RADIOLOGICAL STUDY OF HEREDITARY LYMPHEDEMA IN HEREFORD CATTLE[1]

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Hipoplasia linfática heriditária foi estudada através do exame radiológico do sistema linfático periférico de sete bovinos da raça Hereford com diagnóstico clínico da doença. Quatro bovinos sadios, da mesma raça, pertencentes a um rebanho livre da doença foram utilizados como controle. Dezesseis bovinos sem sinais clínicos, pertencentes a um rebanho experimental, no qual a doença foi reproduzida, foram, também, estudados com o objetivo de detectar-se casos subclínicos da enfermidade. Após a sedação dos animais e anestesia local, foi feita uma incisão na pele, no terço médio da face lateral dos ossos metacarpo e metatarso para exposição dos vasos linfáticos, marcados previamente por azul de metileno. Nos vasos linfáticos foi injetado meio de contraste e foram tomadas radiografias nas regiões anatômicas onde estão localizados os linfonodos pré-escapulares e poplíteos. Nas radiografias eram medidos o calibre dos vasos linfáticos e as dimensões dorso-ventral e crâneo-caudal dos linfonodos. Este estudo demonstrou ser a linfografia direta eficiente para a avaliação do sistema linfático periférico dos membros posteriores de bovinos com hipoplasia linfática. As lesões observadas, de hipoplasia e/ou aplasia do sistema linfático periférico, caracterizaram-se por redução no número e aumento do diâmetro ou ausência de vasos linfáticos e diminuição do tamanho ou ausência dos linfonodos poplíteos. Nos animais que não apresentavam sinais clínicos não foram observadas alterações no sistema linfático periférico que permitissem a detecção de casos subclínicos da enfermidade.

TERMOS DE INDEXAÇÃO: Hipoplasia linfática, doenças hereditárias, linfoedema hereditário.

ABSTRACT.- A radiological study of the fore and hind limb lymphatic system was performed in seven calves with hereditary hypoplasia. Four healthy calves from an unrelated Hereford herd were used as a control group. Sixteen calves, without signs of disease, from an experimental affected herd were also studied to detect subclinical cases of lymphedema. After sedation and local anaesthesia a triangular flap of skin was reflected over the lateral aspect of the metacarpus and metatarsus to expose the subcutaneous lymphatics, which were previously stained by methylene blue injected subcutaneously into the interdigital space. The contrast medium was injected into the stained lymph vessels and lymphographies were taken in the anatomical regions where the popliteal and prescapular lymph nodes are located. The lymphangiograms obtained were used to determine the caliber of lymph vessels and the cranio-caudal and proximo-distal dimensions of the popliteal lymph nodes. It was demonstrated that direct lymphography is a suitable method to study the peripheral lymphatic system in the hind limbs of cattle with hereditary lymphatic hypoplasia. The lesions were hypoplasia and/or aplasia of the peripheral lymphatic system, characterized by decreased number and enlargement or absence of peripheral lymph

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vessels and decreased size or absence of popliteal lymph nodes. In calves without clinical signs the peripheral lymphatic lesions which would allow to detect subclinical cases were not observed.

INDEX TERMS: Lymphatic hypoplasia, hereditary diseases, heriditary lymphedema.

INTRODUCTION

Congenital hereditary lymphedema is a disease characterized by defective development of the peripheral lymphatic system that results in edema of different degrees mainly involving limbs and tail (Leighton & Suter 1979). The disease affects many species including cattle (Donald et al. 1952, Morris et al. 1954), dogs (Patterson et al. 1952) and man (Kinmonth 1965). In Ayrshire cattle the defect is due to an autosomal recessive trait which results in smaller than normal superficial lymph nodes and dilated and tortuous afferent lymph vessels (Morris et al. 1954). In Hereford cattle the disease is characterized by edema of the hind limbs and, sometimes, fore limbs, tail and prepuce and lesions are hypoplasia and aplasia of peripheral lymph vessels and prescapular, iliofemoral and popliteal lymph nodes (Schild et al. 1991). It is transmitted by an autosomal dominant gene with variable expressivity and incomplete penetrance (Schild et al. 1991).

