BLOOD CHEMICAL ALTERATIONS IN EXPERIMENTAL *Setaria cervi* INFECTION IN RABBITS

M. Kumar and H. C. Joshi[1]

Department of Veterinary Medicine, College of Veterinary Sciences
Pantnagar-263145, Nainital, India

Summary

Adult male and female *Setaria cervi* worms were implanted in the peritoneal cavity of rabbits to develop microfilaraemia. These infected rabbits revealed non-significant rise in total protein, significant reduction in albumin content and albumin-globulin ratio and significant increase in globulin and total bilirubin. The levels of total cholesterol, blood urea nitrogen and urea in blood were elevated from day 20 to 40, 25 to 70 and 30 to 70 respectively whereas, uric acid remained high from day 25 to 50 and creatinine from 15th day to 50th day of worm implantation. Biochemical changes suggested the involvement of liver and kidney in the infected rabbits.
(Key Words: Biochemical Changes, *Setaria cervi*, Rabbits)

Introduction

Setaria cervi infection has commonly been reported from developed, developing as well as under-developed countries of the world (Green and Trueman, 1971, Wang, 1981). There are isolated reports of this infection from India also (Anwar et al., 1978) but it is common in buffaloes maintained in tarai area of Kumaon hills of Himalayas (Kumar et al., 1986 and 1989). These worms remain in peritoneal cavity causing peritonitis or intestinal occlusion only (Khatoon et al., 1983) whereas its larval stage commonly referred as microfilariae, produces maximum damage to the different body organs (Kumar et al., 1986 and 1991).

There are isolated reports on biochemical changes in different animals suffering from filariasis but little attention has been paid to report changes in *S. cervi* infection using experimental animal as disease model. The present investigation records the biochemical alterations in experimental *S. cervi* infection in rabbits with an aim to elucidate its pathogenesis.

Materials and Methods

A total of 25 apparently healthy, young and fresh rabbits of either sex weighing around 1 to 1.5 kg were procured from Small Animal Research Division of Indian Veterinary Research Institute, Izatnagar. These were kept under observation for two weeks and their blood and faeces were examined at weekly interval to rule out any infection. From the peritoneal cavity of freshly slaughtered buffaloes, male and female *S. cervi* worms were collected and washed thoroughly in tap water and sterilized normal saline. These worms were transported in sterilized normal saline to the laboratory for further use within 2 hrs of collection.

In the peritoneal cavity of each of 15 rabbits, 9-10 live and motile female and 4-5 male *S. cervi* worms were transplanted whereas 10 rabbits were used as control. Heparinized blood or serum samples were used for biochemical analysis and various parameters were estimated on the day of blood collection or within 24 hours of serum separation. The blood samples were collected on days 3, 5, 7, 9, 11, 13 and 15 and then on every fifth day upto day 90 post implantation and once before implantation from all the rabbits separately.

The serum samples were analyzed for total protein (Henry et al., 1957a), albumin, globulin and albumin-globulin ratio (Green burg, 1929), total bilirubin (White et al., 1958), total cholesterol (MacIntyre and Ralston, 1954), uric acid (Henry et al., 1957b) and creatinine (Bonsnes and Taussky, 1945) and blood samples for urea nit-

[1]Address reprint requests to Dr. H. C. Joshi, Department of Veterinary Medicine, College of Veterinary Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar-263145, Nainital, India.
Received January 27, 1993
Accepted July 8, 1993

rogen and urea in blood (Richter and Lapointe, 1962). The values of these parameters were given in SI units and data were analyzed statistically by Student's t-test as described by Snedecor and Cochran (1967).

Results

There was gradual but non significant increase in total protein in infected rabbits upto day 40 followed by gradual decrease till 90th day (table 1). The values of albumin and albumin-globulin ratio were significantly low from day 30 to 60 and 25 to 50 respectively with minimum values on day 35 (table 1). Globulin and total bilirubin levels were significantly elevated in infected rabbits from day 20 to 70 and 15 to 50 respectively. Maximum rise in globulin content was noticed on 40th day and in total bilirubin on 30th day. On day 90, globulin level and total bilirubin remained nonsignificantly elevated (table 1).

The rabbits in which *S. cervi* worms were implanted, revealed significantly higher values of total cholesterol, blood urea nitrogen, urea in blood, uric acid and creatinine (table 2). Total cholesterol level was high from day 20 to 40 with a peak on 30th day whereas, blood urea nitrogen remained elevated from day 25 to 70 with maximum rise on 35th day. Abrupt rise in urea in blood was noticed from day 30 to 70 and maximum increase was on day 35. The values of uric acid and creatinine were found elevated from 25th and 15th day respectively upto 50th day. Uric acid content was maximum on 25th day and creatinine on day 30 in infected rabbits.

