

Vanadium air pollution: a cause of malabsorption and immunosuppression in cattle

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ABSTRACT

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An epidemiological investigation into an "illthrift" problem occurring on a dairy farm adjacent to an alloy-processing unit, established that the probable cause of the problem was chronic vanadium poisoning. The disease manifested initially in animals 4–18 months old which showed emaciation, chronic diarrhoea and, in some cases, rhinitis, conjunctivitis and recumbency followed by death. Post-mortem ($n = 17$) and clinical-pathology findings ($n = 60$) indicated that malabsorption and immunosuppression were the basis of the pathogenesis in affected animals. Eight months after the commencement of the investigation, adult cows began showing evidence of emaciation, reduced milk production and an apparent increase in the number of abortions, stillbirths and dystocias.

Over a 2-year period, 134 surface-soil samples, 134 subsoil samples and 134 grass samples from the farm were analysed for various fractions of vanadium. Thirty-four of each of these samples were collected at different time intervals (autumn 1990, summer 1991 and winter 1991) and at varying distances and directions from the processing unit, in order to gauge the magnitude of the problem, and the distribution pattern of vanadium, and to identify possible seasonal trends. The remaining 100 of each of these samples were taken at 100-m intervals over an area of approximately 1 140 000 m² directly adjacent to the processing unit so that concentration isolines for vanadium could be drawn and the source more conclusively identified. The levels of vanadium were found to be highest closest to the mine, and surface-soil levels were consistently higher than subsoil levels, suggesting aerial pollution, which was confirmed by air sampling. In addition, washed grass samples were considerably lower in vanadium than the unwashed samples, indicating that most of the vanadium was in the dust on the plants. The highest levels of vanadium were found in the soil during the summer and on the grass during the winter. These analyses confirmed the presence of high vanadium levels (≤ 1122 ppm) in the surface soils and grass (≤ 558 ppm) on the farm and showed that the major source of vanadium was the adjacent alloy-processing unit.

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INTRODUCTION

In April 1990, the Onderstepoort Veterinary Institute (OVI) was approached by a private veterinarian to do an epidemiological study on the cause of "illthrift" occurring in a dairy herd in the eastern Transvaal. This paper describes the investigation and its findings.

MATERIALS AND METHODS

An initial investigation, comprising three farm visits, was conducted. These farm visits were undertaken on 27 April 1990 (late autumn), 15 January 1991 (midsummer) and 17 June 1991 (midwinter). On the first visit, environmental factors were examined, a complete history was obtained and clinical signs were observed. The second visit confirmed the environmental findings, and clinical pathological trends within the population were examined. The final visit confirmed the environmental and population trends previously found. A second investigation was initiated in April 1992, to provide more concrete information on the source of vanadium.

Environment

Soil and plant material

To test an initial hypothesis of copper deficiency due to high background levels of molybdenum (Mo), soil

and grass samples were taken at ten, and later 12, points on the farm (Fig. 1). At each sampling point, approximately 500 g of subsoil, at a depth of 300 mm, and 500 g of surface soil were collected. Grass in the immediate vicinity of the soil-sampling point was then collected. Sampling was done in late autumn, midsummer and midwinter (three times). After the first visit, each specimen was analysed for copper (Cu), Mo and zinc (Zn) as these minerals are generally associated with Cu deficiency.

An alloy-processing unit (APU) was situated immediately adjacent to the farm and it was therefore decided to include iron (Fe), chromium (Cr) and vanadium (V) (which are associated with steel production), in the analysis profile, in order to rule out environmental pollution as a cause. The samples collected in April 1990 were thus analysed for Fe, Cu, Zn, Cr, Mo and V, using standard analytical methods for atomic-absorption spectrophotometry (Perkin-Elmer Corporation, Norwich, Connecticut, USA). The samples collected in January and June 1991 were analysed for Cu, Fe and V only.

A second study, undertaken in April 1992, concentrated on camps 3, 4 and 12 (Fig. 1), immediately adjacent to the APU, and measured V levels only. A 100-m x 100-m grid sampling resulting in 100 sampling points was carried out over this area. At each sampling point surface soil (0–20 mm), subsoil (20–300 mm) and plant material were collected for analysis (Fig. 2 and 3). Analysis of the soil and plant

