



Mycetoma

A neglected disease



SEMTSI meeting Bilbao

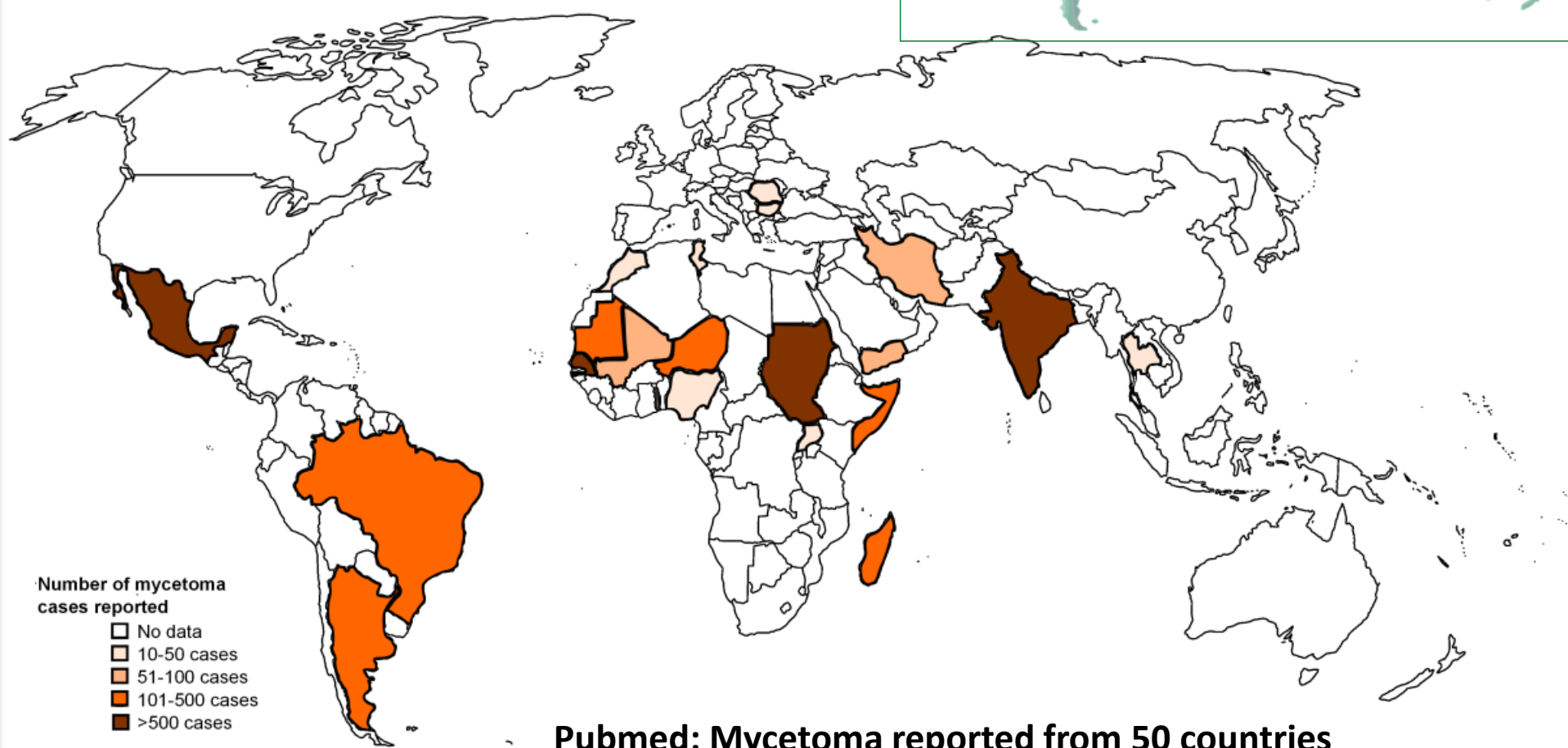
24 October 2017

Ed Zijlstra

DNDi Geneva



Between latitude 30° North and 15° South



Pubmed: Mycetoma reported from 50 countries

DN
Dru



Mycetoma

Definition: **chronic infection of subcutaneous tissues**

Actinomycetoma
(*bacteria*)

Nocardia brasiliensis
Nocardia asteroides
Streptomyces somaliensis
Actinomadura madurae
Actinomadura pelletieri

Eumycetoma
(*fungi*)

Madurella mycetomatis
Falciformispora senegalensis
Trematosphaeria grisea
Scedosporium apiospermum
Acremonium spp.

Mycetoma

Definition: **chronic infection of subcutaneous tissues**

Actinomycetoma
(*bacteria*)

Eumycetoma
(*fungi*)

