

Adenovirus DNA: molekulare menschliche Fußspuren

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Humane Adenoviren (HAdV): Struktur

- non enveloped DNA Virus
- ca. 35 kB dsDNA
- icosahedral capsid

Alle humanen Adenoviren (ja, da gibt es viele!)

- haben die gleiche Struktur und Strukturproteine (große und kleine)
- unterscheiden sich strukturell (nach Spezies) in der Länge der Fibern
- Ausnahme: Spezies HAdV-F: zwei (!!)
- unterschiedliche lange Fibern

Aber:

- haben (je nach Spezies) deutlich unterschiedlich nicht-Strukturproteine!
- sind genetisch hoch divers

Seven species (*not: subgenus*) of human (Mast-)Adenovirus (HAdV-A to -G)

- all seven human adenovirus species belong to the Genus *Mastadenoviridae*
- historic species definition by hemagglutination patterns, DNA hybridization...
- current definition by genome sequence phylogeny (***closely related HAdV types are members of the same HAdV species!***)

Basics on HAdV types

- Serotypes (by definition cross neutralization):
all types up to type 51 (except types 15 and 29 of species D)
- Genotypes (new, alternative definition: complete genomic sequencing, phylogeny of major capsid proteins, recombinant phylogeny of the genome)
since type 53 to 116....

All new types are genotypes
but a few are also (or could be) serotypes

- However: Main rule of typing remained unchanged:
historical order of types (date of isolation of "prototype")

Human Adenoviruses (HAdV):

7 Species with 116 types

Species	types	organotropism	disease
A	12, 18, 31 , 61	gastrointestinal	diarrhea , disseminated disease
B	3 , 7 , 11, 14 , 16, 21 , 34, 35, 50	lung, pharynx, conjunctiva, urogenital	URI/LRI (pneumonia), pharyngoconjunctival fever/conjunctivitis, (shedding with stool) cystitis (shedding with urine)
C	1 , 2 , 5, 6 , 57, 89	pharynx, lymphoid cells	URI (with GI, shedding with stool) disseminated disease
D	8 , 37 , 53 , 54 , 64 many other	conjunctiva/cornea diverse	keratoconjunctivitis Enteritis /subclinical (other types)
E	4	lung	URI/LRI (ARDS, pneumonia) pharyngoconjunctival fever/conjunctivitis
F	40 , 41	gastrointestinal	diarrhea, exclusively?
G	52	gastrointestinal	diarrhea (zoonosis?)

Kindergartenalter
selten, epidemisch

Kleinkinder,
quasi endemisch

HAdV-Übertragungswege

bei akuten Infektionen/Erkrankungen:

- **Schmierinfektion, nosokomiale Übertragung**
 - **Stuhl**
 - **HAdV-F und HAdV-A Typen, sehr hohe Viruslasten im Stuhl!**
 - HAdV-B, -C, -D Typen: fraglich, aber unwahrscheinlich (niedrigere Viruslasten)
 - **Augensekrete**
 - EKC assoziierte HAdV-D Typen (sehr hohe Viruslasten, nosokomiale Übertragung)
 - HAdV-B, -E Typen bei Konjunktivitis: nosokomiale Übertragung selten (niedrigere Viruslasten)
- **Tröpfcheninfektion / direkter Kontakt**
 - **HAdV-B, -C, -E Typen (respiratorische Erkrankungen)**

bei latenter Infektion (asymptomatisch):

HAdV für wenige Jahre nachweisbar in lymphatischem Gewebe, fluktuierend im peripheren Blut

- infektiöses HAdV fluktuierend in resp. Sekreten und Stuhl
 - aber wahrscheinlich zu niedrige Viruslasten für eine Übertragung

Exkursion: Warum gibt es denn HAdV-Latenz?

