

Study of the pathogenicity of *Dermatophilus congolensis* Van Saceghem 1915 on white mice

BY

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Summary — The results of intra-venous injections of *Dermatophilus congolensis* in young white mice are presented. The injection of very high quantities kills the greatest part of a group of twelve mice: the surviving ones show always the same brainsymptoms. Lower quantities appeared to be inoffensive. But repeated injection of low quantities can be pathogenic. It is demonstrated that the cause of death is due to the action of the *D. congolensis* on the organism and not to an embolus nor to a toxin produced in the culture medium nor to the toxicity of the culture medium itself.

Cutaneous streptotrichosis is a cosmopolitan skin disease affecting both wild and domesticated animals. A few cases have been described in man (Dean *et al.*, 1961). The disease has been found widespread in tropical countries, especially among cattle.

It was only in 1914, in the former Belgian Congo, that streptotrichosis in cattle has been described for the first time by Van Saceghem (1914), although we may suppose that the disease has been known since a long time by breeders. The same author (Van Saceghem, 1914, 1915) was also the first to isolate and to describe the causal organism. He called the organism *Dermatophilus congolensis*. He studied also its life cycle (Van Saceghem, 1914, 1915 and 1934).

At first, the causal organism was considered a fungus and afterwards as a bacteria. Now it has been placed in the order of the Actinomycetales (Austwick, 1958; Memery 1961; Vanbreuseghem, 1962).

Subsequently similar organisms have been isolated from sheep. These organisms were called *Actinomyces dermatonomus* Bull, 1929, the causal organism of mycotic dermatitis and *Polysepta pedis* Thompson and Bisset 1957, the causal organism of stawberry foot rot. More recently however, authors have concluded that the three

organisms are not only very related (Austwick, 1958), but that they should be regarded as a single species, called *Dermatophilus congolensis* Van Saceghem 1915 (Gordon, 1964; Roberts, 1965).

The life cycle of *D. congolensis* is quite peculiar. The most recent and most complete description of it is given by Roberts (1961). We can summarize it as follows : on a definite moment, depending from several factors, a motile coccoid spore, called zoospore, on settling germinates. First it loses its motility and swells and then gives rise to one to three germ tubes. Such a germ tube elongates and transforms itself into a hypha. While the hypha elongates, transverse septa are laid down in the oldest part of it and eventually branchings are formed. In this way formed segments are again divided by new septa. At a certain moment the hypha is divided into cubical packets of coccoid forms, as many as eight per transverse row, by the formation of longitudinal septa in vertical and horizontal planes. Those coccoid forms do leave the hypha by their own motility. They can give rise to new hyphae or to zoospores.

Much has been written about the epizootiology and the transmission of the disease but one has not yet come to a definite explanation of all the facts. Moisture, skin-injuries and intense sunlight have been proposed by many authors as factors predisposing to cutaneous streptotrichosis. Many authors (e.g. Bull, 1929; Chodnik, 1956; Egerton, 1964; Kane, Downing and Wilson, 1955; Memery and Thiery, 1960; Pier, Neal and Cysewski, 1963; Van Saceghem, 1914) only report cases of streptotrichosis, or do note a significant increase in the incidence of the disease during the wettest season of the year. Others (e.g. Bentick-Smith, 1961; Macadam, 1961; Schultz, 1955) do not see any influence of humidity on the occurrence of the disease. During our 30 months long stay in the Republic of Rwanda, we (1966) could only note cases of streptotrichosis in cattle during the rainy season. Small skin-injuries, caused e.g. by ticks and rough branches are considered by many authors (Macadam, 1962; Mammerickx, 1961; Thiery and Memery, 1960; Vandemaele, 1961; Zlotnik, 1955) as being absolutely necessary for the penetration of the dermatophilus into the skin, although it has been proved by Richards and Pier (1966) that the disease can be transmitted by *Musca domestica* never disrupting the skin of its host while feeding. Van Saceghem (1915) attaches much importance to microlesions of the skin caused by strong sunbeams.

It is quite easy to infect animals artificially with *D. congolensis*. Typical skin-lesions can be caused in rabbits and mice by applying a dermatophilus culture on the skin (Pier *et al.*, 1963; Dean, 1961).