This report describes a radiological study of the lymphatic system of Hereford cattle with hereditary lymphedema. With the aim to detect subclinical cases, calves without clinical signs from the affected herd were also studied.

MATERIALS AND METHODS

Three groups of calves were used for radiological studies: Group 1 (control) had four healthy Hereford calves; Group 2 (affected) had seven calves with variable degrees of edema in the hind and fore limbs; and Group 3 (suspected) had 16 calves without signs of the disease. Calves of Group 1 were from an unrelated Hereford herd. Calves of Group 2 were from the herd in which the disease was diagnosed and calves from Group 3 were born from the experimental test mating performed to determine the hereditary origin of the condition (Schild et al. 1991). Calves from Group 2 had slight edema at the fetlock and distal metatarsal region of the hind limbs (pattern a); two calves had edema of the hind limbs below the femoro-tibial joint and in the fore limbs below the carpus (pattern b). One calf had slight edema at the fetlock only in the hind limbs. Age and body weight of the calves are presented in Table 1.

In Group 1 and 2, lymphangiograms were performed in the anatomical regions where the popliteal and prescapular lymph nodes are located. The prescapular lymph node is situated at the cranial border of the supraspinatus muscle, 10-12 cm above the level of the shoulder joint and it is covered by the omotransversarius and brachiocephalicus muscles; the popliteal node is situated in a mass of fat on the gastrocnemius muscle, caudal the tibial and peroneal nerves and between the biceps femoris and semitendinous muscles (Sisson & Grassman 1981). In Group 3 the lymphangiographies were carried out only in the hind limbs.

After sedation with 0.2 mg/kg body weight of xylazine chloride 2-3 ml of methylene blue dye in 5% aqueous solution were injected subcutaneously in the interdigital space of the four limbs of calves from Group 1 and 2, and only in the hind limbs of calves from Group 3. Five to 10 minutes later, after local infiltration with xylocaine 2%, a triangular flap of skin was reflected over the lateral aspect of the metatarsus and metacarpus to expose the subcutaneous lymphatics. This flap was formed by two 5 cm incisions, one vertical and the other horizontal, whose ends met at 90° on the caudal aspect of the lateral surface. At this time lymph vessels were evaluated for number and shape. The contrast medium (Hypaque®) at the dose of 8.0 ml mixed with 2 ml xylocaine 2% was injected via an intravenous infusion set (Venescalp® 23 G) into a lymphatic vessel stained by methylene blue.

Lymphangiograms were taken in the medial-lateral position of the hind limbs and the external-lateral position of the forelimbs at three different times: before the injection of methylene blue (time 0); immediately after the contrast medium injection (time 1); and after 15 minutes (time 2). They were taken with a FNX machine, 80Kv and 15 mAs, with an exposure time of 1.5 seconds. The films were Sakura or Kodak, 24x30cm. The area covered by the radiographies is observed in Fig. 1.

Lymphamgiograms obtained in time 1 were used to determine the caliber of lymph vessels and the cranio-caudal and proximo-distal dimensions of the popliteal lymph nodes. For measuring lymph vessels on the lymphographies, a line that passed on tangentially to the distal femur epiphysis, perpendicular of the lymph vessels was considered. The caliber of the vessels was measured in two points located 1 and 2 cm above the intersection of the line with the vessel, and in three points located 1, 2 and 3 cm below the same intersection.