Similar presentation

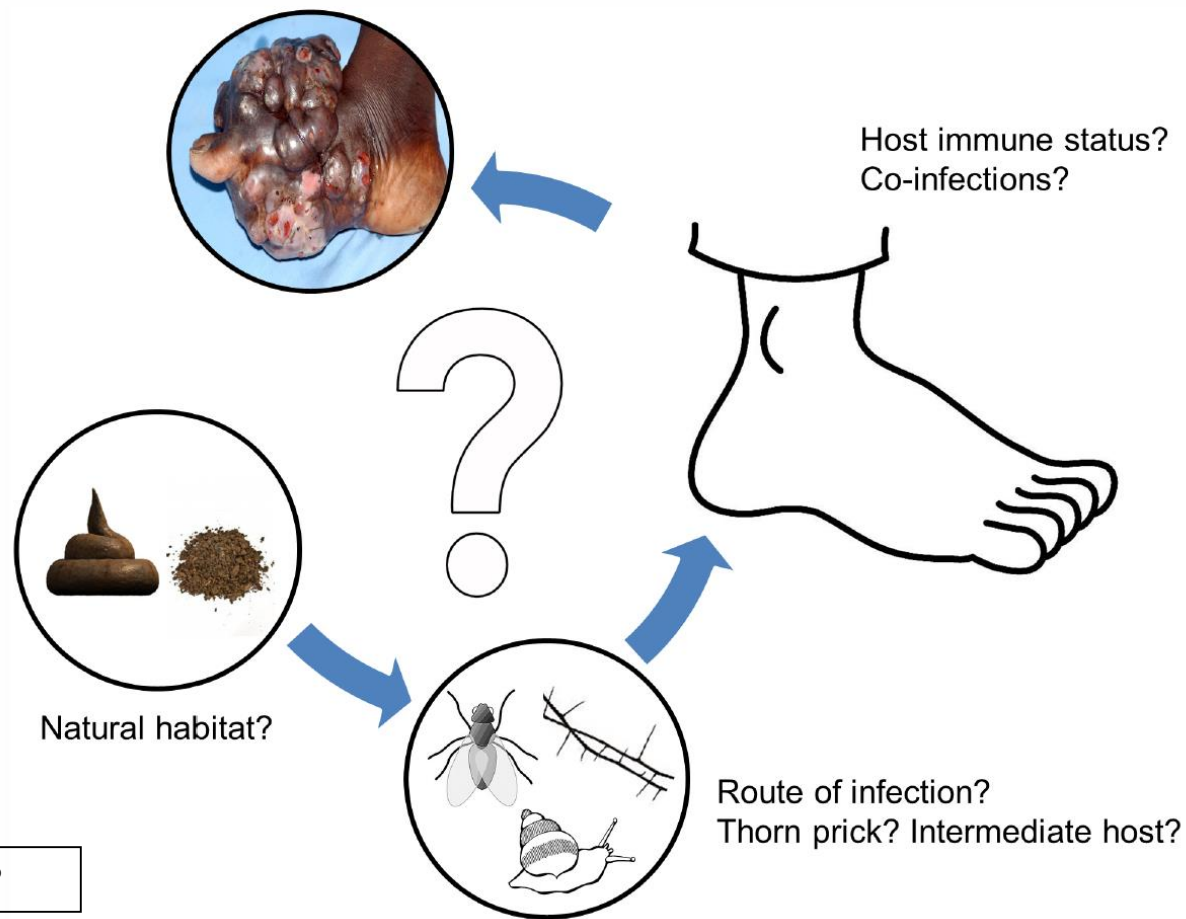
More aggressive

Treatment with antibacterial agents

Slow progression

Treatment with antifungals

The mode of transmission



van de Sande, 2013



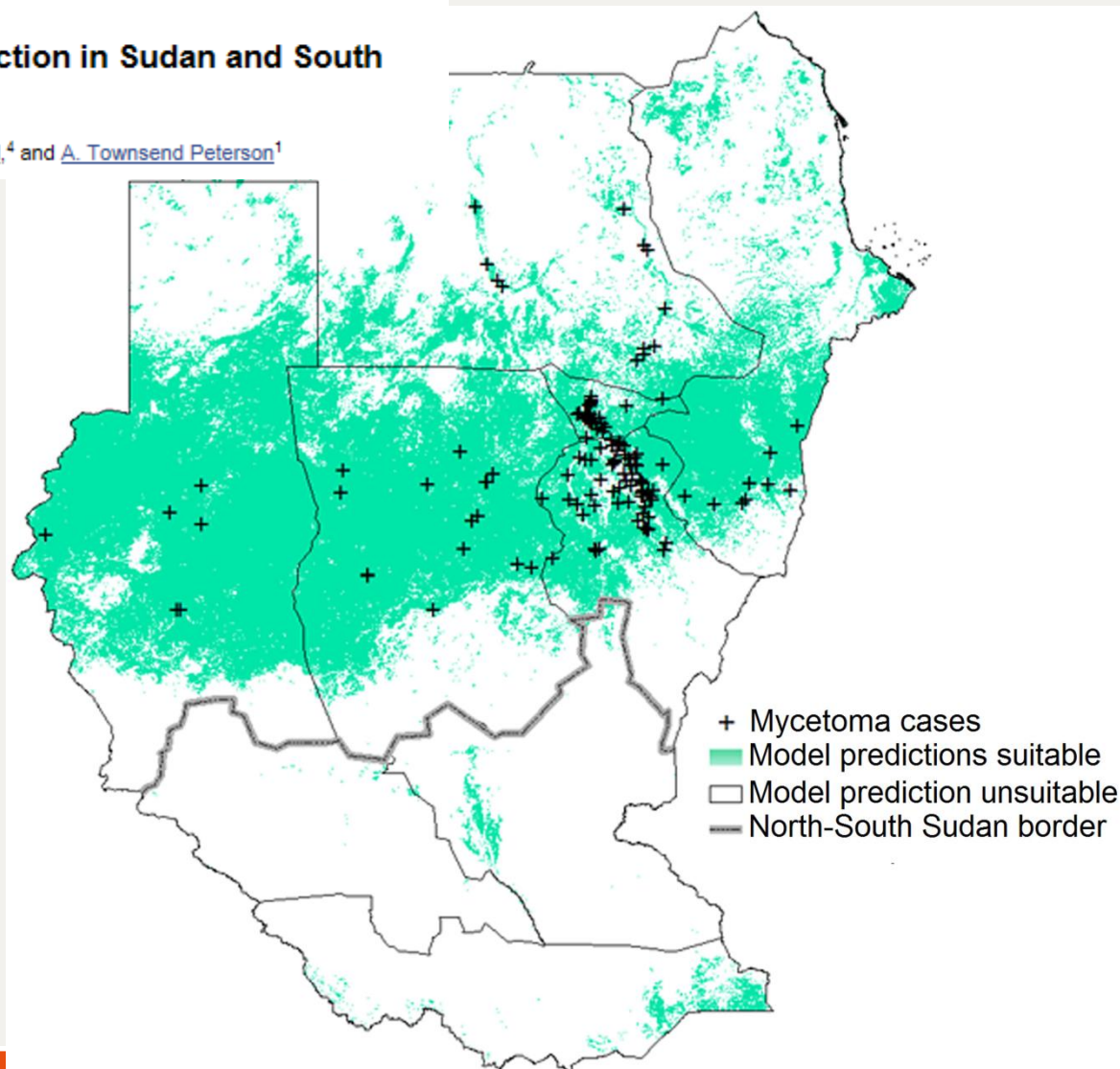
Epidemiology White Nile State Sudan

- Transmission:
 - Thorn prick, (micro-)trauma
 - Most cases on feet, but not exclusively
 - People often barefooted
- Reservoir: unknown
 - Plants, soil, animal dung, other?



Mapping the Potential Risk of Mycetoma Infection in Sudan and South Sudan Using Ecological Niche Modeling

Abdallah M. Samy,^{1,2,*} Wendy W. J. van de Sande,³ Ahmed Hassan Fahal,⁴ and A. Townsend Peterson¹



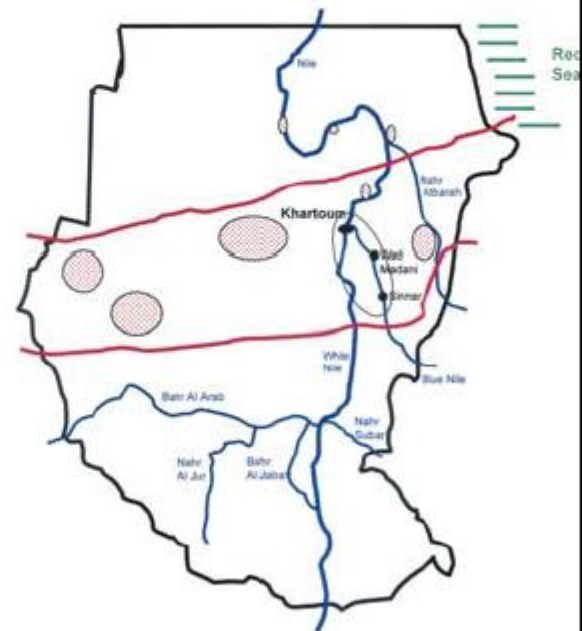
The mode of transmission

- Natural habitat → soil?
- Culture:
 - *M. mycetomatis* was cultured from Indian soil in 1968

