Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 97, No. 6, November 1988, pp. 491–497.


# Haemodynamics of hamsters during *Ancylostoma ceylanicum* infection

J K SRIVASTAVA, SUMAN GUPTA and J C KATIYAR
Division of Parasitology, Central Drug Research Institute, Lucknow 226 001, India

MS received 10 May 1988

**Abstract.** Alterations in haematological and liver glycogen values during the course of *Ancylostoma ceylanicum* ($100L_3$, p.o.) infection in hamsters were investigated. In early phase of infection, there were marginal changes in haematological parameters, albumin-globulin ratio and liver glycogen, which transformed into severe alterations on patency (day 18, post infection). Haemoglobin, packed cell volume, total erythrocyte counts and liver glycogen values had a decreasing trend and reticulocytes increased considerably to compensate the red blood cell loss. Albumin/globulin ratio which was in favour of albumin in the beginning, reversed on day 24 p.i. When the animals were deparasitized with mebendazole (5 mg/kgxl, p.o.), all the parameters started resuming normalcy and on day 12 of treatment of all the parameters except albumin/globulin ratio attained the pre-infection level.

**Keywords.** *Ancylostoma ceylanicum*; hamster; haemodynamics; liver glycogen.

## 1. Introduction

Infection with gastrointestinal helminths inflict a variety of structural and functional changes in the intestine (Symons 1969) and blood of hosts (Ogilvie *et al* 1978; Roth and Levy 1980). Qualitative and quantitative changes in haematological picture constitute some of the most frequent and easily observed manifestations of infection and also reflect the immune response of the host to infection (Boyer *et al* 1971; Ogilvie *et al* 1978; Moqbel 1980). Since, *Ancylostoma ceylanicum* a hookworm of dog, cat and man is of relatively recent introduction to the experimental field (Ray *et al* 1972; Visen *et al* 1984; Gupta *et al* 1987; Srivastava *et al* 1986) and gained favour for experimentation over other hookworm models, *Nippostrongylus brasiliensis* in rats and *Nematospiroides dubius* in mice (Ray *et al* 1972; Misra *et al* 1981; Katiyar *et al* 1982, 1984) there was need to understand haematological and related parameters of the infected host (hamster). A detailed study on this aspect is also important because this laboratory host is the most suitable for *A. ceylanicum*.

Accordingly an attempt has been made to monitor alterations in haematological parameters and liver glycogen reserve of hamsters during the course of infection and following anthelmintic therapy.

## 2. Materials and methods

### 2.1 *Animals*

Laboratory bred male hamsters (70–85 g) maintained under standard animal house conditions, served as experimental host.

CDRI communication No. 4211.

### 2.2 *Ancylostoma ceylanicum*

The procurement of infective larvae, infection to host and recovery of worms have already been dealt in detail elsewhere (Gupta *et al* 1987; Srivastava *et al* 1986).

### 2.3 *Experimental design*

Two groups of animals were examined. Group I (infected) comprising of 60 hamsters, was infected orally with $100 \pm 5L_3$. Three of the survivers from this group were exsanguinated at 3 days interval from day 3–30 p.i. (10 intervals) and their blood samples were collected for examining haematological parameters [Haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), packed cell volume (PCV), reticulocyte percentage (RP), differential leucocyte count (DLC) and albumin/globulin ratio (A/G)]. Liver was isolated under chilled condition for estimating glycogen.

On day 18, when the infection attained patency (Visen *et al* 1984; Gupta *et al* 1987) in the intestine, the remaining animals of Group I (infected) were divided into two sub groups. The animals of one sub group were deparasitized with mebendazole (5 mg/kgxl, per os) and those of the other sub group received sham treatment of normal saline and examination continued till 30 p.i.

Group II (uninfected) having identical number of animals and receiving mebendazole and sham treatment of D.W. was run parallel and examined simultaneously.

On autopsy after collecting blood and liver; intestine of each animal was also examined for observing the worm load. Two to three replicates were carried out.