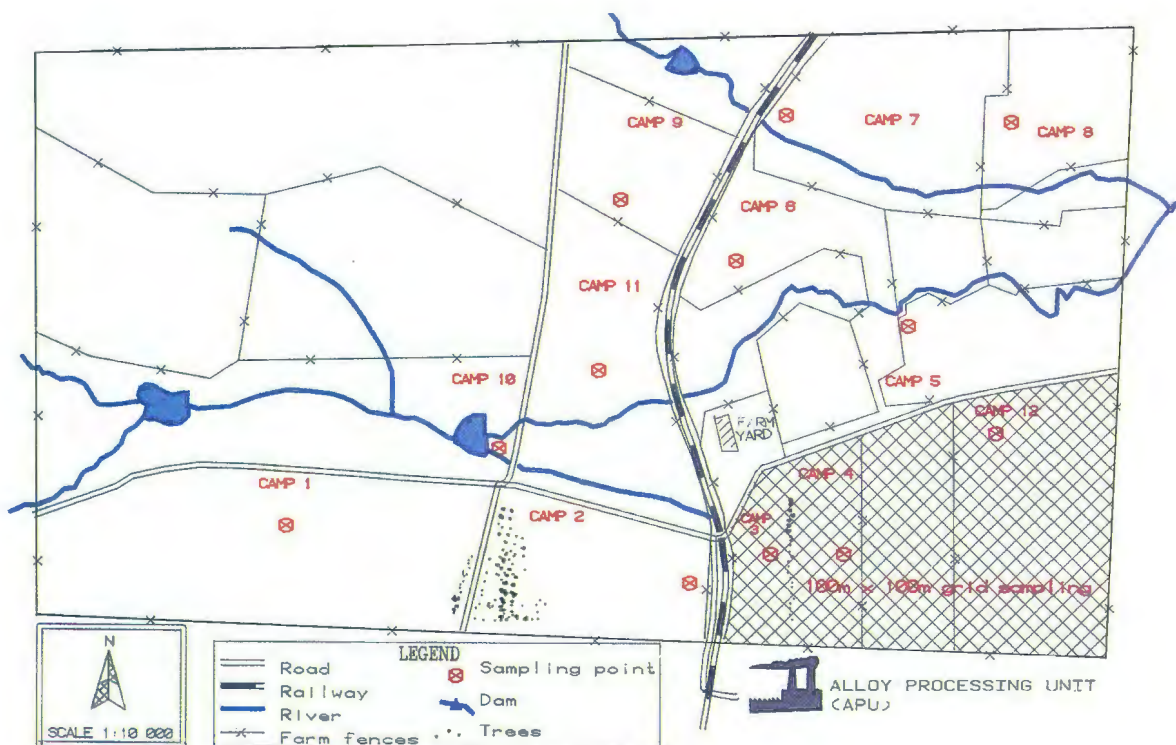


FIG. 1 Map of affected farm showing sampling points and camp numbers

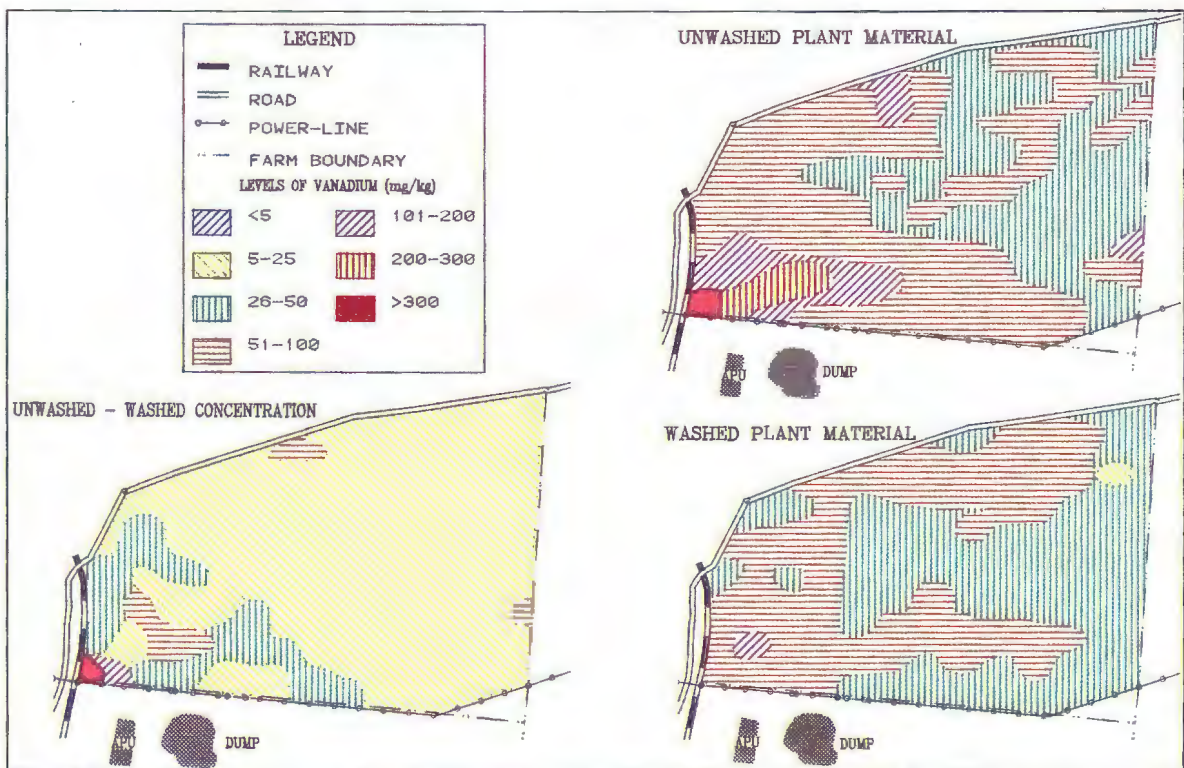
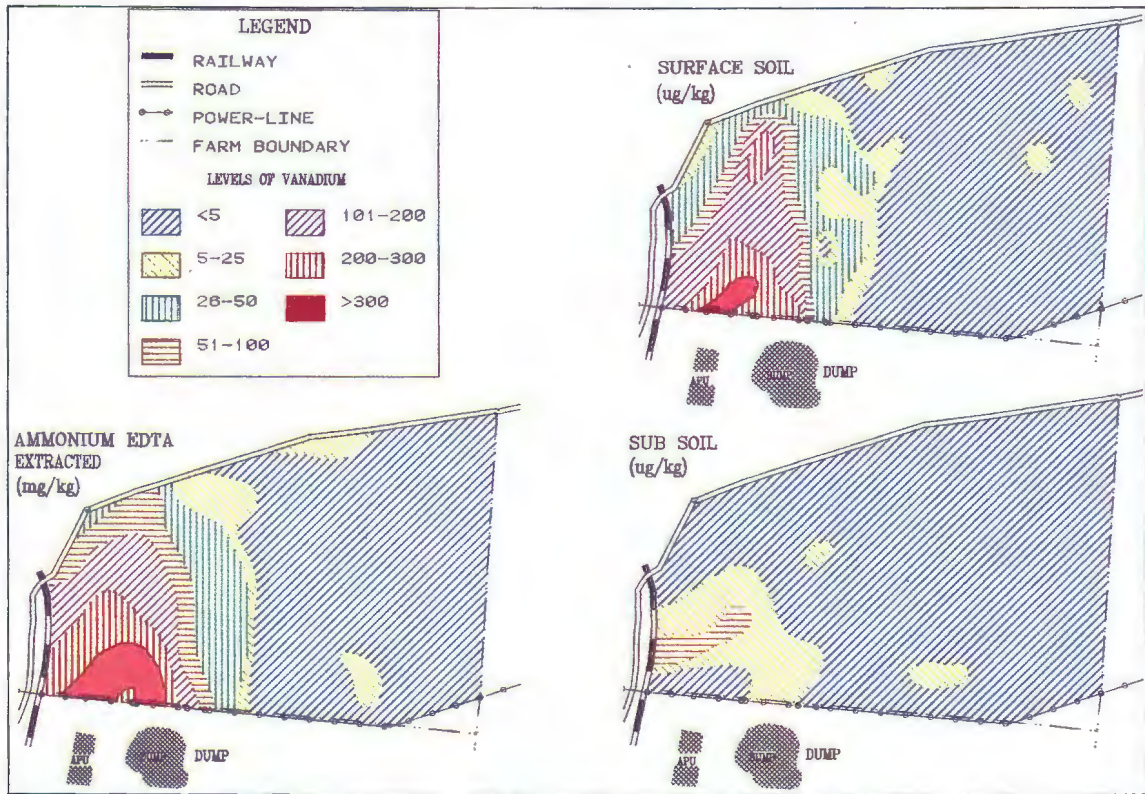


FIG. 2 Diagram of the concentration isolines for vanadium found in the surface soil (water-soluble and ammonium EDTA extracts) and subsoil (water-soluble extract), in the area adjacent to the alloy-processing unit (Fig. 1)

FIG. 3 Diagram of the concentration isolines for vanadium found in and on plant material, before and after washing, in the area adjacent to the alloy-processing unit (Fig. 1)

material was done according to the *Handbook of Standard Soil Testing Methods for Advisory Purposes* (Soil Science Society of South Africa 1990). Two soil extracts were prepared: an ammonium-EDTA (NH_4 -EDTA) extract and a water-soluble extract. Vanadium analysis was carried out on the extracted solutions by means of an inductive coupled-plasma mass spectrometer (VG Plasmaquad PQ2 turbo plus ICPMS) and a high-temperature graphite oven (Perkin Elmer) coupled to an atomic-absorption spectrometer (Varian techtron AA6). Standards and blanks were tested at regular intervals during the analysis.

