Research Article

Haematological alterations in common carp, *Cyprinus carpio* exposed to *Contracaecum* sp.

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Abstract

Contracaecum is a genus ofitic nematodes to the family Anisakidae, commonly found in fish as intermediate hosts to reach their definitive hosts. To assess the effect of this parasite on the haematological parameters of common carp (*Cyprinus carpio*), a total of 60 fish were collected alive from the Parishan basin in Kazerun, Fars Province, Iran. Among these, 50% were infected with L3 larvae of *Contracaecum* sp., with an average intensity of 16.7 ± 2.03 helminths per fish. The fish were analyzed to establish various haematological parameters. The haematological analysis revealed significant reductions in haematocrit (HCT) and red blood cells (RBCs), while lymphocyte counts showed no significant change.

White blood cells (WBCs) increased non-significantly (*p*>0.05), but monocytes and neutrophils exhibited a significant increase (*p*≤0.05). Variations between lymphocyte and WBC counts demonstrated non-significant differences (*p*>0.05) between infected and non-infected groups, whereas the other parameters showed significant differences (*p*≤0.05). Basophil and eosinophil values were not evaluated due to zero counts, resulting in no statistical analysis for these parameters.

Keywords: *Cyprinus carpio*, Hematology, *Contracaecum* sp., Nematodes

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Introduction

Finfish are considered hosts to a majority of ecto- and endo-parasites, which are integral components of every ecosystem and significantly affect fish health (Lieke *et al.*, 2020). In aquaculture, over 50% of productivity losses are attributed to diseases; various causative agents, such as high stocking densities and poor water quality, create optimal conditions for the infestation and reproduction of parasites and other pathogens. The spread of infectious pathogens often results from unhygienic transportation of fish and equipment (Assefa and Abunna, 2018). In recent years, understanding of fish parasites has advanced due to their impact on fish growth and behavior, as well as associated economic losses (Barassa *et al.*, 2003). Fish are frequently infected by either endo- or ecto-parasites, or both, playing important roles in their life cycles and often transmitting infections to other animals, including humans (Timi *et al.*, 2015). Parasitic infections can lead to a variety of pathological changes that severely compromise fish health, including tissue damage, organ obstruction, and nutrient deprivation (Molnar *et al.*, 2006). Consequently, infected fish may exhibit differences from healthy fish in values such as Fulton's condition factor and the length-weight relationship, indicating the severity of the infection. Altered values and ratios can signal future disturbances in growth rates and reproductive success (Olivero-Verbel *et al.*, 2006; Maceda-Veiga *et al.*, 2016). According to Anderson (2000), of approximately 2,272 described genera of Nematoda, at least 33% are well-known vertebrate parasites. Specifically in fish, the genera Anisakis, Contracaecum, Hysterothylacium, and Pseudoterranova are recognized as some of the most prominent nematode parasites, particularly regarding their zoonotic potential (Molnar *et al.*, 2006). Most of these genera belong to the family Anisakidae, except for Hysterothylacium, which is classified under the family Raphidascarididae (Fagerholm, 1991; Nadler *et al.*, 2005). According to Shamsi (2019), the genus Contracaecum is known for its species diversity, containing over 100 species; however, the most recent data from the World Database of Nematodes (Nemys, Ed., 2022) confirm 39 species within this genus. Contracaecum is geographically widespread, successfully utilizing a wide variety of both invertebrate and vertebrate hosts, including terrestrial and aquatic organisms (Al-Zubaidy, 2009; Shamsi, 2019). Haematological parameters are commonly employed as a tool for assessing health status in fish. Furthermore,

