VETERINARSKI ARHIV 73 (5), 247-260, 2003

Iron supplementation therapy in the reversion of the haematological and immunological defects accompanying canine ancylostomosis

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OKEWOLE, E. A., T. O. OMOBOWALE, M. O. AYOOLA: Iron supplementation therapy in the reversion of the haematological and immunological defects accompanying canine ancylostomosis. Vet. arhiv 73, 247-260, 2003.

ABSTRACT

For the purpose of investigating the effects of iron supplementation on the therapy of experimental canine ancylostomosis, twenty 8-week old pups were rando mly divided into four equal groups A, B, C and D. Groups A, B and C were serially infected bi-weekly with infective *Ancylostoma caninum* (L3) larvae up to 98 days. Thereafter, Groups A and B were treated with pyrantel pamoate and dextrose saline infusion with or without iron dextran supplementation, respectively. Pups in Group C were left untreated as were the uninfected control pups in Group D. Clinical observations revealed a serial reversion of the distressing hookworm disease signs in only the two infected and treated Groups A and B, but not in the infected but untreated pups in Group C. Both the haematological, and immunological parameters were most elevated in the iron-treated pups in Group A, with high and positive correlation coefficient (r) of + 0.94, 0.83, 0.41 and 0.98 for the respective Groups A, B, C and D. Iron therapy was suggested as being responsible for the prompt haematological and immunological reversion observed in Group A pups through its facilitation of the haemoglobin synthesis and the iron-dependent mitotic cell division processes.

Key words: ancylostomosis, haematology, immunology, iron supplementation, cell mitosis

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ISSN 0372-5480
Printed in Croatia

Introduction

Ancylostomosis in susceptible dogs is accompanied by the development of a microcytic and hypochromic (iron deficiency) anaemia due to the depletion of the body's iron by both the blood sucking habits of the worms, as well as to bleeding of intestinal bite wounds (KELLY et al., 1977; HOTEZ and CERAMI, 1983). The severity of such anaemia bears a positive correlation to the degree of worm infection (CARROLL and GROVE, 1984) especially in the more susceptible young pups with very low body iron reserves resulting from lower dietary iron intake, which in turn reflects their exclusive feeding on the dam's milk (OFTEDAL, 1984).

Ancylostoma caninum, the most common pathogenic hookworm of dog, can cause a daily loss of 0.01 to 0.2 ml of blood for each adult worm, which can result in the daily loss of 25% or more of the host animal's total blood volume (MILLER, 1966; MILLER, 1971). Given the iron content of whole blood as 40-60 mg per 100 ml (LATNER, 1975), the iron loss in ancylostomosis is colossal.

In addition to the iron deficiency anaemia, ancylostomosis precipitates a gradual state of secondary immunodeficiency which positively correlates with the degree of anaemia (KELLY et al., 1977). Iron deficiency is known to cause tissue defects in mammals and it has been suggested that these might be related to impairment of the iron-dependent enzyme system in cells (other than stem cells) such as those that are involved in DNA synthesis and mitotic process (ROBBINS and PEDERSON, 1970; HERSHKO et al., 1970). JOYNSON et al. (1972) convincingly demonstrated a significant *in vitro* impairment of lymphocyte transformation and macrophage inhibition factor production (Correlates of cell-mediated immune (CMI) responses) in iron-deficient patients suffering from microcytic and hypochromic anaemia. This finding was soon corroborated by HIGGS and WELLS (1972), and KELLY (1973).

Clinically, acquired resistance to infection which accrues to pups by virtue of their age and previous exposure (MILLER, 1964; CARROLL and GROVE, 1985; OKEWOLE and ODUYE, 2001) and the iron supplemented anthelmintic removal of the blood-sucking adult worms have been reported to restore the haematological derangement (PCV or Hb. Conc.) in the hookworm infected pups (FOY and KONDI, 1960; CARROLL and GROVE, 1986).

The ability of the same iron supplementation therapy to reverse the immunological derangement also had been suggested (JOYNSON et al., 1972; KELLY et al., 1977), although its experimental proof has not been convincingly and simultaneously demonstrated in different groups of animals.