The plant material from the second investigation was divided into two equal portions. The first portion was washed with deionized water to remove any dust from the leaves and the second was left unwashed. After it had been dried, the plant material of both portions was ground and digested with HNO_3 and HClO_4 to obtain a solution. The vanadium concentrations of these solutions were then determined according to the methods described above.

Air-sampling methods

An air-sampling site in the farmyard (Fig. 1) was selected, since this offered an available power supply and security for the equipment. Air was drawn through a 47-mm, 8- μm pore-size Nucleopore filter at about 5 ℓ/min . Sampling was carried out over varying time intervals (Table 3) during the winters of 1990 and 1991 and summer of 1991. Deposit samples were collected in open plastic containers 13 cm in diameter, at distances of 150 and 250 m from the processing plant, over 14-d periods, in the summer and winter of 1991.

Animals

Chemical pathology

Ten to 25 animals were examined clinically during each of the three farm visits. On the second and third visits, blood was collected from a total of 60 animals. On the second visit, animals to be bled were randomly selected from various groups as follows:

0–3 m	(n = 3)
4–7 m	(n = 5)
8–11 m	(n = 5)
Heifers	(n = 6)
Dry cows	(n = 6)
Lactating cows	(n = 6)

The animals selected on the third farm visit included those bled on the second visit, as well as additional animals as detailed below:

0–3 m	(n = 9)
4–7 m	(n = 5)
12–15 m	(n = 4)
16–19 m	(n = 5)
Adult cows	(n = 11)

On both visits, venous blood was collected for the determination of the following parameters:

Total serum protein (TSP)
Albumin (Alb)
Globulin (Glob)
Blood-urea nitrogen (BUN)
Gamma-glutamyltransferase (GGT)
Aspartate aminotransferase (AST)
Creatine phosphokinase (CK)
White-cell count (WCC)
Red-cell count (RCC)
Haematocrit (Ht)
Blood glucose

Blood obtained on the third farm visit was also tested for blood-creatinine (Creat) levels and lymphoblast-transformation responses to plant lectins. Standard methods of analysis were used in all cases (Kristensen, Kristensen & Lazary 1982; Jain 1986; Kaneko 1989).

Bacteriology

During the course of the investigation, serum was collected from nine cows which had aborted on the farm, and tested for leptospira antibodies by means of the Microscopic Agglutination Test.

Pathology

A total of 17 animals (cases 1–17) were necropsied between the period May 1990 to January 1992. Tissues for vanadium analysis were collected from 12 of these cases (Table 4). Complete necropsies were performed on 13 of the 17 cases, and the remaining four cases (cases 12–15) constituted formalin-fixed tissues submitted for histopathological examination. Formalin-fixed tissues were sectioned and stained with haematoxylin and eosin (HE) according to standard methods. Where complete necropsies were performed, blood and brain smears were examined for the detection of protozoal blood parasites and heart-water colonies and, when necessary, faeces were collected for the detection of nematode eggs, coccidial oocysts and viral particles. Various tissues were also collected for aerobic and anaerobic bacterial isolation.

The 17 cases were divided into four age groups—groups A, B, C and D—as follows:

- Group A comprised five fetuses/perinatal calves (cases 1–5) which were examined according to standard methods employed at the OVI for the detection of causes of abortion/perinatal mortality in cattle.
- Group B consisted of three suckling calves (cases 6, 7 and 8).
- Group C included seven calves between 3 and 12 months of age (cases 9–15) and was compared with a euthanased healthy calf of about 6 months old obtained from the OVI.

- Group D was made up of two adult cows, 4 and 7 years old (cases 16 and 17).

Vanadium analyses

During the second farm visit faecal samples were collected for V analyses from 19 adult cattle. Tissues and ruminal or abomasal fluid were collected from 12 of the 17 animals necropsied (Table 4). Samples were analysed for V content, by means of standard methods (Boyazoglu, Barrett, Young & Ebedes 1972).

RESULTS AND DISCUSSION

Soil analyses

The soil-V results of the first investigation are shown in Table 1 and those of the second investigation in Fig. 2 and 3. The results of the first farm visit showed that Cu, Cr, Mo and Zn were not present in excessive levels in the vegetation or soil on the farm (Table 2). The pattern of distribution of these elements showed no correlation to distance or direction from the APU (Table 2), implying that these minerals occurred naturally on the farm, and reflecting what would be seen if no air pollution were taking place.

The average level of V in the earth's crust is normally 100–150 ppm (Faulkner Hudson 1964; Richie 1985; Waters 1977). The average levels of V in the surface soil of seven of the 12 camps sampled (camps 3, 2, 4, 5, 12, 7 and 8), were higher than normal (Table 1). Six of the seven camps were situated either adjacent to the APU or north to north-east of the APU, and one camp (camp 2) was more north-west, but bordered on the APU. None of the other camps to the west or north-west of the APU had abnormally high levels

of V. These findings suggest a link between direction from the APU and V levels. The farmer claimed that the wind blew predominantly towards the north for at least 6 months of the year, which supported the postulation that the APU was the probable source of V pollution. To try and verify this, weather data from one of the nearest weather stations (Lydenburg) were examined (unpublished data, Department of Environmental Affairs, RSA 1988). This showed that during the period 1960–1988, the predominant average wind direction was from the south-east for the months December, January, February, March, April and June, which supported the farmer's observations that the wind over the farm blew predominantly in a northerly direction for at least 6 months of the year.

When the surface-soil V levels were examined in terms of distance from the APU, a definite trend could be shown, which indicated that as the distance from the APU increased, so the V levels fell. This was shown most clearly in the detailed study of the area immediately north of the APU, where plotted isolines showed distinct concentration gradients stemming from the APU (Fig. 2). This spatial distribution pattern of V was seen for both water- and NH_4EDTA -extractable V, indicating that the APU was the centre of a V "hot spot". The presence of high levels of water-soluble V is significant, since naturally occurring outcrops of V are unlikely to contain large amounts of water-extractable V. A large amount of the detectable V was therefore most probably due to pollution and not of natural origin.

Camps 7 and 8 appear to have had inconsistent results (Table 1) as they are relatively distant from the APU, yet have high levels of V in the soil. These high levels could probably be ascribed to the topography of the farm, as these camps, though further

TABLE 1 Vanadium levels (ppm) of surface soil, subsoil and grass in relation to approximate distance and direction from plant

Camp no.	Approx. distance (km)	Approx. direction	Autumn			Summer			Winter			Mean V levels		
			Surf. soil	Sub soil	Grass	Surf. soil	Sub soil	Grass	Surf. soil	Sub soil	Grass	Surf. soil	Sub soil	Grass
3	0,32	W	1055	206	290	745	461	27	991	115	180	930	261	166
2	0,49	NNW	514	508	31	313	127	4,9	204	152	85	344	262	40
4	0,52	N	274	198	8	1122	338	9,7	522	155	75	639	230	31
5	1,43	N	149	93	10	333	256	ND	86	67	70	189	139	27
12	1,49	ENE	—	—	—	328	264	4,6	182	127	ND	255	196	2
11	1,56	WNW	—	—	—	67	59	10,0	36	31	ND	52	45	5
6	1,88	NNW	58	41	12	120	80	0,8	67	98	30	82	73	14
10	1,92	WNW	44	18	ND	70	36	10,3	50	36	30	55	30	13
.9	2,14	WNW	42	41	ND	51	51	0,6	48	54	ND	47	49	ND
1	2,40	W	35	22	3	92	57	0,8	61	39	20	63	39	8
7	2,73	NNE	354	215	ND	558	590	4,3	263	247	20	392	351	8
8	2,86	N	385	291	15	494	361	1,0	165	283	30	348	312	15

ND = not detectable (< 0,5 ppm)

— = not determined

from the APU than camps 5 and 6, were at the same altitude as camps 3 and 4. It is possible that pollutants from the APU could overshoot camps 5 and 6, situated in a valley, and settle on camps 7 and 8.

