Heterogeneity among *Dermatophilus congolensis* isolates demonstrated by restriction fragment length polymorphisms *

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FAIBRA (D.T.). Le polymorphisme des longueurs des fragments de restriction démontre l'hétérogénéité parmi des souches de *Dermatophilus congolensis* . *Revue Élev. Méd. vét. Pays trop.*,1993, **46** (1-2) : 253-256

L'existence de différences antigéniques et de virulence entre souches de *Dermatophilus congolensis* est connue. Afin de comprendre l'épidémiologie de la dermatophilose, il est important de pouvoir différencier entre les souches du germe. On a étudié vingt souches isolées sur le terrain à partir de bovins au Tchad et au Cameroun, ainsi qu'une souche américaine de référence, sur le polymorphisme des longueurs des fragments de restriction. Après digestion de l'ADN par l'enzyme de restriction *BamHI* et blotting selon Southern, une sonde d'ADN ribosomal consistant du plasmide pMC5, porteur d'une insertion d'ADN de *Mycoplasma capricolum* de 4,8 kb codant pour les RNA ribosomaux 5S, 23S et une partie des 16S, a permis de distinguer 6 ribotypes chez *D. congolensis*, selon les profils obtenus par hybridation des ADN ribosomaux. Certains ribotypes peuvent avoir une large répartition géographique. Par ailleurs, des souches appartenant à au moins 5 ribotypes peuvent être trouvées dans un même troupeau, ce qui peut partiellement expliquer le peu de succès obtenu lors d'essais de vaccination contre la dermatophilose sur le terrain.

Mots clés : Dermatophilose - *Dermatophilus congolensis* - Souche - ADN - Enzyme de restriction - Polymorphisme enzymatique - Southern blotting - Sonde à ADN - ADN/Ribosome - Hybridation d'ADN - *Mycoplasma capricolum* - Cameroun - Tchad.

INTRODUCTION

Dermatophilus congolensis, an ubiquitous actinomycete, is the causative agent of dermatophilosis, a worldwide cutaneous disease affecting both domestic and wild animals and occasionally man. This disease causes important economic losses in humid and subhumid tropical countries, more particularly West and Central Africa, Madagascar and the Caribbean islands. Dermatophilosis is also a main problem for sheep in Australia and New Zealand.

The genus *Dermatophilus* contains only one species : *D. congolensis* (5). But strain variation is recognized through some studies. In 1975, LLOYD and OJO (7) had found by means of the agar gel precipitation reaction 5 serologically different types among 14 strains (including 7 obtained from donkeys). These strains were isolated from a dermatophilosis outbreak which occurred in Western State of Nigeria. The donkey isolates included three of these types.

More recently in 1990, in the examination of the dose-response of rabbits to *D. congolensis* infection, HOW and LLOYD (6) found a ten-fold difference in the minimum infective dose of zoospores between a Scottish ovine strain (SS 18 C) and a Caribbean bovine strain (FD 11). They also noted an increased severity of the Caribbean strain lesions at the highest dose. These authors concluded that there is a difference in virulence between those strains of *D. congolensis* (FD 11 is more virulent than SS 18 C). In a recent vaccination trial in sheep in Australia differences in terms of virulence and antibody response were demonstrated between the two ovine *D. congolensis* strains used in the study (3).

In a recent biochemical profile of 92 strains of *D. congolensis* we have revealed 7 biotypes based on 3 discriminatory biochemical tests, Gamma Glutamyl Transferase, haemolysis of red blood cells of sheep and hydrolysis of gelatin (4).

There is a need for epidemiological studies to possess and strengthen alternative methods of discriminating among *D. congolensis* strains involved in enzootic and epizootic dermatophilosis. Several molecular typing methods have in recent years gained acceptance for analysing relationships between strains of a wide variety of pathogens. Some of these techniques offer the possibility of strain differentiation at the genomic level and thus are potentially very powerful epidemiologic tools (8). Genes coding for ribosomal ribonucleic acid (rRNA) are among the most conserved genes in procaryotic cells.

The restriction fragment length polymorphism (RFLP) of DNA fragments containing rRNA genes for *D. congolensis* isolates might serve as a distinguishing criterion to investigate the epidemiology of this microorganism.

In this study we show differences between 20 *D. congolensis* strains based on the hybridization pattern of DNA samples cleaved by endonucleases.

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MATERIALS AND METHODS

Dermatophilus congolensis isolates

The designation, the geographic origins and the dates of isolation of 20 field strains and the reference strain ATCC 14637 of *D. congolensis* tested are shown in table I. The field strains were collected from outbreaks of dermatophilosis occurring in some herds of northern Cameroon and southern Chad from 1986 to 1991. All of the field strains were isolated from skin lesions of zebu cattle affected by epizootic dermatophilosis.

