Immune response in spirlins (Alburnoides bipunctatus, Bloch 1782) *infested by* Ligula intestinalis *parasite*

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Summary
Ligula intestinalis parasite is a cestode that can cause remarkable damages to fishes. SDS-PAGE is one of the methods that can be used to determine the immune serum band polymorphism and immune responses in fishes infested by *Ligula intestinalis*. This study reports the results of an investigation conducted using SDS-PAGE focusing on immune serum band polymorphism and on the reaction of the immune system in spirlins (*Alburnoides bipunctatus*) infested by pleurocercoids of *Ligula intestinalis* parasite. Serum samples from infested spirlins revealed a polymorphism band which differed from that reported in sera of roaches (*Rutilus rutilus*), a species of the same *Cyprinidae* family.

Risposta immunitaria in alborelle bipuntate infestate da Ligula intestinalis

Parole chiave
Alborella bipuntata (*Alburnoides bipunctatus*),
Ligula intestinalis,
Rutilo (*Rutilus rutilus*),
SDS-PAGE.

Riassunto
Lo studio descritto in questo articolo utilizza il metodo SDS-PAGE per analizzare bande di polimorfismo in immunosieri di alborelle bipuntate (*Alburnoides bipunctatus*) infestate da pleurocercoidi del parassita *Ligula intestinalis*. Gli immunosieri sono stati ottenuti da alborelle bipuntate infestate da *Ligula intestinalis*. Quando esaminati in SDS-PAGE, i sieri di alborelle hanno evidenziato presenza di bande di 40 e 65 kDa che differiscono con quanto evidenziato nei sieri di rutilo (*Rutilus rutilus*), una specie appartenente alla stessa famiglia *Ciprinidae*.

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Pleurocercoids of the tapeworm *Ligula intestinalis* are important pathogenic agents of spirlins (*Alburnoides bipunctatus*). They are localized in the body cavity of cyprinids, which are supplementary hosts of this parasite. During their growth and life activities they constrict the inner organs causing their atrophy and pathological effects. They also cause morpho physiological shifts in the infested host (2, 3), which mainly include the inhibition of gonadal development resulting from changes in the secretion of releasing hormones by the hypothalamus, and a decrease in the level of particular immunological and biochemical parameters (9, 12, 15, 19, 26, 27). These pathogenic agents may have a strong influence on ecosystem function by inducing a variety of behavioral and physiological changes in their hosts (20). In particular, parasites that impair host reproduction consume high amounts of energy and can have significant impacts on host population dynamics (10, 11). *Ligula intestinalis* is characterised by a life cycle involving three hosts, with copepods as the first and fish as the second intermediate host. The final hosts are piscivorous birds, i.e. gulls (*Larus cachinnans*) or grey herons (*Ardea cinerea*). The parasites persist in the gut of birds for a few days to reach sexual maturity and to reproduce (7). This study aimed to investigate the immune response of spirlins infested by *Ligula intestinalis*.

Preparation of the immune spirlin serum (ISS) was done in the Parasitology Laboratory of Veterinary Medicine of University of Tehran. Immune spirlin serum was recovered from naturally infected spirlins

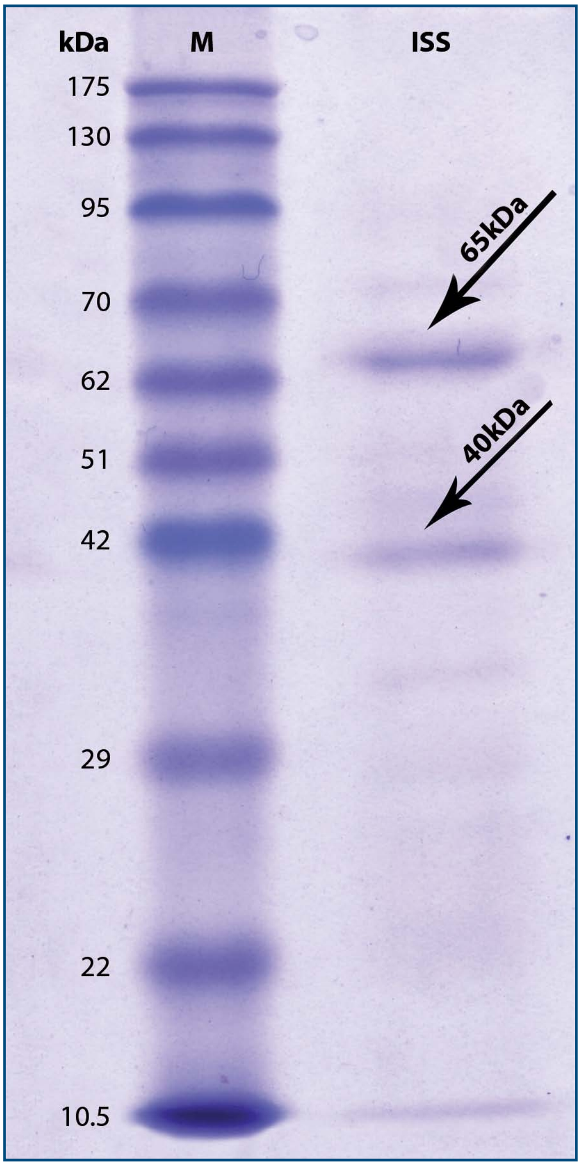

Figure 1. *Results obtained with SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) in immune spirlin serum (ISS).* M = marker, ISS = immune spirlin serum.

immediately after collection from their habitat. We used 6 spirlins (mean weight 23 ± 2 g, mean length 15 ± 2 cm) infected with *Ligula intestinalis* parasite and showing prevalent symptoms of ligulosis disease. In each infected fish, 3 parasites (mean weight 6 ± 1 g) were separated from abdominal cavity and blood samples were taken from the fish by caudal puncture. The blood samples were then centrifuged at 2000 × (g) 5 min, their serum was separated and evaluated by ELISA. Afterward, the sera with high titration were selected and stored at -70 °C.

After preparation of ISS the serum was run on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), composed of 5% resolving gel and 10% stacking gel, under reducing conditions using the discontinuous buffer system (13). For size estimation in SDS-PAGE, a pre-stained protein marker - 10.5-175 kDa molecular weight range (pr0602) - was used.

The results of SDS-PAGE procedure are shown in Figure 1. Two bands of 40 kDa and 65 kDa were observed in the immune spirlin serum.

These findings were in contrast with those found by William and Hoole (33) who observed bands of 65, 90 and 100 kDa in sera of roaches infested by *Ligula intestinalis* and tested by SDS-PAGE. These discrepancies evidence that the immune system of species belonging to same family (*Cyprinidae*) can react in different ways to the infection of *Ligula intestinalis* parasite. The SDS-PAGE also revealed a band polymorphism.

Polymorphism of several immune molecules has been shown to play an important role for defense against parasites. Links between polymorphism and disease resistance have already been studied with the complement C3 (28, 29).

Other studies (14, 21, 22) suggest the involvement of the immune system in determining parasite establishment and population dynamics.

As it has been shown in this article, cestodes can stimulate immune response in fish following infection, it is possible to elicit antibodies to acanthocephalan (30), nematode (8), monogenean (4), digenean (17, 18, 34), and other cestode (23, 25) parasites.

Immunisation with dead parasite material will also capable of eliciting antibody responses to nematodes (8), digeneans (16, 31), and cestodes (24, 32).

In conclusion, this study proved that two different species belonging to different genera but within the same family might have different immune responses although infested with the same parasite.

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