### 2.4 *Haematological examination*

Hb was determined by Sahli-Hellige Haemoglobinometer. Total erythrocyte and leucocyte counts were made on heparinized blood using standard dilution methods (Hayem's fluid for RBC and Turk's fluid for WBC) on a Neubauer haemocytometer (Anon 1978). The differential leucocyte count was made on films of non heparinized blood, fixed in methanol and stained with 10% Giemsa stain. Packed cell volume was determined with heparinized blood in Wintrobe tubes (Anon 1978). The reticulocytes were counted as described by Cheema and Scofield (1984).

### 2.5 *Determination of A/G ratio*

The test sera were first electrophoresced on cellulose acetate membrane in microzone electrophoretic apparatus (Beckman) (Briero and Mull 1964; Jane 1979) and later were scanned over the scanning densitometer (R-112) at 520 nm.

### 2.6 *Estimation of liver glycogen*

Liver glycogen (LG) was extracted from 25–50 mg isolated liver (Good *et al* 1933) and estimated colorimetrically (Montgomery 1957).

## 3. Results

The sequential changes in various parameters that occurred during the course of infection have been depicted in table 1.

### 3.1 *Hb*

Hb level remained unaltered upto day 6 of infection. On day 9 slight decrease was seen which became pronounced as the infection reached patency i.e. on day 18. Maximum drop was observed on day 24 of infection (infected group-4·50 ± 0·94 g%, control group-17·59 ± 0·98 g%).

Mebendazole, knocked out all the worms, and Hb started rising from day 3 post treatment and reached pre-infection level on day 12 post treatment.

### 3.2 *TEC*

TEC showed a gradual fall which was significant on day 12 (when $L_5$ stage was present). On day 18 when adult worms started sucking blood, the fall was more conspicuous and maintained a plateau upto day 30 (last observed period).

### 3.3 *TLC*

Very mild alteration was recorded and the maximum rise (46·5% over the control) was observed on day 12 of infection.

### 3.4 *DLC*

The differential count remained more or less unaltered during the course of infection.

### 3.5 *PCV*

Decrease in PCV started from day 9 and continued till the day of last observation (day 30 p.i.) where the value of infected group was 8·25 ± 1·09% as compared to 48·39 ± 5·31% in control group.

### 3.6 *RP*

The reticulocytes were very few in uninfected hamsters (mean value-1 ± 0·68%) but in the infected animals, a dramatic rise was recorded from day 15 of infection and attained peak (i.e. 78 ± 3·74%) on day 30 p.i.

### 3.7 *A/G ratio*

The A/G ratio which in the initial phase was in favour of albumin (mean value 1·47 ± 0·52) levelled off on day 15 p.i. (mean value 1·05 ± 0·19) and subsequently turned in favour of globulin on day 24 (0·88 ± 0·14).

**Table 1.** Dynamics of blood parameters and liver glycogen reserve of male hamsters during *A. ceylanicum* (100 $L_3$/p.o) infection.