TABLE I List of **Dermatophilus congolensis** *strains used in the study.*

No	Strain	Source
1	Dis. 1	isolated in 1989 from Dissing herd (Chad)
2	Dis. 2	idem
3	Dis. 5	idem
4	Dis. 7	idem
5	Dis. 16	isolated in 1990 from Dissing herd (Chad)
6	Dis. 11b	idem
7	N2	isolated in 1990 from Ngara herd (Chad)
8	N20	idem
9	NG18	isolated in 1990 from Ngondon herd (Chad)
10	D4	isolated in 1989 from Doué herd (Chad)
11	D8	idem
12	NG16	isolated in 1990 from Ngondon herd (Chad)
13	NG12	isolated in 1989 from Ngondon herd (Chad)
14	D22	isolated in 1990 from Doué herd (Chad)
15	F3004	isolated in 1988 from Chari-baguirmi (Chad)
16	ATCC* 14637	reference strain
17	G2	isolated in 1987 from Garoua (Cameroon)
18	NAS32	isolated in 1988 from Nassarwo (Cameroon)
19	W8588	isolated in 1985 from Wakwa (Cameroon)
20	W8753	isolated in 1987 from Wakwa (Cameroon)
21	W9028	isolated in 1990 from Wakwa (Cameroon)

* ATCC : American Types Cultures Collection.

Cultivation of the strains

After isolation, all strains were cloned. Growth of *D. congolensis* for DNA isolation was in tryptose broth with horse serum incubated at 37 °C with shaking and stirring with glass beads. *D. congolensis* cells were harvested after 72 h growth by centrifugation and the pellets were washed once and used for DNA extraction.

DNA extraction

DNA was extracted by lysis of bacterial pellets with lysozym and SDS. Then a standard phenol chloroform extraction with ethanol precipitation was repeated twice.

DNA yield was determinated by spectrophotometric analysis. The DNA concentration of the 21 *D. congolensis* purified DNA samples varied from 0.5 to 3 µg/µl.

Restriction enzyme digestion of DNA and separation of fragments

2 µg of each DNA sample were digested with the following restriction endonucleases : *BamHI*, *EcoRI* and *PstI*.

Digested DNA fragments were separated electrophoretically on agarose gel stained with ethidium bromide and transferred to a nylon filter by the method of SOUTHERN (10).

DNA blots were probed with plasmid pMC5 carrying a 4.8 kilobases insert of *Mycoplasma capricolum* DNA coding for the 5S, 23S, and part of 16S rRNAs (1). Cloned rDNA probes were labelled by using the "random priming" method.

The blots were hybridized, washed, air dried and exposed to X Ray films. Lambda DNA digested by *PvuII* served as molecular weight markers.

RESULTS AND DISCUSSION

In an attempt to find an enzyme which gave a reasonable number of well-separated bands, we examined the restriction patterns generated by *EcoRI*, *PstI* and *BamHI*.

The result with *BamHI* was most encouraging (fig. 1). With most *D. congolensis* strains, this enzyme gave two or three fragments hybridizing with the probe. The strains were grouped in 6 ribotypes according to their hybridized rDNA patterns. The diagrams of each of the 6 ribotypes observed within the isolates were shown in figure 2.

The ribotype 1 is distributed in almost all of the herds from which *D. congolensis* strains were collected (table II). The herds were about 100-200 km distant from each other. Five of the 6 ribotypes (ribotypes 1, 2, 3, 4 and 6) were found in the Dissing herd. Heterogeneity between the *D. congolensis* strains in the same flock is important. These findings agree with the previous observations of LLOYD and OJO in 1975 (7) showing variation of *D.congolensis* strains in the field. In their study, they observed that 3 of the 5 identified *D. congolensis* serogroups occurred in the same outbreak of dermatophilosis in donkeys.

These findings may in part explain the failure of field vaccination trials carried out in the past in Chad by PROVOST et al. (9) and CHENEAU (2) and more recently in Australia by ELLIS *et al.* (3).

In conclusion, this study shows that ribotyping can be a valuable tool for characterization of *D. congolensis*. This

