Emergomyces africanus in Soil, South Africa

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We detected *Emergomyces africanus*, a thermally dimorphic fungus that causes an HIV-associated systemic mycosis, by PCR in 18 (30%) of 60 soil samples from a wide range of habitats in South Africa. Direct and indirect culture techniques were unsuccessful. Experimental intraperitoneal inoculation of conidia induced murine disease.

The newly described thermally dimorphic fungal genus *Emergomyces* comprises human pathogens that cause systemic mycoses in immunocompromised persons globally (*1*). Among these fungi, *Emergomyces africanus* (formerly *Emmonsia* sp. [*2*]) is the species responsible for the most human disease. HIV-associated emergomycosis is the most common endemic mycosis in South Africa and is associated with a high case-fatality ratio (*3*,*4*).

Although an environmental reservoir for *Es. africanus* has not been established, soil is presumed to harbor the mycelial phase (*2*). We tested soils in South Africa for *Es. africanus* by using molecular- and culture-based methods.

The Study

We collected 60 soil samples from various soil habitats around South Africa by convenience sampling; 82% percent of samples came from the Western Cape Province, with the remaining samples from Gauteng (7%), Eastern Cape (7%), KwaZulu-Natal (2%), and Northern Cape (2%) provinces. For each sample, we used sterile, plastic tubes to collect ≈100 mL of topsoil.

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We extracted DNA from soil by using the ZR Soil Microbe DNA Miniprep Kit (Zymo Research, Irvine, CA, USA). DNA extraction was successful for 56 soil samples (93%). We subjected extracted genomic DNA (gDNA) to a nested PCR. To amplify the internal transcribed spacer (ITS) region of the ribosomal RNA, we used the universal primers ITS1 and ITS4 in the first reaction (*5*). We used an Applied Biosystems 2720 Thermal Cycler (Foster City, CA, USA); thermocycling conditions consisted of 95°C for 5 min, 30 cycles of 95°C for 30 s, 52°C for 30 s, 72°C for 45 s, and 72°C for 7 min. We subjected PCR products to amplification by using *Es. africanus*–specific primers (forward, 5′-CCTGGTTTGGGGAGAGGGGT-3′; reverse, 5′-CCGGGGGAGCTCTTGGCTCT-3′), followed by electrophoresis on a 2% agarose gel. We performed amplification as described, except with an annealing temperature of 57°C. PCR mixtures consisted of 10 µL 2× KAPA Taq ReadyMix (KAPA Biosystems, Wilmington, MA, USA); 1 µL of each primer (10 µmol/L; Inqaba Biotechnical Industries, Pretoria, South Africa); and 1 µL of extracted gDNA or ITS PCR product, in a final reaction volume of 20 µL. We sequenced amplified products and compared them using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The PCR could detect as few as 10^2–10^4 conidia/10 g of soil (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/2/17-1351-Techapp1.pdf).

We plotted results of molecular testing and residential postal codes of persons with confirmed infections (Figure 1). We detected *Es. africanus* DNA in 18 (32%) of 56 soil samples representing all types of soil habitats tested (Table).

We used soil dilution plates prepared with Sabouraud agar (40 g/L glucose [Merck, Darmstadt, Germany], 10 g/L peptone [Merck], and 15 g/L agar) supplemented with 0.2 g/L chloramphenicol (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) to culture *Es. africanus* from 4 randomly selected soil samples. We incubated the resulting spread plates at 26°C, inspecting plates daily for 1 week and then twice weekly for an additional 3 weeks. All culture plates were rapidly overgrown by filamentous fungi other than *Es. africanus.*

To overcome rapid contamination, we used indirect culture methods. First, we used the flotation method adapted from Larsh et al. (*7*) to separate the conidia from other particles in the soil (online Technical Appendix). We plated the resulting soil suspensions on Sabouraud agar and brain heart infusion (BHI) plates and incubated them at 26°C, conducting daily examinations for fungal colonies