In the camps to the north and north-east of the APU, the V levels in the subsoil were almost always lower than the surface-soil levels. This was particularly evident in the grid samples taken during the second investigation (Fig. 2). Such a finding is inconsistent with soils formed *in situ* on parent material naturally rich in V, and leads to the assumption of V air pollution, the most likely source of which was the adjacent APU. In those camps north-west of the APU, the difference between the sub- and surface-soil V levels was very small, which is what can be expected when there is no aerial pollution.

Camps 4, 5, 7, 8 and 12 had much higher levels in the soil during summer than during winter. This is the converse of what occurred in the grass samples, and suggests that during the high-rainfall summer months, much of the V which may have been trapped on the grass was washed off into the soil, resulting in higher levels in the soil and lower levels on the grass during the summer. This seasonal variation supported a postulation of air pollution and could explain why there was no cumulative effect over the sampling period.

Grass analyses

Grass V levels showed a decrease with distance from the APU (Table 1, Fig. 3), the highest levels being those closest to the APU. This finding supported the postulation that the source of the V was the APU. The grass iron levels (Table 2) showed no correlation with distance, but a good correlation with soil levels, and illustrate what can be expected when air pollution does not play a role.

The low V levels on grass in camps 7 and 8 (Table 1) suggest that there was little correlation between levels of V on soil and aerial grass. Normal levels of V in higher plants are given as an average of 1 ppm (Faulkner Hudson 1964; Platonow & Abbey 1968; Waters 1977). The average levels on the farm all exceeded 1 ppm, with the exception of camp 9. Most of the farm therefore had abnormally high levels of V in or on the grass. The highest levels occurred in camp 3, closest to the APU, where levels ranged from 32–533 ppm (Fig. 3). Seven of the 12 camps sampled had average levels greater than 10 ppm (Table 1).

Higher levels of V occurred on the grass during winter than during summer (Table 1). This trend supported a postulation of aerial pollution, as it can be assumed that during the rainy season (summer) dust particles will be washed off the grass, thus resulting

TABLE 2 Analytical results of samples collected on the farm during the first farm visit (27/4/1990)

Sample no. and description	Fe ppm	Cu ppm	Zn ppm	Cr ppm	Mo ppm	
Water						
1	1	0	0	0		
2	1	0	0	0		
Grass						
1	<i>Hyperthernia hirta</i>	35	0	5	0	< bl
1	<i>Eragrostis curvula</i>	225	5	5	0	< bl
3	<i>Eragrostis curvula</i>	30	0	0	0	< bl
3b	Dead grass	225	5	5	0	< bl
3c	Jan 1989	95	0	5	0	< bl
3c	Jan 1990	75	5	5	0	< bl
4	<i>Eragrostis curvula</i>	45	0	0	0	< bl
4	Hay bale	50	5	385	0	< bl
5	<i>Hyperthernia hirta</i>	35	0	5	0	< bl
6	<i>Cymbopogon excavatus</i>	25	0	0	0	< bl
7	Grass unidentified	45	0	25	0	< bl
8	<i>Hyperthernia hirta</i>	30	0	5	0	< bl
9	<i>Sporobolus fimbriatus</i>	20	0	10	0	< bl
10	Grass unidentified	10	0	15	0	< bl
Soil						
1	Surface	523	2	11	0	0,5
1	Deep	378	2	28	0	0,5
2	Surface	458	3	12	1	1,1
2	Deep	550	3	2	0	1,7
3	Surface	324	3	20	1	< bl
3	Deep	228	3	2	0	3,7
4	Surface	358	3	12	1	< bl
4	Deep	227	4	2	0	4,0
5	Surface	169	3	14	12	1,1
5	Deep	135	3	3	0	1,1
6	Surface	449	4	6	0	0,5
6	Deep	460	5	3	0	< bl
7	Surface	320	6	11	1	5,5
7	Deep	368	6	1	0	5,5
8	Surface	433	10	10	1	5,5
8	Deep	436	7	2	0	5,1
9	Surface	125	1	10	1	1,1
9	Deep	228	2	2	0	4,4
10	Surface	416	3	6	0	1,4
10	Deep	495	3	2	0	1,4

Soil and plant = dry-mass basis
bl = blank sample

in lower levels on the grass. The difference in V content, between washed and unwashed grass samples, further supported this postulation. The V concentrations on unwashed grass were up to 430 times higher than those on washed grass samples from the same sampling points (Fig. 3).

Little work has been done to determine the toxic level of V for cattle in grass. Fox (1987) specifies that the usual feedlot diet for cattle contains $\pm 0,57$ ppm V. Most of the camps on this farm exceeded 0,57 ppm

TABLE 3 Results of aerial sampling for vanadium

Season	Sampling period		Distance from APU (m)	Concentration of V
Airborne vanadium at farmhouse				
Winter	29/6/1990–29/7/1990	30 d	750 (N)	0,12 µg V/m ³
	29/7/1990–13/8/1990	14 d		0,19 µg V/m ³
Summer	27/1/1991–14/2/1991	18 d	750 (N)	ND*
	14/2/1991–28/2/1991	14 d		0,16 µg V/m ³
	28/2/1991–13/3/1991	14 d		0,15 µg V/m ³
Winter	3/6/1991–7/6/1991	4 d	750 (N)	0,25 µg V/m ³
	7/6/1991–11/6/1991	4 d		0,13 µg V/m ³
	17/6/1991–29/6/1991	12 d		0,64 µg V/m ³
Vanadium deposition rate				
Summer	1/2/1991–14/3/1991		150 (N)	3,90 mg V/m ² .day
	1/2/1991–14/3/1991		250 (N)	3,10 mg V/m ² .day
Winter	29/7/1991–12/8/1991		150 (N)	4,70 mg V/m ² .day
	29/7/1991–12/8/1991		250 (NE)	0,54 mg V/m ² .day
	29/7/1991–12/8/1991		250 (NNW)	0,54 mg V/m ² .day

* ND = not detectable

and levels in the grass in the camps closest to the APU at times reached 533 ppm. Most of the feed trials that describe V toxicity, have been carried out over fairly short periods of time (Faulkner Hudson 1964; Kubena & Phillips 1986; Platonow & Abbey 1968) and there are therefore no available data on the chronic effects of V intake in cattle. It can be concluded that because the V levels in the grass were abnormally high (in terms of accepted cattle-diet V levels) the potential for chronic vanadium toxicity definitely existed on the farm.

