

**Zeitschrift:** Acta Tropica  
**Herausgeber:** Schweizerisches Tropeninstitut (Basel)  
**Band:** 38 (1981)  
**Heft:** 1

**Artikel:** Circulating immune complexes in human fascioliasis : relationship with "Fasciola hepatica" egg output  
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**DOI:** <https://doi.org/10.5169/seals-312803>

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## **Circulating immune complexes in human fascioliasis. Relationship with *Fasciola hepatica* egg output**

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### **Summary**

Circulating immune complexes were investigated by the  $^{125}\text{I}$ -Clq binding test in serum from patients with fascioliasis. Only 36% of all the patients studied showed significant levels of CIC. Nevertheless, when we considered only the patients eliminating *Fasciola hepatica* eggs in the stool and/or with the acute phase of the infection, the detection of CIC was very higher (more than 70% of the cases). In addition, a close relationship was observed between *F. hepatica* egg output and the incidence of CIC. This data suggest strongly the occurrence of specific parasite antigens in the detected CIC. The involvement of CIC in the pathogenesis of the acute hepatic fascioliasis is discussed.

*Key words:* human fascioliasis; circulating immune complexes (CIC);  $^{125}\text{I}$ -Clq binding test.

### **Introduction**

Circulating immune complexes (CIC) have been found in a variety of parasitic diseases and appear to be related to the development of immunopathological lesions (Lambert et al., 1978; Verroust et al., 1979; Santoro et al., 1980). The classical parasitic infection associated with an immune complex disease was schistosomiasis, who CIC was suspected to be involved in the renal injury observed (Digeon et al., 1979; Andrade and Rocha, 1979; Houba, 1979).

Fascioliasis produced by *Fasciola hepatica* is a parasitic disease frequently observed in a variety of mammals, including man. It is clinically characterized by its evolution in two steps: 1. the toxic allergic or hepatic invasion phase that takes place few weeks postinfection, showing an hepatomegalic form and 2. the

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second phase, beginning 7 months after infection, is characterized by the localization of flukes into the bile ducts and the appearance of angiocholitis (Dawes and Hughes, 1964; Biguet and Capron, 1966; Capron and Vernes, 1969; Watre et al., 1978). Most patients with liverfluke also show cell-mediated immunity and specific antibodies against *F. hepatica* antigen (Armour and Dargie, 1974; Watre et al., 1978). This specific humoral immune response could be followed by the formation of localized and/or circulating immune complexes, which would be involved in the immunological or immunopathological mechanisms of the host-fluke relationship.

The purpose of the present work was to investigate CIC in human fascioliasis in relationship to both the *F. hepatica* egg output and to the clinical form of the patients.

## Materials and methods

### *Patients*

In an area of the Braga district (Portugal) endemic for fascioliasis (Silva, 1978), 291 patients were studied. The *F. hepatica* infection was monitored by identification and output of parasite eggs in the stool according to the method of Stoll and Hausheer (1926) and by serological investigations (immunoelectrophoresis, indirect immunofluorescence and hemagglutination) as previously described (Watre et al., 1978). The patients were classified into 3 groups according to the geometric mean egg count/g stool of 3 different examinations: group I, 19 infected patients eliminating less than 100 eggs/g stool; group II, 48 patients eliminating between 101 and 500 eggs; and group III, 12 patients eliminating more than 500 eggs/g stool. The other 212 patients, although presented clinical signs of fascioliasis and/or a positive serodiagnostic, were either negative for *F. hepatica* eggs in the stool or the faecal examination was not performed. According to the clinical form, the patients were also classified into 3 groups: 174 subjects were asymptomatics, 86 were symptomatics, and 31 patients showed an acute fascioliasis. Eighteen subjects without detectable parasitic infection formed the control group (NHS). Blood was collected and was allowed to clot at room temperature for 2 h. Serum was removed by centrifugation and used after storage at  $-30^{\circ}\text{C}$ .

### *Evaluation of CIC*

It was performed by the  $^{125}\text{I}$ -C1q binding test (Zubler and Lambert, 1978). Briefly, C1q was isolated from normal human serum and labeled with  $^{125}\text{I}$ . This preparation was then mixed with test serum, previously treated with 0.2 M EDTA. Free C1q was separated from C1q bound to CIC by precipitation with 3% polyethylene glycol (PEG, mol. wt. 6000). All tests were done in triplicate. Results were expressed as percentage  $^{125}\text{I}$ -C1q precipitated as compared with the protein bound radioactivity precipitable with 20% trichloroacetic acid.

### *Statistical evaluation*

Results were analysed by the chi-square test, analysis of variance, or Student's t test when required.

## Results

Investigation of CIC by the  $^{125}\text{I}$ -C1q binding test in serum from all the infected patients and control subjects (NHS) is indicated in Table 1. The incidence of CIC in infected patients was significantly higher than in control sub-

Table 1. Study of C1q-binding circulating immune complexes (CIC) in human fascioliasis

Subjects	Number of cases	Mean level of CIC	Percentage of positivity	p value**
Infected .....	291	12.6 ± 4.23*	36%	<0.001
Control .....	18	10.4 ± 2.01*	11%	

\* Arithmetic mean ± standard deviation

\*\* The p value in the table is between the percentage of positivity (Student's t test).