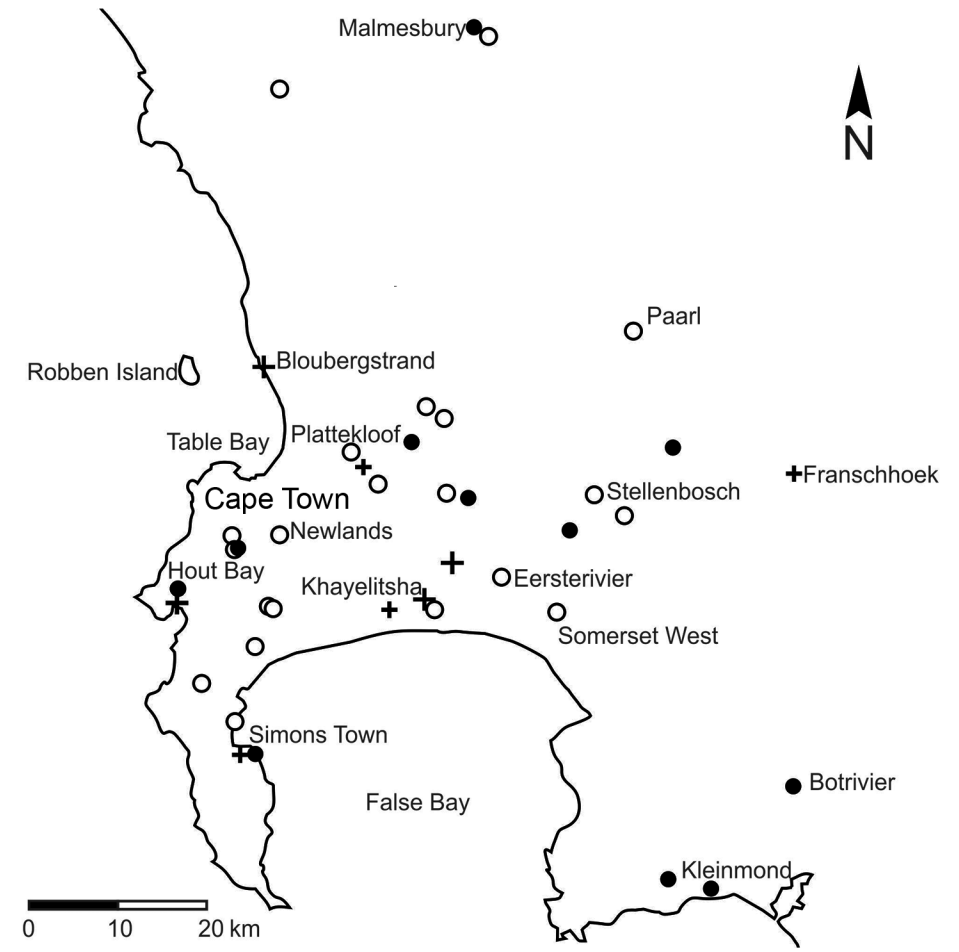

Figure 1. Results of molecular tests for the presence of *Emergomyces africanus* in soil samples in relation to residential locations of 14 patients diagnosed with emergomycosis (*6*), Cape Peninsula, Western Cape Province, South Africa. Black circles indicate *Es. africanus* detected in soil sample; white circles indicate *Es. africanus* not detected in soil sample; plus signs indicate residential locations of patients with emergomycosis. A larger cross indicates >1 infected patient at that particular location.

resembling *Es. africanus* (*1*). This preparation also resulted in rapid contamination of all plates.

Thereafter, we passaged soil suspensions through mice to screen out nonpathogenic soil organisms (*8*; online Technical Appendix). Animal studies were approved by the University of Cape Town's Animal Ethics Committee (protocol 016–002). We created soil suspensions by using the flotation method and sampling from the bottom third of the column; penicillin G (1,000 IU/mL) and gentamicin (0.1 mg/mL) were included in the solution. We inoculated 1 mL of soil suspension intraperitoneally into each of 4 BALB/c or C57BL6 mice. We euthanized the mice after 2 weeks and plated livers, spleens, or both onto Sabouraud agar plates with and without chloramphenicol, which we then incubated at 30°C and 35°C–37°C. We inspected plates as described previously. Pilot studies demonstrated that this method could detect as few as 10^2 conidia in 10 g of soil (online Technical Appendix). Notably, in a pilot study in

