

Congenital Hereditary Lymphedema in the Pig

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Summary

A histopathological investigation was performed in newborn pigs with congenital hereditary lymphedema. The edema varied greatly in degree and extension. Although mostly confined to the hind legs it may involve other parts of the body, and even attain a most severe generalized distribution. It was found due to malformations of the lymphatic system which were general in character in all animals irrespective the grade in their edema. Parallel to the grade of edema however, these lymphvascular abnormalities varied from minor hypoplasia and localized aplasia to a complete agenesis of the whole lymphatic system.

Introduction

The congenital hereditary lymphedema is a well known entity in human pathology, inseparably associated with the names of *Nonne* and *Milroy* (for a review see *Esterely* (4)). The disease is characterized by a persistent edema of the lower extremities present from birth. Other parts of the body are far less frequently involved (4, 12, 15). The edema may vary in grade and extension, and an asymmetrical involvement is not unusual. Except for the time of its onset this disease does not differ essentially from another form of hereditary lymphedema that usually starts at puberty and is associated with the name of *Meigs*.

After the introduction of lymphangiographic techniques both disorders were found to be essentially similar being the results of a localized hypoplasia or aplasia of the lymphatic system. It was suggested that the more severe the malformations of the lymphvessels, the earlier the onset and the more serious the clinical manifestations (6, 7). Studies of all pedigrees available made it highly probable that the disorder is most commonly inherited as a simple dominant trait, but with a female predominance in some families (4). Chromosomal studies revealed no abnormalities (3).

Much less is known about congenital hereditary lymphedema in animals. There are only isolated reports on its occurrence in cattle (2, 9), dog (8, 10) and pig (16, 17). The clinical manifestations varied in different species: the edema was generalised in calves, whereas in dog and pig it was most conspicuous in the hind and fore legs respectively. Adequate histopathologic and lymphangiographic investigations were only performed in dogs. They showed that the lymphatic system of the hind legs was definitely abnormal exhibiting hyperplastic changes distally and hypoplasia or aplasia proximally.

In 1961 the Department of Zootechnics of the University of Utrecht acquired for genetical studies a boar and two of his daughters, which suffered from a congenital and possibly hereditary edema of the hind legs. Ever since, most elaborate mating experiments have been carried out in order to establish its mode of inheritance. In the affected animals which were of Butch Landrace breed, the edema was predominantly localized in the posterior parts, most markedly in the hind legs (fig. 1). It varied in degree and extension involving in some dead born animals the fore legs and the head as well. In grown up pigs the edema was gradually replaced by an extensive fibrosis of the affected parts. By injecting Patent Blue Violet interphalangeally in the hind legs of 7 week's pigs the existence of a serious hypoplasia or aplasia of the lymphatic system in this area was strongly suggested. Since histopathological data about congenital hereditary lymphedema in pigs are lacking completely it seemed worthwhile to set up an investigation.

Material and Methods

Investigations were made in 15 newborn pigs. They were born from matings between two defective animals (3), between a defective boar

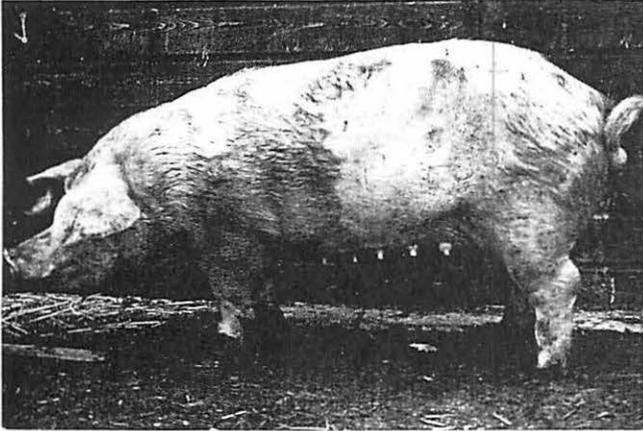


Fig. 1 A soar with congenital hereditary lymphedema. The edema and concomitant fibrosis is most pronounced in the hind legs. Involving also the posterior part of the rump it decreases gradually towards the head. No edematous changes existed in the fore legs and head.

and a normal sow (11), and between two normal animals unrelated to the defective family (1). Four pigs were born dead. The others either died spontaneously within 2 days after birth, or were sacrificed. They varied in crown-rump length from 240 to 300 mm; five were boars. After external and internal examination whole section blocks were taken serially from the fore- and hind-legs, and transverse blocks were taken from the jugular region and the prevertebral region at mid-thoracic, mid-abdominal and pelvic level. In addition tissue was removed from the lungs, liver and mesentery with adjacent intestine. The tissue was fixed in 4 percent formalin or Bouin's fixative, embedded in paraplast and stained with haematoxylin-eosin.