Taking the lymphocyte blastogenesis as a typical representation of the immunological changes in ancylostomosis, *in vitro* mitogenic (antigenic) stimulation tests could be performed on whole blood or separated lymphocytes from experimentally infected and iron treated pups in order to evaluate and compare their haematological and immunological responses.

The aim of this study was to evaluate the role of iron supplemented anthelmintic therapy in the reversal of haematological and immunological derangements that accompany canine ancylostomosis.

Materials and methods

Animals. Twenty, 8-week old local mongrel pups, whelped in our kennel, were used for the study. They were treated twice with pyrantel pamoate (Canex® Pfizer, Australia) at a dose rate of 5 mg kg^{-1} at 2-week intervals. They were negative for nematode ova by the flotation method.

Experimental design. The 20 pups were randomly divided into 4 groups, A, B, C and D of 5 pups each. Each group was kept separately on zinc-floored kennels to facilitate total faecal recovery and they were fed balanced rice-based pasty formulated food; supplemented twice weekly with re-constituted dry full-cream milk (Glaxo Nig. Ltd.) and fish meal. Groups A, B and C pups were subcutaneously infected with 500 infective larvae (L_3) of *Ancylostoma caninum* biweekly from days 0 to 98, while the group D pups were kept as uninfected controls. On day 100, Group A pups were treated with oral pyrantel pamoate (Canex®-Pfizer) at the above stated dosage, intramuscular injection of iron dextran (Ronidex®-Dizengoff.WA Nig. Ltd.) at a dose rate of 10.0 mg of elemental iron kg^{-1} body mass twice daily from days 100 to 114 (DODDS and WARD, 1980). The pups were also intravenously rehydrated with Dextrose-Saline infusion (Unidexral®-Unique Pharma Ltd.) as clinically indicated. Group B pups were treated in

the same way as their Group A counterparts but with no iron dextran supplementation. The infected Group C pups were left untreated, as were the uninfected Group D pups.

Clinical, haematological, coprological and immunological observations were made on all groups until day 126.

Parasites. Infective (3rd stage) larvae (L_3) of *Ancylostoma caninum* were obtained from a culture of ova in faecal samples from 4 naturally infected local pups from the Ibadan metropolis. The detailed culture technique, larval harvesting, washing, dilution, dosaging and the subcutaneous infection methods were as previously described (MILLER, 1964).

Haematology. Blood samples from each pup were taken every other week, anticoagulated with EDTA and analyzed with electronic coulter counter S-plus® (Coulter Electronics, Hialeah, Fla). Haemoglobin (Hb) concentration, haematocrit value (PCV%), total red and white blood cell counts were estimated, while the mean corpuscular volume (MCV) was calculated. Differential leukocyte counts were performed on heparinized blood on an improved Neubauer haemacytometer after staining with new Carpentier's stain (CARROLL and GROOVE, 1984).

Estimation of worm egg production rate. Worm eggs per gramme of faeces were also simultaneously estimated biweekly using the modified Mc Master slide counting technique, according to the Manual of Veterinary Parasitological Laboratory Technique (MAFF, 1986).

Peripheral lymphocyte transformation study. The whole blood method was used to determine the response of dog lymphocyte to stimulation by a T-cell mitogen; soluble phytoheamagglutinin P (PHA) - (Difco laboratory, Detroit) on a biweekly basis for the 4 groups of pups. This method has the advantage of eliminating the necessity for the separation and purification of lymphocytes (COLGROVE, 1978). Also, erythrocytes in the whole blood cultures provide a desirable environment for lymphocyte activation (PAULEY et al., 1973).

Lymphocyte culture. In a preliminary experiment designed to establish optimal culture conditions with normal dogs, serial dilutions of blood from normal dogs expressed in cell density (lymphocyte/ml) were titrated against

varying doses of PHA (1.5 - 250 μg). A dose-response relationship (curve) was established between concentrations of PHA and lymphocyte counts (mean counts per minute from triplicate cultures). The relationship revealed that 20 μg PHA dose level was the most sensitive in detecting small changes in responsiveness and that the curve kurtosis reflects changes in the relative and absolute numbers of reactive cells, thus presenting the curve as a useful device for monitoring the profile of peripheral blood lymphocytes (KENNY, 1975).