Table. Results of molecular-based detection of *Emergomyces africanus* in 60 soil samples, by province and type of soil habitat, South Africa*

Soil habitat	Western Cape	Eastern Cape	Gauteng	KwaZulu-Natal	Northern Cape	Total
Garden	6/30	0/2	1/4	–	–	7/36
Agricultural	3/5	–	–	–	–	3/5
Compost	3/5	–	–	–	–	3/5
Disturbed	1/2	0/2	–	0/1	–	2/5
Fynbos	1/2	–	–	–	–	1/2
Veld	1/1	0/1	–	–	0/1	1/3
Rotting tree	1/1	–	–	–	–	1/1
Unknown	1/3	–	–	–	–	1/3
Total	17/49	0/4	1/4	0/1	0/1	18/60

*Data represent number of samples in which *Es. africanus* was detected by nested PCR/total number of samples. Soil habitats: garden, soil from private gardens; agricultural, soil used for farming purposes; compost, soil rich in compost; disturbed, nutrient-poor uncultivated soil subjected to anthropogenic activities; fynbos, soil from a natural indigenous vegetation type endemic to the Cape Floristic region; veld, soil from grassland or uncultivated land; rotting tree, decaying woody debris; unknown, soil from unknown origin. –, sample not taken.

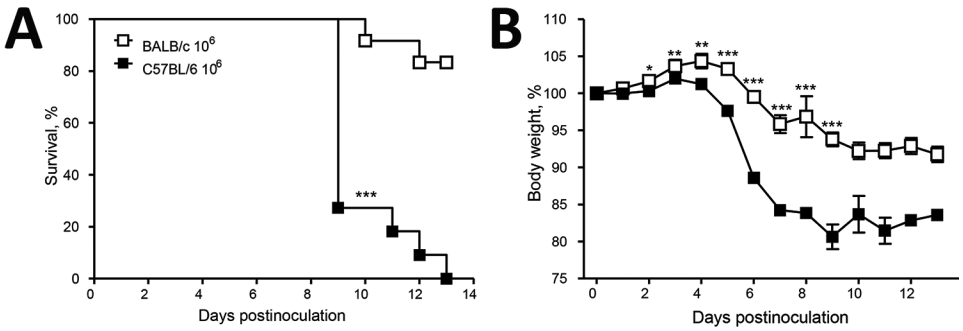

Figure 2. Infection of mice with *Emergomyces africanus*. In a proof-of-principle study, C57BL/6 and BALBc mice were inoculated intraperitoneally with 10^6 conidia (*Es. africanus* CAB 2141, a clinical isolate) in saline. Mice were weighed and monitored twice daily for distress. Both mouse strains had symptom onset, with C57BL/6 mice showing significantly more severe disease pathophysiology in response to the high dose of *Es. africanus* (demonstrated by reduced survival and increased weight loss). Data represent 2 pooled experiments (n = 8 [panel A] and n = 2 [panel B] combined), mean ±SD of the mean. p values were determined by using unpaired 2-tailed Student *t*-test or 1-way analysis of variance using a Bonferroni posttest (GraphPad Prism version 5). Values of p<0.05 were considered significant. *p<0.05; **p<0.01; ***p<0.001 (C57BL/6 compared with BALB/c mice).

which BALB/c and C57BL/6 mice were challenged with graded doses *of Es. africanus* conidia, genetic background of mice influenced host susceptibility to the organism; C57BL/6 mice were more sensitive to infection and had significantly higher mortality and weight loss in response to the high dose of 10^6 conidia compared with BALB/c mice (Figure 2).

We screened 26 soil samples for the presence of *Es. africanus* by using mouse passage. These samples included all 18 soil samples in which *Es. africanus* was detected by nested PCR, as well 8 soil samples that were PCR-negative. None of these samples, however, led to the isolation of *Es. africanus* through mouse passage.

