

## THE RELATIONSHIP BETWEEN SELENIUM DEFICIENCY AND THE DEVELOPMENT OF PULMONARY AND SUBCUTANEOUS EMPHYSEMA IN BOVINE EPHEMERAL FEVER VIRUS-INFECTED CATTLE

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### ABSTRACT

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Bovine ephemeral fever (BEF) was diagnosed on several commercial farms around Harare, Zimbabwe. The affected animals showed signs of fever (40-41.5 °C), depression, ruminal stasis, lameness and recumbency. Eight of those attended had severe respiratory distress and subcutaneous emphysema. Haematological and biochemical results indicated leukocytosis with an attending lymphopaenia. Selenium deficiency was detected only in those animals which showed respiratory embarrassment and subcutaneous emphysema.

### INTRODUCTION

Bovine ephemeral fever (BEF) is a non-contagious viral disease of cattle caused by a pleomorphic virus of the rhabdovirus group (Heuschele, 1970; St. George, 1981). The disease is characterised clinically by an acute onset of fever, depression, salivation, ruminal stasis, muscle fasciculations, lameness and recumbency (Mackerras, Mackerras & Burnett, 1940; Basson, Pienaar & Van der Westhuizen, 1970; Burgess & Spradbrow, 1977). An uncharacteristic clinical sign of severe respiratory distress characterised by a forced expiratory grunt and the development of pulmonary and subcutaneous emphysema has been described only in a small percentage of clinically affected cattle (Sen, 1931; Mackerras *et al.*, 1940; MacFarlane & Haig, 1955; Burgess & Spradbrow, 1977; Theodoridis & Coetzer, 1979). Most of these succumb to the disease with high mortality.

Mulhearn (1937) described this condition in Australian cattle which were affected with BEF. His opinion was that secondary bacterial infection may have accounted for the respiratory signs. Basson *et al.*, (1970) described histopathological lesions in such animals dying of the disease. They observed focal, pale circumscribed areas in skeletal muscles consisting of hyaline necrosis of muscle fibres. Theodoridis & Coetzer (1979) described in detail these changes as pulmonary emphysema resulting from bronchiolar obliteration with cellular debris and necrosis. Other histological findings included Zenker's degeneration of muscular layer and thrombosis of pulmonary vessels.

They suggested that forced movement during the clinical bout could precipitate pulmonary distress. Because only a small percentage of clinically affected animals showed this syndrome, they suggested that studies be directed to the microbiology, blood physiology and nutrition of such animals.

Lober, Thurston, Gander & Goodale (1971) and Stoner (1972) were, however, unable to attribute the pulmonary pathology in such cases to the direct viral activity because BEF virus is non-cytolytic. Cowie & Dear (1976) observed an increase in the incidence of respiratory embarrassment in cattle with clinical selenium deficiency. Their findings, however, did not involve control animals. Blood, Radostits & Henderson (1985) reported that secondary pneumonia seem to be sequela to clinical selenium deficiency

in cattle where muscles of the throat and chest are affected. They described hyaline degeneration and coagulative necrosis of the muscle layer of the bronchioles. Cowie, Dear, Blood and others, therefore, believe that impaired respiratory muscle function might contribute to the increased incidence of pneumonia in cases of responsive nutritional myopathy. However, Philippo, Arthur, Price & Halliday (1987) reported that the incidence of pneumonia in housed calves was not related to selenium deficiency but to herd size and building design. This paper describes the clinical, haematological and selenium status of eight cases of BEF with pulmonary and subcutaneous emphysema studied in Zimbabwe between January 1987 and April 1988.

### MATERIALS AND METHODS

#### *Animals*

A severe outbreak of BEF occurred in several commercial farms around the capital, Harare, during the early months (January to April) of 1986, 1987 and 1988. Those of 1986 have been described (Hill, MacKenzie & Honhold, 1988, in press) while the 1987 ones were sporadic and less severe than those of 1986 and 1988. The cases described fell under three main clinical groups:

- (1) Those which showed transient fever, less severe clinical signs and quick recovery.
- (2) Severely affected animals with pyrexia, salivation, ruminal stasis, knuckling, muscle fasciculations, lameness and recumbency, and the course of illness lasted for about 3-5 days with subsequent recovery.
- (3) A few cases (8 attended) which had similar clinical signs like those in (2) but in addition had very severe respiratory distress characterised by a forced expiratory grunt and on chest auscultation, dry crackling rales were detected suggesting pulmonary emphysema. Subcutaneous emphysema was evident along the trunk, neck region and the hind limbs extending up to the hock joints. All these cases were attended in the fields except for case number 242 which ended up in the University Veterinary Hospital.