RESULTS

Methylene blue staining

The number of lymph vessels stained by methylene blue in each limb of calves from the three groups is presented in Table 1. In the fore limbs of calves from Group 1 the two superficial lymph vessels stained by methylene blue were observed beside the dorsal common digital vein or they were on the deep surface of the dermis when the skin was reflected (Fig. 1). In the hind limbs the two vessels were observed beside the saphenous lateral vein. In calves from Group 2 and 3 the lymph vessels were located in the same place. All lymph vessels observed in the limbs of calves from Group 1 and 3 had an uniform caliber. In calves from Group 2 the lymph vessels were enlarged and had a sacculated shape. In calves that did not have superficial lymph vessels the methylene blue was spread in the subcutaneous edema.

Times 0 lymphographies

In time 0 lymphographies lesions were not observed in calves from the three groups.

Time 1 lymphographies

In the fore limbs of calves from Group 1 and 2 satisfactory lymphangiograms were not consistently obtained and the lymph vessels were not measured. In two calves from Group 1 the lymph node opacification did not occur because the contrasted lymph vessel drained directly to the thoracic wall. It also was not possible to measure the prescapular lymph nodes because they were partially obscured by bone structures.

Table 1. Age and body weight of calves from the three groups and number of lymph vessels stained by methylene blue

Calf	Age (months)	Body weight (kg)	Number of lymph vessels stained by methylene blue			
			Fore limbs		Hind limbs	
			Right	Left	Right	Left
Group 1 (control)						
1	6	150	2	2	2	2
2	6	140	2	2	2	2
3	6	95	2	2	2	2
4	6	85	2	nd[a]	2	nd
Group 2 (affected)						
5	1	42	0	0	0	0
6	18	150	1	1	1	1
7	18	154	1	1	1	1
8	18	125	0	1	0	0
9	10	123	nd	nd	0	0
10	10	125	nd	nd	0	0
11	10	60	1	1	0	0
Group 3 (unknown)						
12	12	56	nd	nd	2	2
13	4	92	nd	nd	1	1
14	3	76	nd	nd	2	2
15	4	55	nd	nd	2	2
16	4	94	nd	nd	2	1
17	3	57	nd	nd	2	2
18	3	61	nd	nd	2	3
19	3	63	nd	nd	2	2
20	4	70	nd	nd	2	2
21	6	78	nd	nd	2	2
22	6	102	nd	nd	2	2
23	3	65	nd	nd	1	1
24	3	58	nd	nd	1	1
25	3	53	nd	nd	1	2
26	3	68	nd	nd	1	1
27	4	85	nd	nd	2	1

[a] Not done.

Lymphangiograms of hind limbs allowed the evaluation of the lymph vessels and popliteal lymph nodes in all calves into which the contrast medium was injected. In calves from Group 1 (control) one or two main lymphatic channels extend from the metatarsal region proximally to the popliteal lymph node (Fig. 2). In Group 2 and 3 the lymph vessels were in the same place. Into five calves from Group 2 the contrast medium was not injected due to aplasia of lymph vessels (Table 2). Lymphographies were performed in two calves. The lymph vessels from these calves were enlarged and had a sacculated shape; one calf had aplasia of the right popliteal node (Fig. 3).

Hypoplasia of lymph vessels and nodes were observed in calves with slight edema of the hind limbs (pattern a) and in calves with edema of the four limbs (pattern b). Aplasia of lymph vessels and popliteal nodes were only observed in calves with edema of all limbs (pattern b). Abnormalities were not detected in lymphographies of calves from Group 3.

The mean and standard deviation of lymph vessel calibers of the calves from the three groups are in Table 2.