Discussion

The rabbits in which *S. cervi* worms were implanted, showed a non significant rise in total protein. This is due to liver and kidney damage by circulating larvae as evidenced by histopathological investigation (Kumar et al., 1991). In severe hepatic dysfunction, protein concentration is affected (Kaneko, 1989). The albumin content was significantly decreased. As liver is the site of synthesis for albumin and fibrinogen, any damage to liver adversely affects the synthesis of these fractions (Kaneko, 1989) and decreased albumin content is usually associated with chronic hepatic damage (Coles, 1967).

The globulin content was elevated in present study which is observed in hepatitis and diffuse fibrosis (Kaneko, 1989). The rise in globulin is indirectly a reflection of increase in gamma globulins (Coles, 1967). Increased globulin content may be correlated with increased antibody production against filarial antigens. Since the elevation of globulins is from increased production and not due to delayed removal, the increased number of plasma cells in bone marrow and kupffer cell proliferation serve as source of gamma globulins (Kaneko, 1989) and kupffer cell proliferation has been noticed in experimental *S. cervi* infection in rabbits (Kumar et al., 1991). Similar changes in total protein, albumin and globulin levels were noticed by Snyder et al. (1967) in dogs showing microfilariae and Baqui and Ansari (1977) in experimental setariasis in rats. The decrease in albumin globulin ratio is as a result of decrease in albumin and increase in globulin fractions.

There was significant elevation in total bilirubin level. The change in bilirubin indicates jaundice and excretory and conjugative dysfunction of liver and bilirubinemia is associated with liver damage and renal parenchymal disease (Kaneko, 1989). Change in bilirubin in present study is due to liver damage and destruction of renal parenchyma as both these changes were reported by histopathology (Kumar et al., 1991). Increased level of total cholesterol as observed here, reflects direct or indirect involvement of liver or kidney (Kaneko, 1989) and this rise is due to over production by liver and not due to retention as noticed in obstructive jaundice (Kaneko, 1989). In natural *S. cervi* infection in buffaloes, Sharma et al. (1981) noticed rise in total cholesterol.

Blood urea nitrogen and urea in blood were increased in infected rabbits. BUN or urea is formed in liver during detoxification of amino acids and these are the end products of nitrogen metabolism. Their levels in circulation are dependent on the rate of nitrogen metabolism and ability of kidney to remove them and in kidney damage they are not removed but remain in higher concentration (Kaneko, 1989). Kidney damage has been noticed by Kumar et al. (1991) in experimental setariasis, which resulted in rise in BUN and urea levels.

Significant elevation in uric acid was recorded in present study. Uric acid in blood depends on

TABLE 1. BIOCHEMICAL CHANGES IN EXPERIMENTAL *S. cervi* INFECTION IN RABBITS (Mean ± SE)

Parameters	Group	Days of observation											
		0	5	15	20	25	30	35	40	50	60	70	90
Total protein (g/l)	I	61.8 ±4.6	63.6 ±5.1	66.7 +5.7	69.1 ±5.3	69.7 +4.9	68.9 ±4.2	69.3 ±5.9	70.5 ±6.3	68.3 ±5.7	68.1 ±4.9	65.8 ±5.3	63.5 ±4.3
	C	62.1 ±4.7	63.7 ±4.6	63.0 ±4.5	62.9 ±5.2	62.1 ±4.8	63.3 ±4.5	62.6 ±5.1	62.9 +4.7	62.2 ±4.2	61.6 +5.1	63.7 +4.3	63.1 ±4.8
Albumin (g/l)	I	37.5 ±2.1	38.1 ±2.3	35.1 +3.3	34.0 ±3.6	31.6 +4.0	28.4** ±4.3	26.4** ±3.6	27.4** ±3.1	27.6** ±3.4	27.9** ±2.8	29.6 ±3.1	32.5 ±2.7
	C	38.1 ±2.4	39.2 ±3.1	38.4 ±2.6	37.1 ±3.3	38.7 ±3.0	39.4 ±3.9	39.6 ±3.2	38.8 +3.5	37.7 +2.9	38.0 +3.8	38.2 +3.0	40.3 +2.7
Globulin (g/l)	I	24.3 ±2.5	25.5 ±2.8	31.6 +2.4	35.1** ±2.1	38.1* ±1.4	40.5* ±1.2	42.9* +2.3	43.2* ±3.1	40.7* ±2.3	40.2* ±2.2	36.2* ±2.3	31.0 ±1.6
	C	24.0 +2.3	24.5 ±1.6	24.6 ±2.0	25.7 ±1.9	23.4 ±1.8	23.9 ±1.4	23.0 ±1.6	24.1 +1.4	24.5 ±1.8	23.6 +1.9	25.5 +1.3	22.8 ±1.4
Albumin globulin ratio	I	1.54 ±0.21	1.49 ±0.22	1.11 ±0.27	0.97 ±0.20	0.83** ±0.18	0.70** ±0.21	0.62* +0.23	0.63** ±0.21	0.68** ±0.19	0.69 ±0.27	0.82 ±0.17	1.05 ±0.22
	C	1.58 ±0.27	1.60 +0.23	1.56 ±0.20	1.44 +0.31	1.65 ±0.37	1.65 ±0.35	1.72 ±0.36	1.61 ±0.25	1.54 ±0.33	1.61 ±0.27	1.50 ±0.27	1.76 ±0.31
Total bilirubin (μmol/l)	I	8.63 ±0.84	9.79 ±0.92	12.35** ±0.97	14.72** ±1.43	16.47** ±1.64	19.75* ±1.49	15.51** ±1.35	14.83** ±1.20	13.37** ±1.45	10.98 ±0.91	9.91 ±0.87	9.97 ±1.06
	C	8.76 ±0.82	9.25 ±0.87	8.71 +0.88	9.22 ±0.86	8.57 +0.91	9.63 ±0.94	10.25 ±1.48	9.46 ±1.32	8.86 ±0.91	9.68 ±0.97	9.70 ±1.27	9.28 ±0.98

