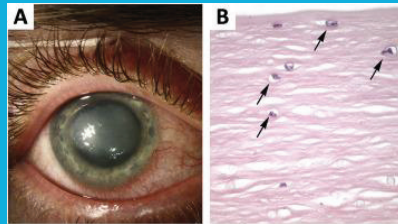


# Acanthamoeba keratitis

## clinical evaluation of qPCR

NWKP  
21-01-2020

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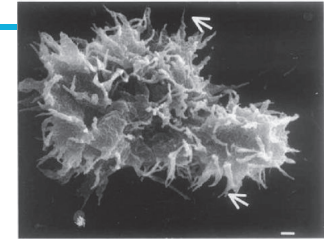
# Acanthamoeba

Free living amoeba (parasite)

cosmopolite !

it is present in:

- air
- water (all types of water)
- rhizosphere (soil around plant roots)
- animals



'Acantha' = spine

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## RESEARCH ARTICLE

The rising incidence of *Acanthamoeba keratitis*:  
A 7-year nationwide survey and clinical  
assessment of risk factors and functional  
outcomes

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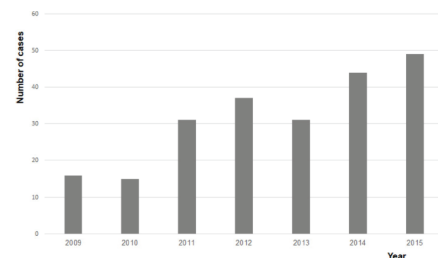


Fig 1. Number of cases per year. The annual number of cases of *Acanthamoeba keratitis* in the Netherlands.  
<https://doi.org/10.1371/journal.pone.0222092.g001>

## Increasing incidence of *Acanthamoeba keratitis* i a large tertiary ophthalmology department from year 1994 to 2018

Stine Elkjær Nielsen, Anders Ivarsen and Jesper Hjortdal

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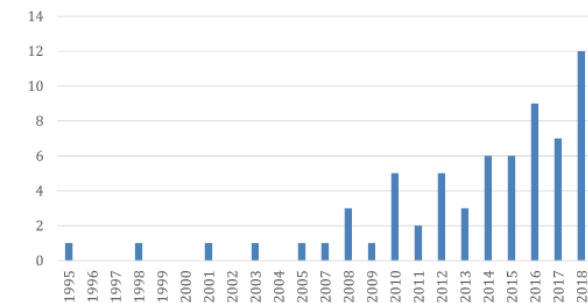
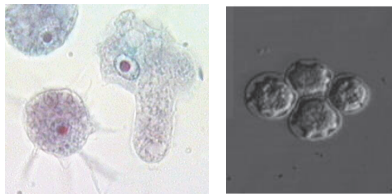


Fig. 1. Year of diagnosis of *Acanthamoeba* (AA) keratitis.

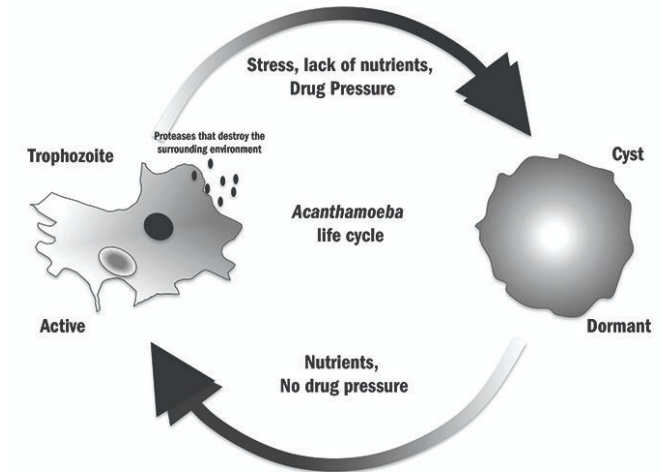
## Symptoms and treatment not evident

1. Early diagnosis is difficult. Often mistaken for herpes simplex keratitis. Often treated with corticosteroids which is unfavorable
2. Two forms – a mobile trophozoite and a dormant cyst – of which the latter is resistant to therapy.