Evolution of HAdV types by

1. immune escape

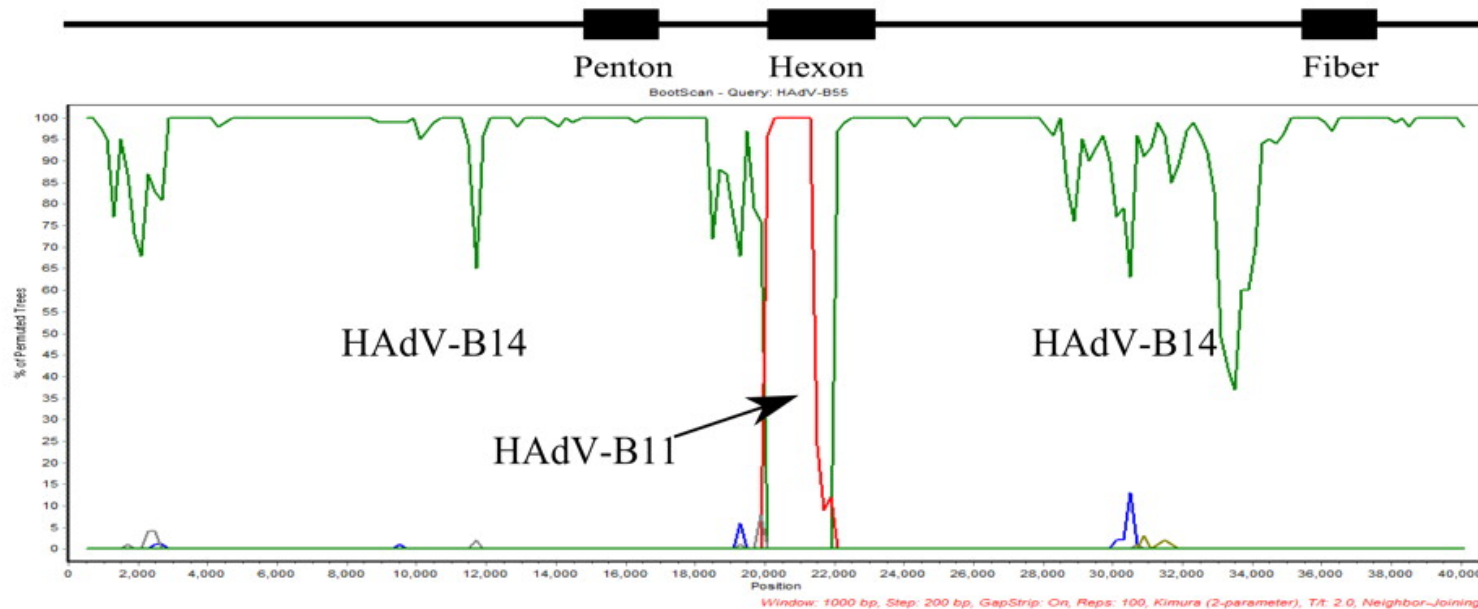
- positive selection of mutations of the ϵ (neutralization) determinant (rather slow in a DNA virus due to low mutation rate)
- homologous recombination of the ϵ (neutralization) determinant (more frequently observed!)

2. gain of fitness/virulence, change of tropism

- positive selection of mutations almost anywhere in the genome (rather slow in a DNA virus due to its low mutation rate)
- homologous recombination of fiber knob, penton base..... genes

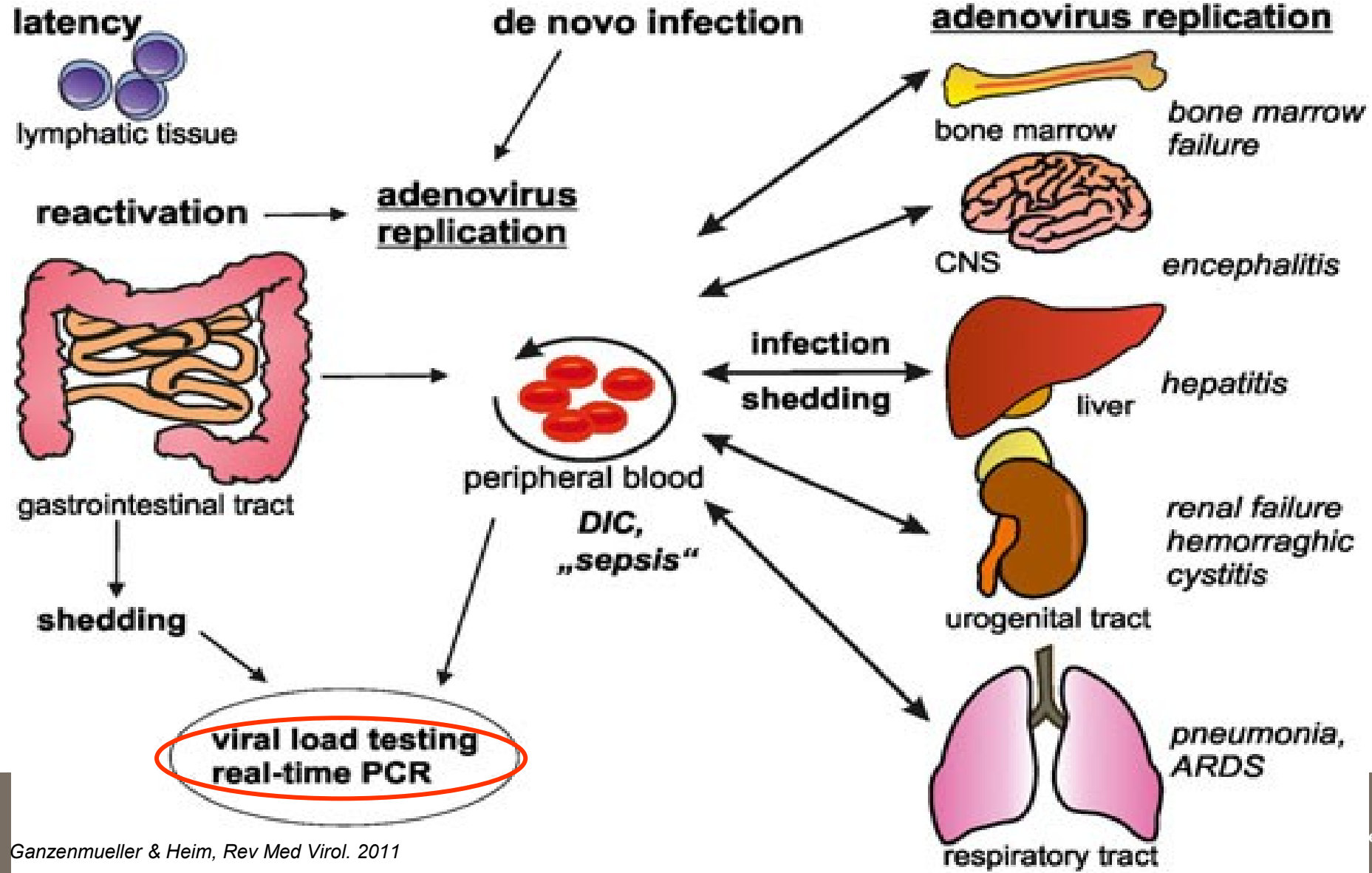
Infection with two different types (of the same HAdV-species) is required for homologous recombination
Superinfection of a latently infected individual!