The best way to proceed it to prepare the skin by shaving it or by plucking the hair first and by scarifying it afterwards. Cattle, sheep, goat and deer also can be infected in this way (Dean, 1961; Pier *et al.*, 1963; Memery *et al.*, 1960). It is important to note that the lesions caused do not usually spread and heal within one or two weeks. This is in contrast to the natural lesions.

Mammerickx (1961) tried to induce lesions in cattle by injecting *D. congolensis* intravenously, but without success. Neither were Memery, G. and Memery, L. (1962) successful in their attempt to infect a calf and a goat in this way. But they obtained surprising results when they injected intravenously, at intervals of eight days, high doses of *D. congolensis* in rabbits. From the twelve rabbits treated in this way, six did not show any lesions; four became ill and died, showing internal but no external lesions and two exhibited typical and generalized lesions of streptotrichosis. Most of them exhibited a swelling and typical streptotrichosis lesions along the vein.

The present experiments were undertaken to investigate the pathogenicity of *D. congolensis* on white mice when injected intravenously.

Experiment I

a) *Methods and Materials*

The following pure *Dermatophilus congolensis* strains (*) were used :

- Strain R.V. 17347 : Isolated in Lubumbashi (ex-Elisabethville, République Démocratique du Congo) from cattle on 19 July 1965.
- Strain R.V. 17450 : Isolated in Lubumbashi (ex-Elisabethville, République Démocratique du Congo) from cattle on 9 September 1965.
- Strain R.V. 17451 : Isolated in Lubumbashi (ex-Elisabethville, République Démocratique du Congo) from cattle on 9 September 1965.
- Strain R.V. 17745 : Isolated in the Somali Republic from a donkey on 4 October 1965 (**).
- Strain R.V. 16126 : Isolated in République Démocratique du Congo from cattle on 10 November 1964.

The strains are maintained on Brain Heart Infusion agar slants (B.H.I. agar) and transferred at approximately two weeks intervals. The strains were incubated aerobically at 37 °C during the first five days and afterwards at room temperature (± 20 °C). The purity of the strains was regularly verified. It is important to note that the strains R.V. 17451 and R.V. 17745 had a smooth aspect when grown

(*) These strains were obtained from the laboratory of Mycology, Institute for Tropical Medicine, Antwerp, and kindly put to our disposal by Professor Dr R. Vanbreuseghem.

(**) This strain has been isolated in the Republic of Somali by Professor Dr R. Vanbreuseghem and is apparently the first isolated from this country. It will be described by R. Vanbreuseghem and M. Takashio.

on B.H.I. agar while the strains R.V. 16126, R.V. 17347 and R.V. 17450 had a rough aspect.

Each group of mice was composed of twelve, four weeks old male white mice, bred in the Tropical Institute of Antwerp (strain N.M.R.I. Hannover).

During the ten days that preceded the experiment we transferred each strain daily on a new B.H.I. agar slant, in order to acquire cultures between one and ten days old. This was done to assure dermatophilus was injected in all its evolutive forms.

Each slant was washed with 2 ml sterile physiological water (0,85 % NaCl). The suspensions obtained from the different cultures of each strain were put together and were shaken. Physiological water was added to obtain a final suspension of 20 ml. Immediately after a group of twelve mice, 0,5 ml of the suspension was injected in the tailvein, with an intradermatic needle. If an animal died immediately after the injection it was discarded and replaced. The post-injection observation period was six weeks. The attempts to isolate *D. congolensis* from dead mice was done on B.H.I. agar slants.

Unless otherwise mentioned, we used the same methods and materials for all the experiments.

b) Results

From the two groups of twelve mice each challenged respectively with strain R.V. 17451 or strain R.V. 17745, ten were dead and one was dying twenty-four hours after injection in each group. Before dying the animals exhibited manifest illness, characterized by dullness, superficial and accelerated respiration and raised hairs. The two dying animals were sacrificed. From the heartblood and liver of the twenty-two dead animals we could isolate *D. congolensis*. The two remaining mice showed a few days after challenge a slight swelling on the tail, near the place of injection. On the fifth day after challenge they started showing manifest brains symptoms characterized by rotary motions, excitation and holding the head sidewise. They were killed on the tenth day. Neither from the heart blood, nor from the liver, nor from their brain could we isolate *D. congolensis*.

