

Contact dermatitis in dairy cattle caused by calcium cyanamide

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Nine of 250 cows on a dairy farm initially developed severe dermatitis on parts of their bodies that touched the floor, and it then spread over their entire body. The cause was suspected to be calcium cyanamide, which had been added to the material spread on the floor to prevent environmental mastitis. Experimental exposure of the skin of a cow to calcium cyanamide induced the same type of contact dermatitis, and histopathological investigations showed that it caused irritant and allergic reactions. To identify the cause of the dermatitis, a patch test with calcium cyanamide and its breakdown products, cyanamide, urea and ammonium bicarbonate, was carried out on four cows. Three of them had a positive reaction to calcium cyanamide and cyanamide; delayed and amplified reactions suggesting an allergic response were observed.

CALCIUM cyanamide is one of the longest-used nitrogenous fertilisers. Its breakdown products have two different functions: urea, ammonia and nitrate are used in fertilisers, and quicklime (calcium oxide) and cyanamide are used as agrochemicals. It has been reported that adding calcium cyanamide to a mixture of manure and sawdust is a very effective method of reducing levels of *Escherichia coli* (Minato and others 2001). In human beings, calcium cyanamide fertiliser has been shown to cause headache, vertigo, vomiting, respiratory disturbances, dermatitis and even death (Hauschild 1953), and two of its breakdown products have been reported to induce dermatological conditions: quicklime causes an irritant contact dermatitis (Winder and Carmody 2002) and cyanamide causes an allergic contact dermatitis (Goday Bujan and others 1994, Kawana 1997, Trebol and others 2005). Cyanamide is a drug used to treat chronic alcoholism because it interferes with the hepatic biotransformation of ethanol by inhibiting aldehyde dehydrogenase (Peachey and others 1981). To the authors' knowledge there has been only one report of toxicity due to calcium cyanamide in animals (Iizuka 1997) and none on the toxicity of cyanamide, probably because these chemicals are not found in nature and there is little chance that animals will come into contact with them.

This paper describes some dairy cattle that developed severe dermatitis that was suspected to have been caused by calcium cyanamide because the chemical had been added to the material spread on the floor of the cattle shed to prevent environmental mastitis. To investigate whether this was the case, a cow was exposed experimentally to calcium cyanamide. Having obtained a positive result, a patch test was conducted to determine which degradation product of calcium cyanamide had caused the dermatitis and to clarify its clinical characteristics. Patch tests are not widely used in veterinary medicine. However, patch tests are indispensable in the definitive diagnosis of contact dermatitis in human beings, and the method was therefore adapted for cattle.

MATERIALS AND METHODS

Crude calcium cyanamide nitrogenous fertiliser contains refined calcium cyanamide and many other substances, such as quicklime, carbon and various cyanide compounds. Its toxicity appears to result from the total effect of all the harmful components in the fertiliser. In this study, the fertiliser rather than refined calcium cyanamide was used, and 'calcium cyanamide' refers to the fertiliser.

Clinical history

Between February and December 2004, nine cows in a dairy herd of 250 developed severe dermatitis characterised by alopecia, erythema and crust formation with pruritus. Six of the

nine cows developed clinical signs from between 40 days before calving to 40 days after calving, and had calved an average of 3.2 times. The dermatitis initially developed on the teats, mammary gland, lower abdomen and dewlap – the regions that could touch the floor – and it spread to the head, neck and face (Fig 1). In severe cases, cows also became weak, anorexic and pyrexia, and suffered from diarrhoea and nasal bleeding. One of the nine cows died and another four were euthanased.