immune escape by recombination: HAdV-B55 causing a prolonged outbreak of LRTI in China



genomic backbone of HAdV-B14, a well known cause of LRTI in the US

klinisch bedeutend: HAdV Latenz / Reaktivierung nur bei Immunsuppression



Zurück zum Thema: Wo müssten Menschen HAdV bzw. HAdV-DNA Spuren hinterlassen?

- im Abwasser: HAdV Ausscheidung mit dem Stuhl (und Urin), aber auch respiratorische Sekrete und Augensekrete gelangen ins Abwasser!
- auf Oberflächen, auf die HAdV verschmiert wird (seltener untersucht, meist Studien zur nosokialen Übertragung)
 - gelangt mit dem Putzwasser auch ins Abwasser...

Das sind stabile, leicht nachweisbare Spuren:

- sehr hohe Viruslasten möglich (1e10/ml bzw. g Sekret oder Stuhl)
- dsDNA in ein stabiles Kapsid verpackt (UV, low pH etc. resistent...)..lang infektiös
- selbst nach Verlust der Infektiosität bleibt die HAdV-DNA geschützt und ist nachweisbar

Die älteste Arbeit zu Abwasser, die ich gefunden habe:

L G Irving, F A Smith. *One-year survey of enteroviruses, adenoviruses, and reoviruses isolated from effluent at an activated-sludge purification plant*
Appl Environ Microbiol. **1981** Jan;41(1):51-9

Virus	Conc IU*/ML Mean	Conc IU/ML Range	Samples positive
Enterovirus	1400	150-6,350	26 (100%)
Adenovirus	1,950	0—6,850	25 (96%)
Reovirus	2,150	0—6, 150	24 (92%)

* infectious units, **sampling: 1977/1978**

Abwasser – "Oberflächenwasser" - Trinkwasser

reports	sample source	methods of detection	findings
Tani et al. 1992, 1995	river water, Japan	tissue culture	low level, year-around (0-25 PFU/liter)
Grohmann et al. 1993	river water, coastal water and stormwater, Australia	tissue culture	12% river water, 9-24% of coastal water and 10-25% of the storm water were positive
Puig et al. 1994	river water, Spain	nested PCR	all 9 samples were positive
Genthe et al. 1995	surface water, South Africa	gene probe	up to 59% samples were positive for enteric adenoviruses
Pina et al. 1998	river and coastal water, Spain	nested PCR	65% of 23 river samples were positive, 7 of 9 coastal waters
Vantarakis and Papapetropoulou 1998	bathing beach water, Greece	nested PCR	were positive
Castignolles et al. 1998	Seine River, France	nested PCR	28% of 120 samples were positive
Chapron et al. 2000	surface water, U.S.	ICC-nested PCR	some estuary samples were positive
Cho et al. 2000	river water, South Korea	RT-multiplex-nested PCR	48% of 29 samples were positive for Ad40 and 41
Jiang et al. 2001	coastal water, Southern California, U.S.	nested PCR	10 of 12 samples were positive
Greening et al. 2002	river water, New Zealand	ICC-PCR and nested PCR	4 of 12 samples were positive
Van Heerden et al. 2003, 2004, 2005	river and dam water used as sources for drinking water, South Africa	ICC-nested PCR, QPCR	4 of 6 samples were positive by nested PCR but none was positive by ICC-PCR