In no case did the *post-mortem* examinations reveal any macroscopically visible lesions.

The mice injected with the strains R.V. 16126, R.V. 17347 or R.V.17450 stayed alive and showed only the above mentioned swelling on the tail. These swellings disappeared about one week after their appearance.

c) Discussion

The fact that almost all the mice injected with strains R.V. 17451 or R.V. 17745 died and that the remaining showed manifested symptoms appears to suggest that *D. congolensis* is pathogenic for mice when injected intravenously. That the animals injected with strains

R.V. 16126, R.V. 17347 or R.V. 17450 stayed alive and did not show any symptom, does not mean that those strains are less or not virulent. Indeed, those three strains do have a rough aspect when grown on B.H.I. agar and were much more adherent on the culture medium and could less easily be removed by washing. In consequence, the suspensions prepared from these strains were less rich. This suggests that a high dose of *D. congolensis* is needed to cause symptoms. This will be further investigated in experiment II. But we may not exclude, however, the possibility that death can be caused by embolus or by a toxical action of the culture medium. But the fact that the mice treated with the rough strains remained in perfect health excluded this possibility for the greater part. These strains have also been maintained on B.H.I. agar and it would be surprising that none of the thirty-six mice challenged with them died, if embolus were the cause of the death. The lower concentration of the suspension would not be a satisfactory explanation for this. Moreover, some authors (e.g. Gordon, 1964) noticed that filaments are predominant in rough colonies and coccoid forms in smooth colonies. The rough could easily lead to embolus. The late appearance of the brainsymptoms supports the belief that they are not caused by embolus.

The lesions on the tail are probably caused by the organisms accidentally injected beside the vein.

Experiment II

This experiment was designed to verify if the injection of high doses of *D. congolensis* is really necessary to provoke death or symptoms.

a) *Methods and Materials*

For the purpose of this experiment we prepared a suspension of strain R.V. 17451 in the same way as for experiment I, the only difference, this time the slants were washed with sterile physiological water in which sterile glass beads have been added. The obtained suspension was diluted three times with an equal volume of sterile physiological water. In this way we obtained from the original suspension (1 : 1) dilutions of 1 : 2; 1 : 4; and 1 : 8. With each dilution and with original suspension we treated a group of twelve mice each.

b) *Results*

The mice treated with the original suspension (1 : 1) became sick a few hours after challenge. On the sixth hour, all but one were dead. The remaining dying animal was sacrificed. From the liver and heartblood of all the dead mice we could isolate *D. congolensis*.

From the group treated with the dilution 1 : 2, six were dead and four were dying twenty-four hours after injection. One of the dying mice was sacrificed. From the heartblood and liver of these seven dead mice we could isolate *D. congolensis*. The three remaining sick mice died during the following night. From them, also, we could isolate the dermatophilus, but with difficulty because the B.H.I slants were overgrown by faster growing germs. On the fifth day after injection the two remaining mice started showing the above described brain symptoms. On the tenth day we killed one of them. From its liver and heartblood, we could isolate *D. congolensis* but not from its brain. The other animal kept showing the symptoms until the end of the observation period.

From the group treated with the dilution 1 : 4, four died twenty-four hours after injection. Here again we could isolate *D. congolensis*. Two mice started showing brain symptoms on the fifth day. We killed one of them on the tenth day and from its liver and heart blood we could isolate *D. congolensis* but not from its brain. The second one kept showing the symptoms until the end of the observation period. The remaining animals remained in perfect health.

All the mice treated with the dilution 1 : 8 stayed alive and remained in perfect health. No attempt was made to recover *D. congolensis* from these animals.

Almost all the animals of each group who lived longer than forty-eight hours showed the previously described swelling on the tail.

The *post-mortem* examination never revealed a macroscopically visible lesion.

c) Discussion

From the results obtained with this experiment we may deduce that *D. congolensis* is only pathogenic for mice when very high doses are injected. Indeed, the higher the injected dose, the sooner the mice died. The highest doses caused also the highest dead-rate, and the greatest number of cases of brain symptoms. The injection of relative low doses, on the contrary, did not provoke any symptoms, except the slight swelling on the tail. It is important to note that the dermatophilus could still be isolated ten days after the injection, from the organs of mice suffering from brain symptoms. That the animals treated with the original suspension (1 : 1) died sooner than the animals in experiment I, can be explained by the fact that the original suspension used in experiment II was much richer, because the slants had been washed with the aid of glass beads.

