ARTICLE / INVESTIGACIÓN

The Babylon River's common carp (*Cyprinus carpio*) gills were used for the histopathological examination and PCR detection of Koi herpesvirus disease (KHVD)

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Abstract: The first outbreak of koi herpesvirus in Iraq occurred in Babylon Province in the floating cages in the Euphrates River from the Musayyib thermal electric power station to the Al-Hindiya Dam. Fish were suffering from gills rot that did not respond to treatment and lesions and ulcers on the fish's body. The fatalities reached 80%. was water temperature 24°C, pH 6.85, Salinity 760 ppt. So the present study aims to highlight the pathological changes in gills after the Koi herpes virus infection in Babylon Province. The study started after the detection of (KHV) by polymerase chain reaction (PCR) from the suspected samples (30) for the detection of virus nucleic acid. Also, study the water characterization during the periods of outbreaks. The gills specimen was collected from the positive cases for gross and histopathological examination. The result showed that ten samples were positive for (KHV) infection. The gross and histopathological examination results showed severe congestion with necrotic foci and sloughing of secondary lamella with increased mucus secretion. In addition, necrosis in the primary lamellae, Edam and inflammatory cells infiltration with hyperplasia of the secondary lamellae with the presence of intranuclear inclusion body in all exanimated slides.

Key words: Koi Herpes, Cyprinus Carpio, Euphrates River, KHV, secondary lamellae, floating cages.

Introduction

Koi herpesvirus disease (KHVD) is a rare virus that can infect koi and carp quickly and widely[1]. The first report of the koi herpes disease outbreak in Iraq occurred in Babylon Province (north of the Province) in the floating cages in the Euphrates River in the area extending from the Musayyib thermal electric power station to the Al-Hindiya Dam. The first death report was on 10/28/2018, and the death continued until 27/11/2018. Affected fish lost their appetite and exhibited abnormal swimming patterns before they died. Discoloration, elevated respiratory rate[2], swollen gills, and grayish and patchy skin lesions are the most constant signs of the sickness[3]. The koi herpes virus is very contagious and causes massive koi (*Cyprinus carpio koi*) and common carp mortality[4]. For at least four hours, the virus is contagious in water. Which explains why it is so contagious in lakes[5]. It's unclear if The virus enters the fish's body through its gills. Then, it multiplies and causes necrosis and mucosal sloughing or enters the intestine[5,6]. During overt infection, KHV is most prominent in the gill, kidney, and spleen[7]. Gill injury is most likely a major factor in fish mortality. The virus replicates in the infected gills before being released into the water and transmitted to the kidney by white blood cells. The virus causes severe interstitial nephritis in the kidneys. This idea fits with the contagious disease's rapid spread and tends to be similar to respiratory viruses in mammals[8].

Materials and methods

Samples collection

Thirty live fish suspected samples were collected randomly from fish breeding cages in the Hilla River in October 2021. The project consists of ten floating cages with a capacity of ten thousand fish. The fish were suffering from gills rot that did not respond to treatment and lesions and ulcers on the fish's body. The fatalities were more than 80%. The samples were sent directly to the central laboratory of the Veterinary Department for PCR detection.

PCR detection

The real-time PCR detection of the KHV nucleic acid was done in the laboratory of the Veterinary Directorate by using Microboss Hightech GmbH /Germany) kit and the following primers:

Water characteristics

Some characteristics of water were measured over five days from sample collection, such as Sa-linity, Ph and Temperature.

Gross and Histopathological examination

All collected samples were examined grossly for recording the gross pathological changes and for taking the

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specimen for histopathological section; the gills were fixed in 10 % formaldehyde pro-cessed routinely by histokinette, embedded in paraffin, sectioned for 5µm stained by hema-toxylin and eosin stain as described by (8).

Results

PCR Detections

The Real-time PCR detection of KHV nucleic acid showed positive results (ct 30.35) according to the manu-facturing kit (up to 30-40 Ct should be taken positive results) as shown in figure (1).

Water characteristics

The mismeasurement of some water characteristics du-ring the study is illustrated in the table(1). The mean result of temperature was (24 ˚c), Ph (6.85), and salinity (760).