Thirumalachar et al. Hindustan Antibiot Bull. 1968

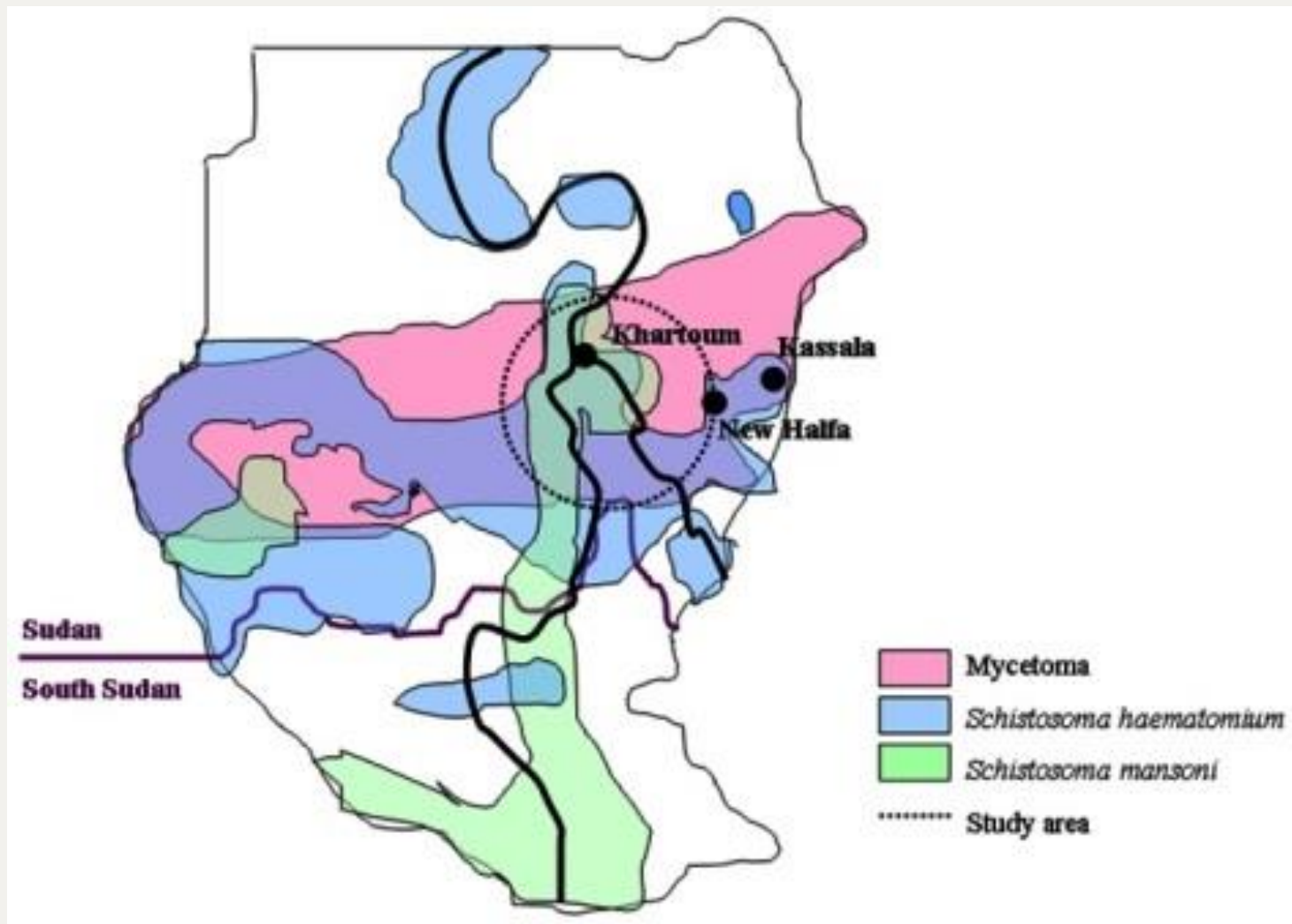
- PCR:
 - 17/74 soil samples positive for *M. mycetomatis*
 - 1/22 thorn samples positive for *M. mycetomatis*