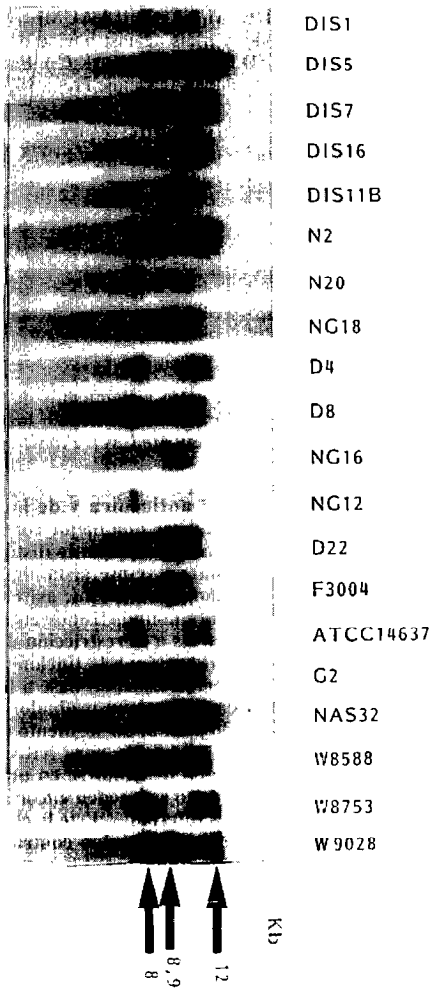

Figure 1 : RFLP patterns of Dermatophilus congolensis *strains studied.*

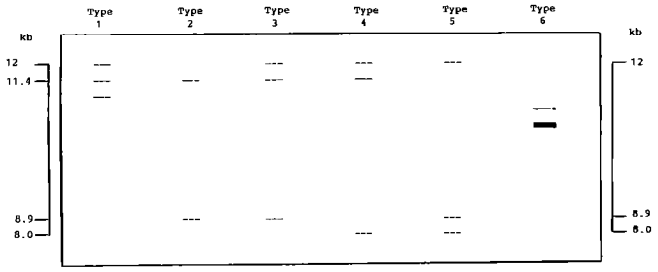

Figure 2 : Pattern of each of the 6 identified ribotypes of Dermatophilus congolensis.

genomic fingerprinting technique emphazises DNA restriction site heterogeneity between isolates, a characteristic presumably more stable than those studied by traditional phenotypic characterization techniques such as serotyping and biotyping.

TABLE II Ribotype grouping of **Dermatophilus congolensis** *strains.*

Ribotype groups	Composition
Ribotype 1 (6 strains)	Dis16, Ng18, Ng16, D22, G2 and Nas32
Ribotype 2 (2 strains)	Dis1, N20
Ribotype 3 (4 strains)	Dis5, D4, Ng12, ATCC14637
Ribotype 4 (4 strains)	Dis7, D8, W8588, W8753
Ribotype 5 (2 strains)	N2, W9028
Ribotype 6 (2 strains)	Dis11b, F3004

In particular ribotyping compares highly conserved rRNA genes and their adjacent sequences, genes not subject to frequent mutation.

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FAIBRA (D.T.). Heterogeneity among *Dermatophilus congolensis* isolates demonstrated by restriction fragment length polymorphisms. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 253-256

There is evidence of antigenic diversity and of differences in virulence in *Dermatophilus congolensis*. For the understanding of the epidemiology of dermatophilosis it is important to distinguish between strains of the organism. Twenty field isolates from cattle in Chad and Cameroon, and an American reference strain, have been examined on restriction fragment length polymorphisms. After restriction enzyme digestion of DNA by *BamHI* and Southern blotting, a rDNA probe consisting of plasmid pMC5 carrying a 4.8 kb insert of *Mycoplasma capricolum* DNA coding for the 5S, 23S and part of 16S rRNA allowed to distinguish 6 ribotypes of *D. congolensis*, based on their hybridized rDNA patterns. Particular ribotypes may be distributed over a wide geographical area. On the other hand, strains belonging to at least 5 different ribotypes may be found in one herd; this may partly explain the lack of success in immunization against dermatophilosis in the field.

Key words : Dermatophilosis - *Dermatophilus congolensis* - Strain - DNA - Restriction enzyme - Enzyme polymorphism - Southern blotting - DNA probe - Ribosomic DNA - DNA Hybridization - *Mycoplasma capricolum* - Cameroon - Chad.

FAIBRA (D.T.). Heterogeneidad entre aislamientos de *Dermatophilus congolensis*, demostrada mediante polimorfismos en los fragmentos de restricción de la longitud. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 253-256

Se ha demostrado la diversidad antigénica y de la virulencia de *Dermatophilus congolensis*. Para lograr una mejor comprensión de la epidemiología de la dermatofilosis, es importante distinguir entre las cepas del organismo. Se examinaron veinte aislamientos de campo provenientes de ganado de Chad y Camerún, así como una cepa Americana de referencia, mediante polimorfismos en los fragmentos de restricción de la longitud. Gracias a la restricción de la enzima de digestión de ADN por parte del *BamHI* y a la tinción "Southern", se identificaron 6 ribotipos de *D. congolensis*, en base a los patrones de hibridación del ADNr. El test consiste en un probador de ADNr, que transporta mediante un plásmido pMC5, un segmento de ADN codificado de *Mycoplasma capricolum* de 4,8 kb, para ARNr de 5S, 23S y parte del 16S. Los ribotipos pueden distribuirse en una amplia zona geográfica. Por otro lado, en un hato se pueden encontrar cepas provenientes de hasta cinco ribotipos diferentes. Este hecho podría explicar parcialmente el fracaso de la inmunización contra dermatofilosis en condiciones de campo.

Palabras claves : Dermatofilosis - *Dermatophilus congolensis* - Cepa - ADN - Enzyma de restrición - Polimorfismo enzimatico - Southern blotting - Sonda de ADN - ADN Ribosomico - Hibridación de ADN - *Mycoplasma capricolum* - Camerún - Chad.