Gross and histopathological examination

The grossest observation in most cases was severe congestion with necrotic foci and sloughing of secondary lamella with an increase in mucus secretion (figure2). However, the primary histo-pathological lesion in most exa-mination sections was necrosis in the primary lamellae, with de-generative changes in the epithelial cells lining the se-condary lamellae, with the presence of an in-tranuclear in-clusion body (figure 3). The presence of viral inclusion was shown in most sections characterized by chromatin migra-tion with vacuolation in the lamellar epithelium (figure 4). Edam inflammatory cell infiltration and hyperplasia of the secondary lamellae are also seen (figure 5). Many exami-ned sections showed congestion of the central venous si-nus with fusion and des-quamation of secondary lamellae (figure 6,7).

Discussion

In poikilothermic species, temperature dramatically in-fluences how the disease progresses[3]. Water temperature has been found to influence the initiation and severity of viral infection by influencing virus multiplication and indirectly by improving the efficacy of the host immune re-sponse[9]. Tem-peratures in the water have a direct impact on cellular and humeral immunity. KHV outbreaks in koi and common carp are influenced by water temperature. Viral load in the en-vi-ronment is not as crucial for infection outbreaks when the temperature of infected fish shifts from lower to warmer tem-peratures (e.g., 23°C); a death occurs quickly (7–12 days from onset to death). This finding matched that of (6), who discovered that water temperature, virus pathogenicity, fish age and condition, high population, and stress conditions all influence sickness patterns (e.g., trans-portation, spawning, poor water quality). Other research has suggested that the infection is tem-perature dependant, occurring between 16 and 25 ° C.[5,6,10,11]. Gilad *et al.*; Ilouze *et al.*[7,12] found that the disease caused high mortality under experimental condi-tions at 28°C but not at 29°C or 30°C, nor at 13°C.

Target	Primer	Sequence (5'→3')
KHV-DNA	KHV-86f	5 '- GAC GCC GGA GAC CTT GTG-3 '
	KHV-109p	5'-CGG GTT CTT ATT TTT GTC CTT GTT-3'
	probe 78 bp	5'-FAM-CTT CCT CTG CTC GGC GAG CAC G-TAMRA3 '

Table 1. Oligonucleotides used in PCR.

