

## A STANDARD METHOD FOR DEMONSTRATION OF THE LIFE CYCLE OF *DERMATOPHILUS CONGOLENSIS*

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### Abstract

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Determination of the life cycle of *Dermatophilus congolensis* is a prerequisite for its diagnosis. Attempts to demonstrate the life cycle of *D. congolensis* in preparations are successful only now and then even when nutrient-rich media for its cultivation are used. Enrichment of the media with sterile rabbit blood serum stimulates typical septation of the hyphae and intratubular development of the spores, which is the unique characteristic of this species. Preparations from the media enriched with blood serum regularly contain all stages of the life cycle of *D. congolensis* and thus make its accurate diagnosis possible.

*Dermatophilus congolensis*, life cycle, enrichment of media with sterile serum.

*Dermatophilus congolensis* Van Saceghem 1915, 357, emend. mut. char. Gordon 1964, the causative agent of dermatophilosis of animals and man, is characterized by its unique life cycle, at the beginning and at the end of which are motile zoospores of 0.5 µm in diameter. Upon germination they lose flagella and develop into tubular forms, generally up to 5 µm wide and extremely long, branching laterally at right angles and slightly conical in form. The substrate mycelium developing on solid medium gives rise to a colony. This process also takes place in liquid medium, being manifested by a membrane and by the development of flocculi or small granules. The internal transverse and longitudinal branching of tubular hyphae creates septa in which new spores develop. After being released from the septa the zoospores are motile for a certain period of time and their germination marks the beginning of a new life cycle (Roberts 1961; Gordon and Edwards 1963; Gordon 1964; Gyles 1986; Gordon 1989). *D. congolensis* is intensively Gram-positive.

Attempts to detect the life cycle of *D. congolensis* in fixed stained preparations often fail to yield positive results because of different multiplication rates of the strains and because of the culture medium employed (Pospíšil et al. 1992). To determine the conditions permitting regular demonstration of the life cycle of this species in fixed stained preparations was the objective of the present study.

### Materials and Methods

#### Bacterial strains

Five strains of *D. congolensis* were used. Of these one strain was isolated by us (Pospíšil et al. 1991), two strains were from the National Collection of Type Cultures (NCTC), London, namely strains 5175 and 7915, and the remaining two from the Norwegian College of Veterinary Medicine, Oslo, namely strains N-A and N-L. All five strains are deposited in the Czechoslovak National Collection of Type Cultures (CNCTC), Prague, under code designation of Dc 1/91, Dc 2/91, Dc 3/91, Dc 4/91 and Dc 5/91, respectively.

#### Culture media

Tryptone Soya Agar (TSA) CM 131, Tryptone Soya Broth (TSB) CM 129, Columbia Blood Agar Base (CBA) CM 331, Brain Heart Infusion (BHI) CM 225 and Brain Heart Infusion Agar CM 375, all of them Oxoid Ltd, were used. The media were used both unsupplemented and supplemented with 25 % (v/v) sterile rabbit serum. The agar media were also supplemented with 5 % (v/v) sheep erythrocytes after three washings.

#### Preparations for microscopic examination

All five *D. congolensis* strains growing in the afore-mentioned media were examined by microscope in fixed preparations stained according to Gram. The materials were collected after 14 h, 18 h, 24 h, 36 h and 48 h

of aerobic incubation at 37 °C. The materials from the solid media were collected directly from the colonies and those from the liquid media, after their centrifugation and subsequent re-suspension of the sediment in saline.

## Results

The five *D. congolensis* strains grew equally well on all media employed. On the solid media their colonies grew into the surface in the form of pits. On the blood agar media they produced zones of complete haemolysis. In the liquid media not supplemented with serum they grew as a sediment of minute flocculi in clear medium. In serum-supplemented broth media they grew in the form of a membrane that was either located at the bottom of the test tube or was floating in the clear medium, being reminiscent of a very delicate lace.

Preparations from the solid media without serum, both from those unsupplemented and from blood agar media, contained only Gram-positive spores and merely rudimentary filaments if the material was withdrawn carefully from the surface of the medium (Fig. 1). Where the materials were collected from the disrupted surface together with parts of the colonies or with the flocculi from the liquid medium sediment, they contained branching mycelial forms showing no marked tendency to the intratubular formation of septa and spores, this phenomenon being observed only exceptionally. The above-described microscopic picture was seen in preparations made within 24 h of incubation, whereas those made after a longer period of time contained only spores.

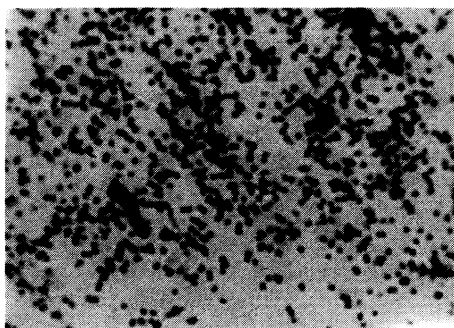


Fig. 1. Zoospores of *D. congolensis*.  $\times 1\ 800$ .



Fig. 2. Filamentous forms of *D. congolensis* showing incipient septation and intratubular development of spores.  $\times 1\ 800$ .