I: Experimentally infected, C: Healthy control. *: $p < 0.01$, **: $p < 0.05$.

TABLE 2. BIOCHEMICAL CHANGES IN EXPERIMENTAL *S. cervi* INFECTION IN RABBITS (Mean ± SE)

Parameters	Group	Days of observation											
		0	5	15	20	25	30	35	40	50	60	70	90
Total cholesterol (m mol/l)	I	1.09 ±0.15	1.27 ±0.16	1.70 ±0.23	1.95** ±0.25	2.17* ±0.23	2.36** ±0.29	2.21* ±0.27	2.12* ±0.19	1.77 ±0.21	1.53 ±0.22	1.42 ±0.17	1.49 ±0.18
	C	1.16 ±0.14	1.09 ±0.09	1.18 ±0.16	1.19 ±0.12	1.25 ±0.17	1.33 ±0.15	1.36 ±0.18	1.17 ±0.09	1.41 ±0.23	1.27 ±0.19	1.19 ±0.09	1.33 ±0.19
Blood urea nitrogen (m mol/l)	I	9.17 ±1.27	9.19 ±1.45	10.91 ±1.25	11.37 ±1.19	13.61** ±1.17	18.75* ±1.92	22.36* ±2.36	21.65* ±2.49	20.75** ±2.17	21.19* ±1.92	17.66** ±1.82	14.45 ±1.81
	C	9.43 ±0.97	11.10 ±1.38	9.65 ±1.28	9.58 ±0.98	9.93 ±1.87	10.37 ±1.46	10.72 ±1.15	11.16 ±1.31	9.84 ±0.98	9.27 ±1.43	8.77 ±1.04	10.35 ±1.37
Urea in blood (m mol/l)	I	19.62 ±2.78	19.67 ±3.31	23.35 ±3.25	24.33 ±3.19	29.12 ±4.23	40.13** ±4.74	47.85* ±5.54	46.33* ±5.79	44.40** ±5.61	45.35* ±3.76	37.79* ±3.89	30.92 ±3.87
	C	20.18 ±2.17	23.75 ±2.83	20.65 ±2.63	20.51 ±2.10	21.25 ±3.17	22.19 ±3.14	22.94 ±2.76	23.88 ±2.80	21.05 ±2.06	19.84 ±3.43	18.77 ±3.02	22.15 ±3.48
Uric acid (mg/dl)	I	2.63 ±0.26	2.87 ±0.31	3.75 ±0.35	4.30 ±0.47	5.67* ±0.43	5.29* ±0.39	5.43** ±0.48	5.56** ±0.42	5.28* ±0.37	3.92 ±0.35	4.17 ±0.31	3.26 ±0.28
	C	2.72 ±0.27	3.17 ±0.38	2.81 ±0.34	2.89 ±0.29	2.75 ±0.25	3.18 ±0.31	3.45 ±0.33	2.86 ±0.27	3.06 ±0.26	2.62 ±0.27	2.84 ±0.29	2.78 ±0.33
Creatinine (μmol/l)	I	81.7 ±5.6	82.3 ±5.3	92.3** ±4.6	97.7* ±4.3	98.4* ±5.2	98.6* ±5.1	97.1* ±4.7	95.3** ±6.3	91.2** ±4.9	92.6 ±5.3	90.2 ±4.7	84.8 ±4.7
	C	83.3 ±4.7	84.7 ±5.0	84.5 ±4.7	84.4 ±4.9	85.6 ±5.1	86.0 ±5.8	84.2 ±4.6	82.5 ±4.8	82.2 ±5.0	84.7 ±4.3	84.2 ±4.9	83.1 ±4.5