Sawdust had been spread on the floors of the loose-housing cattle barns on the farm, and mastitis had occurred frequently, caused by coliform bacteria, especially *Klebsiella* species. Calcium cyanamide (COOP Chemical) was then added to the sawdust at 5 kg calcium cyanamide per m³ sawdust, and the number of coliform bacteria in the sawdust decreased, at most, from 1.3×10^7 /g to 7×10^4 /g, and the incidence of mastitis was reduced. However, when the concentration of calcium cyanamide was increased to 10 kg per m³ sawdust, dermatitis developed. Symptomatic treatments, including the systemic administration of antimicrobials and the local application of skin moisturisers, proved ineffective. The external iliac lymph node of a cow that died was collected, fixed in 10 per cent neutral phosphate-buffered formalin solution, and paraffin sections were prepared and stained with haematoxylin and eosin, periodic acid-Schiff, Gram and Ziehl-Neelsen stains.

Experimental exposure

One five-year-old Holstein-Friesian cow on the farm developed dermatitis on December 10, 2004. It calved on January 16, 2005 and was hospitalised on February 17. The cow's contact with calcium cyanamide was eliminated until the signs of dermatitis had disappeared and its general clinical signs and blood tests were normal. On July 31, it was housed in a pen for 10 days, with sawdust bedding material containing 2.5 kg calcium cyanamide per m³, and it was provided with a constant supply of water and feed. Haematological and serum biochemical assays were performed each day, including haemoglobin and haematocrit, red blood cell count, white blood cell count, the serum activities of aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase, lactate dehydrogenase and creatine kinase, and the serum concentrations of total cholesterol, triglycerides, bilirubin, urea nitrogen, creatinine, glucose and total protein. Biopsies of skin from the mammary gland were taken each day and the tissues were examined histopathologically.

Patch test

A patch test was conducted on four Holstein-Friesian cows that had had dermatitis on the farm in June 2006. Patch test units (Finn Chamber, standard 7.5 mm filter paper disc; Epitest) were applied with adhesive (Aronalpa; Toagosei) to the rump and the mammary gland after they had been shaved. The test substances were as follows: calcium cyana-

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FIG 1: (a) Severe dermatitis in a Holstein-Friesian cow characterised by alopecia, erythema and crust formation on the femoral region, abdomen and mammary gland. **(b)** Thickened and lichenified skin with alopecia around the neck of a cow that died

mide (COOP Chemical); cyanamide (Sigma), which is produced by the hydrolysis of refined calcium cyanamide; urea (Wako), which is a degradation product of cyanamide; and ammonium bicarbonate (Wako), which is a degradation product of urea. They were dissolved in distilled water at concentrations of 0.1, 0.5, 1 and 5 per cent, and 20 µl of the prepared solutions was titrated on to filter paper discs. The patch test units were removed 48 hours after they had been applied and the results were measured one, 72 and 96 hours later. They were measured according to International Contact Dermatitis Research Group standards, widely accepted in human medicine (Wilkinson and others 1970).

The experiments were approved by the Animal Care and Use Committee at Azabu University School of Veterinary Medicine, Japan.

RESULTS

Histopathology on the dead cow

The external iliac lymph node from the dead cow showed signs of granulomatous inflammation (Fig 2). The node was

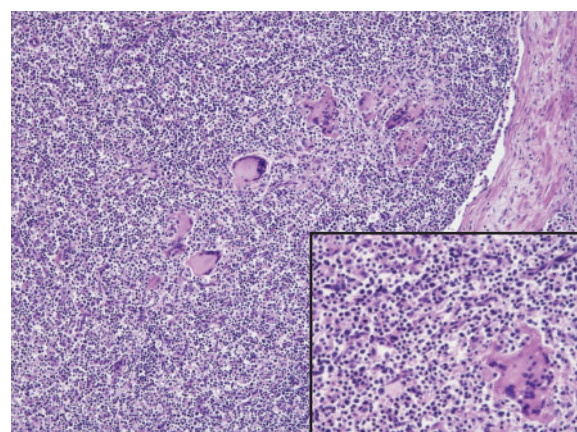


FIG 2: Histological section of the external iliac lymph node of the cow that died, showing lymphocytes, macrophages, plasma cells and multinucleate giant cells. Haematoxylin and eosin. x 70. Inset: Cellular infiltrates. Haematoxylin and eosin. x 280

heavily infiltrated by lymphocytes, macrophages and plasma cells, and several multinucleate giant cells were observed. No causative bacteria were detected with any of the stains used.