| Parameters observed (pooled control values) | | Observation intervals (days) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group II | Group I | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 |
| Hb g% | Infected | 16·73 ± 0·81 | 16·70 ± 1·31 | 14·63 ± 1·17 | 10·57 ± 0·77 | 8·30 ± 1·13 | 5·11 ± 1·94 | 4·52 ± 0·90 | 4·50 ± 0·94 | 4·60 ± 0·5 | 4·60 ± 0·37 |
| (17·59 ± 0·98) | Infected treated | — | — | — | — | — | — | 8·88 ± 2·05 | 10·3 ± 2·69 | 15·1 ± 2·80 | 18·1 ± 1·1 |
| TEC (× $10^6$) | Infected | 5·40 ± 0·09 | 5·39 ± 0·10 | 5·64 ± 0·19 | 3·42 ± 0·25 | 2·65 ± 0·19 | 1·45 ± 0·45 | 1·26 ± 0·47 | 1·09 ± 0·16 | 0·97 ± 0·14 | 1·07 ± 0·19 |
| (6·13 ± 0·42) | Infected treated | — | — | — | — | — | — | 3·45 ± 0·53 | 3·39 ± 0·26 | 4·8 ± 1·24 | 5·09 ± 0·47 |
| TLC | Infected | 5637 ± 543 | 5859 ± 421 | 5791 ± 264 | 7607 ± 393 | 5908 ± 175 | 6427 ± 1006 | 5557 ± 1581 | 6250 ± 425 | 5050 ± 1148 | 5437 ± 499 |
| (5189 ± 1475) | Infected treated | — | — | — | — | — | — | 5808 ± 896 | 5587 ± 589 | 5800 ± 699 | 5000 ± 331 |
| PCV % | Infected | 48·83 ± 0·95 | 49·0 ± 2·15 | 39·66 ± 4·41 | 25·67 ± 1·74 | 19·50 ± 0·66 | 13·75 ± 3·9 | 12·43 ± 3·58 | 9·33 ± 1·49 | 9·17 ± 1·95 | 8·25 ± 1·09 |
| (49·39 ± 5·31) | Infected treated | — | — | — | — | — | — | 27·33 ± 3·25 | 35·5 ± 2·29 | 40·5 ± 6·73 | 45·0 ± 5·6 |
| Reticulocyte % | Infected | 0·90 ± 0·2 | 0·67 ± 0·32 | 1·66 ± 0·45 | 4·58 ± 2·04 | 33·15 ± 2·71 | 67·02 ± 10·01 | 61·45 ± 11·32 | 57·92 ± 3·37 | 59·79 ± 0·9 | 78·0 ± 3·74 |
| (1·00 ± 0·68) | Infected treated | — | — | — | — | — | — | 28·66 ± 16·83 | 5·65 ± 1·75 | 2·63 ± 1·04 | 1·49 ± 0·39 |
| A/G | Infected | 1·47 ± 0·52 | 1·38 ± 0·65 | 1·35 ± 0·21 | 1·17 ± 0·32 | 1·05 ± 0·19 | 1·01 ± 0·15 | 1·02 ± 0·31 | 0·88 ± 0·14 | 0·80 ± 0·5 | 0·92 ± 0·06 |
| (1·50 ± 0·35) | Infected treated | — | — | — | — | — | — | 1·07 ± 0·11 | 1·10 ± 0·19 | 0·97 ± 0·05 | 0·97 ± 0·23 |
| Liver glycogen mg/g | Infected | 68·86 ± 4·37 | 66·27 ± 13·50 | 39·39 ± 7·61 | 26·74 ± 3·50 | 17·63 ± 5·88 | 8·17 ± 5·76 | 1·25 ± 0·72 | 2·25 ± 0·75 | 1·84 ± 1·38 | 1·90 ± 0·75 |
| (74·73 ± 8·76) | Infected treated | — | — | — | — | — | — | 16·77 ± 4·9 | 51·87 ± 4·5 | 58·41 ± 18·04 | 75·53 ± 5·42 |

Mean value ± SE.

### 3.8 *LG*

From day 6 of infection, there was gradual depletion of glycogen reserve from the liver and on day 9, almost half of the reserve was exhausted (39·39 ± 7·61 mg/g vs 74·73 ± 8·76 mg/g). The depletion was proportionate to the age of infection and almost all the glycogen was utilized by day 27 p.i. (1·84 ± 1·38 mg/g).

## 4. Discussion

Haematological changes represent a facet of the host's overall response to infection. In gastrointestinal helminthiases this aspect has been well documented, firstly from a description point of view (Ruitenberg *et al* 1977; Moqbel 1980; Ogilvie *et al* 1980; Przyjalkowski 1980; Cheema and Scofield 1984) and secondly in an attempt to pin-point the cells that may be important for host defence (Ogilvie *et al* 1980; Wakelin and Donachie 1983).