The grass analyses showed that the northern side of the APU had high V levels and that the V on the grass probably came from the air. The detection of V in the aerial samples (Table 3) supported the postulation of aerial contamination of the grass. The fact that the V levels in the grass samples were highest closest to the APU, indicated that the APU was the most likely source of aerial V. Since animals spend most of their lives in the outdoors, the potential exists for them to breathe in large quantities of V from the air and, furthermore, when grazing, to breathe in V present in the dust near the ground. Inhalation is recognized as one of the major routes of V toxicity in humans (Waters 1977) and cannot be ignored as a possible route of V intake in cattle. The high levels of V on the grass indicate that V was ingested with the feed, but it could not be conclusively determined for this report whether, or in what quantities, V had been inhaled.

Air sampling

The results of the air sampling are given in Table 3 and show that V was present in the air over the farm at levels which exceeded those measured at general sites in South Africa or elsewhere in the world (Environmental Options report 1993). They could serve as a basis for introducing legislation pertaining to maximum permissible emission levels for V, since such legislation currently appears to be lacking in South Africa (Environmental Options report 1993).

Clinical signs

Signs of illthrift occurred primarily in 0–12-month-old calves and comprised poor growth, emaciation, intermittent diarrhoea, sub-mandibular oedema, pot-belly, lacrimation/conjunctivitis, rhinitis, congested mucous membranes, intermittent fever, dull staring hair coats and stiff gait. Animals became progressively weaker over varying periods of time before dying of cachexia. Force-feeding failed to reverse the course of the disease. Some animals have survived for more than 2 years despite being stunted. They tend to walk stiffly and are not as active as other animals of similar age.

The above signs are consistent with those described for V toxicity (Fox 1987; Faulkner Hudson 1964; Hansard, Ammerman, Fick & Miller 1978; Hansard, Ammerman, Henry & Simpson 1982a; Hansard, Ammerman & Henry 1982b; Kubena & Phillips 1986; Platonow & Abbey 1968; Van Vleet, Boon & Ferrans 1981;

Waters 1977). Similar clinical signs can be associated with a variety of conditions, and considerable effort was made to exclude the following differential diagnoses: poor management practices, poor genetic material, poor ration quality and quantity, verminosis, paratuberculosis, arsenic poisoning, gossypol poisoning, Vitamin E/Se deficiency, bovine viral diarrhoea, *Brucella*, *Campylobacter*, *Leptospira* infections and Cu, Zn, Mo toxicities and deficiencies. It is postulated that the underlying cause of the signs was a malabsorption problem coupled to an immunosuppression phenomenon. Frank, Kristiansson & Petersson (1990), describing field cases of acute toxicity in dairy cattle, reported seeing facial paralysis and blindness, and put forward the hypothesis that there may be some effect on the central nervous system (CNS) as well. However, no clinical evidence of facial paralysis or blindness was seen in the current investigation.

At the onset of the investigation, no clinical signs were noted in the adult cows. However, during the last 12 months there was a dramatic rise in abortions, stillbirths and neonatal mortalities. Pregnant cows have never been experimentally exposed to V, so there is no evidence to show whether V can cause stillbirths in cattle. Wide (1984) reports an increased frequency of spontaneous abortions in Finnish women, and this was correlated with exposure to Al, Co, Mo and V in metal industries. As no evidence could be found that any of the common causes of abortion were playing a role on the farm, it was assumed, by a process of elimination, that V was the cause of the stillbirths. The farmer also reported that at about the time the abortions occurred, there was a drop in milk production in his herd. These findings correlated with a previous report from the Bon Accord area of South Africa (1962) of V toxicity (unpublished OVI archival data). It is postulated that V may result in a decreased contractability of muscle, resulting in the cow being unable to adequately expel the foetus, causing it to suffocate in the birth canal.

Pathology results

The gastro-intestinal tract (GIT), lymphoid and haemopoietic tissues, and respiratory system in calves and adults were primarily affected.

Group A (foetuses/perinates)

The standard tests/examinations on all five cases yielded negative results, as did the MAT for antibodies to leptospirosis.

Group B (suckling calves)

Case 6 was weak from birth and death was ascribed to an *Escherichia coli* endotoxaemia due to *E. coli* O26:K60. Case 7 developed acute bloat and died as a result of *Clostridium perfringens* type A enterotox-

aemia. Case 8 developed a watery diarrhoea and was dehydrated and emaciated at the time of death. No pathogenic bacteria could be isolated from this calf and no viral particles could be detected in the faeces. Gross and microscopic lesions in the gastro-intestinal tract (GIT), lymphoid tissues, and lungs were similar to those described for cases in group C and D (*vide infra*).

Group C (calves 3–12 months)

Case 9 was a 10-week-old calf and cases 10–16 were 6–12-month-old calves. The calves were all emaciated and pot-bellied with harsh, staring haircoats. Signs of diarrhoea were evident in cases 9 and 11. The presence or absence of diarrhoea could not be determined in the other five cases.

No pathogenic aerobic bacteria were isolated from cases 9–11, and blood and brain smears were negative. Signs of cachexia were present in all three calves. Gross lesions were evident in the GIT, lymphoid tissues and respiratory systems of these three cases. The GIT lesions included watery ruminal contents or ruminal stasis, mucoid small-intestinal contents, ileal dilatation and rectal dilatation with hard, mucoid-covered faecal balls. Lesions of the lymphoid tissues comprised moderate to severe atrophy of the spleen, lymph nodes and gut-associated lymphoid tissue. The respiratory lesions were mild and characterized by multifocal lobular areas of pulmonary atelectasis and/or emphysema.

Histopathological examination revealed significant lesions in the GIT, lymphoid tissues and lungs. The GIT was examined in six cases and lesions included a mild to moderate granulomatous and eosinophilic enteritis and typhlocolitis (six cases), moderate villous atrophy characterized by fusion and stunting of villi and dilatation of the lacteals (four cases) (Fig. 4), and mild parakeratosis of the ruminal and omasal mucosa (one case).

The spleen and the prescapular and prefemoral lymph nodes were examined in five cases. There was moderate to severe splenic and lymph nodal atrophy, characterized by absence or paucity of lymphoid follicles and hypocellularity of the medullary and red-pulp cords (Fig. 5). In the cases where lymphoid follicles were evident, they appeared inactive, with depleted centres, or occasionally showed evidence of active necrosis. The thymus revealed moderate to advanced signs of regression.

Sections of the lungs were examined in seven cases. Moderate bronchiolar ectasia with flattening and, occasionally, necrosis or desquamation of the bronchiolar epithelium, and associated alveolar and septal emphysema were present in three cases (Fig. 6 and 7). Mild, multifocal areas of atelectasis, interstitial pneumonia and alveolar emphysema were evident in two cases, and mild alveolar oedema in two cases.