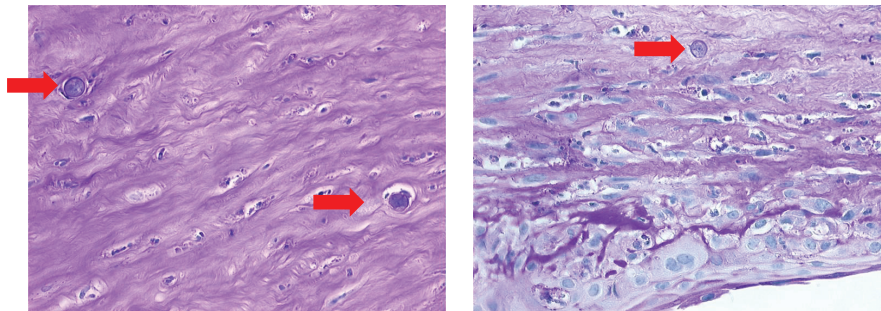
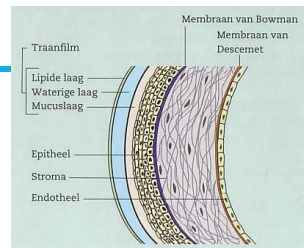
- Cysts; develop when conditions are unfavorable
- Two walls, ectocyst & endocyst
- Resistant to treatment

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## Cornea



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## Acanthamoeba keratitis symptoms



Pain in the Eye

**Symptoms and Signs of Acanthamoeba Keratitis**



Redness of the Eye



Watering of Eyes



Photophobia

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Table 4. Multivariable logistic regression analysis on 'treatment failure'.

Exposure factor	No. of patients	Odds ratio (Exp (B))	95% C.I. for Exp (B)
<b>Referral by</b>			
Other ophthalmologist (reference)	142	-	-
General practitioner	47	0.311	0.115–0.841
Patient	8	2.308	0.417–12.791
Other	1	0.000	0.000
<b>Severity at diagnosis</b>			
Stage 1 (reference)	50	-	-
Stage 2	87	1.467	0.624–3.450
Stage 3	61	3.847	1.544–9.584
<b>Corticosteroid use before diagnosis</b>			
No (reference)	66	-	-
Yes	132	3.308	1.375–7.963
Age at presentation, years	196	1.052	1.029–1.075

Exp (B) = exponentiation of the B coefficient, C.I. = confidence interval.

<https://doi.org/10.1371/journal.pone.0222092.t004>

## Validation of qPCR for *Acanthamoeba* species

## Methods

### Culture

- Nutrient agar with *E.aerogenes* (ATTC 13048)
- Incubated at 27°C
- Inspection under inversion microscope during 7 days
- Trophozoites, cysts, feeding track



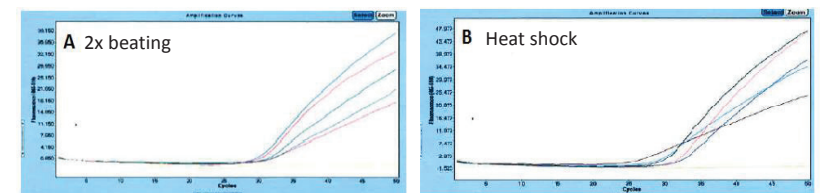
### qPCR

- 18S rRNA gene (~180 bp)



## Pre-treatment

- 2x Beating versus Heatshock



- MagNa Lyser beating 2x at 6500 rpm, 20s

## qPCR

- Standard Lightcycler 480 program
  - 15 s → 95°C
  - 60s → 60°C
- Primers;
  - AcantF900 (5-CCC AGA TCG TTT ACC GTG AA-3)
  - AcantR1100; (5-TAA ATA TTA ATG CCC CCA ACT ATC C-3)
- Probe;
  - Cy5-labeled AcantP1000 (5-Cy5-CTGCC ACC GAA TAC ATT AGC ATG G-BHQ3-3).