Ahmed et al. J. Clin Microbiol. 2002

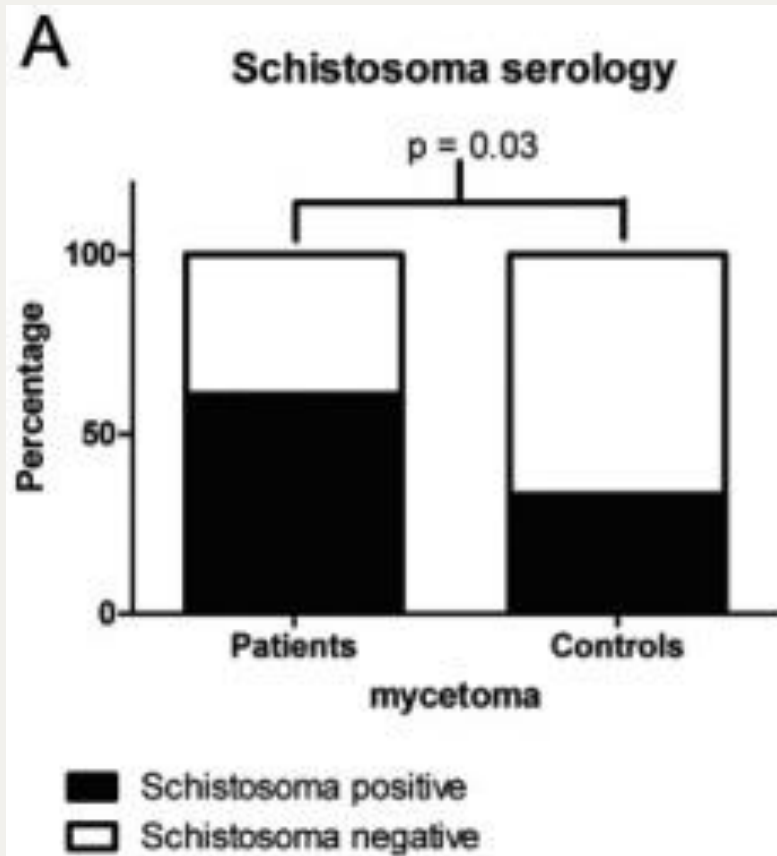


Erasmus MC

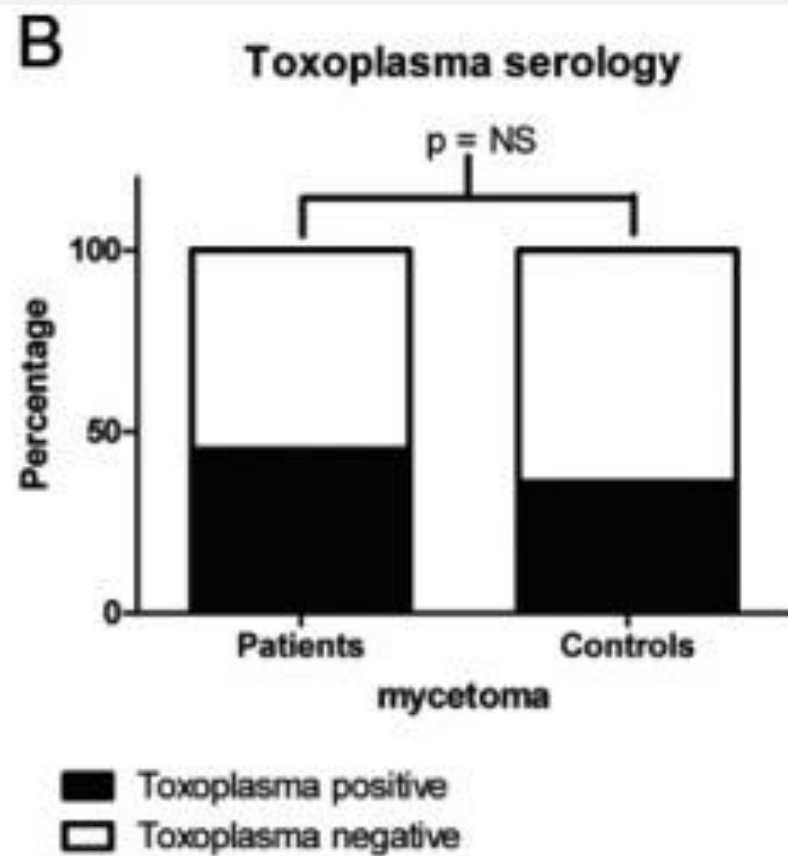
Кэри



N=53



N=27



Van Hellemond, PLoSNTD 2013



Spread along the lymphatics



Mycetoma diagnosis

- Clinical
- Microbiological
- Serological
- Radiological

Mycetoma diagnosis

- Clinical
 - subcutaneous mass
 - foreign body, ganglion, fibroma, neurofibroma, rhabdomyosarcoma, Kaposi's sarcoma, sporotrichosis, lobomycosis, etc
 - sinuses
 - tuberculosis
 - malignancy
 - discharge – grains colour

Mycetoma diagnosis – clinical: grains colour

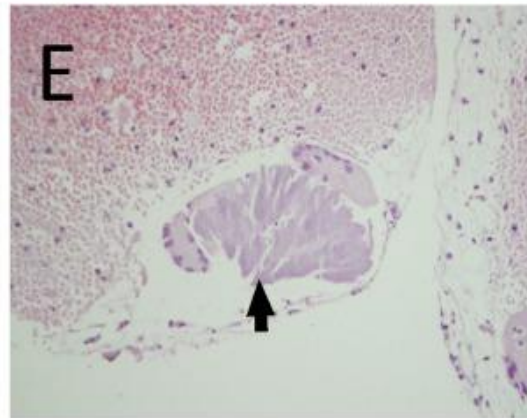
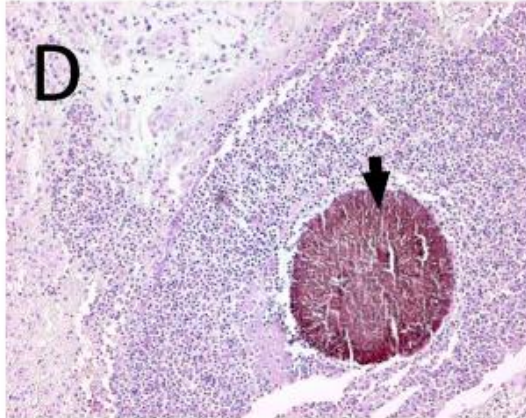
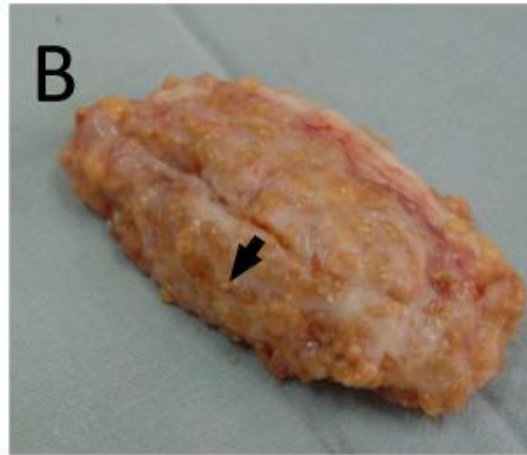
- Black
 - *Madurella mycetomatis*
 - *Trematospheria grisea*
 - *Exophiala jeanselmei*
 - *Medicopsis romeroi*
 - *Falciformispora senegalensis*
 - *Curvularia lunata*
- Red
 - *Actinomyces pelletieri*
- Yellow-brown
 - *Nocardia brasiliensis*
 - *Nocardia otiditiscaviarum*
 - *Actinomadura madurae*
 - *Streptomyces somaliensis*
- Yellow
 - *Pleurostomophora ochracea*



Grains are easily demonstrated either by spontaneous discharge or by FNA



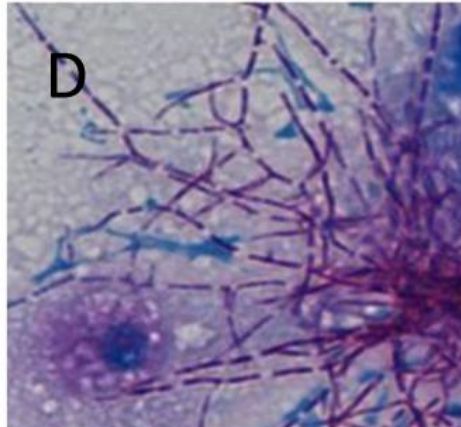
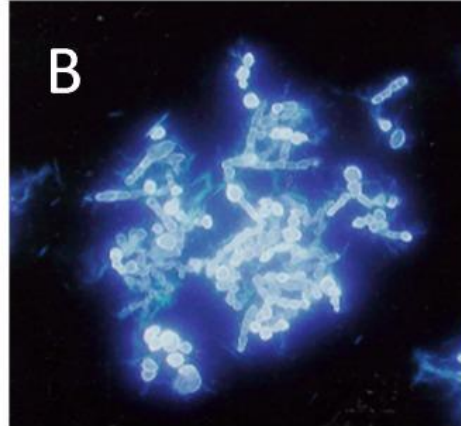
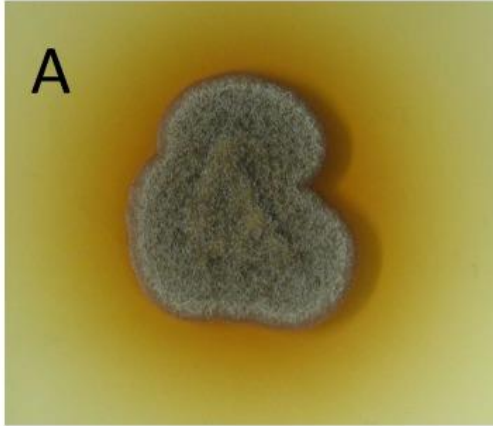
Microbiological diagnosis – grains



- A. Black grains - *M. mycetomatis*
- B. Yellow grains – *S. somaliensis*
- C. *M. mycetomatis* – in formalin
- D. *M. mycetomatis* – grain in tissue
- E. *S. somaliensis* – grain in tissue

van de Sande, PLoS NTD 2014

Microbiological diagnosis – culture: macroscopic, microscopic



Disadvantages

- Time consuming
- Contamination
- Experience needed

Misclassifications occur!