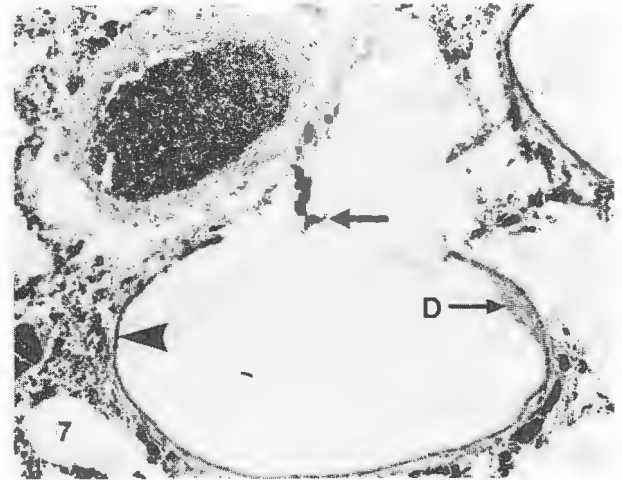
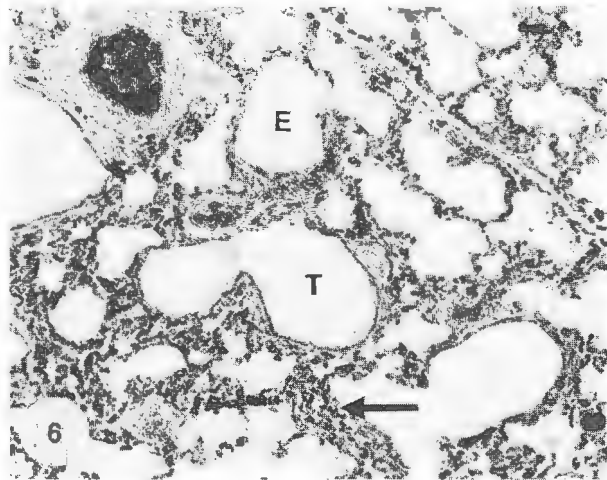
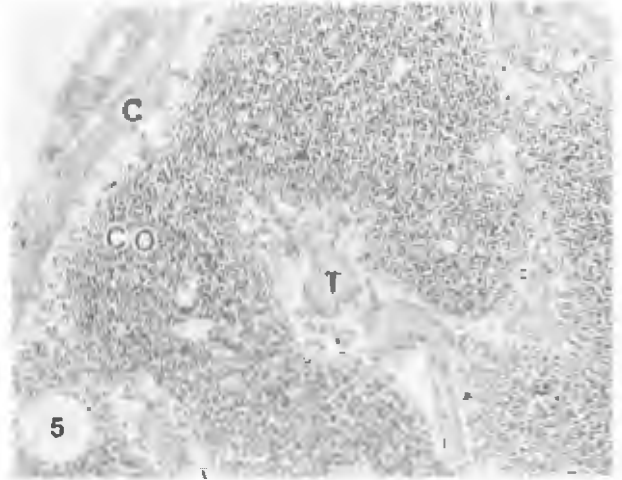


FIG. 4 Case 17. Small intestine. Villous atrophy characterized by short, blunted villi and lacteal dilatation (L). Note also round-cell infiltration in mucosa, irregular proliferating basal glands (G) and oedema of the submucosa (S). HE x 90

FIG. 5 Case 8. Lymph node. Atrophy of lymph node evidenced by prominence of the capsule (C) and trabeculae (T), cortical (CO) hypocellularity and absence of lymphoid follicles. HE x 90

FIG. 6 Case 8. Lung. Ectasia of the terminal bronchioles (T) with multifocal alveolar atelectasis (arrow) and emphysema (E). HE x 90

FIG. 7 Case 8. Lung. Ectasia of a terminal bronchiole. Note flattened epithelium (arrowhead), desquamated cell debris (D) and rupture of alveolar walls (arrow). HE x 200

Group D (adult cows)

The clinical symptoms in the two cows necropsied, included emaciation, soft to watery faeces, tachycardia or cardiac arrhythmia, and anasarca of the neck and brisket in case 16. Case 16 (4 years old) had experienced a dystocia a few weeks prior to presentation for necropsy. Case 17 calved normally in May 1989 and then returned to oestrus several times. She produced a stillborn calf in July 1990 and again returned to oestrus several times before being necropsied in September 1991.

Blood and brain smears and faecal flotation tests were negative in both cows. No pathogenic aerobic bacteria were isolated from case 17. *Actinomyces*

pyogenes was isolated from the pericardial pus from case 16, which showed lesions of a traumatic reticulo-pericarditis. Evidence of a previous dystocia was present in case 16 and moderate lesions of a chronic hepatic fascioliasis, in both cows. Gross lesions, not associated with the above conditions, involved the gastro-intestinal tract, lymphoid tissues and lungs. In both cows the ruminal ingesta contained moderate amounts of whole-maize kernels, whilst copious amounts of whole-maize kernels were present in the abomasum. In case 16, the spleen and superficial lymph nodes were moderately atrophic, and in case 17, the lungs revealed mild multifocal areas of septal and alveolar emphysema. In case 17, pneumonic lesions attributable to the traumatic reticulo-pericarditis

TABLE 4 Results of organ analyses for V (ppm WM)

Animal group	Animal necropsy case no.	Age	Tissue levels				Date
			Liver	Kidney	Bone	Ruminal fluid	
A	1	Foetus (6 months)	0*	0	ND**	ND	June 1991
	4	Stillborn foetus	0	0	0	ND	November 1991
	5	Neonate (1-2 h)	0	0	ND	ND	December 1991
B	8	2 weeks	0	0	ND	4	June 1991
C	9	10 weeks	0,3	0	1,3	4	October 1990
	10	11 months	0,4	2,1	2,8	1,3	May 1990
	11	11 months	0,1	0	2,4	2,0	May 1990
	13	6-12 months	0,7	0,2	ND	1,0	January 1991
	14	6-12 months	2,0	0	ND	0	January 1991
	15	6-12 months	ND	ND	ND	2,6	January 1991
D	16	4 years	0	0	ND	23,0	September 1991
	17	7 years	0	0	ND	27,0	September 1991

* 0 = negative (limit of detection = 0,05 ppm)

** ND = not determined

obscured any other pathological lesions which may have been present. Histopathological examination revealed GIT, lymphoid-tissue and respiratory lesions similar to those described for cases in group C.

Pathology discussion

The pathological lesions indicated that exposure to toxic levels of V over a prolonged period, had chronic, wide-ranging effects in older calves and adult cattle, evidenced by cachexia, GIT, respiratory and reproductive disorders, and atrophy of the lymphoid and haemopoietic tissues.