Observations

For descriptive purposes it proved to be convenient to make a division into 4 categories of animals relative to the condition of their lymphatic system.

Category 1 (normal)

This category comprised 3 newborn animals which died from unrelated diseases or were sacrificed.

Macroscopical findings

Except for anal atresia in one of them no gross abnormalities were seen on external and internal examination.

Microscopical findings

It proved to be very difficult to obtain a complete and detailed survey of the distribution and structure of the whole lymphatic system. While it was fairly easy to locate the larger trunks it was very hard to find the networks of smaller vessels and capillaries (Fig. 3a). But as far as could be concluded from these observations the distribution, pattern and structure did not seem to differ essentially from those described in adult animals (1).

Category 2 (minor hypoplasia)

This category comprised 8 animals which died from unrelated diseases within a few days or were sacrificed.

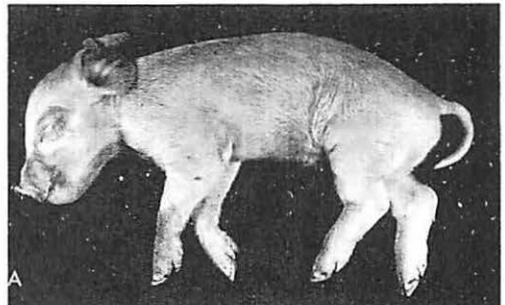


Fig. 2 Neonatal pig showing a moderate lymphedema that is confined to the hind legs and adjacent part of the rump (Category 2).



Fig. 3a Cross-section through the skin of the metacarpal region in newborn pigs. Normal configuration of the lymph vessels on the fore limb which form superficial extensions in the dermis (not visible) and a deeper network (11) just under the epidermal appendages (Ap). E. epidermis.



Fig. 3b Malformed lymph vessels in a non-edematous fore limb of an animal with congenital hereditary lymphedema. The lymphatics (11) have increased considerably in number and wideness especially in the subcutaneous layer which shows a slight increase of finely fibrillar connective tissue. HE. X. 63.

Macroscopical findings

All showed a swelling of the posterior part of the body. It was most marked in the rear legs, but involved also the posterior part of the rump though gradually decreasing towards the head (fig. 2). No edema was seen in the anterior part of the rump, the fore legs and the head. There was a considerable variability in the degree of this edema in the various animals. After the incision of the rear legs and the posterior bodywall the subcutaneous layer was found to be markedly broadened, up to 6 times its normal thickness. The cut surface was glistening and oozing some clear fluid.

Except for some unrelated diseases like peritonitis and incomplete expending of the lungs no gross abnormalities were seen.

Microscopical findings

The edema proved to be far more extensive than was expected from the macroscopical examination. In fact it was more or less generalized, although very slight in the anterior half and the more central area of the posterior half of the body. The edema was always associated with an increase of finely fibrillar connective tissue, which in the anterior and central parts was often more conspicuous than the edema itself. Changes were far more striking in the subcutis and adjacent intermuscular stroma of the rear legs.

The lymphatic system was distinctly abnormal in its more peripheral part. The abnormalities were best demonstrated in the extremities. Here, unlike the situation in the normal animals the lymphatics were rather easy to find because of wide lumina and relatively thick walls.



Fig. 4a

Fig. 4 Cross-section through the skin of the proximal portion of the hind legs of newborn pigs with congenital hereditary lymphedema. They show two different patterns of malformation of the lymph vessels.



Fig. 4b

- "Lymphangiectatic" configuration with numerous very wide lymph vessels (II) more or less evenly distributed in the cutis and subcutis.
- "Lymphangiomatous" configuration with very intricate vascular structures (II) surrounding and parting bundles of the skin muscle (M) HE. X. 40.

In the fore legs changes were far less marked than in the hind legs although essentially similar (fig. 3b). The dermal lymphvessels were irregularly distributed forming too large-meshed networks. But as in the normal, they extended up to the tips of the claws, were provided with valves and were continuous with some large longitudinally running trunks although the last were too few in number.