Twenty-five microlitres of heparinized blood from each pup were placed in a polypropylene tube containing 0.5 ml of culture medium: [RPMI 1640 buffered with 28 mM, HEPES-N-2-hydroxyethyl piperazine-N-2-ethane sulfonic acid, (Sigma Pty Ltd.) plus penicillin G (100 μg/ml) streptomycin (100 μg/ml), and 25 microlitres of various stock solutions of PHA (Difco labs., Michigan)] to give the following final concentrations; 2.5 μg, 5.0 μg, 10.0 μg and 20.0 μg per culture. Triplicate cultures for each dose of PHA were set up and 25 microlitres of normal saline in tubes provided unstimulated control for each set. Tubes were tightly capped, kept upright and incubated at 37 °C, under an atmosphere of 5% CO_2 in air for 24 hours before its radiochemical labelling to monitor DNA synthesis in the T-cells.

Labelling and harvesting procedures. At the end of the first 24-hour incubation period, 2.0 μCi of tritiated thymidine (TRA-120 Radiochemical Centre, Amersham, UK) (specific activity 5 ci/mM) was added to each culture before being further incubated for another 24 hours. The detailed descriptions of the harvest procedures were a modification of those previously given by PAULEY et al. (1973). In brief, cells were resuspended and 6.0 ml of normal saline were added to each. Erythrocytes were lysed with 2.0 ml of 2% acetic acid by Vortex mixer under agitation. Supernatant was removed and the pellet was suspended and spun in 5% trichloroacetic acid (TCA) and methanol. Pellet was finally dissolved in 0.5 ml hyamine hydroxide (Hyamine 10x-P), incubated at 56 °C for 20 minutes before transfer into scintillation vials. The incorporation of [3H] thymidine (which correlates with the rate of DNA synthesis) was determined with the aid of a scintillation spectrometer (Tri-carb liquid scintillation spectrometer-Packard Instruments, Downers Grove, Illinois) with external

standardisation. The PHA dose-response curves were drawn for group mean counts of lymphocytes per minute against PHA concentrations, and the 20 μg dose response was statistically fixed as the optimum for group comparison of responses. An index of responsiveness (IR) takes into account the different lymphocyte counts on a biweekly basis and is expressed as mean counts per minute per 1×10^6 lymphocytes.

Statistics. All results were expressed as means ± standard deviations. All tests of significance were performed, using the two-tailed Student's *t*-test, at 5% level of significance, unless otherwise indicated. Statistical analysis was done on a SAS (1987), ver. 6.03 Statistical Analysis System Institute, Cary, North Carolina, U.S.A.

Results

Clinical observations. Days 0 to 28 were uneventful in the 3 infected Groups, A, B and C, but from days 65 to 100, 4, 3 and 4 pups from groups A, B and C, respectively came down with watery and slightly bloody diarrhoea. Groups A and B pups in particular were severely dehydrated, weak, inappetent, emaciated, pale and with cold extremities of the legs (group mean rectal temperature (GMRT) was 37.4 ± 1.8 °C). Group C pups also had intermittent bloody diarrhoea, dyspnea, weakness, pallor of visible mucosae and fairly cold extremities, GMRT was 37.8 ± 1.2. Diarrhoea became more bloody and regular in Group C pups from days 103 to 126, with 4 of the 5 pups being seriously dehydrated, weak, anorexic, dyspnoeic and in sternal recumbency. In contrast, Group D pups were healthy, alert, groomed and growing, especially from day 50 to day 126. GMRT was 38.7 ± 1.6 °C.

However, the clinical picture in pups in Groups A and B became reversed from days 107 to 126, with improved appetite, alertness, vigour and the groomed nature of hair coat. Extremities were normally warm, with GMRT at 38.8 ± 1.3 °C. Visible mucosae were pink and liveweight gain was clinically evident, especially in Group A pups.