The gross GIT lesions in the adult and sub-adult cattle and a 2-week-old calf are suggestive of impaired ruminal and intestinal motility. Stasis and impairment of GIT motility would ultimately lead to inadequate nutrient uptake and absorption from the GIT, with subsequent malabsorption and cachexia. Platonow & Abbey (1968) noted adherence of food material to the mucosa of the entire GIT, which could also imply impairment of GIT motility. The detection of high levels of V in the ruminal fluid, compared with non-detectable or low levels in other organs (Table 4), suggests that a high proportion of the ingested V is retained within the GIT lumen where it could adversely affect the absorption of nutrients. These findings are supported by those of Frank *et al.* 1990 who recorded similar high levels of V in the rumen, reticulum and abomasum. Vanadium is known to interfere with the active transport of Na⁺ and K⁺ across membranes, and high levels within the GIT would conceivably in-

terfere with the motility of the GIT and uptake and absorption of nutrients, with consequent malabsorption and eventual cachexia (Bracken & Sharma 1985; Kubena & Phillips 1986; Macara, Kustin & Cantly 1980; Ramasarma, Mackellar & Crane 1981).

Recent *in vitro* work has shown that glucose absorption rates from the jejunum of rats decrease to a point of no absorption of glucose within 60 min after exposure to a Krebs' solution containing V (J. Keegan 1993, unpublished data). These findings add further support to the postulation that V can lead to malabsorption. The histopathological findings of villous atrophy and a granulomatous enteritis are consistent with the theory of a malabsorption syndrome. Similar intestinal lesions have been reported in pigs fed high levels of V (Van Vleet *et al.* 1981).

Bone-marrow atrophy was present in two calves and splenic and lymph-nodal atrophy in six of the nine sub-adult and adult cattle necropsied and the 2-week-old suckling calf. These lesions may be a reflection of the cachexic state of the animals, but on histopathological examination there were indications of immune hypoactivity as evidenced by lack or paucity of lymphoid follicles, depletion of germinal centres and hypocellularity of the medullary and splenic cords. The lesions of hypoactivity correlate with the finding of suppressed lymphocyte transformation responses in the circulating lymphocytes (see later). The finding of an *E. coli* endotoxaemia and *C. perfringens* type A enterotoxaemia in two of the suckling calves may be indicative of a bacterial infection secondary to

immunosuppression as a result of the ingestion and uptake of V. The presence of V in the ruminal fluid of the 2-week-old calf (Table 4) indicates that V was indeed ingested shortly after birth, and the inability to detect V in the younger calves may be a reflection of the limitations of the detection methods employed.

The respiratory lesions were mild in nature and characterized by multifocal areas of oedema, interstitial pneumonia and atelectasis, and septal and alveolar emphysema. Of interest at the microscopic level, was the presence of multifocal bronchiolar ectasia with flattening and metaplasia of the epithelium in three cases. According to Symanski (1939, cited by Waters 1977), bronchiectasis, following prolonged exposure to V, can occur in humans. Various respiratory symptoms and lesions such as conjunctivitis, rhinitis, pharyngitis, bronchiectasis, bronchospasm, bronchitis, emphysema and a chronic, productive cough have been recorded in humans exposed to V dust in industrial concerns. These effects are ascribed to the extremely irritant effect of V dust (usually the pentoxides) on the mucous membranes of the eyes, nose, throat and respiratory tract (Waters 1977). The presence of respiratory lesions, in particular bronchiolar ectasia and emphysema, in the animals in this series, implies V uptake via the respiratory passages.

No significant pathology was evident in the foetuses and perinatal calf necropsied. Aerobic bacteria, leptospirae, *Brucella*, *Chlamydia* and *Campylobacter* spp. were eliminated as possible causes. No V could be found in the abomasal fluid, livers or kidneys of the three cases analysed. This suggested that there was no transplacental absorption of V but, on the other hand, it might be a reflection of the limitations of the detection methods employed. Uterine fluids or specimens of the uterine wall were not analysed for their V content. As high levels of V were present in the tissues of the two adult cows necropsied (Table 4), V may indeed have been present in the uterine lumen or tissue.

Vanadium analyses

At the time of the second farm visit, the V levels in the faeces of the heifers, and the dry and lactating cows ranged from 2,1–8,8 ppm with a mean of 4,5 ppm \pm 2 (n = 19). There were no significant differences between the groups. The V levels obtained in the organs during the period of investigation are shown in Table 4.

Normal-tissue V levels for cattle are reported to be in ppb (ng/g) and the level for liver is given as 0,006–0,007 ppm (WM) (Puls 1988). The levels of V in bone and liver were consistent with those described for V toxicity, while the levels in the kidney were not (Faulkner Hudson 1964; Hansard *et al.* 1978; Hansard *et al.* 1982a; Hansard *et al.* 1982b; Nechay,

Nanninga, Nechay, Post, Grantham, Macara, Kubena, Phillips & Nielsen 1986, Puls 1988). No reported reference values for rumen content could be found, but the fact that V was consistently present in the rumen of the dead, and the faeces of the living, animals, supported a postulation of V exposure and uptake via the GIT.

Clinical pathology

The main thrust of the clinical pathology was to try and establish underlying causes for the symptomatology and pathological findings. The areas concentrated on were:

- evidence that may indicate organ damage;
- evidence to support a malabsorption syndrome; and
- evidence to support an immunosuppressive effect.

Evidence to support organ damage

The enzymes used to assess liver damage were GGT and AST, and the activities of neither were abnormally high for any of the groups studied. There was therefore little evidence of acute liver damage in the herd.

The enzyme used to test for muscle damage was CK. Raised CK activity was seen in the 0–3 (CK: \bar{x} = 74 \pm 15 u/l excluding one value = 2895; range: 47–2895 u/l) as well as the 12–15-month-old group (CK: \bar{x} = 68 \pm 14 u/l; range: 52–83 u/l). This would support evidence of muscle breakdown as would occur with a cachexic state.

Indicators used to assess kidney function were BUN and Creat. A decrease in BUN (< 3,6 mmol/l) was seen in individuals in all the age groups. The prevalence of low BUN levels increased with increasing age. All animals 12–19 months old or which had aborted, showed low BUN levels. This finding supports the postulation made by others that V causes an increased glomerular filtration rate (Faulkner Hudson 1964; Heinz, Rubinson & Grantham 1982; Patterson, Hansard, Ammerman, Henry, Zech & Fisher 1986). The Creat levels increased with increasing age and all the animals in the 12–19-months-old group (Creat: \bar{x} = 150 \pm 14 μ mol/l; range: 135–194 μ mol/l) had abnormally high Creat levels. High Creat levels, together with high BUN levels, are usually an indication of glomerular damage. However, non-Creat chromogens may cause false high values and the most significant of these are ketones (Duncan & Prasse 1986). Animals that are energy deficient, such as would occur with malabsorption, could therefore have false high Creat levels. Since the BUN levels were not raised, the latter explanation for high Creat is more feasible than that of glomerular damage and would give added evidence for malabsorption.