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qualitative and quantitative variations in these parameters are significant for diagnostic purposes (Martins *et al.,* 2004). According to Rolbiecki (2006), fish of different length classes exhibit varying degrees of exposure to parasites; thus, the abundance and composition of parasitic fauna change with age. Parasites can often induce anemia, characterized by reduced hemoglobin concentration, haematocrit, and erythrocyte counts (Martins *et al.*, 2004). Therefore, evaluating changes in haematological parameters associated with various parasites is crucial for establishing a database that allows for precise diagnostics and serves as a reliable tool for interpreting treatment or preventive measures, both of which are vital in fish farming. The objectives of our study were to identify the parasites of *Sander lucioperca* and to determine changes occurring in blood parameters in association with parasitism. The common carp (*Cyprinus carpio*) is a widely distributed freshwater fish inhabiting eutrophic waters in lakes and large rivers across Europe and Asia. It is notably the most geographically widespread fish species in Iran and many other regions worldwide. Due to its adaptability to a wide range of climatic and geographical conditions, a considerable number of parasites from almost all major taxa have been found in both wild and domestic carp. It is generally acknowledged that external parasites constitute the largest group of pathogenic organisms in warm-water fish (Snieszko and Axelrod, 1971). The Parishan Basin is located in the southern part of the Zagros Mountains, approximately sixty kilometers west of Kazerun, Fars Province. To date, the L3 larvae of *Contracaecum* sp. and their haematological effects on common carp in Iran have not been studied. Therefore, the objective of the present study was to analyze and describe the potential haematological alterations caused by a high intensity of L3 larvae of *Contracaecum* sp. in the abdominal cavity of common carp (Fig. 1).

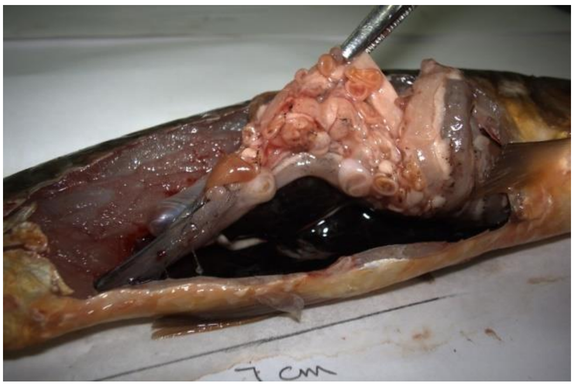

Figure 1. Infected common carp to *contracaecum* sp.

Materials and methods

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A total of 60 common carp (*Cyprinus carpio*) were collected from the Parishan Basin early in the morning by local fishermen using gill nets and angling techniques. The fish were transported alive to the laboratory in aerated tanks and housed in an equipped aquarium. To minimize sampling stress, the fish were anesthetized with tricaine methane sulfonate (MS 222). Blood samples were obtained from the caudal vessels of the anesthetized specimens using syringes containing 10% EDTA, then stored until analysis. The counts of red blood cells (RBC) and white blood cells (WBC) were determined using Neubauer chambers with a Rees diluting solution, which comprised 1 g of Brilliant cresyl blue, 31.3 g of sodium citrate, 10 mL of 37% formalin, and 1000 mL of distilled water (Rowley and Ratcliffe, 1988). A differential leukocyte count was performed on blood smears stained with Giemsa solution, which were then examined under light microscopy (Olympus, Tokyo) at 1000× magnification. Haematocrit values were assessed using micro-haematocrit capillaries filled with blood, centrifuged at 5000 rpm for 5 minutes, and expressed as a percentage of the total blood volume (Smith *et al.*, 2007) Fish identification was conducted based on morphometric and meristic characteristics as outlined by Coad (2010). Following identification, the fish were dissected from the ventral side. The body cavity, stomach, spleen, liver, kidneys, heart, muscles, swim bladder, and gonads were thoroughly examined for anisakid cysts. The gastrointestinal tract was excised from the rectum to the esophagus, opened longitudinally, and carefully observed under a stereoscope (Amlacher, 1970). The cysts were collected and rinsed with physiological saline solution (0.9%) in a glass Petri dish. Each cyst was opened under a stereoscope with a fine needle to release the Contracaecum larvae, which were subsequently washed with saline solution, counted, and preserved. The SPSS Biostatistics Program (version 20) was utilized for statistical analysis. The means were compared using independent samples, and two-way ANOVA was conducted to determine significant differences ($p \leq 0.05$) between the non-infected and infected groups.