Experiment III

This experiment was designed to ascertain if the injection of relative low doses at intervals can provoke symptoms.

a) *Methods and Materials*

A suspension of strain r.v. 17451, prepared in the same way as for experiment II was diluted with sterile physiological water to obtain a final dilution of 1 : 8. A group of twelve mice was challenged with it. Eight days later, six of the mice were rechallenged with approximately the same quantity of a freshly prepared suspension. The six other mice were retained as control.

b) *Results*

After having received the first challenge, the mice remained in perfect health and exhibited only the usual swelling on the tail. The six mice who were rechallenged exhibited manifest illness after five days and died a few hours later. From their heartblood and liver we could isolate *D. congolensis*. The *post-mortem* examination did not reveal any lesions.

The six control mice remained in perfect health.

c) *Discussion*

That the animals challenged with relative low doses of *D. congolensis* stayed alive, corresponds with the observations made in experiment II. A rechallenge eight days later with about the same dose is fatal, but the signs of illness appeared much later than when treated with one high dose.

This observation excludes the possibility that death would be caused by embolus and points again to a toxical action of *D. congolensis*.

Experiment IV

This experiment was designed to ascertain that the cause of death is not a toxical action of the culture medium or a toxin produced by the organism in the culture medium.

a) *Methods and Materials*

Strain r.v. 17451 was cultured in beef infusion broth and incubated aerobically during the first five days at 37 °C and at room temperature (± 20 °C) during the following days. We proceeded again in such a way to obtain cultures between

one and ten days old on the day of the experiment. These cultures were mixed and divided into three equal parts. Part I was left untreated; part II was filtrated through a Seitz filter and part III was heated during forty-five minutes in a boiling water bath. After treatment, part II and III were found to be sterile.

With each part, a group of twelve mice was treated. A group of control mice was treated with a sterile, freshly prepared beef infusion broth.

b) Results

From the twelve mice treated with part I, ten died twenty-four hours after injection, after having shown signs of manifested illness. The two remaining mice on the second day showed the usual swelling on the tail, and on the fifth day they started showing brain symptoms. One of them was killed on the tenth day. From its liver and heart blood and from the liver and heart blood of those who died twenty-four hours after challenge, we could isolate *D. congolensis*. The presence of *D. congolensis* could not be demonstrated in the brains.

The one remaining mouse kept showing brain symptoms until the fourth week after challenge, when it died. This time the *post-mortem* examination revealed the following lesions : degenerated kidneys, haemorrhagic inflammation of the colon descendens, degenerated liver and a slight pneumonia. The faeces were mixed with blood. The *D. congolensis* could be isolated from the heart blood.

The mice treated with parts II and III and with the freshly prepared beef infusion broth remained in perfect health. None of them showed a swelling at the place of injection.

c) Discussion

From the results of this experiment it is concluded definitely that *D. congolensis* is pathogenic for mice when injected intravenously. The intra-venous injection of a killed or filtrated culture does not have any effect on mice and refutes the possibility that death would be caused by embolus or by a toxin produced in the culture medium. We may also exclude the toxic action of B.H.I. agar, because the *D. congolensis* strain cultured in beef infusion broth was as noxious as when cultured on B.H.I. agar. Furthermore, the injection of a freshly prepared beef infusion broth appeared to be inoffensive.

It is important to note that the culture in beef infusion broth was more numerous in small coccoid forms than the culture on B.H.I. agar. This corresponds with the observations of Chodnik (1956) who noted that coccoid forms are predominant in a fluid medium. The fact that the injection of a culture rich in coccoid

forms was as noxious as the injection of a culture richer in filaments is also an argument against death by embolus.

It is interesting to note that the *D. congolensis* could still be isolated four weeks after injection.