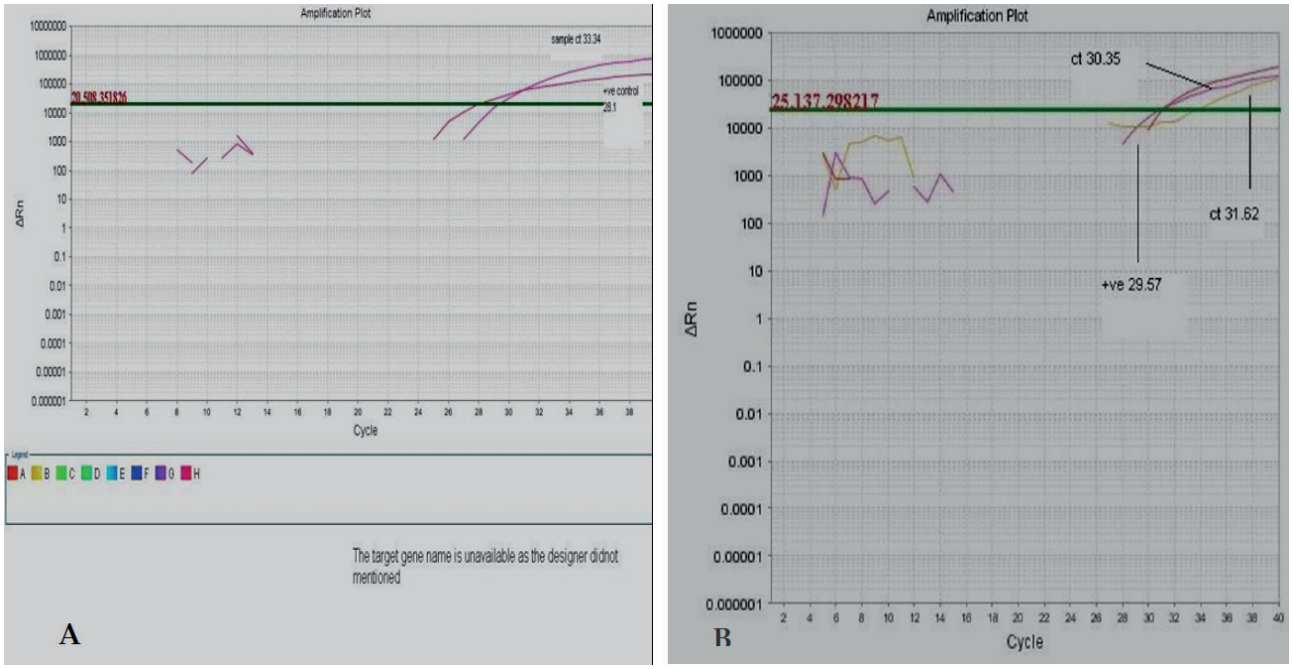
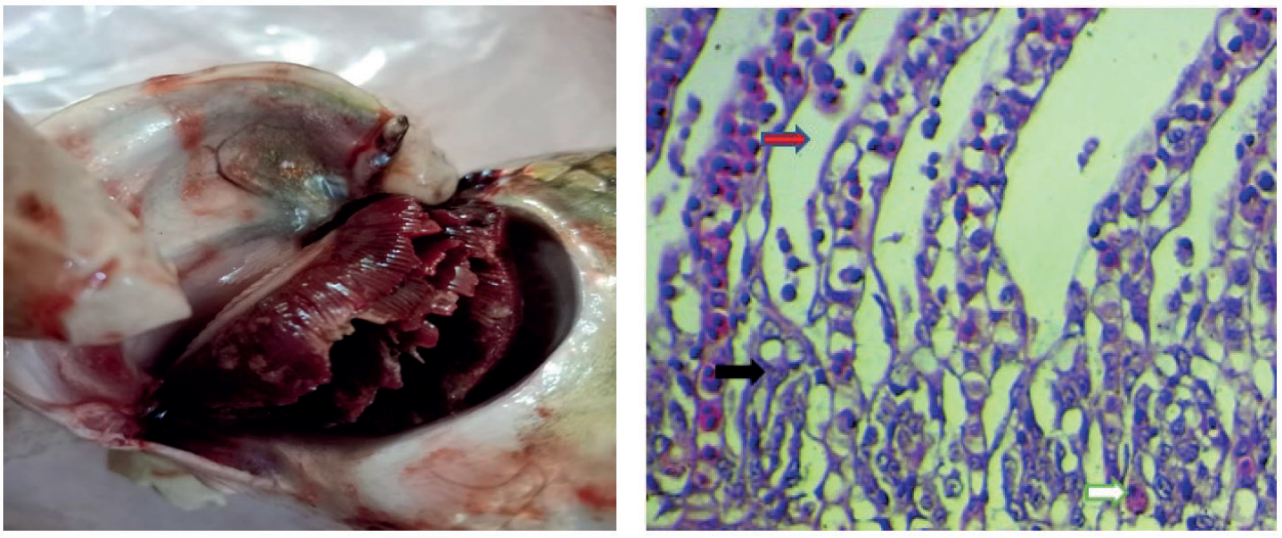


Figure 1. Amplification plot for Real time detection of KHV (A)for control(Ct33.34) and(B) for collected samples(Ct 30.35).