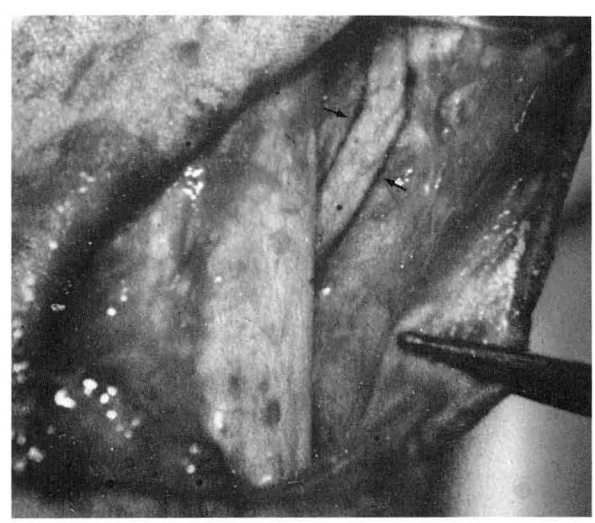

Fig. 1. Calf from Group 1 (Control). Two lymph vessels are observed in the deep surface of the dermis (small arrows) when the skin was reflected, 5 minutes after methylene blue injection into the interdigital space.

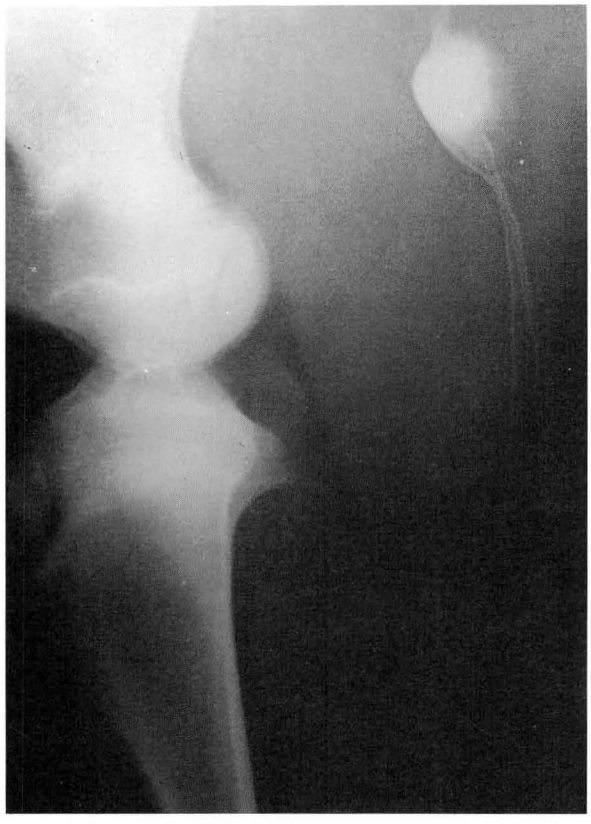

Fig. 2. Calf from Group 1 (Control). Time 1 lymphography. Three lymph vessels and the popliteal lymph node are observed.

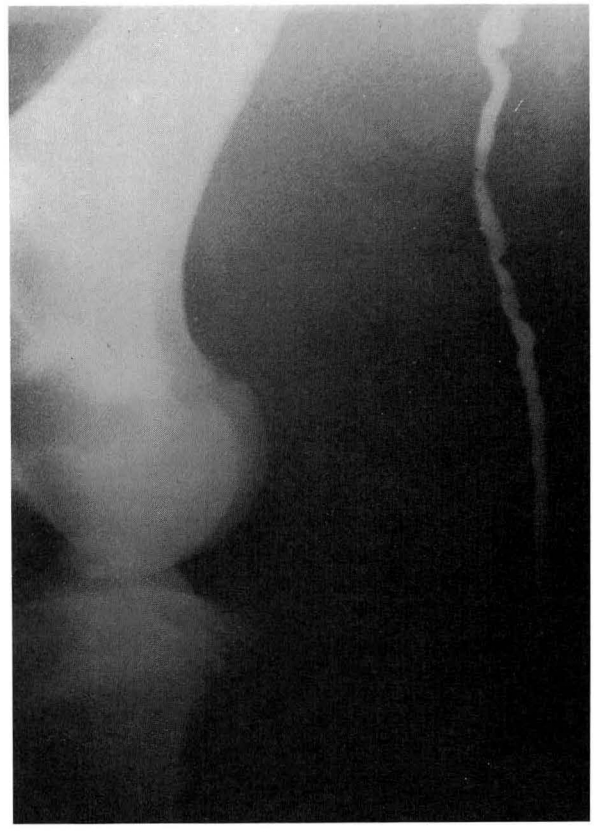

Fig. 3. Calf from Group 2 (Affected). Time 1 lymphography. An enlarged lymph vessel with a sacculated shape is observed. The popliteal lymph node is absent.

