

## Cat Leprosy in the Netherlands<sup>1</sup>

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In 1962, Brawn *et al* (<sup>1</sup>) reported the finding of nontuberculous cutaneous granulomata in nine cats in New Zealand. Ulcerating and nonulcerating nodules were found on the limbs, lips, neck, abdomen and back. Multiple nodules were found in some cats. The lesions were located in the cutis and subcutis. Epithelioid cells, with large numbers of acid-fast bacilli in the cytoplasm, were present. The acid-fast bacilli did not grow on Löwenstein medium after incubation for eight weeks at 37°C. Attempts to grow the bacilli in guinea pigs failed. The authors concluded that the disease in cats resembled both human and murine leprosy.

In 1963, Lawrence and Wickham (<sup>2</sup>) showed that acid-fast bacilli collected from similar skin lesions and from regional lymph nodes of cats in Australia could be transmitted to rats and that the clinical and histopathologic picture resembled murine leprosy. In one guinea pig, epithelioid cells and a single acid-fast bacillus were found in the regional lymph node four months after inoculation. In a young cat, three small foci of epithelioid cells, with central caseation and acid-fast bacilli, were found on the peritoneal surface of the small intestine and in the omentum ten months after intraperitoneal infection. They introduced the designation "cat-leprosy" for this disease.

In 1964, Wilkinson (<sup>4</sup>) reported three additional cases in England. One cat presented numerous ulcerating lesions with very large numbers of acid-fast bacilli, located on the eyelids and the face. The bacilli were situated mainly within macrophages. Cultures on Löwenstein medium were negative. Inoculation of the bacilli in guinea pigs did not produce lesions, either at the site of inoculation or elsewhere.

In 1964, "cat-leprosy" was diagnosed in a domestic cat in Utrecht, the Netherlands. Experiments with this strain are here re-

ported. Leiker and Poelma (<sup>3</sup>) produced further evidence on the etiology of this cat leprosy strain.

### MATERIALS AND METHODS

**History.** A two and a half year old castrated, tabby short hair male cat had an ulcer located above the left eye, one centimeter in diameter, for four weeks, which had a raised indurated margin. Abundant acid-fast bacilli, 2.5–5  $\mu$  long, slightly curved and mostly beaded, were found in a smear stained by the Ziehl-Neelsen method. Histopathologically, large numbers of acid-fast bacilli were found mainly within the macrophages (Fig. 1). Treatment consisted of daily application of cod-liver oil ointment to the ulcer. Two weeks later, the ulcer had become slightly smaller but three new ulcers appeared on the back of the left paw, the back of the right hind leg and the medial side of the left hind leg, respectively.

The cat was killed at the request of the owner. At autopsy, no pathologic changes

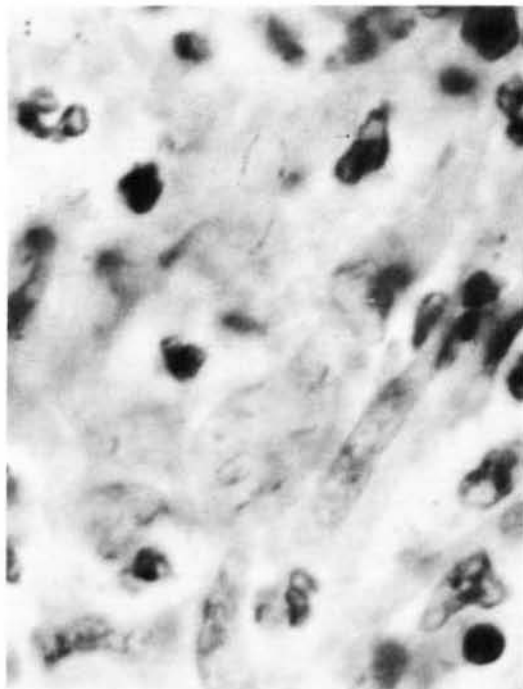


FIG. 1. Macrophages with acid-fast bacilli. Section of an ulcer of the leg from the cat. Triff stain;  $\times 1200$ .

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were found apart from the four skin ulcers and bilateral enlargement of the popliteal lymph nodes. Smears made from the ulcers revealed numerous acid-fast bacilli. In the popliteal lymph nodes only a few bacilli were found. Histopathologically, the ulcers showed extensive granulomatous infiltration of the dermis and the subcutis with numerous epithelioid cells, lymphocytes and neutrophils diffusely scattered in the infiltrates (Fig. 2). Focal accumulations of large epithelioid cell-like vacuolated macrophages and some multinucleated giant cells were present. Within these cells the numbers of acid-fast bacilli varied from one to fifty (Fig. 3).