Experimental exposure

Clinical findings During the test period, the activity, appetite, body temperature, pulse rate and respiratory rate of the cow remained normal. Severe pruritus was observed on day 1, evidenced by the cow frequently licking its forelimbs and mammary gland. The first lesions observed were erythema and erosions around the teats and mammary gland on the morning of day 1, and erythema was observed on the pastern on the afternoon of day 1. On day 2, erythema and crusts were observed on the entire lower abdomen, and the lesions became worse until day 4, after which the region gradually became thickened and keratinised by day 10.

Blood tests The results of the complete blood counts before the trial and on day 10 are shown in Table 1. The haemoglobin concentrations and haematocrits were slightly low before the trial and on day 10. The number of white blood cells decreased from $7.2 \times 10^9/l$ to $4.3 \times 10^9/l$, with a reduction in neutrophils but an increase in eosinophils. The blood biochemical parameters were within their reference ranges and not markedly different after the calcium cyanamide challenge.

Histopathology On day 1, necrosis and haemorrhage were observed in the epidermis, and neutrophils and eosinophils had infiltrated under the necrotic layer (Fig 3). On day 2, spongiosis and advanced spongiotic microvesicles were observed in the epidermis where there was no necrosis, and cellular infiltration, mainly of lymphocytes and eosinophils, was observed in the upper layer of the dermis. On day 4 there was less spongiosis, and on day 10 irregular epidermal hyperplasia (acanthosis) and hyperkeratosis were observed.

Patch test

One of the four cows (cow 1) had a negative patch test reaction and the three other cows (cows 2, 3 and 4) reacted positively to calcium cyanamide and cyanamide (Table 2). These results were obtained only from the patch test units applied to the rump (Fig 4a) because the units applied to the mammary glands had fallen off. Cow 2 had a positive reaction not only to calcium cyanamide and cyanamide but also to urea, and 1 per cent and 5 per cent ammonium bicarbonate. A delayed reaction to 5 per cent cyanamide was observed in

TABLE 1: Complete blood counts of the cows exposed experimentally to calcium cyanamide

	Before exposure	After 10 days	Reference range*
Red blood cells ($\times 10^{12}/l$)	6.08	6.03	5.0-10.0
Haemoglobin (g/l)	81	77	80-150
Haematocrit (l/l)	0.25	0.245	0.24-0.46
White blood cells ($\times 10^9/l$)	7.20	4.30	4.0-12.0
Lymphocytes ($\times 10^9/l$)	2.79	2.44	2.5-7.5
Neutrophils ($\times 10^9/l$)	4.01	1.10	0.60-4.12
Eosinophils ($\times 10^9/l$)	0.338	0.679	0.2-4.0

* Meyer and Harvey (1998)