In the hind legs however a very irregular lymphvascular pattern was found. The lymphvessels were far less in number and wider than in the fore legs. They were often grouped together, sometimes to such an extent that angioma-like structures were formed (fig. 4). Their extensions did not reach beyond the tibiometatarsal joint or the metatarsal region.

The more distal parts were totally devoid of lymph vessels (fig. 5). Although very inconsistent in form and position even in one and the same animal they were nearly always situated on the medial side with a predilection for the vicinity of the muscle of the skin. Lymphatics were also present along the deep blood vessels but only around their most proximal parts. No valves were observed.

These differences as shown between the anterior and posterior extremities were more widespread and were actually present between all anterior and posterior parts of the peripheral lymphatic system. In the lungs and mesentery the lymph vessels seemed but slightly wider than normal. Only sporadically were lymph nodes present in the axillary, gluteal and inguinal regions.



Fig. 5 Cross-section through the skin of the metatarsal region of a hind limb of a newborn pig with congenital hereditary lymphedema. The cutis and subcutis are considerably thickened by edema and fibrosis. No lymph vessels have developed. HE. X. 63.

No abnormalities could be established in the main prevertebral lymph trunks which exhibited a great variability in form and patterns as under normal circumstances (fig. 6). Nevertheless there seemed to be a deficit in number and volume of the lymph nodes.

Even in the small number of pigs studied, considerable variations in grade of the malformation existed. In one animal for example slight edematous changes were associated with but minor abnormalities of the lymphatic system. This pig showed in the fore legs an almost normal configuration while in the hind legs a pattern existed that compared well with the relatively slight changes in the fore legs of the other affected animals.

Category 3 (major hypoplasia)

The three pigs of this group were born dead.

Macroscopical findings

The animals showed moderate post-mortem maceration. Their external appearances differed from the preceding group in degree rather than in nature (fig. 7). The edema was apparently general in character. But as in the former group, it was far less serious anteriorly than posteriorly. The subcutaneous layer had broadened considerably and a notable amount

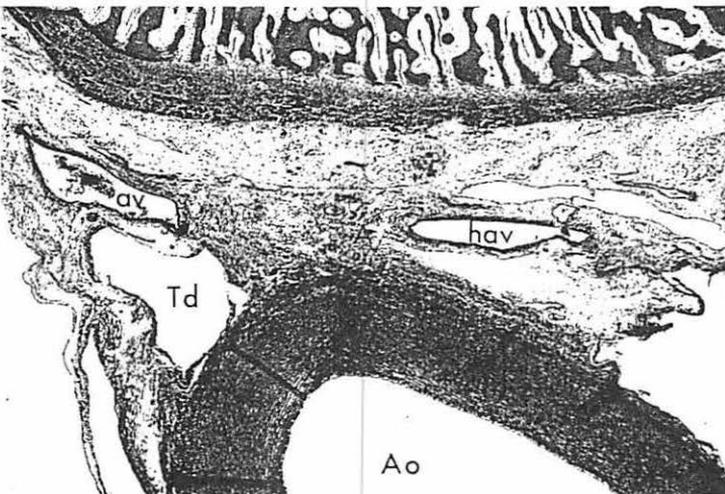


Fig. 6 Cross-section through the lower thoracic region of a newborn pig with congenital hereditary lymphedema. Except for the finely fibrillar connective tissue surrounding the aorta (ao), azygos vein (av), hemi-azygos vein (hav) and thoracic duct (td) no striking abnormalities are shown. HE. X. 25.

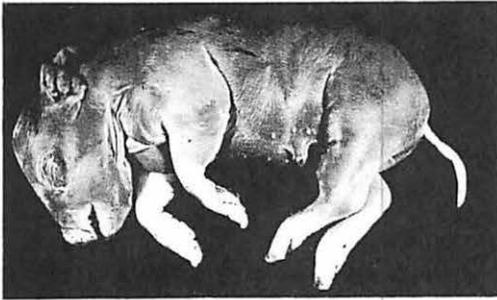


Fig. 7 Still-born pig with severe generalized lymphedema. The edematous changes are slightly more marked posteriorly than anteriorly (category 3).

of fluid escaped from its surface. Besides some straw-coloured fluid in the serious cavities no other gross abnormalities were found.

Microscopical findings

As to the edema the gross findings were confirmed. The lymphvascular abnormalities were much more conspicuous than in the former category. In the fore legs they showed a greater variability in form and distribution and were distinctly less numerous. Although the distal parts of these extremities were reached, the number of lymphatics here was often very small. Valves were scanty.