Conclusions

Es. africanus is a newly described dimorphic fungal pathogen and causes an important HIV-associated systemic mycosis in South Africa (*9*). Many aspects of this organism remain unknown, including its ecologic niche. Our findings demonstrate that *Es. africanus* is present in a high proportion of soil samples collected from a range of habitats in South Africa, suggesting that soil might be a natural reservoir for this pathogen.

The isolation of pathogenic fungi from soil is challenging. Soil naturally contains a vast array of bacteria, viruses, fungi, and protozoa, all of which can interfere with or contaminate culturing the organism of interest (*8*). Since 1932, when Stewart and Meyer first cultured *Coccidioides immitis* from soil (*10*), flotation and animal passage has been the most robust method to isolate pathogenic fungi from soil. However, animal passage is laborious and expensive, can take months of turnaround time, requires special animal facilities, and results in discomfort and loss of life to laboratory animals, necessitating stringent ethics review (*11*).

Molecular detection is a valuable tool for establishing the presence of genetic material in environmental samples (*11*). In addition to high sensitivity, molecular detection has the advantages of being easy to apply, inexpensive, and rapid, and it can be performed in most laboratories. Alternatively, molecular detection lacks specificity because it cannot determine the viability (and hence infectivity) of the detected target (*11*). In our study, mouse passage of soil samples shown by nested PCR to contain *Es. africanus* genetic material did not result in the isolation of this fungus.

We have demonstrated that experimental infection with *Es. africanus* can produce pathology in mice. Moreover, susceptibility to disease appears to be mouse strain–dependent, with C57BL/6 mice being more susceptible than BALB/c mice.

This study has some limitations. The number of samples, and especially those from outside Western Cape Province, was relatively small, limiting inferences about the geographic range of *Es. africanus* in the environment. Moreover, our method of convenience sampling is prone to sampling bias. Nonetheless, this study is instructive for future ecologic studies, which should use random sampling to refine knowledge of the ecologic niche of this fungus.

In conclusion, this study demonstrates that *Es. africanus* can be frequently detected in a wide range of soils in South Africa. Moreover, our findings support the hypothesis that soil serves as a reservoir for this pathogen.

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Dr. Schwartz is an infectious diseases physician and researcher with interests in emerging fungal infections, immunocompromised hosts, and global health. This work comprises part of a doctoral thesis at the Global Health Institute, Faculty of Medical Sciences, University of Antwerp.

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Technical Appendix

Establishing Sensitivity of Molecular Detection Method

We used artificial inoculation of soil to determine the limits of the PCR. First, to remove microbial biomass including possible *Emergomyces africanus* cells, 1 kg of sandy soil was acid-washed by submerging in 0.1 M HCl (Merck, Darmstadt, Germany) for 14 h, whereafter the soil was rinsed 3 times with distilled water and oven dried at 80°C. To confirm the absence of *Es. africanus* gDNA in the acid-washed soil, DNA extractions were conducted on triplicate soil sub-samples (0.25 g). Extracted gDNA was subjected to nested PCR and tested for presence of a PCR product by visualization using agar gel electrophoresis. The lack of nested PCR product indicated the absence of *Es. africanus* DNA in the acid-washed soil.

A suspension of *Es. africanus* conidia was subsequently prepared using a 28-day-old fungal culture of *Es. africanus* CAB 2141 (a clinical isolate) grown on brain heart infusion (BHI; Merck) agar at 26°C. In brief, 5 mL saline Tween 80 solution (0.89% sodium chloride and 0.01% Tween 80) was added to the BHI agar plate (diameter, 90 mm), whereafter an inoculation loop was used to gently scrape the surface of the culture. The resulting conidial suspension in the Tween 80 and saline solution was aseptically filtered through a loose plug of glass wool (*1*) in a Pasteur pipette into a sterile test tube, whereafter the conidia in the filtrate were counted using a hemocytometer (Improved Neubauer, Marienfeld Superior, Germany). Samples of acid-washed soil (10 g) were then added to conical flasks (50 mL) and artificially inoculated with 10^2, 10^3, 10^4 and 10^6 conidia, respectively. A negative control consisted of 10 g of acid-washed soil without added conidia. All flasks were incubated at 26°C for 24 h with regular shaking to allow time for conidial binding to soil particles (*2*). After incubation, DNA extractions were conducted in duplicate on each of the artificially inoculated soil samples. Thereafter, extracted DNA was

subjected to nested PCR. Amplified products were sequenced and compared to the available sequences on the GenBank database.