A. *M. mycetomatis*

B. *M. mycetomatis*, microscopic calcofluor white stain

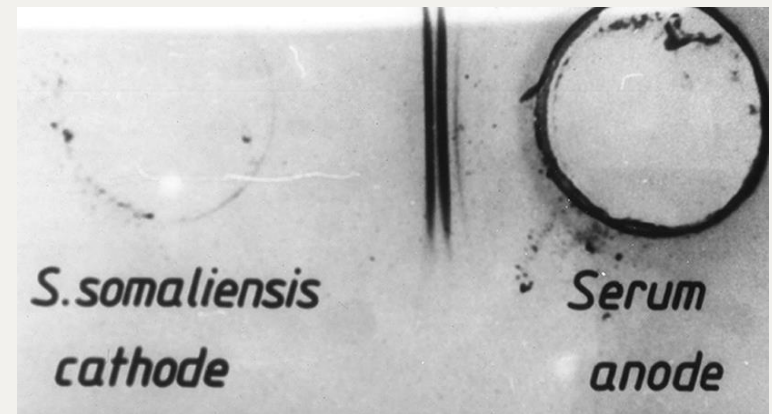
C. *Nocardia brasiliensis*

D. *N. brasiliensis*, microscopic

van de Sande, PLoS NTD 2014

Serodiagnosis

- Actinomycetoma

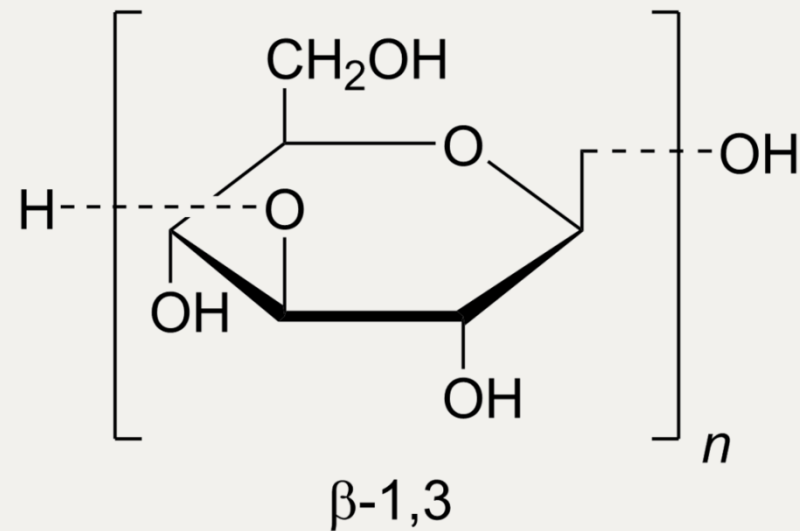


Photograph showing a counterimmunoelectrophoresis test with positive bands.

- Eumycetoma
 - Crude antigens
 - Recombinant antigens
 - No distinction between patients and endemic controls

Serology

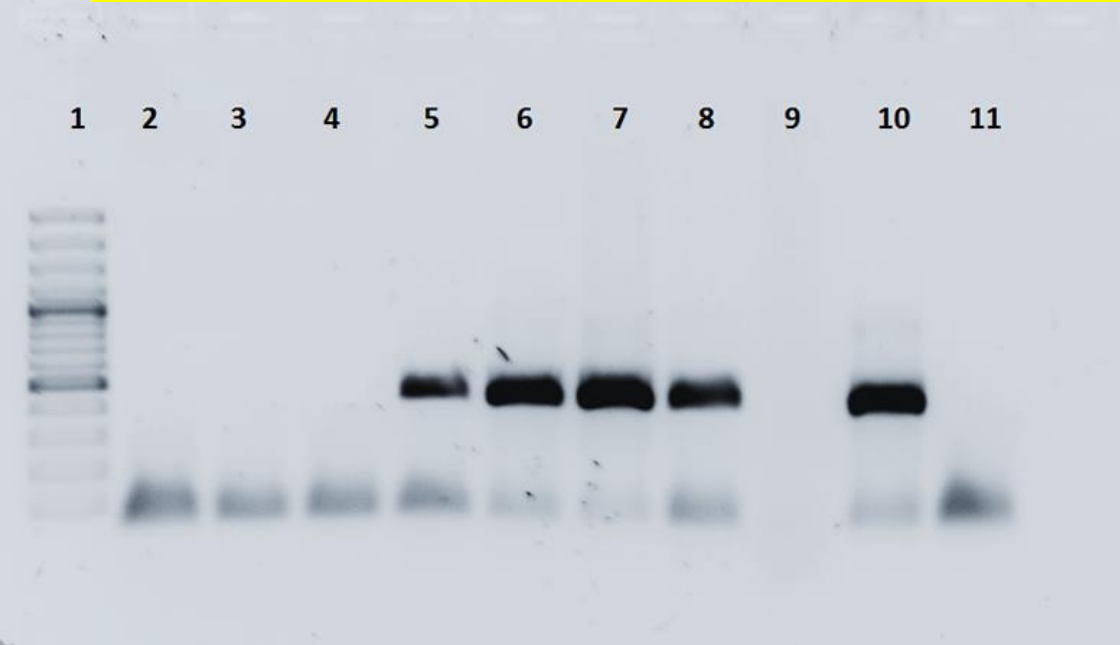
- Antigen
 - β -D-glucan
 - Fungus cell wall
 - Not specific
 - Commercially available



Molecular diagnosis

- **Standard:** Amplification of ITS region and sequencing
 - Expensive equipment needed:
 - Thermocycler
 - Sequencer
 - Time consuming

PCR restriction fragment length polymorphism (RFLP) analysis



Ahmed AO. J Clin Microbiol 1999

Lane 1 contain a 100 bp DNA ladder.

Lanes 2 to 4: PCR products for three samples which were negative for *Madurella mycetomatis*.