#### *Blood and serum sample analysis*

Blood samples were taken by venipuncture at the height or near peak temperature reaction into plastic tubes containing dipotassium diethane-tetraacetate (EDTA) and stored at -20 °C until analysed or analysed within hours of collection. Blood for serum was collected in plain plastic tubes and kept at

TABLE 1 Glutathione peroxidase activity, haematological and biochemical results of five animals from group (2) and eight animals from group (3)

Animal No.	rbc	wbc	seg	Lymph	Ca	Mg	Inorg. phos.	Glutathione peroxidase activity
	$\times 10^{12}/\ell$	$\times 10^9/\ell$	%	%	mmol/ $\ell$	mmol/ $\ell$	mmol/ $\ell$	Adequate/low
71**	6,31	12,64	18	79	2,26	1,0	ND	Deficient
52	6,40	15,44	48	44	1,87	0,9	0,50	Adequate
61	5,40	12,31	18	82	1,84	1,9	1,0	Adequate
346*	5,34	15,67	63	21	1,60	1,1	1,00	Deficient
109	7,72	8,50	42	41	2,40	1,0	1,20	Adequate
15*	9,39	18,73	70	29	1,93	ND	2,10	Deficient (died)
242*	9,67	21,02	75	24	1,82	ND	ND	Deficient
4*	9,89	16,39	51	47	1,10	ND	4,30	Deficient
21*	8,69	23,76	50	50	1,90	1,2	1,00	Deficient
3*	8,24	13,66	59	33	1,86	ND	3,10	Deficient
106*	6,90	16,12	60	36	1,89	1,0	2,10	Deficient
89	6,16	14,36	43	33	2,60	0,9	2,10	Adequate
66*	5,85	13,31	59	36	1,78	0,7	1,40	Deficient

\* Animals which showed pulmonary distress with subcutaneous emphysema

\*\* Animals with low blood selenium levels but never showed any respiratory distress

ND = Not done

4 °C, centrifuged and serum obtained. Blood cell counts were determined using Coulter Counter machine (Coulter Counter<sup>R</sup>, ZM). Blood selenium levels were determined by estimating glutathione peroxidase (GSHPx) activity according to the methods of Board & Peter (1976). This method estimates the blood selenium levels semiquantitatively. A sample was said to have low enzyme activity (hence low selenium) when there was retention of fluorescence for more than 30 min, and adequate when fluorescence disappeared between five and thirty min. Blood calcium, magnesium and inorganic phosphate levels were measured calorimetrically (Gemini<sup>TM</sup>, Electro nucleonics Inc. New Jersey). The animals with pulmonary distress were treated intramuscularly each with 20 ml of dexamethasone<sup>R</sup> (Byk Gulden, Netherlands), 10 ml of Injacom E-selenium (Roche) preparation containing 0,5 mg selenium and 150 mg vitamin E acetate per ml. They were also given tetracycline injectable solution (8 mg/kg).

### RESULTS

Most animals showed pyrexia (40–41, 5 °C). There was a general tendency to leukocytosis with a shift to the left and an accompanying lymphopaenia (Table 1). The red blood cell counts of the affected animals were within normal range. Hypocalcaemia was detected in those animals which were bled at the height of the temperature reaction; however, some of them (numbers 71, 109 & 89) had normal serum calcium levels. There was no significant change in magnesium and phosphate levels. Glutathione peroxidase activity was low in all the animals with respiratory distress and subcutaneous emphysema, except for animal 71 which was low on selenium but never had any respiratory problems. Some of the animals (52 & 61) had low calcium levels but adequate selenium and never showed any respiratory embarrassment. No autopsy was performed on the dead animal (15) as death was reported two days later.

The regimen of treatment described above seemed to have resolved the respiratory crisis since the animals had an uneventful recovery.

### DISCUSSION

Respiratory distress and subcutaneous emphysema were seen only in animals which had low GSHPx activity, except in animal number 71 which

had low blood selenium levels but without the development of respiratory distress. The leukocytosis with an accompanying leukopaenia and hypocalcaemia reported here are similar to those reported by previous authors (Mackerras *et al.*, 1940; Basson *et al.*, 1970; Burgess & Spradbrow, 1977; Young & Spradbrow, 1988; Uren & Murphy, 1985). Normal calcium levels in animal numbers 71, 109 and 89 may have been related to the time when the blood samples were taken.