A nested PCR product of the expected size (≈400 bp) was obtained from extracted DNA originating from 10 g of soil artificially inoculated with 10^2 conidia. The product was visualized on a 2% agarose gel but its concentration was too low for sequencing. Nested PCR products, as visualized by agarose gel electrophoresis, were also obtained from DNA originating from 10 g of soil inoculated with 10^4 and 10^6 conidia, respectively (Technical Appendix Figure). Sequencing and BLAST analysis of the nested PCR product, representative of the soil inoculated with 10^4 conidia, confirmed the presence of *Es. africanus*. From these results, it seems that the detection limits of the method used in the experimentation was between 10^2 and 10^4 conidia per 10 g of soil.

Flotation Method

In an attempt to separate *Es. africanus* conidia from other particles in the soil, we used the flotation method adapted from Larsh *et al* (*3*). The method entails preparing 100 mL soil suspension in a saline Tween 80 solution, from 10 g of soil within a 100-mL measuring cylinder. First, a pilot experiment was conducted to localize where in the column the conidia settled 1 hour after mixing with the saline Tween 80. For this, we spiked sterilized soils with known quantities of conidia produced by *Es. africanus* CAB 2141. Conidia of a 21-day old plate culture, on Sabourad (SAB) agar incubated at 26°C, were harvested using the filtered saline Tween 80 method described above. After using a hemocytometer to count conidia suspended in filtered saline Tween 80, 10^6 conidia were inoculated into 10 g of sterilized soil contained in a measuring cylinder. Also, dilution plates were prepared with SAB agar to determine the numbers of culturable conidia in the conidial suspension. These viability plates were incubated at 26°C; developing fungal colonies were counted twice weekly for 4 weeks.

Two soils were used in the experimentation: sandy soil collected from and representative of that which predominates in the Cape Flats, an area of the Western Cape where clinical cases of *Es. africanus* infection have been diagnosed (*4*); and compost soil from the Winelands area, Western Cape. Both soils were sterilized by autoclaving twice at 121°C for 20 min. In each case,

the autoclaved soil was cooled to ca. 25°C, whereafter it was transferred to a measuring cylinder and inoculated as described above.

Each measuring cylinder, containing the 10 g of inoculated soil, was aseptically filled to a total volume of 100 mL with saline Tween 80. The cylinder was subsequently covered with sterilized aluminum foil and agitated with a Vortex Genie 2 (Scientific Industries, Bohemia, NY, USA) for 3–5 minutes. The resulting soil suspension was allowed to settle for 1 hour, whereafter 5 mL sub-samples of the suspension was collected from the top, middle, and bottom of the column. For the compost soil, these aliquots were each aseptically filtered through a loose plug of glass wool in a Pasteur pipette; this step was omitted for the sandy soil because few grossly visible soil particles remained suspended. The remaining soil suspension in each measuring cylinder was subdivided in 2 and concentrated via centrifugation (2500G; 30 min), whereafter 10 mL combined concentrated suspension (5 mL from the bottom of each centrifuge tube) was retained; the remainder was discarded. Finally, ≈1 mL of soil was removed from the bottom of the cylinder, suspended in 9 mL saline Tween 80, and agitated. This suspension was passed through glass-wool for the compost soil, but the filtering step was omitted for the sandy soil, as above. The culturable conidia, suspended in each of the above-mentioned 5 sub-samples, were enumerated using plate counts. From each sub-sample, 0.1 mL was plated onto each of 10 BHI plates and incubated at 26°C. Colonies were counted after 8 days. The rate of recovery of conidia was calculated as total colonies from all sample locations divided by the expected number based on colony counts on the viability plates (Technical Appendix Table 1).