The changes that occurred in the TEC, PCV and Hb parameters suggested the development of microcytic anaemia which is characteristic of blood sucking hookworms (Chatterjee 1967; Roach 1970; Hoagland and Schad 1978). These parameters showed the abducting trend after day 12 of infection when cutting armature of parasite was fully developed and they started removing the blood.

The marked increase in reticulocytes during patency may be due to heavy destruction of erythrocytes, which consequently forced the reticulocytes to accumulate in the blood to compensate erythrocytic loss.

The elevated leucocytes in early phase of infection was in conformation with the previous observations of Baker (1962), Ogilvie *et al* (1978) and Roth and Levy (1980) but dipping at the later stage of infection could be due to the severe loss of cellular components of the blood because of haemophagy by parasite.

The reversion of A/G ratio at the later stage of infection depicted the development of protective immunity (Gupta and Katiyar 1985). Carroll and Grove (1985) also observed increased immunoglobulins in dogs following *A. ceylanicum* infection. Non resuming of A/G ratio after mebendazole therapy may be explained on the basis of definite half life of antibodies. Therefore, as soon as the antigenic source is removed, the globulin levels are not supposed to reach preinfection level.

Excess of carbohydrate is stored in the liver as glycogen and is released during emergencies. The excessive blood loss during *A. ceylanicum* infection and consequent drainage of glucose along with, necessitated an additional demand of glycose by the host. Hence, the liver glycogen was mobilized to meet the host requirement (Kaul *et al* 1982). As such, the reserve glycogen in the liver was depleted and showed decreasing pattern.

It may be inferred that *A. ceylanicum* like other hookworms of men and animals, is highly pathogenic to its laboratory host, hamster, suggesting the suitability of this host parasite combination for experimental studies.

## Acknowledgements

The authors thank Dr S K Puri for his help in Electrophoresis of sera. The financial assistance to one of the authors (JKS) from the Council of Scientific and Industrial Research, New Delhi is gratefully acknowledged.

## References


Anon 1978 *Manual of Veterinary investigation laboratory technique, Part 4. Haematology* 2nd edition (London: Ministry of Agriculture, Fisheries and Food)

Baker N F 1962 The nature and ćtiology of the leukocyte response of Webster mice infected with *Nematospiroides dubius*; *J. Parasitol.* **48** 438–441

Boyer M H, Spry C J F, Beeson P B and Sheldon W H 1971 Mechanism of eosinophilia IV. The pulmonary lesion resulting from intravenous injection of *Trichinella spiralis*; *Yale J. Biol. Med.* **43** 351–356

Briero R O and Mull J D 1964 Electrophoresis of serum proteins with cellulose acetate; *Am. J. Clin. Pathol.* **42** 547–551

Carroll S M and Grove D I 1985 *Ancylostoma ceylanicum*: immunization with soluble worm extract and responses to challenge infection of dogs; *Exp. Parasitol.* **60** 263–267

Chatterjee K D 1967 *Phylum nemathelminthes—Ancylostoma duodenale parasitology (protozoology and helminthology) in relation to clinical medicine* (Calcutta, India: Chatterjee Medical Publishers) VI edition

Cheema K J and Scofield A M 1984 The influence of level of infection of rats *Nippostrongylus brasiliensis* on the haematology and phospholipaseactivity and mast cell numbers in the small intestine and colon; *Int. J. Parasitol.* **15** 55–60

Good A, Kramar H G and Somagyi M 1933 The determination of glycogen; *J. Bio. Chem.* **100** 485–491

Gupta S and Katiyar J C 1985 Immunological response of hamster to *Ancylostoma ceylanicum* infection; *Helminthologia* **22** 181–184

Gupta S, Srivastava J K and Katiyar J C 1987 *Ancylostoma ceylanicum*: migratory behaviour in golden hamsters after oral and parenteral infection; *Ann. Trop. Med. Parasitol.* **81** 412–442