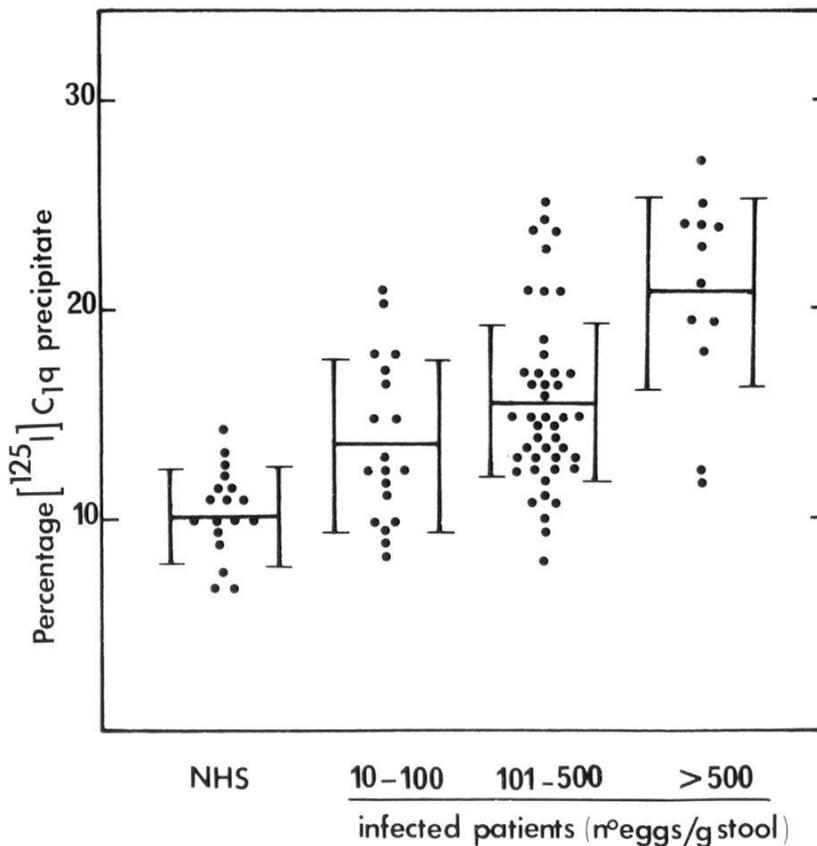


Fig. 1. C1q-binding circulating immune complexes (CIC) in human fascioliasis. Relationship to the mean of three determinations of *F. hepatica* egg count. NHS: control subjects.

jects ( $p < 0.001$ ). In fact, significant levels of C1q-binding material were found in 105 out of 291 (36%) serum from patients with fascioliasis.

The results for the detection of CIC in the patients classified according to the *F. hepatica* egg output are showed in Fig. 1. A highly significant difference was noticed between CIC levels in the different groups of infected patients. Moreover, a direct relationship was observed between egg output and incidence of CIC in human fascioliasis (Table 2). In addition it is noteworthy that more than 70% of the patients eliminating *F. hepatica* eggs in the stool showed significant levels of C1q-binding CIC.

Table 2. Relation of incidence of C1q-binding circulating immune complexes (CIC) in infected patients to *F. hepatica* egg output\*

CIC	Number of eggs/g stool			
	10-100	101-500	>500	Total
+ .....	9	37	10	56
- .....	10	11	2	23
Total	19	48	12	79

\* Chi-square test:  $\chi^2 = 6.886$ ;  $df = 2$ . Significant at the level  $0.02 < p < 0.05$

Table 3. Study of C1q-binding circulating immune complexes (CIC) in the different clinical forms of human fascioliasis

Clinical form	Number of cases	Mean level of CIC	Percentage of positivity
Asymptomatic .....	174	$10.9 \pm 2.3^*$	20.1%
Symptomatic .....	86	$13.7 \pm 3.9^*$	54.6%
Acute .....	31	$17.6 \pm 6.1^*$	74.2%

\* Arithmetic mean  $\pm$  standard deviation

When the patients were classified according to the clinical form (Table 3) a high significant difference was also observed between the three groups. In fact, the patients with an acute fascioliasis showed high levels of CIC.

## Discussion

The present study demonstrated the presence of CIC in patients with fascioliasis. The results were obtained using the  $^{125}\text{I}$ -C1q binding test (Zubler and Lambert, 1978) which have been used to demonstrate CIC in a wide variety of parasitic diseases such as schistosomiasis (Bout et al., 1977; Santoro et al., 1980), trypanosomiasis (Fruit et al., 1977), malaria (Houba et al., 1976) and leishmaniasis (Desjeux et al., 1980).

Significant levels of C1q-binding CIC were only found in 36% of all the patients studied (Table 1). By comparison with the control group, in which C1q-binding activity was high in 11%, the detection of CIC in all the patients suspected to have a fascioliasis was relatively weak. Nevertheless, if we consider only patients eliminating *F. hepatica* eggs in the stool and/or with an acute infection, the occurrence of CIC was very higher (more than 70% of the cases).

Thus, the appearance of CIC in human fascioliasis is probably associated with the activity of the infection.

A close relationship was observed between *F. hepatica* egg output and the detection of CIC in human fascioliasis (Table 2). As the fecal egg count is presumably dependent of the number of flukes in the host, one can postulate that CIC levels in patients infected with *F. hepatica* are directly related to the number of adult parasites. These findings suggest strongly the involvement of specific parasite antigens in the detected CIC. Nevertheless, it is possible that CIC of non-parasitic origin could also be formed during the infection. Further studies on the antigens making up the CIC in human fascioliasis are under way.

High levels of CIC were specially found in patients with an acute fascioliasis (Table 3). In human schistosomiasis also, CIC appeared more frequently in the acute phase of the infection (Lawley et al., 1979). The formation of CIC in the acute phase of several parasitic infections could be involved in their pathogenesis.

*Acknowledgments.* The authors wish to thank Mrs L. Boutry, M. A. Bastos and M. N. Luis, and Mr. J. L. Neyrinck for their expert technical assistance. We also thank Miss C. Colson and Mrs M. F. Massard for typing this manuscript.

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