Glutathione peroxidase is a selenoenzyme (Rotruck, Pope, Ganther, Swanson, Hafeman & Hoekstra, 1973) and its activity in blood is related to blood selenium concentration and dietary intake. It has, therefore, been considered a sensitive indicator for selenium status in rats, sheep and cattle (Hoekstra 1974; Allen, Parr, Anderson, Barrett, Bradley & Petterson, 1975; Thompson, McMurray & Blachflower, 1976; Wilson & Judson, 1976).

Considered together, the only monitored biochemical difference between animals in group (2) and (3) is the selenium concentration in the blood. On the other hand, the major clinical difference between these two groups is the presence of pulmonary and subcutaneous emphysema in animals from group (3) and none in group (2).

Warso & Lands (1983) reported that when GSHPx activity is low (i.e. low blood selenium levels) the platelets metabolise their membrane arachidonic acid differently with altered production of its metabolites to increased levels of hydroxyeicosatetraenoic acids (HETES) via the lipoxygenase pathway. Ali & McDonald (1977) and Kawaguchi, Ishibashi & Imai (1982) reported that decreased GSHPx activity in blood resulted in increased synthesis of thromboxane B<sub>2</sub> (TXB<sub>2</sub>). The HETES and TXB<sub>2</sub> are potent chemoattractants for other polymorphonuclear (PMN) leukocytes with resultant release of leukocytic proteases (Turner, Tainer & Lynn, 1975; Stenson & Parker, 1980).

Leukocytic elastase can cause pulmonary emphysema in goats (Janof, Sloan, Weibaune, Damiano, Sandhaus, Elias & Kimbel, 1977). Masukawa, Goto & Iwata (1983) reported what they termed 'arachidonic acid-induced respiratory distress' in selenium-deficient rats. In their study, they found that platelets from such animals showed increased tendency to aggregate with resultant thrombus formation in pulmonary vessels.

The pathogenesis of pulmonary emphysema has been described (Spencer, 1985) as resulting from obliteration and destruction of respiratory bronchioles causing 'air-trapping' and also due to inflammatory weakening of the walls of either respiratory bronchioles or more distally situated structures including peribronchiolar alveolar walls.

The histological findings described by the above author are similar to those described for BEF by Basson *et al.* (1970) and Theodoridis & Coetzer (1979). They described bronchiolar obliteration with inflammatory cell infiltrates and cellular debris, destruction of respiratory bronchioles and thrombus formation in pulmonary blood vessels of BEF cases with pulmonary and subcutaneous emphysema.

The findings reported here would suggest that selenium deficiency in cattle infected with BEF virus is a factor in the pathogenesis of pulmonary distress in cattle. It is the author's opinion that the occurrence of respiratory distress and subcutaneous emphysema in these animals was related to reduced GSHPx activity (selenium deficiency) and that the infection was superimposed on this nutritional stress. Work is in progress to verify this hypothesis.

