red arrow (→). Alcian blue stain [10x].



Fig. 10. Hyperplasia of the epithelial cells with vacuative degeneration green arrow (→) hypertrophy of mucus cells red arrow (→) and chloride cells black arrow (→) and pillar cells blue arrow (→) PAS stain [40x].

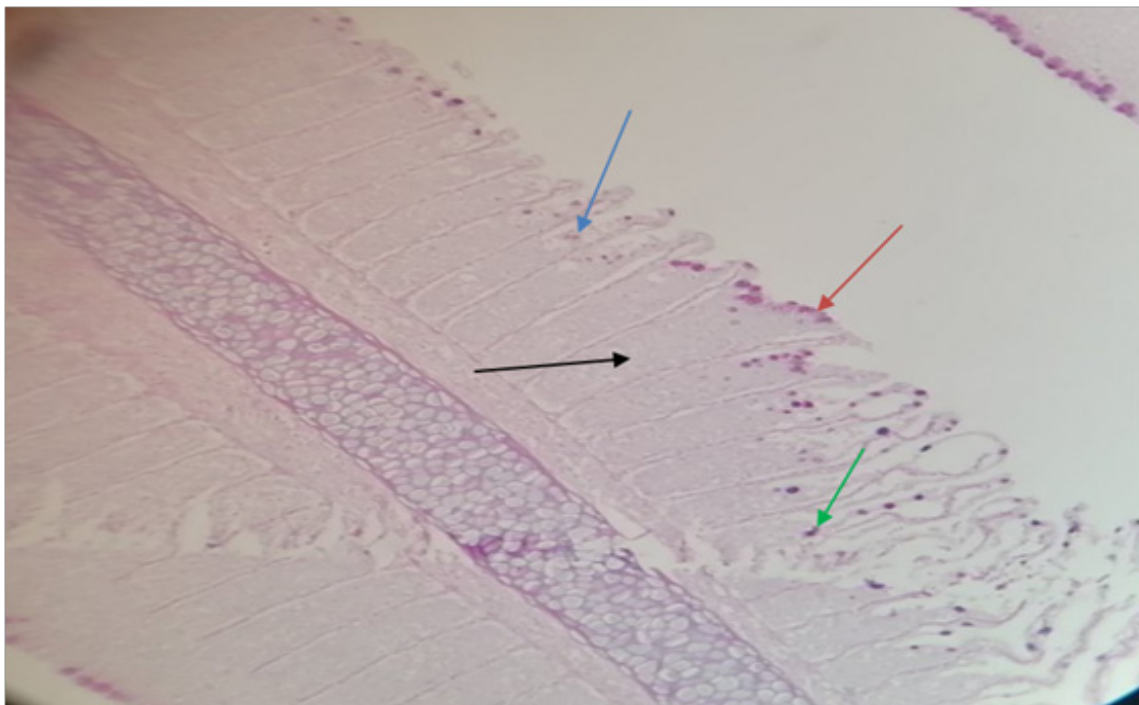


Fig. 11. Adhesion of the secondary lamellae with vacuolation of epithelial cells black arrow (→) hyperplasia of the mucus cells red arrow (→) vacuolation of pillar cells blue arrow (→) increase in number of chloride cells green arrow (→), PAS stain [40x].

Discussion

Gills are essential for several additional serious physiological functions as well as gaseous exchange, including osmoregulation's, nitrogenous waste excretions, productions hormones and regulating the pH [24], so the gills were the most seriously damaged organ in naturally sick trout, with enlargement of the gills, hyperplasia of the epithelial cells of secondary lamellae, and widespread proliferation of interlamellar cells, which led to secondary lamella fusion, also fish with BGD (Bacterial gill disease) typically exhibit lamellar fusion and hyperplasia lamellar epithelial cell [25] . These lesions were recorded in this study and similarly the histopathological changes of the gills reported in juvenile turbot [26], the adhesions of the gills filaments degradation. With severe coagulation necrosis in the gill filaments, there was an immature granulomatous inflammatory response between the gill filaments.

The infiltration of inflammatory cells could be seen in chronic infection, particularly parasites, can stimulate the responses of inflammation through the immigration of macrophage, lymphocyte neutrophil, thrombocyte, and granulocytes of the eosinophilia alongside of the gills filaments and in the essential filaments vessels [27, 28]. In this study, each of these lesions was identified as fish can get stressed due to a variety of causes, including changes in water quality, high stocking density, poor management, or improper feeding, making them more susceptible to various infection and infestations [29, 30] . Nile tilapia was found in the Jansen lagoon in Brazil, and researchers noticed gill abnormalities such lamellar epithelial detachment, lamellar fusion, and infiltration inflammation. They came to the conclusion that these changes were the fish's adaptive reaction to the poor microbiological water value. The occurrence of this alteration in the trout gills of the present study may be justified by the fact that it may also cause vasodilation of the vascular axis, which leads to pillar cell rupture and the onset of telangiectasia [31]. Similarly, [32] suggested that morphological changes in the gills may occur during environmental changes as adaptive efforts to reserve physiological functions, which supports the findings of the present study. Though a protecting reaction, any increases in respiratory surface thickness due to epithelial hyperplasia lead to larger distances for the exchanges of gases and metabolites with water.