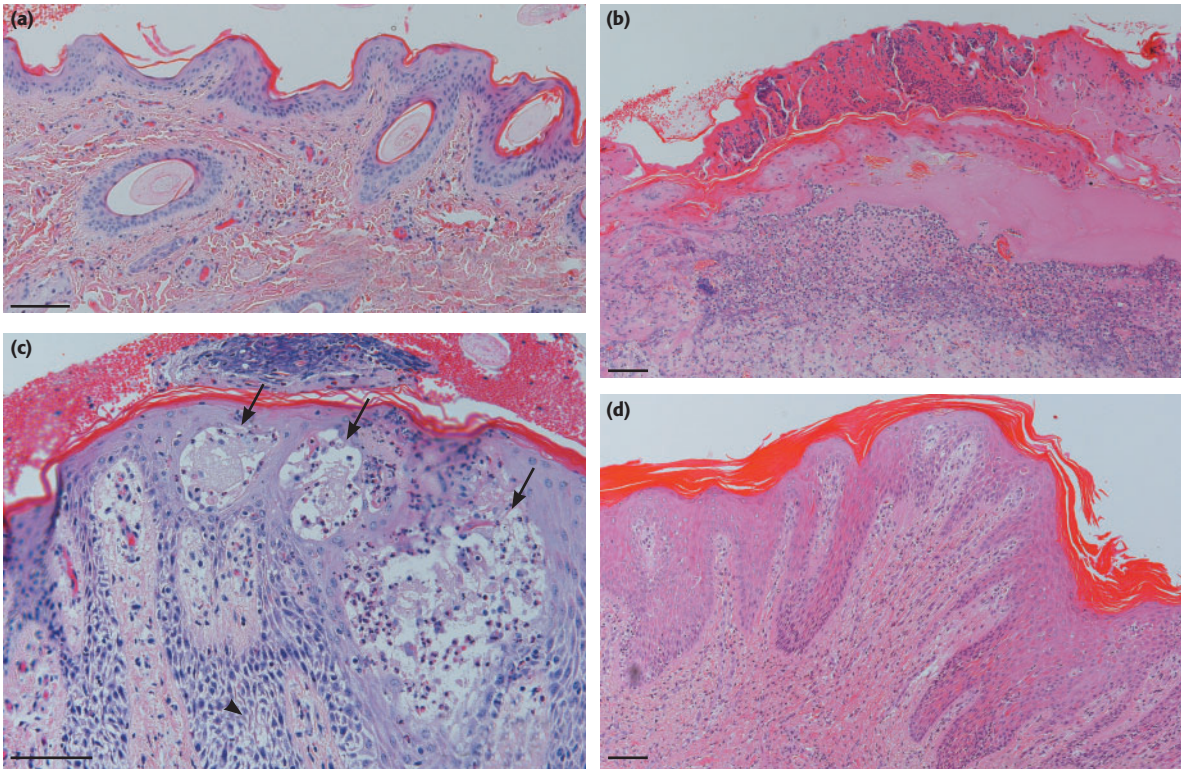


FIG 3: Histological sections of mammary gland skin from a cow exposed experimentally to calcium cyanamide. (a) Before exposure. (b) Day 1 Necrosis and haemorrhage were observed in the epidermis; neutrophils and eosinophils had infiltrated below the epidermis. (c) Day 2 Spongiosis (arrowhead) and advanced spongiotic microvesicles (arrows) were observed in the epidermis, and cellular infiltration, mainly of lymphocytes and eosinophils, was observed in the upper layer of the dermis. (d) Day 10 Spongiosis was less evident and irregular epidermal hyperplasia (acanthosis) and hyperkeratosis were observed. Haematoxylin and eosin. Bars=100 µm

cow 3, with no reaction after 48 hours and a weak reaction after 72 hours. In cow 4 there was a weak reaction to 5 per cent calcium cyanamide and 1 per cent and 5 per cent cyanamide after 48 hours, and a strong reaction after 72 hours (Fig 4b). In cows 2 and 3, the positive reactions to 5 per cent cyanamide were stronger than to 5 per cent calcium cyanamide after 48, 72 and 96 hours. In contrast, in cow 4, the positive reactions to calcium cyanamide at 0.5, 1 and 5 per cent were weaker than to cyanamide at the same concentrations after 72 and 96 hours. Cow 4 reacted positively to 0.5 per cent cyanamide, whereas cows 2 and 3 had negative reactions. The four cattle all reacted negatively to distilled water, which was used as the solvent to dissolve the chemicals. Another two cattle that had been exposed to calcium cyanamide on the farm, but had not developed dermatitis, reacted negatively to all the chemicals.