Flotation of Sterilized Soil Seeded with *Es. africanus* Conidia

Flotation resulted in a low recovery of *Es. africanus* conidia (Technical Appendix Table 1). The total proportions of inoculated conidia recovered from the sterilized sandy and compost soil samples were 4.7% and 10.0%, respectively. The total proportions of conidia that were floated away from the sedimented soil were 96.1% and 85.9% for the sandy and compost soil, respectively.

For selected soil samples that were positive by molecular detection, we performed the flotation method, as described above but only sampling from the bottom third of the column based on the results of the pilot experiment. The sample was then plated on SAB and BHI plates

and incubated at 26°C, with daily examination for developing fungal colonies resembling those of *Es. africanus* (*1*).

Mouse Inoculation

Animal studies were approved by the University of Cape Town's Animal Ethics Committee (protocol 016–002).

To overcome rapid overgrowth of soil dilution plates by filamentous fungi other than *Es. africanus*, we passaged filtered soil suspensions through mice obtained from the University of Cape Town Research Animal Facility (*5*). First, we tested whether BALB/c and C57BL/6 mice could be infected with *Es. africanus* by inoculating them intraperitoneally (i.p.) with varying doses of conidia (from 10^2-10^6 in 1 mL saline). Mice were euthanized after 2- and 4-weeks and livers and spleens were plated onto SAB incubated at 30°C and 35–37°C, respectively. Plates were inspected daily for developing fungal colonies.

Conidia suspended in saline and inoculated into wild-type mice could be detected as developing colonies upon culture of their spleens and livers. Here, the genetic background of the mice susceptibility to the organism: C57BL/6 mice were more sensitive to infection with significantly higher mortality and weight loss in response to the high dose of 10^6 conidia compared to BALB/c mice (Figure 2 in main manuscript). The organisms were visualized in the spleen of C57BL/6 mice that were euthanized after reaching 20% weight loss (data not shown). C57BL/6 mice inoculated with 10^2-10^5 conidia in saline handled the infection well (i.e., they did not lose weight and did not die or require euthanasia) but *Es. africanus* could still be cultured from spleen and liver after i.p. inoculation with as few as 10^2 conidia.

Inoculation of Mice with Soil Suspensions Derived from Sterilized and Unsterilized Soils Seeded with *Es. africanus* Conidia

Next, we tested whether the flotation method plus mouse passage could recover *Es. africanus* from unsterilized soil. In each case, 10 g of soil aliquots were artificially inoculated with 10^2, 10^3, 10^4 and 10^6 *Es. africanus* conidia, respectively. A soil suspension of each aliquot was subsequently prepared in a 100-mL measuring cylinder as described above, but with the addition of penicillin G (1000 IU/mL) and gentamicin (0.1 mg/mL) to the saline Tween 80. After

an hour, *Es. africanus* conidia were obtained from the suspension, by extracting a 5-mL sample from the bottom third of the column (based on the pilot data from the flotation experiment), whereafter the 5-mL conidial suspension was filtered through a loose plug of glass wool in a Pasteur pipette. We then inoculated 1 mL of the filtered suspension i.p. into each of 4 C57BL/6 mice and 4 BALB/c mice. The mice were pre-treated with subcutaneous carprofen (5 mg/kg), a non-steroidal anti-inflammatory analgesic, based on a previous report that suggested that this may have mitigated adverse reactions in mice exposed to Arizona soil (*6*). Mice were euthanized after 2- and 4-weeks and livers and spleens were plated onto SAB (with and without chloramphenicol) and BHI plates, incubated at 30°C and 35–37°C, respectively. Plates were inspected daily for developing fungal colonies resembling *Es. africanus*. The experiment was terminated after 4 weeks and the identities of colonies resembling *Es. africanus* were confirmed by sequence analyses of ITS region.

The limit of detection for mouse inoculation of unsterilized soil suspensions appeared to be 10^2 and 10^4 conidia per 10 g of soil for BALB/c and C57BL/6 mice, respectively (Technical Appendix Table 2). The procedure was tolerated by the mice, and no animals died or required euthanasia before the planned chronological endpoint.