**Transmission experiments** (inbred rat strain WAG/Cpb and inbred mouse strain CPB-S were used in the experiments). *First transmission from cat to rat.* Tissue from the ulcers was minced in a mortar, treated with 5% oxalic acid, incubated at 37°C for 30 minutes, and then centrifuged at approximately 3,000 RPM for 10 minutes. The supernatant was discarded. The sediment was washed with physiological saline solution

and again centrifuged. This was repeated twice. The sediment was then suspended in an equal volume of saline solution.

Two rats were inoculated subcutaneously with 0.5 ml of this suspension (Nos. D 66/164, D 66/195) and one rat intraperitoneally (D 67/20). The rats died 12, 13 and 24 months respectively after infection.

Suspensions of material from each of these rats were prepared as described above. The sediments were cultured (at 27°C and 37°C) on Löwenstein medium, with and without the addition of glycerol and on Stonebrink's medium without glycerol. Even after two years of incubation no growth was found.

*Second transmission from rat D 66/195 to rat, mouse and cat.* Four mice and six rats were inoculated subcutaneously with a 0.1 ml suspension made of bacilliferous subcutaneous granulomatous tissue of rat D 66/195. One cat was inoculated subcutaneously in a front leg, a hind leg and a shoulder region with a 0.1 ml suspension at each site.

*Third transmission A.* Material from rat D 67/488 was inoculated into two other rats and into two guinea pigs.

*Third transmission B.* Material from a subcutaneous tumor of the cat, from trans-



FIG. 2. Granulomatous infiltration of the dermis and subcutis. Section of the edge of the ulcer above the left eye of the cat. H & E stain; X54.

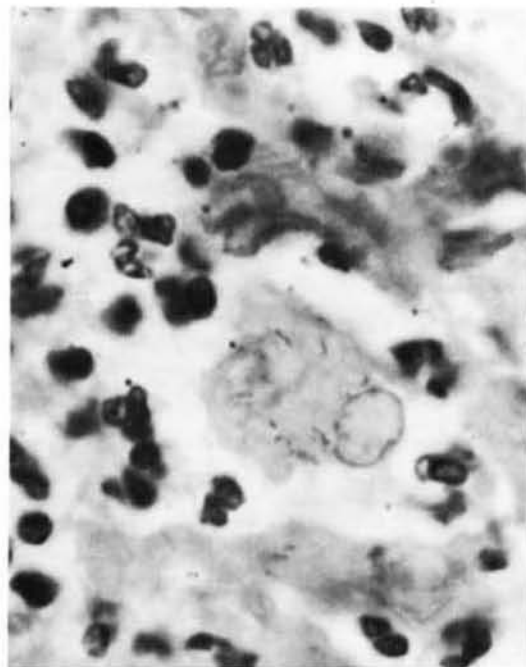


FIG. 3. Large epithelioid cell-like macrophage with vacuolated cytoplasm, and many acid-fast bacilli. Section of an ulcer of the leg from the cat. Triff stain; X1500.

mission 2, was inoculated subcutaneously into three rats, two guinea pigs, and one rabbit.

*Fourth transmission A.* Transmission from rat (D 69/348) to rat (D 70/322) and from rat (D 69/327) to mice were successful. Material from one (D 70/480) of these mice was successfully transmitted to three other mice.

*Fourth transmission B.* Material from rat (D 68/126) was inoculated into rats, mice, two guinea pigs, and two chickens.

**Culture attempts.** Material from the second, third, fourth and fifth transmission in animals was inoculated on Smith's medium, Stonebrink medium and on Löwenstein medium, with and without glycerol, and incubated at temperatures of 27°C, 33°C and 37°C.

Even after two years, no growth was found on any of the media. In order to assess the viability of the acid-fast bacilli which were still present on the media, material from a 23 month old culture was inoculated subcutaneously into three mice. After nine months, acid-fast bacilli were found in granulation tissue at the site of inoculation and in foci in liver and spleen.