As a result, impairments to the respiratory, osmoregulatory, and excretory systems may result, especially if the reaction is diffuse and impacts a significant portion of the gill [27] as was seen in the trout used in this investigation, where the intensity of the organ's modification was high. This information is of significant commercial significance since any health issues with farmed fish are concerning because the animals can use their energy to maintain homeostasis instead of growing and gaining weight, in addition alteration in tissues. Inflammations are a protecting response of live animals, and happen when biochemical, physiological and parasitological stimulation intensely disturb native tissues. In this instance, the blood flow is hindered by hyperplastic blood capillaries, which release fluids and defense cells that move to the site of inflammation [33].

The fish gill epithelium's chloride cells are characterized as specialized structural components that actively transfer ions between the inner and outer media in order to maintain ion and osmotic balance. Na⁺ and Cl⁻ are excreted outside by seawater fish, but freshwater fish take these ions in from their surroundings [34] in this study there was increase in number of chloride cells and hypertrophy of these cells these results agree with [35] Despite the limited range of pathologic responses in gill tissue, lesions such as chloride cell hyperplasia, mucous cell hyperplasia, lamellar cell degeneration and necrosis, epithelial lifting, lamellar or filament fusion, lamellar adhesions, and epithelial cell hypertrophy and hyperplasia have all been documented. [36] and all these lesions have been observed in this study .

Conclusion

In conclusion the common microscopical lesions recorded in the gills tissue were epithelial cells hyperplasia of the primary and secondary lamella with haemorrhage and congestion of the blood vessels, facultative degeneration of epithelial cells could be demonstrated by the routine stain (H&E)but the changes in the special cells which include (pillar cell ,mucus cells and chloride cells) could be demonstrated very clear by special stains like periodic acid Schiff's reagent and Alcian blue stain .

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Conflict of Interests

The authors declare no conflict of interest.

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الآفات المرضية تمرض الغلاصم المعقد في اسماك التروت سلمون المرقط (*Oncorhynchus mykiss*) المستزرع في محافظة دهوك

شعبان طيار صديق^١، الاء حسين الحمداني^٢ و زانان محمد امين طه^٣

١. فرع الامرض و الاحياء المجهرية - كلية الطب البيطري - جامعة دهوك - دهوك - العراق.
٢. فرع الامرض و امراض الدواجن - كلية الطب البيطري - جامعة الموصل - الموصل - العراق.
٣. مركز بحوث دهوك - كلية الطب البيطري - جامعة دهوك - دهوك - العراق.

اصبحت اضطرابات الغلاصم مشكلة كبيرة خلال فترة تربية السلمون المرقط (*Oncorhynchus mykiss*) ان مصطلح مرض الغلاصم المعقد «CGD» (complex gill disease) يطلق الى العديد من العلامات المرضية لإصابات الغلاصم والتي تظهر في جميع مواسم السنة خلال تربية اسماك السلمون المرقط. تمثل الآفات المرضية العيانية والنسجية النهائية لكل الإصابات الناتجة عن المسببات المرضية. إن تقييم الحالة الطبيعية والمرضية ممكن ان يكون عن طريق دراسة التراكيب النسجية للغلاصم. اجريت هذه الدراسة لتقييم الحالة الصحية لغلاصم ٢٠٠ سمكة مأخوذة من مزارع تربية اسماك السالمون المرقط في محافظة دهوك عن طريق دراسة الآفات النسجية باستخدام الصبغة الروتينية الهيموتوكسلين والايوسين والصبغات الخاصة صبغة البيريديك الاسيد شفت وصبغة الاليشين الزرقاء باستخدام المجهر الضوئي. شملت الآفات الملاحظة النزف في الصفائح الغلصومية الابتدائية والاحتقان للأوعية الدموية في الصفائح الأولية والثانوية ووجود التنكس الفجوي للخلايا الظهارية وارتشاح الخلايا الالتهابية وحيدة النواة ووجود النخر للخلايا الظهارية للصفائح الغلصومية الثانوية كما سجل الالتصاق للصفائح الغلصومية الثانوية وتضخم خلايا الكلورايد والخلايا المخاطية ووجود التنكس الفجوي لخلايا بلير وتكاثر خلايا الكلورايد.

الكلمات المفتاحية: آفات الغلاصم المرضية، مرض الغلاصم المعقد، اسماك السالمون المرقط.