DISCUSSION

Poisoning by calcium cyanamide has been reported in cows that licked calcium cyanamide directly from fertilised grass-

land or ate forage to which calcium cyanamide had adhered (Iizuka 1997). Its toxic effects were roughly classified as local, in the form of lesions on the oral mucous membranes caused by its strong alkalinity, and systemic, as a result of cyanide poisoning. However, the pathological signs of calcium cyanamide poisoning differ from those of cyanide poisoning (Murakami 1959), and the mechanism of toxicity of calcium cyanamide is still unclear. In the present study, some of the cows became weak and anorexic, in addition to developing dermatitis. It is possible that calcium cyanamide may be absorbed through the skin, but it is likely that the cows showing systemic signs had ingested calcium cyanamide present in the sawdust. The cows that developed dermatitis licked the affected regions vigorously and must have ingested some of the sawdust that adhered to their skin. However, it is not clear why the dermatitis spread to the entire body, that is, whether the calcium cyanamide that came directly into contact with parts of the cows' bodies caused the dermatitis to spread, or whether the spread was caused by the ingestion of calcium cyanamide.

To avoid causing systemic signs, the concentration of calcium cyanamide to which one cow was exposed experi-

TABLE 2: Macroscopic scores of the lesions observed on the four cows subjected to a patch test																	
Cow	Time (hours)	Calcium cyanamide (%)				Cyanamide (%)				Urea (%)				Ammonium bicarbonate (%)			
		0.1	0.5	1	5	0.1	0.5	1	5	0.1	0.5	1	5	0.1	0.5	1	5
1	48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	72	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	96	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	48	–	–	?+	++	–	–	–	+	–	–	–	+	–	–	–	+
	72	–	–	–	+	–	–	–	?+	–	–	–	–	–	–	+	–
	96	–	–	–	?+	–	–	–	–	–	–	–	–	–	–	–	–
3	48	–	–	–	++	–	–	–	–	–	–	–	–	–	–	–	–
	72	–	–	–	++	–	–	–	+	–	–	–	–	–	–	–	–
	96	–	–	?+	++	–	–	?+	+	–	–	–	–	–	–	–	–
4	48	–	+	+	+	–	+	+	+	–	–	–	–	–	–	–	NT
	72	–	?+	+	++	–	+	++	++	–	–	–	–	–	–	–	NT
	96	–	–	+	+	–	+	+	++	–	–	–	–	–	–	–	NT

++ Strong oedematous or vesicular reaction, + Weak non-vesicular reaction, ?+ Doubtful reaction, – No reaction, NT Not tested

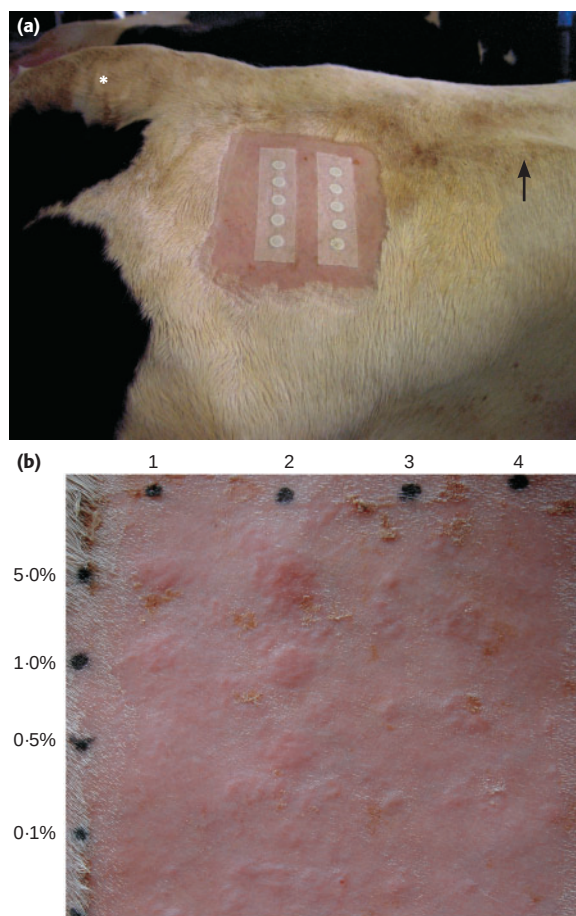


FIG 4: (a) Patch test units applied to the right rump of cow 4. Tail (*) and hook bone (arrow) are indicated. (b) Strong positive reactions observed after 72 hours. 1 Calcium cyanamide, 2 Cyanamide, 3 Urea, 4 Ammonium bicarbonate