## RESULTS

*First transmission.* Macroscopically, rat (D 66/164) showed yellow granulomatous tissue at the site of inoculation. In sections of the liver and spleen a few foci with numerous acid-fast bacilli were present. Rat (D 66/195) presented, in addition to bacilliferous granulomata in the cutis and subcutis at the site of inoculation, foci with acid-fast bacilli in liver, spleen, lung, heart and kidney. Rat (D 67/20), after 1.5 years, developed an edged ulcer in the umbilical region at the site of the intraperitoneal injection. This might represent the result of an inoculation by the passage needle. In this ulcer, numerous acid-fast bacilli were found. Liver and spleen were enlarged and presented macroscopically visible, pinhead-size white lesions. Histopathologic examination revealed bacilliferous granulomata in cutis, subcutis, liver, spleen, kidney and adrenal glands.

*Second transmission.* The mice died eight to ten months after inoculation. Numerous acid-fast bacilli were found at the site of inoculation and in the liver and spleen. The

rats died six to fourteen months after inoculation. Numerous acid-fast bacilli were present both subcutaneously and in the skin at the inoculation site. Additionally, bacilli were present in the liver, spleen, lymph nodes and kidneys. Remarkably, in some rats numerous acid-fast bacilli were also found in the subcutaneous tissue all over the body. The cat died from an undetermined cause five months after inoculation. At the three inoculation sites, subcutaneous tumors with a diameter of about 1.5 cm were present. At one site the cutis was attached to the subcutaneous tumor. In the granulomatous tissue groups of acid-fast bacilli were found. Bacilli were also found in the enlarged right popliteal lymph gland.

*Third transmission A.* The two rats (D 69/348, D 69/327) showed a 2 mm layer of bacilliferous, granulomatous tissue in the subcutis all over the body. Yellow-white foci were also found in subcutaneous lymph nodes, liver and spleen. One guinea pig was found to be negative two years after inoculation. The second guinea pig was reinoculated intraperitoneally after 20 months. Seven months after this second inoculation, foci with numerous acid-fast bacilli were found in the omentum. Histologically, the foci consisted of epithelioid cells and giant cells with large numbers of acid-fast bacilli. No bacilli were found in sections of liver and lung. Material from this guinea pig was successfully transmitted to mice.

*Third transmission B.* The rats (D 67/684, D 67/709 and D 68/126) died 15-18 months after infection. In all three rats, bacilliferous foci were found in the subcutis and in liver, spleen, and lymph nodes. In one guinea pig, no evidence of transmission was found. The second guinea pig was killed after four years. In the retropharyngeal lymph nodes and in the liver, spleen and lungs, many calcified foci with a diameter of about 3 mm were found. No acid-fast bacilli were found in these foci. Suspension of the liver of this guinea pig was inoculated into three mice. In two mice no acid-fast bacilli were found. In the third mouse, however, 22 months after inoculation granulomata with acid-fast bacilli were found in the spleen, liver, lung and axillary lymph nodes. The rabbit developed a subcutaneous abscess. Acid-fast bacilli were still present but transmission to mice failed.

**Fourth transmission B.** In one of the two guinea pigs, the results were negative after six months. The second guinea pig, inoculated intraperitoneally, died 16 days after inoculation from hydrothorax. Acid-fast bacilli were demonstrated in the lung and regional lymph nodes, spleen, liver, and in one focus in the omentum. In sections of liver and spleen, epithelioid cells with acid-fast bacilli were found. One of the two chickens, inoculated in the breast muscle, presented a 4 cm sized granuloma at the site of inoculation after six months. Acid-fast bacilli were present in the granuloma. Transmission to mice failed. In the second chicken, a few foci with acid-fast bacilli were found six months after intraperitoneal inoculation, in the wall of the abdominal air sac. Acid-fast bacilli were located in epithelioid cells. Transmission to mice was not successful.

**Fifth transmission.** Transmission from rat (D 70/322) to mice and from mouse (D 70/480) to other mice were successful. The latter strain was used in the experiments to produce further evidence regarding the etiology of cat leprosy (3). Material from rat (D 70/322) was also inoculated into two Syrian hamsters. The transmission failed in one hamster. In the second hamster, acid-fast bacilli were found at the site of inoculation and in liver and spleen eight months after inoculation. Many epithelioid cells were present in sections of liver and spleen.