Using Mouse Passage to Screen Soil Samples

A subset of 26 soil samples, from a set of 60 samples collected from around South Africa were subsequently screened for the presence of culturable *Es. africanus* via the above-mentioned mouse passage. Each soil sample was prepared using the flotation method as described above, inoculated i.p. into each of 4 C57BL/6 mice, 4 BALB/c mice, or both. Mice were pretreated with carprofen, as above, and euthanized after 2 weeks whereafter spleens and/or livers were plated onto SAB agar, with and without chloramphenicol, and incubated at 30°C and 35–37°C. Plates were monitored and fungal growth identified as above.

Monitoring and Euthanasia of Mice

After inoculation, mice were monitored at least daily for signs of illness or need for euthanasia. We defined the humane endpoint for animals as loss of 20% bodyweight; loss of 10% bodyweight sustained over 3 days plus another sign of clinical stress, such as hunching,

tachypnea, anorexia; or, clinical signs of distress such as lethargy and absence of response to being approached or touched by the monitor. Upon reaching the humane or chronological endpoints, mice were anesthetized with inhalation of halothane (5% in air), and euthanized by inhalation of CO_2 (10%–20% fill rate per minute) followed by cervical dislocation.

Epidemiologic Data

Residential postal codes of incident cases of disease caused by *Es. africanus* diagnosed in the Western Cape between Dec 2014 and Feb 2016 were collected. The locations representing these postal codes were plotted in comparison to study sample sites to ensure appropriate sampling in areas where persons are affected by *Es. africanus* infection. Patient enrolment and data collection was approved by human research ethics committees of University of Cape Town (HREC 138/2014), Stellenbosch University (N14/02/011), Institute of Tropical Medicine and University of Antwerp (ITG 926/14), and the institutional review boards of provincial hospital involved.

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Technical Appendix Table 1. Efficiency of the flotation method in recovery of culturable *Es. africanus* CAB 2141 from sterilized soil artificially inoculated with conidia

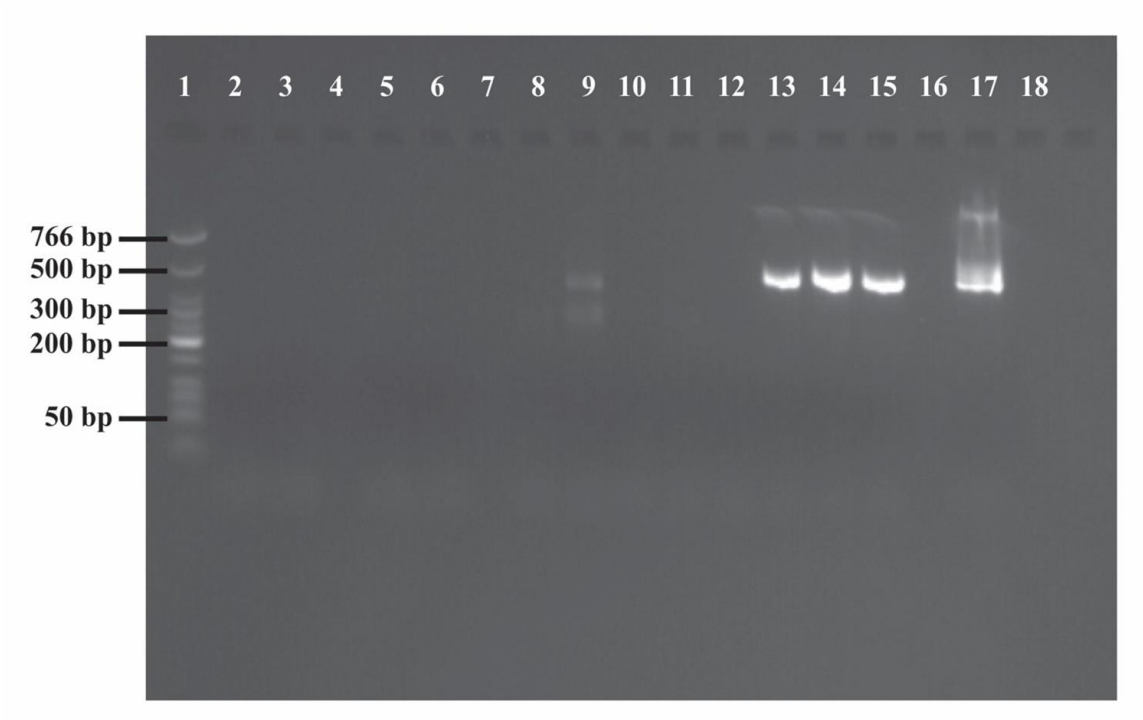
	No. of recovered colonies (% of total recovered)	
Sample location from column	Sandy soil	Compost soil
Top of column	239 (10.3)	373 (15.8)
Middle of column	655 (28.2)	673 (28.4)
Bottom of column	597 (25.7)	896 (37.9)
Centrifuged soil suspension	743 (32.0)	92 (3.9)
Sedimented soil	91 (3.9)	333 (14.1)
Total	2325 (100)	2367 (100)