Evidence for malabsorption

The parameters used to assess malabsorption were TSP and its various fractions. The TSP levels were low in all the 0–3-months-old calves (TSP: \bar{x} = 55 ± 9,6 g/l; range: 43–68 g/l) and in the majority of animals in the 4–7-months-old group (TSP: \bar{x} = 63,8 ± 8 g/l; range: 50,5–70,5 g/l); 8–11-months-old group (TSP: \bar{x} = 67 ± 5 g/l; range: 59–71,4 g/l), 12–15-months-old group (TSP: \bar{x} = 68 ± 4 g/l; range: 65–73 g/l) and 16–19-months-old group (TSP: \bar{x} = 69 ± 3 g/l; range: 65–73 g/l) groups. The adult cattle had normal TSP (70–78 g/l) levels. The albumin:globulin (A/G) ratio in the 0–3-months-old group (A/G: \bar{x} = 1 ± 0,4; range: 0,62–1,86) and 8–11-month-old group (A/G: \bar{x} = 1,1 ± 0,2; range: 0,84–1,29) was close to normal (0,9), indicating that the low TSP levels were probably the result of low levels of both alb and glob. This would occur with protein-losing enteropathies, malabsorption, malnutrition or chronic liver disease, together with immunodeficiency and/or failure of passive colostral transfer (Duncan & Prasse 1986). Hence the low TSP values in animals < 12 months old, appeared to confirm a diagnosis of malabsorption as supported by the pathology. It is interesting to note that it was only the calves that showed an overall decrease in TSP, and it was also only these animals that showed severe clinical signs.

Animals 4–7 months old and > 11 months old had low A/G ratios. In the 4–7-months-old group (A/G: \bar{x} = 0,87 ± 0,2; range: 0,62–1,86), the 12–15-months-old group (A/G: \bar{x} = 0,72 ± 0,09; range: 0,64–0,84) and the 16–19-months-old group (A/G: \bar{x} = 0,86 ± 0,12; range: 0,73–1,09), this decrease was primarily due to a decrease in alb, suggesting a protein-losing enteropathy in this group, but not immunosuppression, as the glob fraction was normal. The adult animals had low A/G ratios (A/G: \bar{x} = 0,75 ± 0,15; range: 0,42–0,99) but these appeared to be due to an increase in glob levels rather than a decrease in alb levels, suggesting an over-stimulation of the immune system in adult cattle.

Glob fractions were also examined for each age group. The 0–3-month-old group showed a deficiency in gamma-globulin (γ -glob) levels (γ -glob: \bar{x} = 9,2 ± 6 g/l; range: 2,7–21,6 g/l), which supported the findings of malabsorption and immunodeficiency. The 4–7-month-old and adult cattle groups showed a low γ -glob fraction (< 16 g/l) and a high β -glob fraction (> 9 g/l). The β -glob fraction represents transferrin, β -lipoprotein, complement-3 and some immunoglobulins (Duncan & Prasse 1986). It is reported that V competes with iron for transferrin (Nechay 1984; Nechay *et al.* 1986; Patterson *et al.* 1986; Ramasarma *et al.* 1981), and it can therefore be assumed that with V toxicity there would be an extra demand for transferrin, causing more of it to be produced, and therefore higher serum levels. The high β -globs

could therefore be the result of increased transferrin which would be consistent with a diagnosis of V toxicity. Similar findings were seen in the 12–15- and 16–19-month-old groups with the additional finding of a low α -glob fraction (< 9 g/l) which, together with the low γ -glob fraction, strengthens the argument for immunosuppression.

Evidence for immunosuppression

Cellular immunity was examined by means of differential WCC. The WCC were above normal (> 10 × 10³ cells/l) in all the age groups studied, with the exception of the 0–3-month-old group (WCC: \bar{x} = 9,5 ± 3 × 10³ cells/l), implying that some form of chronic immune stimulation was taking place.

From 4–12 months old there is evidence of a lymphocytosis which becomes less common in the adult cattle. High lymphocyte counts are usually a reflection of white-cell production and function and are associated with chronic infections (Duncan & Prasse 1986). Hence there appears to be evidence of a chronic stimulation of the immune system in the majority of the animals in the herd. Persistent exposure to V dust could act as a chronic irritant with subsequent chronic inflammation. An increase in immature neutrophils was seen in the 0–3 (N_i: \bar{x} = 4,2%) and 4–7-months-old groups (N_i: \bar{x} = 4%). These findings indicate that there is an increase in demand for neutrophils which is usually associated with an inflammatory condition (Duncan & Prasse 1986) such as rhinitis, conjunctivitis and enteritis.

A monocytosis (> 7%) was seen in all the age groups < 15 months old and > 19 months old, but was most obvious in the 0–3-months-old group (\bar{x} = 11,7%). Causes of monocytosis include protozoal infections, suppuration, haemolysis and immune injury (Duncan & Prasse 1986). A monocytosis therefore fits in with evidence to support some form of immune injury.

The absence of eosinophils in all the groups gave added evidence that helminths were not playing a significant role on the farm.

A puzzling question was why there were so many white cells if the immunity of the animals was compromised? In order to solve this problem, *in vitro* tests were carried out to establish how active the lymphocytes were (Kristensen *et al.* 1982). Lymphocytes from 20 cattle were examined: ten calves < 7 months old and ten cows > 19 months old. Not one of the adult cows had normal lymphoblast-transformation responses to plant lectins and only four of the calves showed a response, but this was suppressed. Hence, although the lymphocytes were present, they were not active, implying that V was possibly preventing them from responding normally.

A further finding supporting V toxicity, was the presence of Heinz bodies in the erythrocytes of cattle

under the age of 7 months, while it was absent in the adults. Heinz bodies usually indicate an impairment of the glucose-6-phosphate dehydrogenase pathway or a depletion of glutathione (Duncan & Prasse 1986). Glutathione is thought to reduce vanadate to vanadyl and thus to render it less toxic (Faulkner Hudson 1964; Hansen, Aaseth & Alexander 1982; Heinz *et al.* 1982). Continual exposure to V could therefore lead to a deficiency in glutathione and result in denaturation of haemoglobin, leading to Heinz bodies.

Although the clinical pathology results alone cannot be used to make a diagnosis, it becomes clear from them that they support evidence for malabsorption and immunosuppression as well as V toxicity.

CONCLUSION

Owing to the vague nature of the clinical signs of V toxicity and the lack of definitive tools for diagnosing this disease, it is very difficult to prove conclusively that an animal has died from V toxicity or is suffering from it. What this investigation did, was to accumulate a library of circumstantial evidence which led us to believe that the animals on the affected farm were suffering from the effects of excessive levels of V. Where it was thought that another known disease might result in a similar picture, steps were taken to ensure that no such disease was playing a role. We therefore feel confident that chronic V toxicity was the underlying cause of the illthrift problem experienced on the farm.

Because little has been published on the effects of V air pollution on animals, there are few precedents upon which to base our results. We have accumulated circumstantial evidence suggesting that air pollution was taking place on the farm and that the source of the air pollution was the adjacent APU.

On the basis of all our findings, we conclude that there was sufficient evidence to make a diagnosis of chronic V toxicity which was caused by air pollution from the nearby APU.

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