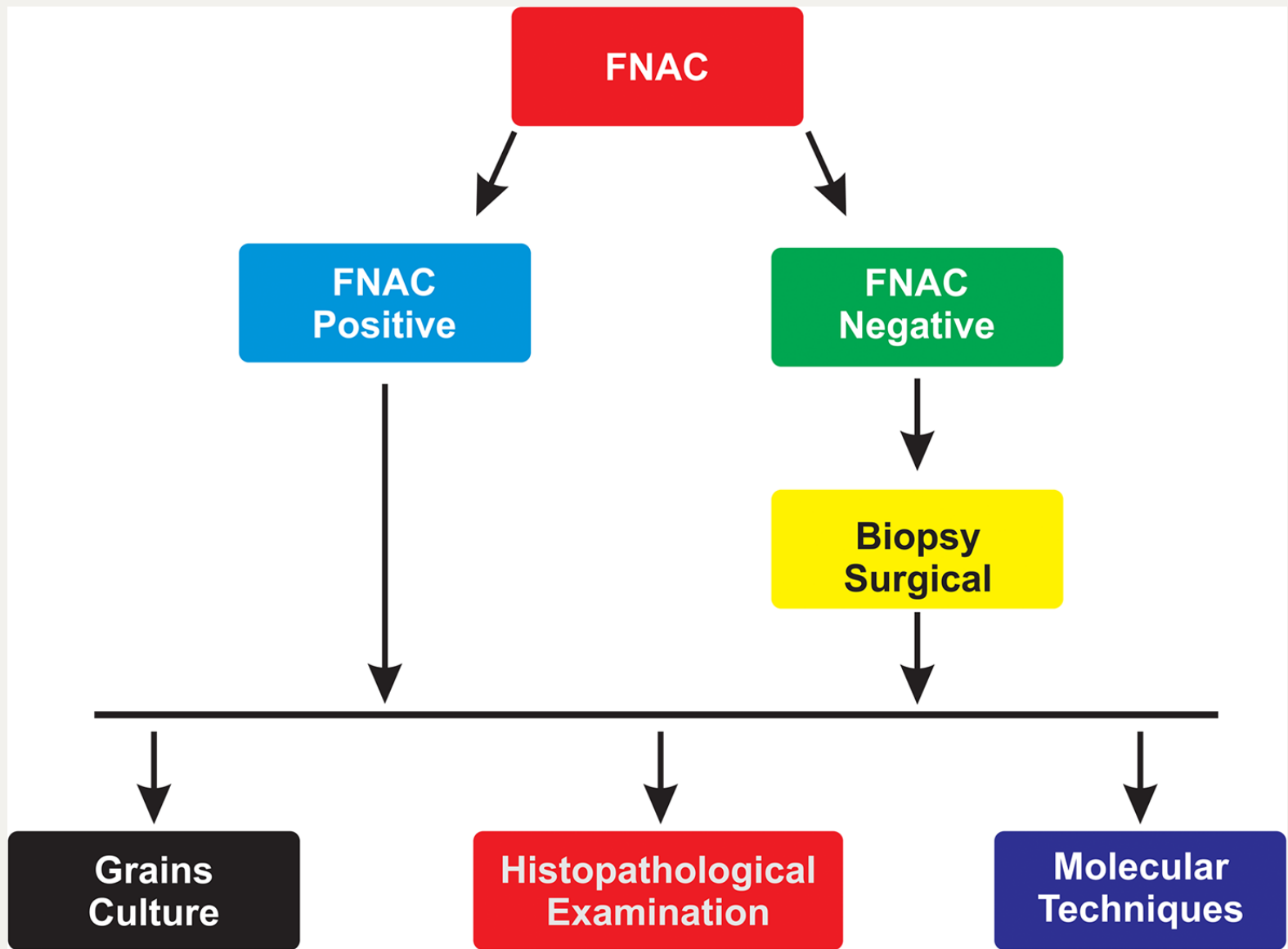
Lane 5 to 8 :PCR products for four samples which were positive for *Madurella mycetomatis*.

Lane 10 : positive control

Lane 11 : negative control

Molecular diagnosis

- **Isothermal amplification** to identify black grain causative agents of mycetoma
 - Loop-mediated isothermal amplification (LAMP)
 - Recombinase polymerase amplification (RPA)
- **Genome sequencing**
 - Smit 2016 : *M. mycetomatis* strain
 - Lucio Vera-Cabrera 2014 : *Actinomadura* strain



Ahmed AA. PLoS NTD 2017

Key characteristics of eu- and actino-mycetoma

	Eumycetoma	Actinomycetoma
Causative agent	Fungi	bacteria
Main endemic area	Africa	Middle- and South America
Treatment	Antifungal + surgery	antibiotics
Current regimen	Ketoconazole	amikacin (IV) + cotrim (PO)



U.S. Food and Drug Administration
Protecting and Promoting Your Health

Drug Safety Communications

FDA Drug Safety Communication: FDA limits usage of Nizoral (ketoconazole) oral tablets due to potentially fatal liver injury and risk of drug interactions and adrenal gland problems

or itraconazole
12 Months + mass removal

Cure Rate

37% → 25.9%

> 90% (in Mexico)

DN

No randomized trials

	Organism (number of cases)	Dose	Outcome	Country
Ketoconazole ¹²⁹	<i>Madurella mycetomatis</i> (13 [8 from Sudan and 5 from Saudi Arabia])	200–400 mg once a day; median treatment duration is 13 months (range 3–36 months)	5 cured and 4 improved	Sudan and Saudi Arabia
Ketoconazole ¹³⁰	<i>M mycetomatis</i> (50)	200 mg twice a day for 3–36 months	36 (72%) were cured or had notable improvement; 10 (20%) had some improvement; 4 (8%) had no response or deteriorated	Sudan
Ketoconazole ¹³¹	<i>M mycetomatis</i> (4), other (4)	400 mg once a day for 8–24 months	6 cured, no recurrence after 3 months (2 years follow-up); 2 improved	India
Itraconazole ¹³²	<i>M mycetomatis</i> (13)	200 mg twice a day for 3 months, then 200 mg once for 9 months	12 improved and cured after 3 months; 1 recurrence	Sudan
Terbinafine ¹³³	<i>M mycetomatis</i> (10), <i>Falciformispora senegalensis</i> (3), other (3), not known (7)	500 mg twice a day for 24–48 months	11 improved	Senegal
Voriconazole ¹³⁴	<i>Scedosporium apiospermium</i> (1)	400 mg twice daily for 12 months	Cured	Côte d'Ivoire
Voriconazole ¹³⁵	<i>S apiospermium</i> (1)	200 mg twice daily for 12 months duration	Cured	India
Voriconazole ¹³⁵	<i>Trematosphaeria grisea</i> (1)	200 mg twice daily for 6 months duration	Little change	India
Voriconazole ¹³⁶	<i>M mycetomatis</i> (1)	200 mg twice daily for 3 months, then 300 mg twice daily for 13 months	Cured	Mali
Voriconazole ¹³⁷	<i>Madurella</i> spp (1)	200 mg twice daily for 12 months	Cured	Senegal
Voriconazole ¹³⁸	<i>S apiospermium</i> (1)	200 mg twice a day; unknown duration	Cured (after 3 years follow-up)	Brazil
Posaconazole ¹³⁹	<i>M mycetomatis</i> (2), <i>T grisea</i> (3), <i>S apiospermium</i> (1)*	400 mg twice daily for a maximum of 34 months	Initially 5 were cured and 1 had no improvement; 2 were successfully retreated after interval of >10 months	Brazil
Liposomal amphotericin B ¹³⁰	<i>T grisea</i> (2), <i>Fusarium</i> spp (1)	Total dose 3–4 g and 2–8 g (<i>T grisea</i> cases), and 4–2 g (<i>Fusarium</i> spp case); maximum daily dose is 3 mg/kg	All showed temporary improvement but relapsed within 6 months	Not specified

*All refractory cases.