Haematological findings. Haemoglobin concentration (Hb.conc), haematocrit value (PCV%) and total red cell counts (RBC counts) were almost invariably constant from days 0 to 28 in the 4 groups, but the same

Table 1. Haemogram and worm egg production rates in dogs serially infected with *Ancylostoma caninum* larvae and treated with or without iron supplementation

Pup group	Parameter	Unit	Days post infection								
			0	14	28	42	56	70	84	112	126
A (n=5)	Hb. Conc.	gm%	8.30 ± 1.12	8.31 ± 2.14	8.20 ± 1.62	8.00 ± 1.21	7.70 ± 1.02	7.60 ± 1.22	7.20 ± 0.53	8.01 ± 1.03	8.80 ± 1.04
	PCV	%	24.80 ± 1.21	24.90 ± 2.03	24.80 ± 1.03	24.00 ± 2.01	23.20 ± 1.22	22.70 ± 2.01	21.40 ± 0.02	24.24 ± 1.04	26.03 ± 0.05
	RBC. Counts	10^6/ml	5.20 ± 0.03	5.20 ± 0.11	5.30 ± 0.12	5.20 ± 0.13	5.21 ± 0.03	5.10 ± 0.03	4.80 ± 0.01	5.30 ± 0.72	5.50 ± 0.25
	MCV	μ^3	47.69 ± 0.03	47.88 ± 1.02	46.80 ± 0.11	46.20 ± 1.13	44.53 ± 0.10	44.30 ± 0.13	44.0 ± 0.11	45.73 ± 0.23	47.32 ± 0.10
	Egg Count	x10^3epg	-	-	-	7.82 ± 0.35	8.64 ± 2.13	12.40 ± 2.85	14.50 ± 2.52	14.10 ± 0.37	12.03 ± 2.05
B (n=5)	Hb. Conc.	gm%	8.00 ± 1.10	8.10 ± 0.31	8.20 ± 0.04	8.10 ± 1.13	7.51 ± 2.01	7.21 ± 2.11	7.01 ± 1.12	7.32 ± 2.11	7.62 ± 2.21
	PCV	%	24.01 ± 1.20	24.50 ± 0.52	24.60 ± 1.12	24.30 ± 0.02	22.53 ± 0.52	21.63 ± 0.72	21.03 ± 0.41	21.96 ± 1.34	22.86 ± 0.06
	RBC. Counts	10^6/ml	5.10 ± 0.02	5.12 ± 0.21	5.20 ± 1.02	5.10 ± 0.03	5.10 ± 0.64	5.00 ± 0.04	4.93 ± 0.21	5.00 ± 0.24	5.11 ± 0.02
	MCV	μ^3	47.01 ± 0.02	47.12 ± 0.12	47.31 ± 0.02	47.64 ± 0.11	44.12 ± 0.32	43.26 ± 0.03	42.70 ± 0.03	43.92 ± 1.01	44.74 ± 0.12
	Egg Count	x10^3epg	-	-	-	8.24 ± 0.63	8.86 ± 0.54	11.96 ± 0.64	14.32 ± 0.72	14.00 ± 0.84	12.41 ± 0.74
C (n=5)	Hb. Conc.	gm%	8.41 ± 2.10	8.46 ± 1.02	8.51 ± 1.13	8.20 ± 2.01	7.40 ± 1.13	6.28 ± 1.23	6.20 ± 1.03	6.32 ± 1.32	6.48 ± 1.45
	PCV	%	25.23 ± 1.03	25.38 ± 2.14	25.53 ± 2.16	24.6 ± 2.11	23.10 ± 2.30	22.23 ± 1.63	21.31 ± 1.10	18.60 ± 2.02	19.56 ± 1.03
	RBC. Counts	10^6/ml	5.30 ± 0.01	5.30 ± 0.12	5.21 ± 0.04	5.10 ± 1.04	5.10 ± 0.13	5.01 ± 0.11	4.90 ± 0.03	4.42 ± 0.13	4.52 ± 0.12
	MCV	μ^3	47.60 ± 0.21	47.89 ± 0.04	49.10 ± 0.05	48.24 ± 0.04	45.29 ± 0.11	44.37 ± 0.21	43.49 ± 0.21	42.08 ± 0.13	43.27 ± 0.03
	Egg Count	x10^3epg	-	-	-	8.99 ± 0.74	9.47 ± 0.86	10.86 ± 0.67	14.57 ± 0.52	13.6 ± 0.84	11.62 ± 0.84
D (n=5)	Hb. Conc.	gm%	8.12 ± 0.11	8.20 ± 0.13	8.32 ± 0.14	8.52 ± 0.16	8.67 ± 0.31	8.81 ± 0.08	8.92 ± 0.14	9.04 ± 0.61	9.14 ± 0.41
	PCV	%	24.32 ± 0.22	24.41 ± 0.06	25.02 ± 0.14	25.56 ± 0.41	26.01 ± 0.34	26.43 ± 0.53	26.76 ± 0.31	27.12 ± 0.33	27.42 ± 0.24
	RBC. Counts	10^6/ml	5.00 ± 0.02	5.00 ± 0.13	5.10 ± 0.11	5.12 ± 0.13	5.20 ± 0.34	5.26 ± 0.04	5.30 ± 0.03	5.35 ± 0.03	5.40 ± 0.04
	MCV	μ^3	43.64 ± 0.11	48.82 ± 0.02	49.06 ± 0.02	49.92 ± 0.12	50.02 ± 0.14	50.25 ± 0.13	50.49 ± 0.03	50.69 ± 0.02	50.78 ± 0.05
	Egg Count	×10^3epg	-	-	-	-	-	-	-	-	-