#### DISCUSSION

The findings in a cat, showing acid-fast bacilli in skin lesions, are compatible with those described by Lawrence and Wickham as "cat leprosy." It appears that rats and mice are highly susceptible to this strain of "cat leprosy," that hamsters are less susceptible and that guinea pigs are rather resistant. Another cat that was infected subcutaneously was rather resistant and showed only local reactions at the site of infection and in the regional lymph node. In rats and mice, macroscopically, conspicuous bacilliferous lesions were found in liver, spleen, lung and lymph glands in addition to skin lesions. Microscopically, lesions were also present in kidney, pancreas, testis, epididymus, cardiac muscle, adrenal glands, and less frequently in the mucosa and submucosa of the stomach, small intestine and colon.

Large numbers of bacilli appeared to be scattered at random in macrophages in the granulomata.

In mice and rats the infection became generalized, irrespective of route of inoculation, and the disease was usually lethal after 6-18 months.

The bacilli failed to grow on the usual artificial media. These findings are compatible with those that are known for murine leprosy. It is therefore likely that the mycobacteria from cat leprosy is identical with *M. lepraemurium*.

#### SUMMARY

Acid-fast bacilli found in cutaneous granulomata and regional lymph nodes in a cat in the Netherlands correspond with those found in "cat lepra." The bacilli failed to grow on the media of Stonebrink, Smith and Löwenstein, with and without glycerol, even after two years of incubation at temperatures of 27°C, 33°C and 37°C. Transmission into another cat gave local reactions in the subcutaneous site of infection and in the regional lymph node. In mice and rats, the infection became generalized irrespective of the route of inoculation and the disease was usually lethal after 6-18 months. These findings are compatible with those that are known from murine leprosy. It is therefore likely that the mycobacteria from cat leprosy is identical with *Mycobacterium lepraemurium*.

#### RESUMEN

Los bacilos alcohol-ácido resistentes que se encontraron en un granuloma cutáneo y en los ganglios regionales de un gato en Holanda, corresponden a los que se encuentran en la "lepra del gato." Estos bacilos no crecieron en medio de Stonebrink, de Smith y de Löwenstein, con y sin glicerol, aún después de dos años de incubación a 27°C, 33°C y 37°C. La transmisión a otro gato dió reacciones locales en el tejido subcutáneo del sitio de infección y en los ganglios linfáticos regionales. En ratones y ratas la infección se hizo generalizada, independientemente de la vía de inoculación, y la enfermedad generalmente fué fatal después de 6-18 meses. Estos hallazgos son compatibles con los que se observan en lepra murina. Por lo tanto, es probable que la micobacteria obtenida de la lepra del gato sea idéntica al *Mycobacterium lepraemurium*.

#### RÉSUMÉ

Des bacilles acido-résistants recueillis au ni-

veau d'un granulome cutané, ainsi que dans des ganglions lymphatiques régionaux, chez un chat, aux Pays-Bas, correspondent avec ceux qui ont été trouvés dans la lèpre du chat. On n'a pas réussi à faire pousser des bacilles en milieu de Stonebrink, Smith et Löwenstein, avec ou sans glycerol, et cela même après deux ans d'incubation à des températures de 27°C, 33°C et 37°C. La transmission à un autre chat a entraîné des réactions locales à l'endroit d'infection, en tissu sous-cutané, ainsi que dans les ganglions lymphatiques régionaux. Chez les souris et chez les rats, l'infection est devenue généralisée, et cela quelle que soit la voie d'inoculation. La maladie chez ces animaux était habituellement fatale après 6 à 18 mois. Ces observations sont compatibles avec celles qui ont été faites dans la lèpre murine. Il est dès lors vraisemblable que les my-

cobactéries de la lèpre du chat sont identiques à *Mycobacterium lepraemurium*.

#### REFERENCES

1. BROWN, L. R., MAY, C. D. and WILLIAMS, S. E. A nontuberculous granuloma in cats. NZ Vet. J. **10** (1962) 7-9.
2. LAWRENCE, W. E. and WICKHAM, N. Cat leprosy: infection by a bacillus resembling *Mycobacterium lepraemurium*. Aust. Vet. J. **39** (1963) 390-393.
3. LEIKER, D. L. and POELMA, F. G. On the etiology of cat leprosy. Int. J. Lepr. **42** (1974) 312-315.
4. WILKINSON, G. T. A nontuberculous granuloma of the cat associated with an acid-fast bacillus. Vet. Rec. **76** (1964) 777-778, 833-834.