Table 3: Treatment of eumycetoma in endemic cases and immunocompetent patients

Zijlstra et al. Lancet ID 2016

Determinants of clinical response

- **Host**
 - spectrum
 - size, duration
 - immune response
 - co-infection; super-infection
- **Fungus**
 - type
 - melanin



Blood supply and vasculature in advanced mycetoma

- Arteriography (n=9)
 - Normal femoral, popliteal, tibial arteries
 - Circulation brisk and excessive (in contrast to tumours)
 - Early venous filling, increased veins
 - No AV shunts
- Doppler (n=60)
 - No difference in BP between affected and normal limb (ankle-brachial index)
- Histology (n=30)
 - Endarteritis obliterans in 7% (low in granulomatous disease)
 - Arteritis 7%
 - No thrombosis, fibrinoid necrosis, ischaemia or invasion of vessels by microorganisms



Fahal et al. J Med Vet Mycology 1997



Post-operative mycetoma surgical specimen (H&E x 40)

- colonies of black grains
- surrounded by thick fibrous capsule
- intensive fibrosis

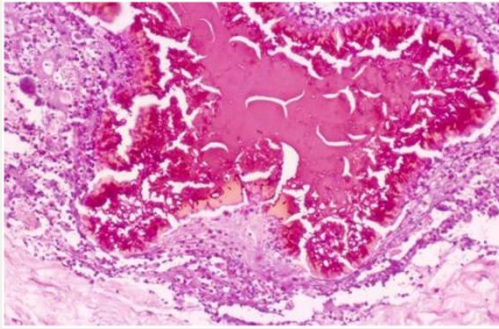
Sudan: study with itraconazole: n=13

Treatment for 12 months

1 cured

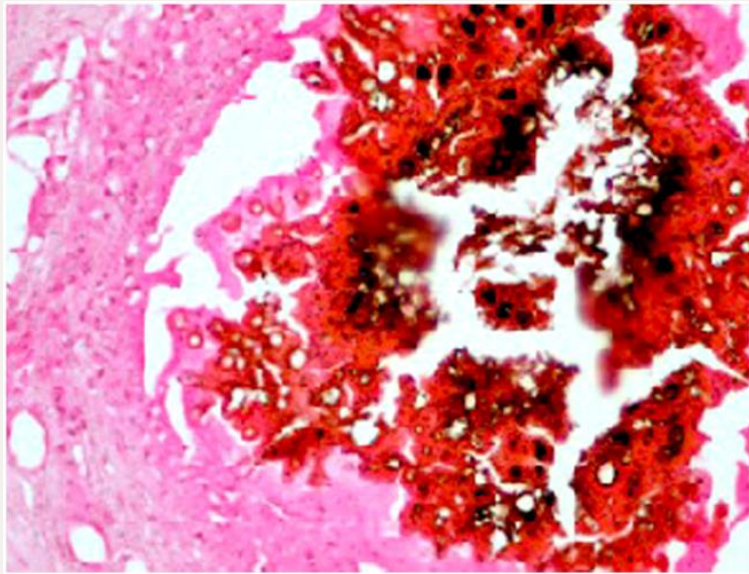
9 partial response

Fahal et al. TRSTMH 2010

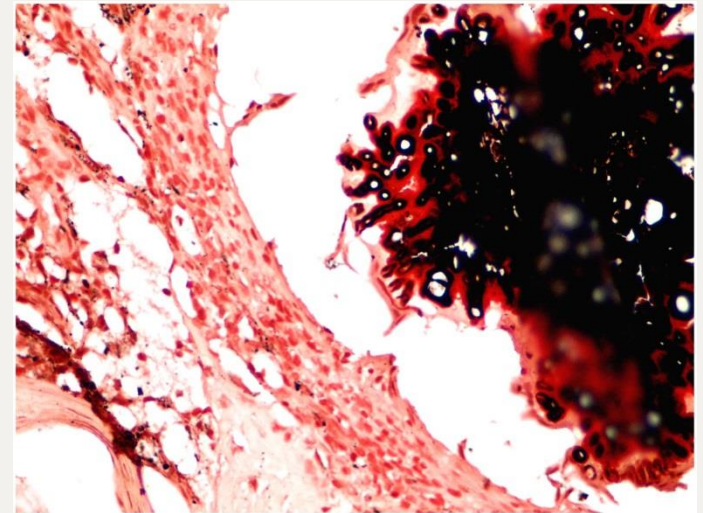


HE stain

Rich in lipid, protein, Zn, Cu, Ca and melanin



Calcium stain



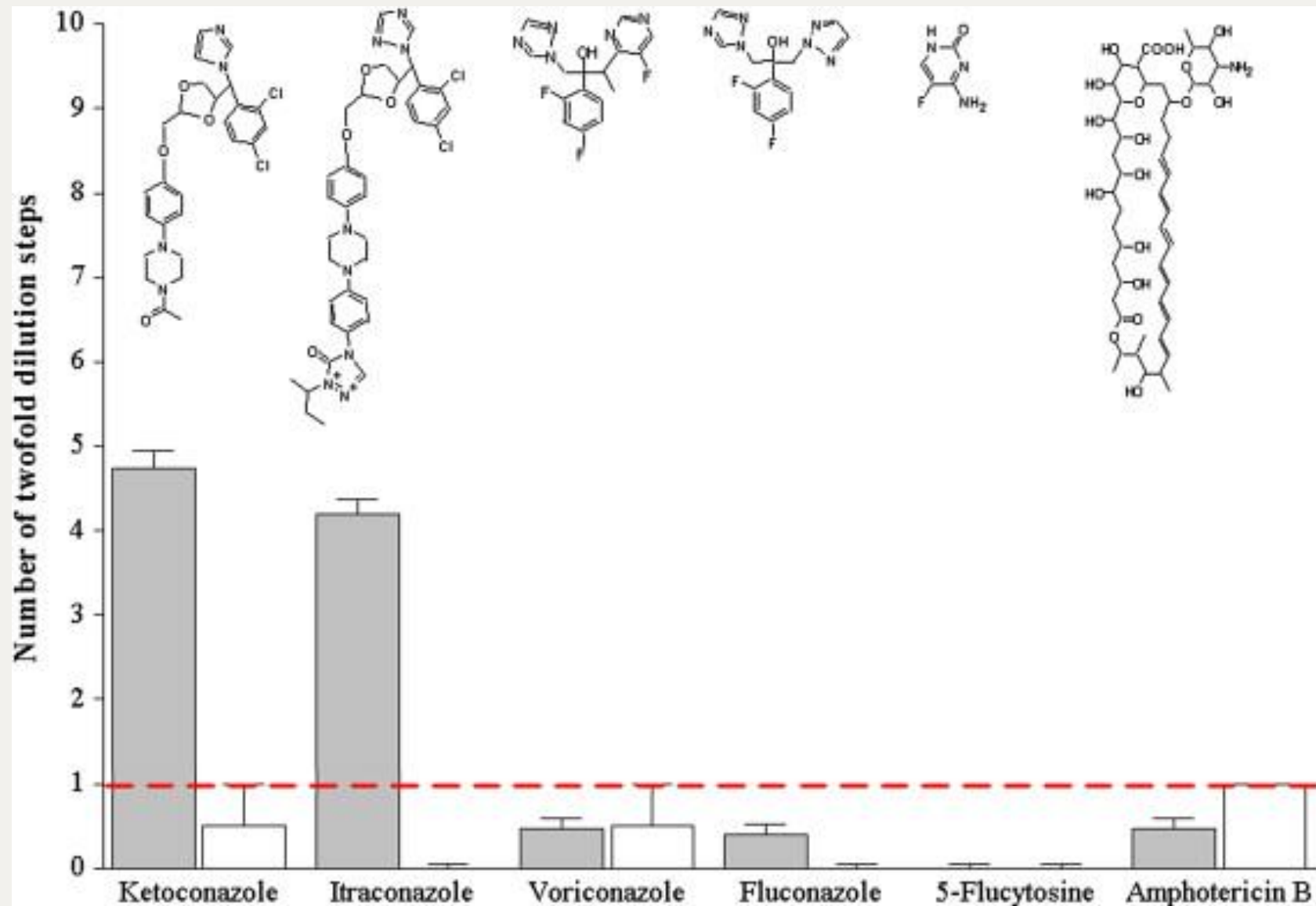
Melanin stain

A Histopathological Exploration of the *Madurella mycetomatis* Grain

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¹ Faculty of Medical Laboratory Sciences - University of Khartoum, Khartoum, Sudan, ² Mycetoma Research Center, University of Khartoum, Sudan, Khartoum, Sudan,