Values are expressed as means ± standard deviations

parameters, as well as the MCV, steadily declined from days 29 to 98, only to increase variously prior to day 126, especially in Group A pups. (Table 1 and Fig. 1). However, the Hb. Conc. rose rapidly in response to the iron therapy in Group A pups to about its initial value at day 126. These parameters decreased considerably from days 56 to 126 in Group C pups, contrasting greatly with the situation in Group D, where they increased steadily until day 126. Reticulocytosis and thrombocytosis were observed from days 47 to 112 in pups in Groups A, B and C. Leukocytic changes consisted of typical left shift with neutrophilic leukocytosis, as well as persistent eosinophilia in the three infected groups from days 54 to 98, more particularly in Group C pups.

Table 2. Index of responsiveness (IR) expressed as group mean counts of peripheral lymphocyte in response to 20 μg dose of phytohaemaglutinin (PHA) in dogs serially infected with *A. caninum* larvae

Groups	Unit	Days post infection								
		0	14	28	42	56	70	84	112	126
Group A (n=5)	$\times 10^3$	155.62 ±1.82	158.88 ±1.62	145.51 ± 0.72	87.76 ± 0.36	82.22 ± 0.51	80.12 ± 0.29	78.81 ± 0.39	81.22 ± 0.52	138.23 ± 0.43
Group B (n=5)	$\times 10^3$	157.82 ± 1.02	161.77 ± 1.01	143.41 ± 0.61	92.81 ± 0.52	87.62 ± 0.41	84.34 ± 0.26	83.41 ± 0.32	80.52 ± 0.28	88.31 ± 0.34
Group C (n=5)	$\times 10^3$	160.31 ± 0.72	168.41 ± 0.54	155.48 ± 0.37	74.84 ± 0.48	40.86 ± 0.39	39.31 ± 0.29	28.45 ± 0.32	24.51 ± 0.22	22.89 ± 0.27
Group D (n=5)	$\times 10^3$	152.64 ± 1.02	156.81 ± 1.13	154.61 ± 0.58	156.17 ± 0.42	163.80 ± 0.51	165.51 ± .0.63	167.61 ± 0.67	168.27 ± 0.74	172.83 ± 0.54

Values are expressed as means ± standard deviations

Worm egg production rate. Worm egg production rate was divisible into three phases in this study viz. the pre-patent phase when no eggs were seen in faeces (days 0 to 28), the early patent phase when eggs were seen at an increasing rate (days 29 to 110), and the late patent phase when egg production declined slightly (days 111 to 126). These 3 phases were identifiable in the 3 infected groups. The control pups had no eggs in faeces (Table 1).