Hoagland K E and Schad G A 1978 *Necator americanus* and *Ancylostoma duodenale*: Life history parameters and epidimiological implication of two sympatric hookworms of humans; *Exp. Parasitol.* **44** 36–49

Jane M P 1979 *Beckman, microzone electrophoresis manual* (California: Beckman Instrument. Inc. Fullerton)

Katiyar J C, Visen P K S, Gupta S, Sen A B, Dubey S K and Sharma S 1982 3, 5-Dibroma-2'-chloro-4'-isothiocynato salicylanilide, a potent anthelmintic; *Experientia* **38** 457–459

Katiyar J C, Visen P K S, Misra A, Gupta S and Bhaduri A P 1984 Methyl 5(6)-4-2-pyridyl piperazino carbamoyl benzimidazole-2-carbamate-a new broad spectrum anthelmintic; *Acta Trop.* **41** 279–286

Kaul G L, Talwalker P K, Sen H G and Grewal R S 1982 Changes in carbohydrate metabolism in golden hamsters infected with *Necator americanus*; *Ann. Trop. Med. Parasitol.* **76** 475–482

Misra A, Visen P K S and Katiyar J C 1981 Comparative efficacy of standard antihookworm drugs against various nematodes; *J. Helminthol.* **55** 273–278

Montgomery R 1957 Determination of glycogen; *Arch. Biochem. Biophys.* **67** 378–386

Maqbel R 1980 Histological changes following primary secondary and repeated infections of rats with *Strongyloides ratti*, with special reference to tissue eosinophils; *Parasite Immunol.* **2** 11–27

Ogilvie B M, Hesketh P M and Rose M E 1978 *Nippostrongylus brasiliensis*: peripheral blood leucocyte response of rats, with special reference to basophils; *Exp. Parasitol.* **46** 20–30

Ogilvie B M, Askenase P W and Rose M E 1980 Basophils and eosinophils in three strains of rats and in athymic (nude) rats following infection with nematodes *Nippostrongylus brasiliensis* or *Trichinella spiralis*; *Immunology* **39** 385–389

Przyjalkowski Z, Kontny E and Cabaj W 1980 Haematological changes evoked by *Trichinella spiralis* as an index of the course of trichinellosis in germ free and conventional mice; *Bull. Acad. Pol. Sci.* **28** 87–93

Ray D K, Bhopale K K and Srivastava V B 1972 Migration and growth of *Ancylostoma ceylanicum* (Looss 1911) in golden hamsters *Mesocricetus auratus*; *J. Helminthol.* **46** 357–362

Roach M 1970 Ancylostomiasis and anaemia; *Biol. Sanit. Panam.* **69** 221–228

Roth R L and Levy D A 1980 *Nippostrongylus brasiliensis*: peripheral leukocyte responses and correlation of basophils with blood histamine concentration during infection in rats; *Exp. Parasitol.* **50** 331–341

Ruitenberg E J, Elgersma A, Kruizinga W and Leenstra F 1977 *Trichinella spiralis* infection in congenitally athymic (nude) mice. Parasitological, serological and haematological studies with observations on intestinal pathology; *Immunology* **33** 581–587

Srivastava J K, Gupta S and Katiyar J C 1986 Behavioural studies on the arrested *Ancylostoma ceylanicum* larvae in hamsters; *Riv. Parasitol.* III (XLVII) 447–454

Symons L E A 1969 Pathology of gastrointestinal helminthiases; *Int. Rev. Trop. Med.* **3** 49–100

Visen P K S, Katiyar J C and Sen A B 1984 Studies on infectivity, longevity and fecundity of *Ancylostoma ceylanicum* in golden hamsters; *J. Helminthol.* **58** 159–164

Wakelin D and Donachie A M 1983 Genetic control of eosinophilia. Mouse strain variation in response to antigens of parasitic origin; *Clin. Exp. Immunol.* **51** 239–246