The regular incidence of typically septed filaments was recorded for all five *D. congolensis* strains in preparations from both solid and liquid media enriched with blood serum. After as few as 14 h of incubation, filaments showing incipient septation and development of spores were observed (Fig. 2). After 18 and 24 h incubation, septation of the hyphae and intratubular development of the spores became increasingly distinct and this phase of the life cycle outnumbered the other stages detectable in the preparations (Fig. 3). Released zoospores, often arranged as they were observed in the septed hyphae, were seen in preparations from these media after 36 h and 48 h incubation (Fig. 4), although the remaining phases of the life cycle of *D. congolensis* were also present.



Fig. 3. Septed hyphae of *D. congolensis* showing developed intratubular spores. x 1 800.

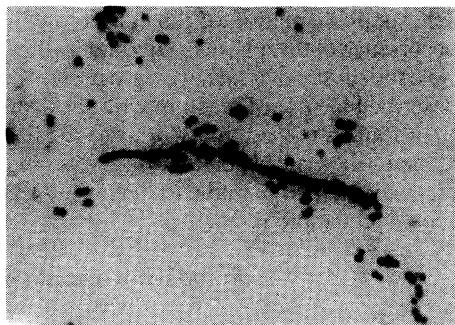


Fig. 4. *D. congolensis* zoospores recently released after disappearance of the hyphae. x 1 800. (Note: The strain shown in Figs. 1 to 4 is *D. congolensis* strain Dc 1/91.)

## Discussion

Dermatophilosis of animals and man is still regarded as a skin disease posing diagnostic problems; for its accurate diagnosis, detection of the causative agent is a necessity (Pospíšil et al. 1991, 1992; Vestweber 1992). Recent isolations in Europe from both farm animals (Baustad et al. 1989; Hannes et al. 1991) and man (Hänel et al. 1991; Pospíšil et al. 1991) have drawn attention to the fact that this species can also be found under European conditions. Microbiologists should therefore be aware about all the *D. congolensis* characteristics that make its accurate diagnosis possible.

Although *D. congolensis* grows even on common nutrient agar, recommendations have been made for its isolation and passage on nutrient-rich media (Gyles 1986; Gordon 1989). In a previous study we found that *D. congolensis* strains grew better on Columbia agar (CBA) than on BHI agar (Pospíšil et al. 1992). This was confirmed in the present study where this moderate difference, however, was reduced by the supplementation with blood serum. A marked difference was observed only in the form of growth in the liquid media with blood serum.

With the aim to make macroscopic diagnosis of *D. congolensis* on culture media more accurate our previous reports have described haemolysin interactions of this species with exosubstances of other bacterial species, particularly with CAMP factor of *Streptococcus agalactiae*, equi factor of *Rhodococcus equi* and staphylococcal beta and delta haemolysins (Skalka and Pospíšil 1992) as well as the possibility of serum diagnosis on the basis of the demonstration of antigenic identity of haemolysin produced by various *D. congolensis* strains (Skalka and Pospíšil 1993a).

Nevertheless, attempts to detect all phases of the life cycle of *D. congolensis* in fixed stained preparations from strain cultures may not necessarily succeed; this applies particularly to the septation of hyphae and to the intratubular development of zoospores. The dependence of the detection upon culture conditions has been described (Gordon 1989). This phase, however, was regularly detected in histological preparations from pathological lesions in both natural and experimental infection (Pospíšil et al. 1991; Buček et al. 1992). In the relevant literature the occurrence of septed hyphae was reported mainly in histological preparations (Roberts 1965; Gyles 1986; Vestweber 1992) and only rarely in materials from culture media (Gordon 1964).

Our previous problems with demonstration of this *D. congolensis* life cycle phase which is of paramount diagnostic value (Gordon 1989) gave an impetus to the present study.

The supplementation of the culture media with blood serum was inspired by the regular occurrence of this phase *in vivo* and by the fact that none of the published studies on this species, the survey of which we have presented elsewhere (Skalka and Pospíšil 1993b), described the use of medium with sterile blood serum.

In stained preparations from the media containing 25 % sterile rabbit serum the occurrence of the typical septed forms was a regular finding. Therefore media supplemented in this way make it possible to diagnose *D. congolensis* accurately on the basis of the microscopic preparation.

### Standardní metoda pro průkaz životního cyklu *Dermatophilus congolensis*

Určení životního cyklu *Dermatophilus congolensis* je základním požadavkem pro diagnostiku tohoto druhu. Úplný průkaz životního cyklu se daří jen nepravidelně v preparátech i při použití nutričně bohatých médií pro kultivaci *D. congolensis*. Obohacení médií nativním krevním sérem stimuluje typickou septaci hyf a intratubulární vznik spór, což je jedinečnou vlastností tohoto druhu. Preparáty z médií obohacených krevním sérem obsahují pravidelně všechna stadia životního cyklu a tak umožňují přesnou diagnostiku *D. congolensis*.

### Стандартный метод определения жизненного цикла *Dermatophilus congolensis*

Определение жизненного цикла *Dermatophilus congolensis* является основным требованием диагностики данного характера. Полное доказательство удается в определении жизненного цикла не всегда в препаратах даже при использовании питательно обильных средств для культивирования *D. congolensis*. Обогащение средств нативной кровяной сывороткой стимулирует характерную септацию гифов и интраклубулярное возникновение спор, что является уникальным свойством данного вида. Preparáty, obohacené krvavou sывороткой, содержат, как правило, все стадии цикла жизни, способствуя, таким образом, точной диагностике *D. congolensis*.

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