Results

In a comprehensive examination of 60 common carp (*Cyprinus carpio*) from the Parishan, it was found that 50% of the fish were infected with L3 larvae of *Contracaecum* sp. (Moravec, 1998), with an average intensity of 16.7 ± 2.03

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helminths per fish. Haematological analyses indicated significant changes between infected and non-infected samples regarding red blood cells (RBCs), white blood cells (WBCs), thrombocytes, and haematocrit (HCT) levels. The results demonstrated reductions in HCT, RBC counts, and lymphocyte percentages in the infected groups compared to the non-infected group. Notably, the total WBC count in the infected group was higher than that in the non-infected group; however, this difference was not statistically significant (p>0.05). In contrast, counts of monocytes and neutrophils increased in the infected fish. While variations in lymphocyte and WBC counts showed non-significant differences (p>0.05) between the two groups, changes in RBC counts, thrombocyte levels, monocyte counts, neutrophils, and HCT levels exhibited significant differences (p≤0.05). Basophils and eosinophils were not evaluated due to having zero results, thus no statistical analysis was performed on these parameters (Table 1).

Table 1. Haematological Parameters in Infected and Non-Infected Common Carp with *Contracaecum* sp.

Parameters	Non-infected	infected	P value
RBCs (mm3)	2.194000×106 ± 8.1	1.825681×106 ± 11.1	p≤0.05
WBCs (mm3)	421563×104 ± 9.2	442033×104 ± 9.2	p>0.05
HCT %	2.879×101 ± 9.2	2.009×101 ± 9.2	p≤0.05
TC (µL)	52.67±1.45	79.33±3.84	p≤0.05
Lymphocytes %	90.45±11.23	86.35±9.49	p>0.05
Monocytes %	3.13±0.44	3.83±1.68	p≤0.05
Neutrophils %	6.42±2.17	11.91±5.18	p≤0.05
Eosinophils %	0	0	-
Basoophils %	0	0	-

RBCs: Red Blood Cells, WBCs: White Blood Cells, HCT: Hematocrit, TC: Thrombocytes.

Discussion

The observed infection of *Contracaecum* sp. in the present study may be attributed to the presence of piscivorous birds, which are definitive hosts. Many parasites coexist with their hosts without causing overt harm, a process referred to as cohabitation. However, this equilibrium can be disrupted by environmental changes within the parasite-host system (Tavares-Dias *et al*., 1999). When this balance is disturbed due

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to an increased parasitic load, the resulting effects on haematological variables can be significant (Corrêa *et al*., 2013; Panjvini *et al.*, 2015). In this study, the infection by L3 larvae of *Contracaecum* sp. correlated with alterations in blood parameters, potentially associated with hemorrhage resulting from larvae migration from the stomach to the mesentery. Our findings indicate that parasitic infections negatively impact haematological parameters in common carp, leading to reductions in RBCs and haematocrit levels. Previous studies have also demonstrated declines in RBC count and hemoglobin (Hb) associated with parasitism (Martins *et al*., 2004; Panjvini *et al.*, 2015; Movahed *et al*., 2016; Nashaat and Maghawri, 2022; Alhayali *et al*., 2023). Furthermore, other studies have highlighted that parasites serve as stressors, which can initiate primary stress responses that affect haematocrit. Parasitic infections can stimulate the release of catecholamines, leading to red blood cell mobilization from the spleen (Wells and Webber, 1990) or even induce RBC swelling due to fluid shifts into intracellular compartments (Chiocchia and Motais, 1989). Corrêa *et al.* (2013) reported low haematological parameters in *Hoplias malabaricus* infected by L3 larvae of *Contracaecum* sp. Similarly, Movahed *et al.* (2016) found reduced haematocrit, hemoglobin, mean corpuscular volume (MCV), and RBC counts in *Sander lucioperca*. Furthermore, Nashaat and Maghawri (2022) observed reductions in hemoglobin, RBCs, and haematocrit levels in red tilapia infected with *Capillaria* sp. Alhayali *et al.* (2023) also indicated significant decreases in hemoglobin concentration, total RBC count, and packed cell volume in fish affected by protozoan blood parasites. The haematological analysis in our study further corroborates these findings, demonstrating reductions in HCT and RBC counts in infected fish groups compared to non-infected groups which confirmed the previous studies (Chiocchia and Motais, 1989; Corrêa *et al.*, 2013; Movahed *et al.*, 2016; Nashaat and Maghawri, 2022; Alhayali *et al*., 2023). The observed elevation of serum WBCs is well-documented as an immune response to various infections. Thus, the increased WBC count in infected common carp may reflect a cellular immune response to parasitic infection. WBCs are crucial during such infestations, stimulating hematopoietic tissues and the immune system, resulting in the production of antibodies and chemical substances acting as defenses against infection (Lebelo *et al.*, 2001). Consistent with our findings,