In the very edematous hind legs lymph vascular structures were only seen in their most proximal portion and never distal to the tibiomatarsal joint. More often than in those of category 2, cystic and angioma-like configurations had formed. There were no true dermal lymphplexuses. In the skin of the back lymphatics were only observed in the lateral parts. They extended from wide intercostal and lumbar lymph vessels and were often lying in groups just superficially to the deep muscles of the back. Here too the malformations were more marked caudally.

In the lungs and mesentery the lymphatic networks had a too coarse pattern and consisted of too wide vessels lying in slightly fibrotic connective tissue.

In these pigs there were also distinct abnormalities in form, pattern and structure of the main prevertebral lymphtrunks. These were too wide, too thin walled and too few in number. There were fewer and smaller lymphnodes than normally.

Category 4 (agenesis)

Only one animal could be brought into this category. From its length (240 mm. C.R. length) and external appearance it was thought to have died several weeks before birth.

Macroscopical findings

This female animal was extremely edematous. In this case the edema was cranially as severe as caudally (fig. 8). In addition a fluctuating swelling was visible in the anterior back region. Cutting of the skin revealed in most parts a dark gelatinous subcutaneous layer. In the back region it showed a series of smooth-lined cysts. They had a largest diameter of about 7 cm. and were filled with a straw-coloured fluid. They extended from the level of the neck to the lower thoracic region becoming gradually smaller caudally. There was no continuity with the central nervous system or any other system.

All cavities of the body contained a considerable amount of slightly turbid light-brown fluid. The lungs were hypoplastic.

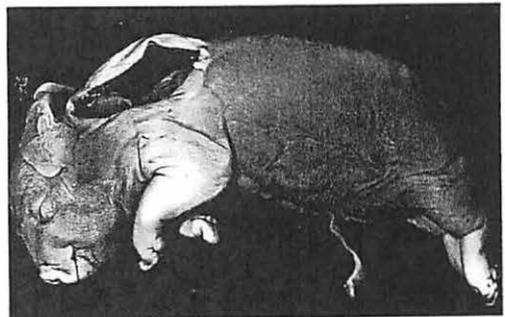


Fig. 8 Still-born pig showing a most severe lymphedema in all parts. A large cystic cavity in the subcutis of the shoulder region has been opened (category 4).

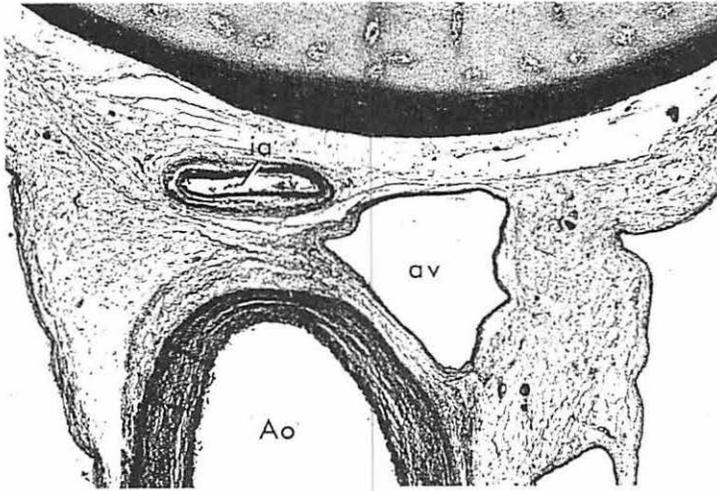


Fig. 9 Cross-section through the lower thoracic region of the still-born pig with complete agenesis of the lymphatic system. In the edematous stroma of the mediastinum only the aorta (ao), azygos vein (av) and an intercostal artery (ia) are present. Lymphvascular structures are totally absent. HE. X. 32.

Microscopical findings

The edema was far more severe than in the specimens of the former group. It was equally distributed and associated with a matrix of finely structured connective tissue elements. The subcutaneous cysts of the back were well defined by a thin layer of dense connective tissue. Lining cells could not be identified.

Most remarkable however was the absence of a lymphatic system. For notwithstanding very careful examination not a single lymphatic structure was found (fig. 9). Sometimes groups of irregular and ill-defined cavities were observed but neither their structures nor their positions had any bearing to the normal or abnormal patterns of the lymphatic system found in the other categories.

The bloodvascular system had a normal configuration and structure. The thymus looked normal, but lymphnodes were absent.