T-lymphocyte transformation responses to PHA stimulation. The group mean index of responsiveness (GMIR), measured as group mean of T-cell counts per minute per 10^6 lymphocytes of triplicate culture, correlated positively with the group mean haemoglobin concentration (GMHC) at the patent phase of the serial infection (days 42-126) with the following correlation coefficients (r) +0.94, +0.83, +0.49 and 0.98 for Groups A, B, C and D pups, respectively, at 5% level of significance. There was an initial elevation of response to PHA stimulation from days 0 to 14 in the three infected groups, followed by an increased impairment as the infection load increased and anaemia intensified with time (Table 2). However, the IR, like the Hb. Conc. values, increased terminally from days 110 to 126 in the iron-treated Group A pups than they did in the other two infected Groups (B and C) without iron supplementation. Group D pups had consistently high IR values, as with the corresponding Hb. Conc. values, until day 126 to justify its positive and highest coefficient (r) (Table 2).

Table 3. Statistical comparison of the pooled group mean patent values (GMPV) of some haematological and immunological parameters for pup groups

	Effect of	Pup group pairing	p-Value	t-Value	Interpretation
a.	Infection of *A. caninum* on the haemoglobin concentration (Hb. Conc.) values. GMPV of Hb. Conc. in the group	C versus D	0.001	-5.920	D was very significantly higher than C
b.	Serial infection of *A. caninum* on the IR of T-cells to PHA stimulation, GMPV of the IR in the group	C versus D	0.001	-15.493	D was very significantly higher than C
c.	General anthelmintic treatment on Hb. Conc. values, GMPV of the Hb. Conc. in the group	B versus C	0.003	1.775	B was significantly higher than C
d.	General anthelmintic treatment on the IR of T-cells to PHA stimulation, GMPV of the IR in the group	B versus C	0.002	5.787	B was very significantly higher than C
e.	Iron supplementation therapy on the Hb. Conc. values, GMPV of the Hb. Conc. in the group	A versus B	0.150	1.573	A was not significantly higher than B
d.	Iron supplementation therapy on the IR of T-cells to PHA stimulation, GMPV of the IR in the group	A versus B	0.609	0.543	A was not significantly higher than B

Statistical analysis. The pooled group mean patent values (GMPV) from days 42 to 126 in Tables 1 and 2 were used for the tabulated statistical comparisons in Table 3.

Discussion

This study has corroborated the previous observations of MILLER (1966), KELLY et al. (1977), and OKEWOLE and ODUYE (2001) in that serial infection of pups with the infective larvae of *A. caninum* culminated in the development of a microcytic and hypochromic (iron-deficiency) type of anaemia. The severity of anaemia, as revealed by the GMHC depletion, increased with infection load and time, the development was terminable with an anthelmintic therapy and reversible when the latter is combined with iron supplementation therapy.

The high and positive correlation coefficient (r) between the GMHC and the GMIR of the T-cells to the PHA stimulation, especially in Group A pups (Fig. 1), was statistically suggestive of the possibility of the above treatment protocols similarly affecting both haemoglobin and IR variables. The iron depletion that went concomitantly with whole blood loss as infection intensified with time had been agreed as the link between these two variables (HERSHKO et al., 1970; JOYNSON et al., 1972; KELLY, 1973; KELLY et al., 1977; VALL and PARRY, 1991) but the relative sensitivity of each to iron depletion and replenishment has not been adequately elucidated. According to JAIN (1986), iron depletion retards the synthesis of haemoglobin through the impairment of DNA synthesis, thus leading to a condition where the nucleus is retained longer than in the normal erythrocytic maturation sequence. This allows for further cell division and smaller than normal definitive red cells (microcytic) with less haemoglobin (hypochromic) than normal. Similarly, ROBBINS and PEDERSON (1970) suggested a crucial role for iron in mitosis. These workers observed that most cellular iron is bound to polysaccharides and that the addition of an iron chelating agent to living cells selectively inhibited the DNA synthesis, when monitored by rate of [3H]-thymidine incorporation, as done in this study also. They concluded that the interphase nucleolus and metaphase chromosomes are particularly enriched iron depots, and that iron moves from one site to the other at

different phases of the cell cycle for successful DNA synthesis and cell division, through the election transferring activities of the iron-dependent cytochrome oxidase systems. The serial iron depletion in these infected pups might have reduced the iron needed for cytochrome oxidative activities and so retarded or arrested the mitosis of the T-cells, while its therapeutic replenishment reversed these trends.