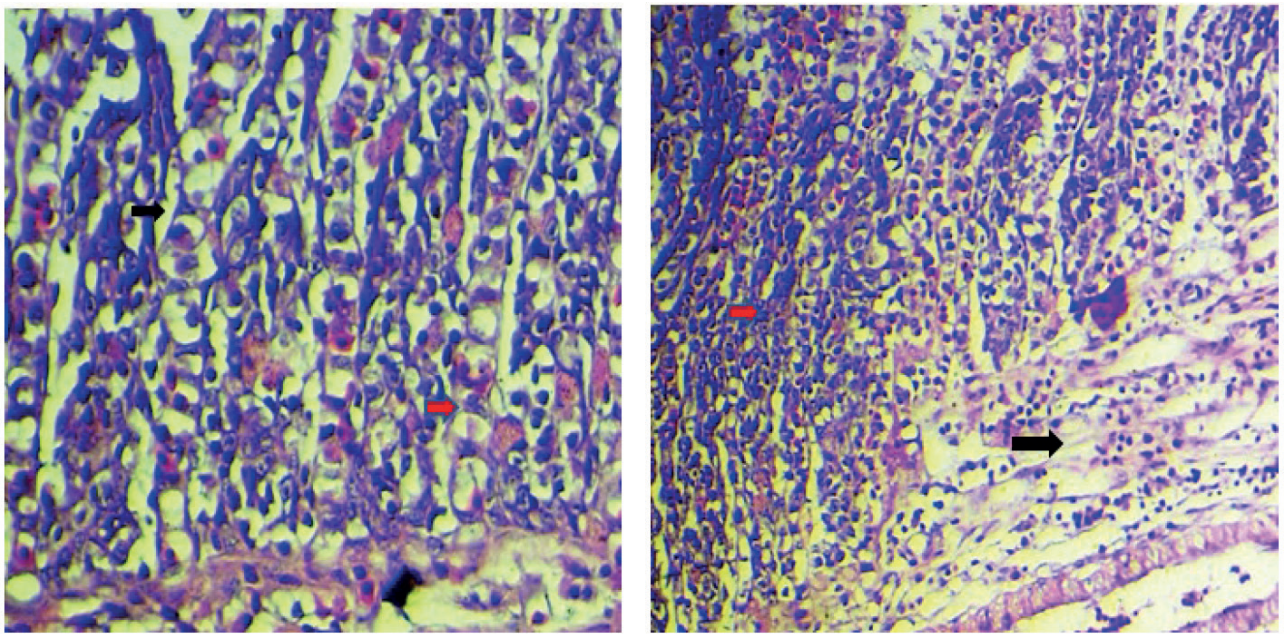
Day	Temperatures(˚c),	Ph	Salinity (ppt)
1st day	23.9	6.9	757
2nd day	23.8	7	760
3rd day	24.1	6.75	762
4th day	24.2	6.8	759
5th day	24	6.8	762
mean	24	6.85	760

Table 2. Measurement of the Temperatures (˚c), Ph, and Salinity (ppt) during the sample collection period.



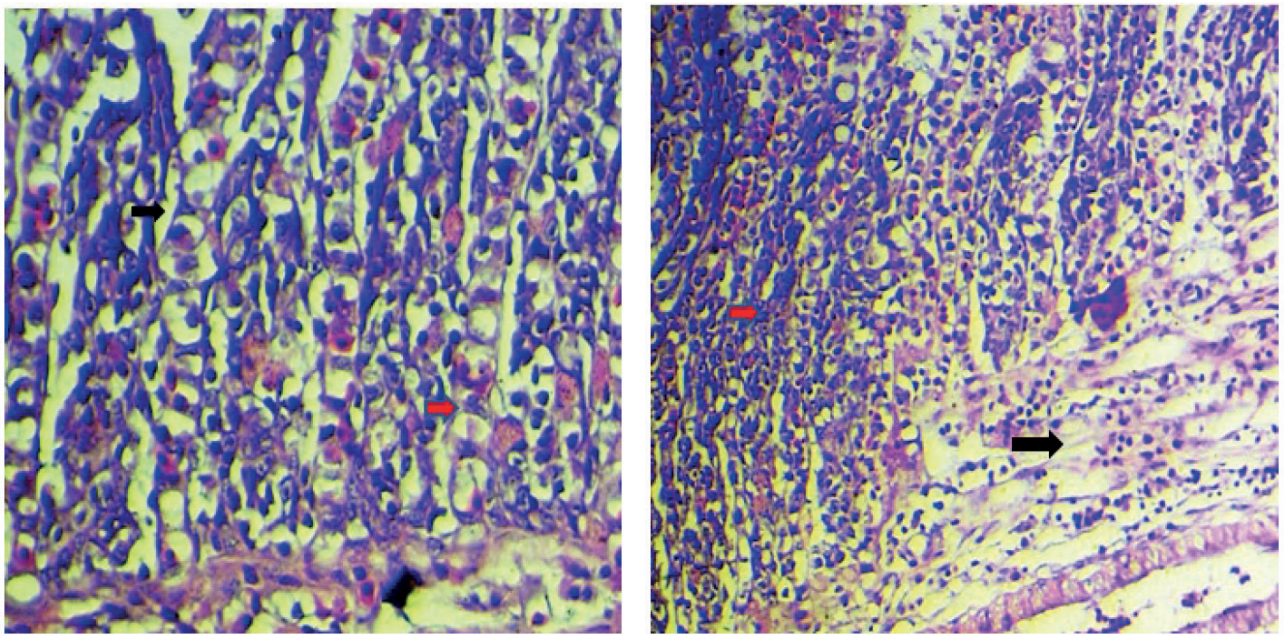
(a) (b)