I: Experimentally infected, C: Healthy control *: $p < 0.01$, **: $p < 0.05$

the function of liver and kidney both as the two specific enzymes of purine metabolism-uricase and xanthine oxidase are located in these organs (Kaneko, 1989) and damage to either liver or kidney or both the organs, is good enough to over whelm this uric acid metabolism and its subsequent rise in blood (Coles, 1967). Rise in uric acid is due to liver and kidney damage both.

The present studies suggest that during *S. cervi* infection, the microfilariae damage the liver and kidneys of the host and for its early detection, liver or kidney function tests may be some value.

Acknowledgements

We are thankful to the Dean, College of Veterinary Sciences, and the Director, Experiment Station for providing the necessary facilities and funds.

Literature Cited

Anwar, N., A. K. Srivastava and S. Gratak. 1978. Effect of antifilarials on the metabolic activity of *Setaria cervi*. Indian Journal of Parasitology. 2:101-105.

Baqui, A. and J. A. Ansari. 1977. Electrophoretic patterns of white rat sera infected with *Setaria cervi*. Indian Journal of Helminthology. 27:106-115.

Bonsnes, R. W. and H. H. Taussky. 1945. The colorimetric determination of creatinine by Jaffe reaction. Journal of Biological Chemistry. 158:581-591.

Coles, E. H. 1967. Veterinary Clinical Pathology. W. B. Saunders Co., Philadelphia.

Green, P. E. and K. E. Trueman. 1971. The occurrence of *Setaria labiatopapillosa* in Queensland. Australian Veterinary Journal. 47:624.

Greenburg, D. M. 1929. The colorimetric determination of serum proteins. Journal of Biological Chemistry. 82:345-350.

Henry, R. J., C. Sobel and S. Berkman. 1957a. Interferences with biuret methods for serum proteins - use of Benedicts' qualitative glucose reagent as a biuret reagent. Analytical Chemistry. 29:1491-1495.

Henry, R. J., C. Sobel and J. Kim. 1957b. Modified carbonate-phosphotungstate method for the determination of uric acid and comparison with the spectrophotometric uricase method. American Journal of Clinical Pathology. 28:152-160.

Kaneko, J. J. 1989. Clinical Biochemistry of Domestic Animals. 4th ed. Academic press, New York.

Khatoon, H., Waijhullah, A. Baqui and J. A. Ansari. 1983. Histopathological study of *Setaria cervi* infection in rabbits. Indian Veterinary Journal. 60:698-699.

Kumar, B., H. C. Joshi and M. Kumar. 1986. Biochemical changes in buffaloes naturally infected with microfilariae of *Setaria cervi*. Indian Journal of Animal Sciences. 56:1009-1012.

Kumar, M., H. C. Joshi and K. C. Varshney. 1991. Pathological changes in experimental *Setaria cervi* infection in rabbits. Indian Journal of Animal Sciences. 61:924-932.

Kumar, V., H. C. Joshi and M. Kumar. 1989. Therapeutic trials in buffaloes naturally infected with microfilariae of *Setaria cervi*. Journal of Veterinary Parasitology. 3:125-129.

MacIntyre, I. and M. Ralston. 1954. Cited in Microanalysis in Medical Biochemistry. Kind, E. J. and Wooten, I. P. J. (eds). A. Churchill Ltd., London.

Richter, H. and Y. Lapointe. 1962. New reagent for use in determination of blood urea nitrogen with special reference to manual analysis. Clinical Chemistry. 8:335-338.

Sharma, V., R. Narayan and P. C. Choudhuri. 1981. Biochemical changes in blood of buffaloes suffering from microfilariasis. Indian Journal of Veterinary Medicine. 1:58-65.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods. 6th ed. Oxford and IBH Publishing Co., New Delhi.

Snyder, J. S. I., L. Kwang and J. T. Robert. 1967. Blood chemical and cellular changes in canine dirofilariasis. American Journal of Veterinary Research. 28:1705-1710.

Wang, Q. Q. 1981. A survey of *Setaria* spp. in cattle and preliminary study on experimental infection of laboratory animals. Chinese Journal of Veterinary Medicine. 7:2-5.

White, D., G. A. Haider and J. G. Reinhold. 1958. Spectrophotometric measurement of bilirubin concentrations in the serum of newborn by the use of microcapillary method. Clinical Chemistry. 4:211-215.