*Number of culturable conidia in inoculum for sandy and compost soil samples were 4.96 x10^4 and 2.37 x10^4, respectively

Technical Appendix Table 2. Recovery of *Es. africanus* CAB 2141 from mouse passage with unsterilized soil artificially inoculated with conidia*

	30°C								37°C			
Group, x 4 mice	1	1 (drugs)	2	2 (drugs)	3	3 (drugs)	4	4 (drugs)	1	2	3	4
BALB/c												
Control	X	X	X	X	BC + FC	C	FC	X	X	FC	FC	FC
10^2	X	X	BC	X	C	BC	EA	EA	BC	X	BC	X
10^3	EA	EA	EA	EA	EA	EA	EA	EA	EA	FC	BC	EA
10^4	EA	EA	EA	EA	EA	EA	EA	EA	EA	EA	EA	EA
10^6	EA	EA	EA	EA	EA	EA	EA	EA	EA	BC	EA	EA
C57BL/6												
Control	FC	BC	BC	FC	X	FC	BC	BC	BC	BC	X	X
10^2	X	BC	FC	FC	X	X	X	BC	BC	X	BC	X
10^3	FC	X	BC	X	X	X	C	X	FC	FC	FC	FC
10^4	EA	EA	EA	EA	EA	EA	EA	EA	FC	EA	EA	EA
10^6	BC	BC	EA	EA	EA	EA	EA	EA	X	EA	EA	EA

*Livers and spleens of each mouse were plated onto 3 different isolation media that were subsequently incubated at different temperatures: Sabouraud's agar with dextrose with and without chloramphenicol (0.05 g/L; designated here as "drugs") incubated at 30°C, and Sabouraud's agar with dextrose incubated at 37°C. EA, *Es. africanus* isolated; X, no growth; BC, bacterial contamination, FC: fungal contamination, C: contamination not specified.



Technical Appendix Figure. UV image of nested PCR products originating from gDNA of acid-washed soil subsamples (10 g) artificially inoculated with 10^2, 10^3, 10^4 and 10^6 *Es. africanus* conidia, respectively. Products were separated with electrophoresis on a 2% agarose gel. Lane 1, Quick- Load Low Molecular Weight DNA Ladder (New England Biolabs, Massachusetts). Lanes 2 and 3, PCR negative controls containing no DNA. Lane 4, blank. Lanes 5 and 6, artificial soil inoculation negative controls (without added conidia). Lane 7, blank. Lanes 8 and 9, acid-washed soil inoculated with 10^2 conidia. Lanes 10 and 11, acid-washed soil inoculated with 10^3 conidia. Lanes 12 and 13, acid-washed soil inoculated with 10^4 conidia. Lanes 14 and 15, acid-washed soil inoculated with 10^6 conidia. Lane 16, blank. Lane 17, PCR positive control containing gDNA of *Es. africanus* CAB 2141. Lane 18, blank.