Discussion

From injection with Patent Blue Violet it became clear that aplasia of the lymph trunks in the distal parts of the extremities would prevent the application of lymphangiographic technics. Therefore only histopathological methods were applied. Although unsuitable

for demonstrating the small compressed lymph vessels in normal animals it proved to be quite accurate to show the often dilated abnormal vessels lying in a surplus of edematous stroma.

The results of this study pointed to a maldevelopment of the whole lymphatic system. This was most spectacularly demonstrated in the extremely edematous pig without a lymphvascular system at all (other, embryological, investigations proved that the cysts in the back had nothing to do with lymphvascular structures). But even in those animals in which the clinical manifestations were confined to the hind legs distinct vascular abnormalities were found in other parts of the body. The slight increase of connective tissue in these areas may well be considered to be the result of a preceding lymphedema. Apparently the lymphvascular malformations here were not as serious as to prevent the re-establishment of an adequate lymph drainage later. These observations are in accordance with those in dog in which lymphatic abnormalities were found also in animals or regions without clinical edema (8, 10). A more general character of this disorder is also suggested by the observations in calves (9), but their data about the morphology of the lymphatic system were too scanty to allow reliable conclusions. As for man the histopathological (13, 15) and lymphangiographic (7, 14)

investigations provided very scarce information, and concerned the lower extremities only. Some clinical reports however, mentioning the occurrence of edema in the upper extremities (4, 12, 15), certainly suggest the possibility of a more general involvement.

The true nature of this lymphatic malformation in pigs is most clearly demonstrated in the posterior parts. Here the lymphovascular system showed an incomplete outgrowth with aplasia in the most distal parts. In addition the vessels were very irregularly distributed forming either too coarse though more or less evenly distributed networks, or a concentration of often very intricate plexuses which leave the rest of the extremity devoid of lymphatics. Although to a far lesser degree, essentially the same changes were seen in the anterior parts. The main prevertebral lymph trunks did not seem to differ from normal in the less affected pigs, but from the hypoplasia of their lymph nodes and the distinct abnormalities in the more seriously affected animals it is concluded that they too are apparently involved.

These findings differ from those in dogs (8, 10). In the latter a kind of a reverse situation was observed in the hind legs. They found a very distended lymph vascular plexus in the most affected distal parts that was drained by a very hypoplastic system proximally. A more detailed comparison comprising the whole lymphatic system could not be made because only data about the hind legs and lateral thoracic wall were available. Nevertheless this discrepancy in findings is very interesting in the light of the more recent subdivision of the lymphovascular hypoplasia in man into an "obstructive" and "non-obstructive" subgroup (5).

Knowledge about the morphological alterations of the lymph vessels in congenital hereditary lymphedema in man however is too defective for reaching more definitive conclusions. Histopathological data comprise only information from a few biopsies of the skin of the lower legs. These showed an increase of finely fibrillary connective tissue with or without channels (13). Because of the rarity of the

disease and technical problems lymphangiographic studies often could not be performed (3). In other investigations their results, if any, are not reported apart from others obtained in different sorts of primary lymphedema (5, 7). But as far as may be concluded from this incomplete information, in man — as in pigs — an "un-obstructive" type of hypoplasia associated with distal aplasia seems to be present. In this investigation in pig no bloodvascular abnormalities were seen as observed by *Schroeder* et al. (15), in man, nor were there muscular (degenerative) changes as found by *Luginbühl* et al. (8) in dog. In many respects the results of this investigation in pig compare quite well with recent observations in human fetuses with an equally generalized malformation of the lymphatic system (11). There are also many features in common with other congenital malformations as hygromata, lymphangiomas, pulmonary and intestinal lymphangiectasis and the abnormalities underlying other sorts of primary lymphedema. This points to the possibility that in the pathogenesis of these diseases, the same mechanism is operating. And it certainly leaves the possibility that also in these disorders lymphovascular abnormalities are more widespread than the clinical manifestations would suggest.

A c k n o w l e d g e m e n t s

I am greatly indebted to the staff of the Department of Zoötechnics of the University of Utrecht for their co-operation. I am most grateful to Drs. *F.A. Neeteson* who preserved this precious herd of pigs and provided me with all the help I needed. I wish to thank Mr. *J. Berends*, Mrs. *S.P. Tan* and Mrs. *A. Jansen* for their technical and Mr. *E.E.W. Dumernit* and Mr. *F.J.C. Jansen* for their photographic assistance. For the typing of this manuscript I would like to thank Mrs. *M.M.M. Verzuu*.

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