mentally was 0.25 per cent. The cow had had a history of dermatitis at the farm and it was thought that a concentration less than 0.5 per cent was appropriate for a preliminary examination. The cow developed the same sort of dermatitis, with pruritus, but no systemic signs. In the early stages of its exposure, histopathological examinations revealed necrosis and neutrophil infiltration, suggesting irritant contact dermatitis, and spongiosis and lymphocyte infiltration, suggesting the acute phase of allergic contact dermatitis. In the later stages of its exposure, irregular epidermal hyperplasia was observed, suggesting the chronic phase of contact dermatitis. These findings, and the fact that only a few of the cows in the herd had been affected strongly, suggested that an allergic reaction was involved. A patch test was therefore applied to four of the cows to try to identify the allergen.

The recommended sites for patch testing in human beings are the area behind the ear or the outer aspect of the arm immediately above the elbow (Rietschel and Fowler 2001). In animals, body size and skin colour vary greatly even in the same species. In the present study, the patch test units were attached to the skin of the rump, because of its width, stability and inaccessibility by the cow, and to the posterior mammary gland, as described by Holzhauer and others (2004). The units attached to the mammary gland fell off, but the units attached to the rump remained in place and up to 20 units were applied at a time.

The patches were left in place for the periods used in human medicine (Wilkinson and others 1970). One cow had a delayed reaction and another cow had an amplified reaction between 48 and 72 hours after the start of the test, suggesting that these periods were satisfactory for patch tests on cattle.

Three of the four cattle reacted positively to 5 per cent cyanamide, but only one reacted positively to 1 per cent cyan-

amide. In human beings, cyanamide at 1 per cent is an appropriate concentration for patch testing patients with contact dermatitis (Trebol and others 2005), and even at 0.1 per cent a positive reaction has been elicited (Goday Bujan and others 1994). However, the three cattle that tested positive for 5 per cent cyanamide did not react positively to 0.1 per cent cyanamide. Two of the three cattle that reacted positively showed stronger reactions to a 5 per cent concentration of calcium cyanamide, which contained a lower total cyanamide concentration, than to cyanamide alone. It is possible that because the calcium cyanamide contained other materials (including quicklime) in addition to refined calcium cyanamide, these other materials functioned synergistically with cyanamide and enhanced the positive reaction.

The histopathological findings in the lymph node of the cow that died did not correspond to the findings in the skin of the cow exposed experimentally; for example, granulomatous inflammation was observed in the lymph node, but not in the skin. There were many differences between the original and experimental exposures, but the low concentration and short period of experimental exposure to calcium cyanamide could explain the difference in findings between the lymph node and the skin. The granulomatous inflammation was presumably caused by the transition from allergic degeneration to the chronic phase.

Kamo and others (2003) reported that cyanamide is biosynthesised in the plant hairy vetch (*Vicia villosa*) and suggested that cyanamide was the toxic compound responsible for hairy vetch poisoning in cattle (Panciera and others 1992). Hairy vetch poisoning in cattle is characterised clinically by dermatitis, pyrexia, diarrhoea and haemorrhage, and the disease has a low morbidity and a high mortality. Histopathological analysis of tissues from cattle poisoned by this plant showed granulomatous inflammation and infiltrations of lymphocytes and multinucleate giant cells, suggesting an allergic response. There are many similarities in the clinical signs and histopathological findings between hairy vetch poisoning and calcium cyanamide dermatitis. The lymph node from the cow that died showed granulomatous inflammation similar to that associated with hairy vetch poisoning. These findings strongly suggest that cyanamide is the toxic agent in both syndromes.

Calcium cyanamide is completely broken down in soil and utilised by growing plants, and is said to be an environmentally sound agrochemical or fertiliser. However, it should not be used for anything other than its original or intended purpose, and should not be allowed to come into contact with an animal's skin or contaminate drinking water.

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