Table 2. Mean and standard deviation of the afferent lymph vessel calibers (cm) of calves from the three groups

Group	n[a]	X	±	S	Range
1	50	0.0972A[b]	±	0.0055	0.09-0.11
2	20	0.4175B	±	0.0396	0.35-0.50
3	205	0.1069A	±	0.0252	0.08-0.20

[a] n= number of measurements.
[b] Means followed by different letters have significant difference by Tukey test (P<0.01).

Table 3. Dimensions of the popliteal lymph nodes observed radiologically in calves from the three groups

Group	Mean of cranio-caudal and dorso-ventral dimensions of popliteal lymph nodes from all calves of each group				
	Nº of measurement	X	±	S	
1	14	3.054	±	0.568	A[a]
2	6	2.243	±	0.415	B
3	62	2.575	±	0.572	A

[a] Means followed by different letters have significant differences by last significance difference of Fisher (LSDF) (P<0.01).

The lymph vessel calibers of calves from Group 2 were significantly larger than the lymph vessel calibers of calves from Group 1 and 3. No differences were observed in the lymph vessel calibers between calves from Group 1 and 3 (Table 2). The size of the popliteal lymph nodes of calves from the three groups are presented in Table 3.

Time 2 lymphographies

In time 2 lymphograms no lymphatic structures were observed in calves from Group 1. In calves from Group 2 opacification of popliteal lymph nodes was observed also at this time. In calves from Group 3 time 2 lymphangiograms were not performed.

DISCUSSION

It was shown that direct lymphography is a suitable method to study the peripheral lymphatic system in the hind limbs of cattle with hereditary lymphatic hypoplasia. The radiological changes observed in the affected animals are similar to those previously reported in other species (Kinmonth et al. 1955, Skelley et al. 1964, Patterson et al. 1969, Leighton & Suter 1979, Davies et al. 1979).

In the affected cattle the technique allowed a previous evaluation of the peripheral lymphatic system, before the contrast medium infusion. The absence of lymph vessels and the observation of methylene blue dye spread into the

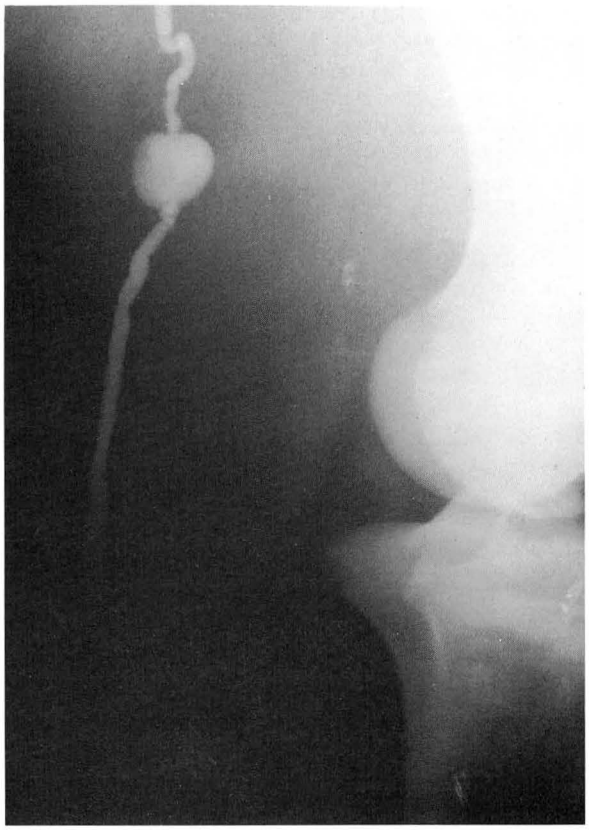

Fig. 4. Calf from Group 2 (Affected). Time 1 lymphography. An enlarged lymph vessel with a sacculated shape is observed. Note the decreased size of the popliteal lymph node.