The return of both the GMHC and the GMIR of T-cells to about their initial values in Group A pups underscores the crucial adjunctive role of iron supplementation therapy in the management of canine ancylostomosis. By implication, such valuable iron disposition also stresses the need for an adequate dietary source of iron, especially to recently weaned pups that might have been fed exclusively on iron-deficient dam's milk (OFTEDAL, 1984) as recently emphasized (VARAYAN and BANWELL, 1982; CHANDRA, 1991).

Clinically, a diagnostic advantage of the high and positive correlation coefficient (r) between the GMHC and GMIR could be taken, since the former is practically easier to determine in order to estimate the latter, which represented the cell-mediated component of the total protective immunity (SMITH et al., 1970). However, the results of such correlation-derived values of GMIR should be interpreted with caution since previous medical treatment (TAJIMA et al., 1989) and the physiological state of the animals (KUHN and HARDEGG, 1992) have been reported to influence its values.

The statistically insignificant difference between Groups A and B values for the effects of iron therapy on both the GMHC and GMIR (Table 3) might be apparent and actually relate to the very late administration of iron during patency, which statistically reduced its enhancement effects on both variables on a D.42 to D.126 patency range. The choice of the intramuscular route for such an enhancing iron therapy (as opposed to the recommended safer and practical oral route (HARVEY et al., 1982) was made taking into account the caution to avoid the possible absorptive barrier that might be created by the inflamed intestinal mucosae. However, such route could be toxic, if unnecessarily prolonged (HARVEY et al., 1982).

Acknowledgements

The technical assistance of Mr. B.A. Bamigbola of the Chemical Pathology Department, College of Medicine, University of Ibadan, on the methodology of lymphocyte blastogenesis is gratefully acknowledged.

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Received: 9 August 2002
Accepted: 5 September 2003

OKEWOLE, E. A., T. O. OMOBOWALE, M. O. AYOOLA: Dodatna terapija željezom poboljšava hematološke i imunološke poremećaje kod ankilostomoze pasa. Vet. arhiv 73, 247-260, 2003.

SAŽETAK

U pokusu određivanja učinka dodatka željeza na terapiju pokusno izazvane ankilostomoze pasa, 20 štenadi u dobi 8 tj. bilo je podijeljeno u četiri skupine označene A, B, C i D. Skupine A, B i C bile su svaka dva tjedna invadirane invazijskim ličinkama *Ancylostoma caninum* (ličinke 3. stupnja) u razdoblju 98 dana. Nakon invazije skupine A i B bile su liječene pirantel pamoatom i infuzijama fiziološke otopine dekstroze s dodatkom ili bez dodatka željeza i dekstrana. Skupina C i kontrolna skupina D nisu bile liječene. Ustanovljeno je znatno poboljšanje kliničkih znakova bolesti u štenadi skupine A i B, ali ne i u invadirane i neliječene štenadi skupine C. Vrijednosti imunoloških i hematoloških pokazatelja bile su mnogo više u skupini A liječenoj željezom s visokim i pozitivnim korelacijskim koeficijentom (r) od +0,94, 0,83, 0,41 i 0,98 za odgovarajuće skupine A, B, C i D. Smatra se da je terapija željezom, putem olakšane sinteze hemoglobina i o željezu ovisnih procesa mitoze, dovela do brzog poboljšanja hematoloških i imunoloških pokazatelja u štenadi skupine A.

Ključne riječi: ankilostomoza, hematologija, imunologija, terapija željezom, mitoza