Conclusion

From the results obtained from these experiments we may conclude that *D. congolensis* is pathogenic for mice when high doses are injected intra-venously. Indeed, the doses we injected may be considered as being very high. Memery, G. and Memery, L. (1962) considered the doses they injected in rabbits as very high. They used for each injection the amount of suspension obtained from one slant. We used approximately 1/4th, 1/8th, 1/16th and 1/32th of this amount for young mice and only a single injection of the first three appeared to be lethal. We do agree with Memery, G. and Memery, L. (1962) that it is possible that *D. congolensis* produces a toxin in the organism.

Almost all the mice challenged with the high dose died or became ill after a short delay, whereas according to Memery, G. and Memery, L. (1962) a rechallenge appeared to be necessary in rabbits. However, the results of experiment III point to the possibility that a single injection of a higher dose could also be lethal for rabbits. It is striking that we never could observe generalized lesions of cutaneous streptotrichosis. This is in contrast to Memery, G. and Memery, L. (1962) observations in rabbits.

The brain symptoms produced have always been the same. As well as the lesions described in the one mouse, in experiment IV, they should be further investigated.

The failure to isolate *D. congolensis* from the organs of the two mice in experiment I could be considered as an accident. Indeed, the slants were contaminated with fast growing bacteria, which could have made the isolation of *D. congolensis* difficult.

This study may be helpful for the investigation of the immunology and treatment of streptotrichosis.

Résumé — Cet article concerne les résultats des inoculations de jeunes souris blanches par voie intraveineuse avec *Dermatophilus congolensis*. L'injection de très fortes doses tue la majorité d'un groupe de douze souris; les survivantes présentent des symptômes cérébraux typiques. Des doses relativement faibles s'avèrent inoffensives. Mais l'injection répétée de doses relativement faibles cause la mort. On a démontré que la mort fut provoquée par une action toxique de *D. congolensis* sur l'organisme et non pas par embolie ou par une action toxique du milieu de culture.

Samenvatting — Intraveineuze inoculatie van jonge witte muizen met *Dermatophilus congolensis* gaf volgende resultaten: Injektie met zeer hoge doses doodt de meeste van een groep van twaalf muizen; de overlevende dieren vertonen typische cerebrale symptomen. Relatief zwakke doses blijken inoffensief, doch herhaaldelijke injectie van relatief zwakke doses veroorzaakt de dood. Zulks is het gevolg van de toxische inwerking van *D. congolensis* op het organisme en niet, zoals wordt aangetoond, van embolie of toxische inwerking van de voedingsbodem.

Zusammenfassung — Dieser Beitrag bezieht sich auf die Resultate von intravenösen Inokulationen junger weisser Mäuse mit *Dermatophilus congolensis*. Die Injektion von sehr hohen Dosen tötete die Mehrzahl einer Gruppe von zwölf Mäusen, die überlebenden Tiere zeigten typische cerebrale Symptome. Relativ schwache Dosen erwiesen sich als inoffensiv. Die wiederholte Injektion relativ schwacher Dosen verursachte aber den Tod. Es wurde nachgewiesen, dass der Tod durch eine toxische Einwirkung von *D. congolensis* auf den Organismus hervorgerufen wird und nicht durch Embolie oder durch eine toxische Wirkung des Nährbodens.

Resumen — Este artículo contiene los resultados de las inoculaciones de ratones jóvenes blancos por vía intravenosa con *Dermatophilus congolensis*. La inyección de dosis muy elevadas mata a la mayoría de un grupo de estos animales; los supervivientes presentan síntomas cerebrales típicos. Dosis relativamente débiles compruébanse inofensivas. Pero la inyección repetida de este tipo de dosis causa la muerte. Se ha demostrado que la muerte fué provocada por una acción tóxica de *D. congolensis* sobre el organismo y no por embolia ó acción tóxica del medio de cultivo.

Acknowledgements — I am indebted to Professor Dr R. Vanbreuseghem and Professor Dr J. Mortelmans for their interest and advice and to Mr. H. O. Marshall for the review of the text.

Laboratory of Mycology (Dir.: Prof. Dr R. Vanbreuseghem). Laboratory of Veterinary Medicine (Dir.: Prof. Dr J. Mortelmans). Institute for Tropical Medicine, Antwerp, Belgium (Dir.: Prof. Dr P. G. Janssens). Received for publication 24th April 1967.

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