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#### REFERENCES

- ALLEN, M. W., PARR, W. H., ANDERSON, P. H., BARRETT, S., BRADLEY, R. & PETTERSON, D. S. P., 1975. Selenium and the activity of glutathione peroxidase in bovine erythrocytes. *Veterinary Record*, 96, 360.
- ALI, M. & MCDONALD, J. W. D., 1977. Effects of Sulfinpyrazone on platelet prostaglandin synthesis and platelet release of Serotonin. *Journal of Laboratory and Clinical Medicine*, 89, 868-875.
- BASSON, P. A., PIENAAR, J. G. & VAN DER WESTHUIZEN, B., 1970. The pathology of ephemeral fever: a study of the experimental disease in cattle. *Journal of South African Veterinary Medical Association*, 40, 385-397.
- BLOOD, D. C., RADOSTITS, O. M. & HENDERSON, J. A. (ed.), 1985. Selenium and/or Vitamin E deficiency. In: *Veterinary Medicine* 6th ed., pp. 1043-1054.
- BOARD, P. G. & PETER, D. W., 1976. A simple test for glutathione peroxidase and selenium deficiency. *Veterinary Record*, 99, 144-145.
- BURGESS, W. G. & SPRADBROW, P. B., 1977. Studies on the pathogenesis of bovine ephemeral fever. *Australian Veterinary Journal*, 53, 363-368.
- COWIE, R. S. & DEAR, J. P., 1976. Pneumonia in single suckled calves. *Veterinary Record*, 98, 283.
- HEUSCHELE, W. P., 1970. Bovine ephemeral fever. I. Characterization of the causative virus. *Archives für die gesamte Virusforschung*, 30, 195-202.
- HILL, F. W. G., MACKENZIE, R. L. & HONHOLD, N., "In press". Haematological and biochemical finding in nineteen cases of bovine ephemeral fever (three-day sickness). *Zimbabwe Veterinary Journal*.
- HOEKSTRA, W. G., 1974. Selenium. In: Trace element metabolism in animals. Volume 2, p. 61 Eds, HOEKSTRA, W. G., SUTTIE, J. W., GANTHER, H. E. & MERTZ, W. University Park Press, Baltimore.
- JANOFF, A., SLOAN, B., WEIBAUNE, G., DAMIANO, V., SANDHAUS, R. A., ELIAS, J. & KIMBEL, P., 1977. Experimental emphysema induced with purified human neutrophil: tissue localization of the instilled protease. *American Review of Respiratory Diseases*, 115, 461-478.
- KAWAGUCHI, H., ISHIBASHI, T. & IMAI, Y., 1982. Increased Thromboxane B<sub>2</sub> biosynthesis in platelets. *Lipids*, 17, 577-584.
- LOBER, D. T. M., THURSTON, S., GANGER, O. & GOODALE, F., 1971. The fate of circulating leukocytic pyrogen in the rabbit. *Proceedings of Society for experimental Biology and Medicine*, 137, 896-901.
- MACFARLANE, I. S. & HAIG, D. A., 1955. Some observations on three-day sickness in the Transvaal in 1954. *Journal of South African Veterinary Medical Association*, 26, 1-7.
- MACKERRAS, I. M., MACKERRAS, J. & BURNETT, F. M., 1940. Experimental studies of ephemeral fever in Australian cattle. *Council for Scientific and Industrial Research. Melbourne, Australia. Bulletin*, 136, 1-116.
- MASUKAWA, T., GOTO, J. & IWATA, H., 1983. Impaired metabolism of arachidonate in selenium-deficient animals. *Experientia*, 39, 405-406.
- MULHEARN, C. K., 1937. Ephemeral or three day fever in Northern Queensland: Its diagnosis and some preliminary investigations. *Australian Veterinary Journal*, 13, 186-191.
- PHILIPPO, M., ARTHUR, J. R., PRICE, J. & HALLIDAY, G. J., 1987. The effects of selenium, housing and management on the incidence of pneumonia in housed calves. *Veterinary Records*, 121, 509-512.
- ROTRUCK, J. T., POPE, A. L., GANTHER, H. E., SWANSON, A. B., HAFEMAN, D. G. & HOEKSTRA, W. G., 1973. Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, 179, 588-590.
- SEN, S. K., 1931. Three day sickness of cattle. *Indian Journal of Veterinary Science*, 1, 14-23.
- SPENCER, H., 1985. Emphysema. In: Pathology of the lung. Volume 1. pp. 557-594. Ed. H. SPENCER. Pergamon Press Ltd. London.
- ST. GEORGE, T. D., 1981. Ephemeral fever. In: Virus diseases of food animals. Volume II. Disease monographs, Ed. E. P. J. GIBBS. Academic Press, London.
- STENSON, W. F. & PARKER, C. W., 1980. Monohydroxyeicosate-tra-eionic acids (HETES) induce granulation of human neutrophils. *Journal of Immunology*, 124, 2100-2104.
- STONER, H. B., 1972. Microbial pathogenicity in man and animal. Cambridge University Press, London, p. 113.
- THEODORIDIS, A. & COETZER, J. A. W., 1979. Subcutaneous and pulmonary emphysema as complications of bovine ephemeral fever. *Onderstepoort Journal of Veterinary Research*, 46, 125-127.
- THOMPSON, R. H., MCMURRAY, C. H. & BLACHFLOWER, W. J., 1976. The levels of selenium and glutathione peroxidase activity in blood of sheep, cows and pigs. *Research in Veterinary Science*, 20, 229-231.
- TURNER, S. R., TAINER, J. A. & LYNN, W. S., 1975. Biogenesis of chemotactic molecules by the arachidonate lipoxygenase system of platelets. *Nature*, 39, 680-681.
- YOUNG, P. L. & SPRADBROW, P. B., 1980. The role of neutrophils in bovine ephemeral fever virus infection of cattle. *Journal of Infectious Diseases*, 142, 50-55.
- UREN, M. & MURPHY, G. M., 1985. Studies in the pathogenesis of bovine ephemeral fever in sentinel cattle. Haematological and biochemical data. *Veterinary Microbiology*, 10, 505-515.
- WARSO, M. A. & LANDS, W. E. M., 1983. Lipid peroxidation in relation to prostacyclin and thromboxane physiology and pathophysiology. *British Medical Bulletin*, 39, 277-280.
- WILSON, P. S. & JUDSON, G. J., 1979. Glutathione peroxidase activity in bovine and ovine erythrocyte in relation to blood selenium concentration. *British Veterinary Journal*, 132, 428-434.