Figure 2. (a) Gross photograph of the fish gills infected with (KHV) shows severe congestion with necrotic foci and sloughing of secondary lamella with the increase in mucus secretion.; (b) Histopathological section of the fish gills shows necrosis in the primary lamellae (black arrow), with degenerative changes in the epithelial cells lining the secondary lamellae (red arrow), with the presence of intranuclear inclusion body (white arrow). H&E stain, 400X).



(a) (b)

Figure 3. (a) Histopathological section of the fish gills showing intranuclear inclusion body with chromatin migration (black arrow) with vacuolation in the lamellar epithelium(red arrow). H&E stain, 400X).; (b) Histopathological section of the fish gills showed edam inflammatory cells infiltration (black arrow) and hyperplasia of the secondary lamellae (red arrow). (H&E stain, 200X).



(a) (b)

Figure 4. (a) Histopathological section of the fish gills shows fusion of the secondary lamellae (black arrow) with sloughing of the others (red arrow). (H&E stain, 400X).; (b) Histopathological section of the fish gills shows severe congestion of the central venous sinus (black arrow) with fusion and desquamation of secondary lamellae (red arrow). (H&E stain, 200X).

However, viral DNA was identified in the fish by PCR at 13°C, suggesting that virus reservoirs may exist among infected fish surviving at low temperatures[7].

The primary histological abnormalities in gill filaments were confirmed by (13). He discovered that most of the gill lamellae's respiratory epithelial cells were enlarged or vacuolated, with nuclear degeneration. Among the nuclear alterations seen were pale coloration, karyorrhexis, and the formation of an intranuclear inclusion body (IIB), defined as basophilic material within the nucleus with marginal hyperchromatic generated by heterochromatin deposition on the inner nuclear membrane. The injured respiratory epithelial cells seemed to have combined with cells from nearby lamellae, causing lamellar fusion and gill filament clubbing. According to to (5–7), the primary target cells in the per-gill infection were respiratory epithelial cells of the gill lamellae, where nuclear degradation and IIB were indicators of infection. The formation of IIB was shown to be caused by an increase in filamentous nucleoproteins and the construction of multiple viral capsids and nu-cleocapsids.

Conclusions

We concluded that the disease eruptions occurred during seasonal and sudden temperature changes and caused a severe mortality rate of fish of various weights, especially in marketing weight. Also, KHVD can cause severe pathological changes in fish gills with the presence of an intranuclear inclusion body in histological exanimated sections.

Author Contributions

Conceptualization, Sadeq. Al-Haider. Ghusoon .Alneamah And Samer Alshkarchy.; methodology, Ghusoon A. A. Alneamah; software, Samer .Alshkarchy; validation, Samer. Alshkarchy, Sadeq. Al-Haider. and Ghusoon .Alneamah; formal analysis, Sadeq. Al-Haider. Ghusoon .Alneamah And Sa-mer Alshkarchy; investigation, Ahmed Farhood.; resources, Ahmed Farhood.; data curation, Samer Alshkarchy.; writing—original draft preparation, Ghusoon .Alneamah.; writing—review and editing, Ghusoon .Alneamah. Samer. Alshkarchy; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

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Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results".

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