³ Erasmus MC, Department of Medical Microbiology & Infectious Diseases, Rotterdam, The Netherlands



MIC shift with melanin from *M. mycetomatis* (grey) and melanin from *C. neoformans* (white)
 From: vd Sande, Microb Infect 2007)

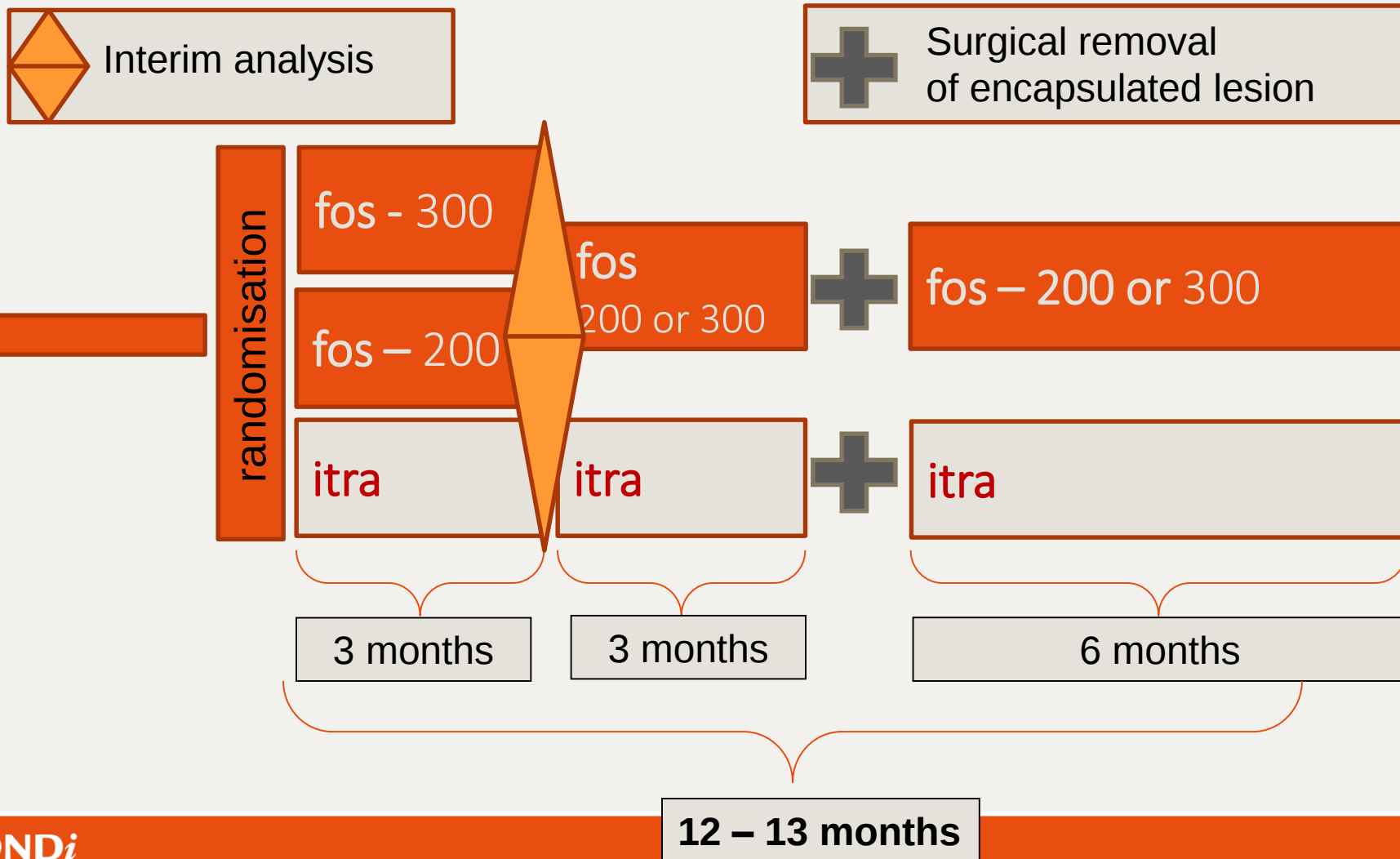
M. mycetomatis

Antifungal susceptibility

Antifungal	MIC ₅₀ (range) µg/ml	Antifungal	MIC ₅₀ (range) µg/ml
Ketoconazole	0.125 (<0.01-1)	Amphotericin B	2 (<0.01-4)
Itraconazole	0.06 (<0.01-0.5)	Terbinafin	8 (1->16)
Posaconazole	0.06 (<0.03-0.125)	5-flucytosine	>128 (<128)
Fluconazole	16 (0.25->128)	Caspofungin	128 (16->128)
Voriconazole	0.125 (<0.01-1)	Anidulafungin	>128 (0.5->128)
Isavuconazole	0.06 (<0.01-0.125)	Micafungin	>128 (8->128)
Ravuconazole	0.004 (<0.002-0.03)		

Teafung

Study design: «drop the loser»



Alternative drugs

	MIC		
	range	50%	90%
Artemisinin	0.03->16	>16	>16
Tea tree oil	0.008-0.25	0.06	0.25
Itraconazole	< 0.002-0.06	0.008	0.03

vd Sande, JAC 2007

NSAIDs

as adjuvant therapy to antifungals

influence inflammation – cytokine profile; Th2 → Th1

Dupont, LID 2016

Secondary bacterial infection



63% have secondary bacterial infection
of those: 56% *S. aureus*; 34% *S. pyogenes*, 10% *P. mirabilis*

Ahmed et al. J Clin Microbiol 1998

Conclusion and Priorities

- No alternatives for azoles in eumycetoma
- Global burden
- Collection of strains
 - *in vitro* sensitivity
- Itraconazole generic formulations
- (Open access) drug discovery including medicinal plants