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Panjvini *et al.* (2015), Movahed *et al.* (2016), and Alhayali *et al.* (2023) reported elevated WBC levels in infected fish compared to their non-infected counterparts. In our study, despite increased neutrophil and monocyte counts, lymphocyte percentages decreased. Notably, the WBC count was higher in infected fish than in healthy individuals. Neutrophils did not show significant differences between the two groups, aligning with Tavares-Dias *et al.* (1999), who found no significant changes in neutrophil levels in the blood of *Piaractus mesopotamicus* parasitized with *Argulus* sp. This finding may indicate that *Capillaria* sp. exerts an extracellular effect on host cells. Monocytes exhibited significant differences ($p \leq 0.05$), with higher values in the infected group, consistent with Furtado *et al*. (2019), who recorded increased monocyte counts in *Oreochromis niloticus* parasitized by *Argulus* sp., *Lamproglena* sp., and *Epistylis* sp. Basophils and eosinophils were not evaluated in this study due to zero results, although Alhayali *et al.* (2023) reported significant increases in the number of inflammatory cells, including lymphocytes, eosinophils, and basophils. Conflicting reports exist regarding eosinophils and basophils; for instance, Klontz (1972) noted the absence of eosinophils in rainbow trout, whereas Loewenthal (1930) claimed they comprised 8% of the total leukocyte population, increasing under stress conditions such as fishing, high density, and starvation. Basophils, which can be stained with basic dyes (e.g., toluidine blue pH 9.0), are infrequently found in teleost blood (Tavares-Dias, 2006). While Yokoyama (2005) found no basophils in fish blood, Loewenthal (1989) reported that basophils constituted 2% and 9% of the blood in *Carassius vulgaris* and common carp, respectively. The role of thrombocytes in fish remains a subject of debate (Lopes *et al.*, 1997). They are present in birds, reptiles, amphibians, and fish and play a discrete role in blood coagulation. Although thrombocytes are not derived from leukocyte lineage, their involvement in inflammatory responses and phagocytic activity suggests an organic defense function, as observed in various animal species (Matushima and Mariano, 1996). Consequently, leukocytes and thrombocytes are often regarded as a collective unit, termed organic defense blood cells (Tavares-Dias and Sandrim, 1998). According to Ueda *et al.* (1997), the average number of total thrombocytes in freshwater teleosts ranges from 2,000 to 68,400 μL of blood. In the current study,

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thrombocyte counts in infected fish were higher than those in non-infected fish, likely due to the immune defense mechanism in response to parasitic infection, although this finding contrasts with those of Nashaat and Maghawri (2022). Their research suggested that thrombocyte counts and differential cell populations might contribute to inflammatory responses and phagocytic activity.

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Conflict of interest

The authors declare that has no conflicts of interest.

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