subcutis demonstrated the aplasia of the peripheral lymph vessels in some calves, and the presence of only one lymph vessel (hypoplasia) in others. Similar findings are reported in man (Kinmonth 1965).

The lymphangiograms performed in calves from Group 2 showed that the main peripheral lesions of the lymphatic system were hypoplasia or aplasia of the popliteal node and enlargement of lymph vessels. These changes apppear to be similar to those reported in dogs and man (Kinmonth 1965, Patterson et al. 1967, Davies et al. 1979, Leighton & Suter 1979). Nevertheless in dogs the lymph vessels ended blindly in the region normally occupied by the popliteal lymph node, which was absent (Patterson et al. 1967, Davies et al. 1979). In lymphedema of Ayrshire cattle, enlarged afferent and efferent lymph vessels were observed at necropsies, but aplasia of lymph nodes is not reported (Morris et al. 1954).

In man and dogs with lymphedema increase in size and number of lymphatic vessels are reported as an hyperplasia (Kinmonth 1965, Olszewski et al. 1972, Davies et al. 1979). In Hereford cattle the enlargement of lymph vessels was not considered an hiperplasia, but a consequence of the arrested lymph flow.

The sacculated shape observed in lymph vessels of calves from Group 2 was probably due to the proliferation of endothelium of valves normally present in lymph vessels. In affected Hereford calves endothelial proliferation forming valve-like structures in lymphatic vessels was also observed histologically (Schild et al. 1991). Morris et al. (1954) studying two affected Ayrshire calves mentioned extensive proliferation of the endothelium leading to the formation of strands of tissue that in many cases divided the lumen of vessels.

In a previous report it was shown that the the disease is transmitted by an autosomal dominant gene with variable expressivity and incomplete penetrance (Schild et al. 1993). Different degrees of edema observed in Hereford cattle showed the variable expressivity of the gene and could indicate the occurrence of calves without clinical signs of the disease but with minimal lesions of the peripheral lymphatic system. Nevertheless, in calves from Group 3, without clinical signs of the disease, lesions of the peripheral lymphatic system were not evidenced by lymphangiographies. In man cases of lymphatic hypoplasia escape clinical detection, resulting in generations that do not have clinical signs (Esterly 1955).

Luginbühl et al. (1967) observed absence of prescapular nodes in dogs without edema of the fore limbs and absence of popliteal nodes in dogs with transient edema of the hind limbs. They suggested that the lack of regional lymph nodes was one of the manifestations of a more generalized defect in the development of the peripheral lymphatic system, and that in a few cases morphogenesis apparently may continue after birth, with formation of abnormal lymphatic vessels and nodes, but with functionally adequate lymphatic drainage. This apparently does not occur in Hereford cattle because the aplasia of lymph vessels and popliteal nodes was observed only in calves with severe degree of edema (pattern b).

In the affected calves popliteal lymph nodes still were opacified in time 2 lymphangiographies suggesting that edema is a consequence of a deficient lymph flow due to the hypoplasia of lymph vessels and nodes. In man and dogs the aplasia and hypoplasia is also considered to be responsible for the insufficiency of lymph fluid removal and edema formation (Esterly 1965, Leighton & Suter 1979). On the other hand, Patterson et al. (1967) mentioned the obstruction of lymph vessels as the main cause of edema. In Hereford cattle the obstruction of lymph channels was not observed in the lymphographies.

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