## Detection limit

	1st run		2nd run	
Dilution	No trophozoïtes (Burker Tûrk)	Cp value	No trophozoïtes (Burker Tûrk)	Cp value
1:1	103.594	24.57	121.875	23.73
1:10	10.359	26.11	12.187	26.93
1:100	1036	29.71	1218	28.57
1:1000	103	31.72	121	31.51
1:10000	10	-	12	35.55
1:100000	1	-	1	-

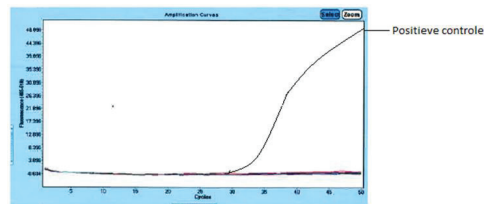
Detection limit ~ 5 trophozoïtes

No distinction possible between trophozoites and cysts

## Specificity

- The most prevalent causes of keratitis did not show a reactivity in the Acanthamoeba specific qPCR.

Sample	Cp waarde Acantha
Neg iso	-
HSV	-
VZV	-
Adeno	-
Fusarium	-
Chlamydia	-
Neigon	-
Staaue	-
Strpne	-
Strvirg	-
Entbclo	-



## Clinical evaluation of qPCR versus culture

- Period: 1 July 2017 – 1 January 2018
- Patients prospectively presenting at the Ophthalmologic outpatient clinic with keratitis suspected for Acanthamoeba keratitis
- N=41 qPCR
  - N=16 (39%) positive
  - N=24 (56%) negative
  - N=1 nonreactive due to interference
- N=39 cultured
  - N=9 (23%) positive
  - N=26 (67%) negative
  - N=4 (10%) bacterial overgrowth

## Results clinical evaluation

- qPCR pos N=16

N=8 (50%) culture positive

N=4 (25%) culture negative

N=4 (25%) bacterial overgrowth

- Culture pos N=17

N=16 qPCR positive

N=1 qPCR negative

		Acan +	Acan -	Total
PCR	Positive	16	0	16
	Negative	1	24	25
	Total	17	24	41

*Pus, 1 of 3 samples.  
Sampling error or difficult  
material*

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### Culture

- Sensitivity 53%
- Turn-around-time 2-7 days
- Expert technicians

### qPCR

- Sensitivity 94%
- Turn-around-time 1 day
- Routine molecular diagnostics

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## Take home message

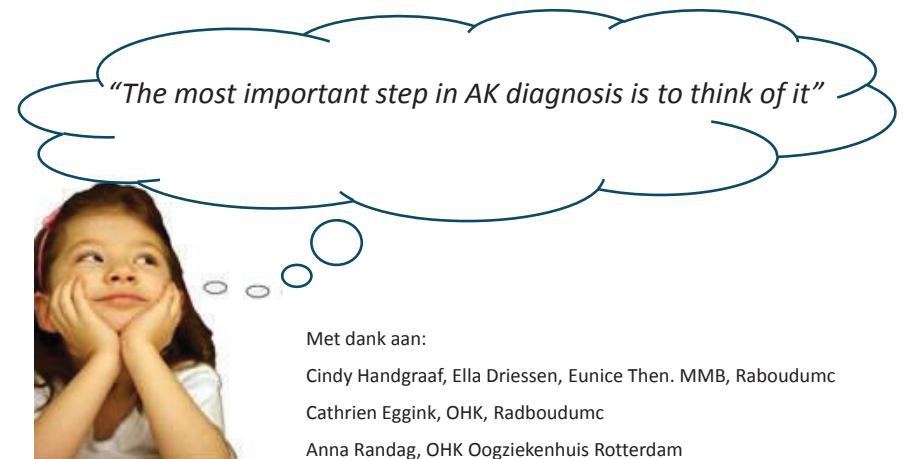
Acanthamoeba keratitis is .....

- ..increasingly seen in people wearing contactlenses
- ..a debilitating infection with a poor prognosis

Therefore rapid and sensitive diagnostics is important

Currently qPCR is the most sensitive, rapid and accurate diagnostic method

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Met dank aan:

Cindy Handgraaf, Ella Driessen, Eunice Then. MMB, Raboudumc

Cathrien Eggink, OHK, Radboudumc

Anna Randag, OHK Oogziekenhuis Rotterdam

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## FUSACA

- Nationwide study on *Fusarium* en *Acanthamoeba* keratitis in people wearing contactlenses
- Coordinated by the Dutch National Institute for Public Health and Environment
- Includes several specialists in epidemiology, ophthalmology and microbiology from academic and large peripheral hospitals