Prevalence, quantification and typing of adenoviruses detected in river and treated drinking water in South Africa

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Over a 1-year period (June 2002 to July 2003), HAdVs were detected in **5.32% (10/188) of the treated drinking water and 22.2% (10/45) of river water samples**... The original treated drinking water and river water samples had an estimate of **less than one copy per litre of HAdV DNA present**. In treated drinking water samples, species D HAdVs predominated.... Most (70%) of the HAdVs detected in river water samples analysed were enteric HAdVs (serotypes 40 and 41)...

Table 4 PCR and typing results for river water samples

Sampling date	Conventional nested PCR	LightCycler (viruses ml ⁻¹)	Virus DNA copies l ⁻¹	First round Hex-PCR	Nested Hex-PCR	Virus serotypes	Cloning followed by sequencing
2002-07-08	Pos	23 100	9.24×10^{-3}	Pos	Pos	HAd 40	ND
2002-07-22	Pos	25 300	1.01×10^{-2}	Pos	Pos	HAd 40	ND
2002-08-05	Pos	244	9.76×10^{-5}	Pos	Pos	ND	HAd Species D
2002-08-29	Pos	5296	2.12×10^{-3}	Pos	Pos	HAd 41	ND
2002-09-16	Pos	<30	$<1.2 \times 10^{-5}$	Neg	Pos	ND	HAd Species D
2003-01-13	Pos	106	4.24×10^{-5}	Pos	Pos	HAd 41	ND
2003-01-20	Pos	1041	4.16×10^{-2}	Pos	Pos	HAd 2	ND
2003-02-04	Pos	Neg	Neg	Pos	Pos	HAd 40	ND
2003-02-24	Pos	3264	1.31×10^{-3}	Pos	Pos	HAd 40	ND
2003-04-14	Pos	4864	1.95×10^{-3}	Pos	Pos	HAd 41	ND

Pos, Positive; Neg, Negative; ND, Not done; HAd, human adenovirus.

Table 5 PCR and typing results for treated drinking water samples

Treatment unit	Sampling date	Conventional nested PCR	LightCycler (viruses ml ⁻¹)	Virus DNA copies l ⁻¹	First round Hex-PCR	Nested Hex-PCR	Virus serotypes	Cloning followed by sequencing
Unit B	2002-07-29	Pos	<30	$<1.5 \times 10^{-6}$	Neg	Pos	HAd 40	ND
Unit A	2002-07-29	Pos	273	1.37×10^{-5}	Pos	Pos	HAd Species D	ND
Unit A	2002-08-05	Pos	<30	$<1.5 \times 10^{-6}$	Neg	Pos	ND	ND
Unit A	2002-09-09	Pos	228	1.14×10^{-5}	Pos	Pos	HAd Species D	ND
Unit A	2002-09-09	Pos	59	2.95×10^{-6}	Neg	Pos	ND	HAd Species D
Unit A	2002-09-16	Pos	104 920	5.25×10^{-3}	Pos	Pos	HAd 2	ND
Unit A	2002-09-23	Pos	123	6.15×10^{-6}	Pos	Pos	HAd 41	ND
Unit B	2002-09-23	Pos	11 920	5.96×10^{-4}	Pos	Pos	HAd Species D	ND
Unit B	2002-09-23	Pos	1180	5.9×10^{-5}	Pos	Pos	HAd Species D	ND
Unit A	2002-09-30	Pos	1966	9.83×10^{-5}	Pos	Pos	HAd Species D	ND

Pos, Positive; Neg, Negative; ND, Not done; HAd, human adenovirus.

HAdV-Übertragung durch Trinkwasser ?

*The consumption of (HAdV-)contaminated food or water **may be an important source of enteric (HAdV-)illness, although there is no substantial evidence supporting this route of transmission...***

Confirmed outbreaks of adenovirus infections associated with water have been limited to pharyngitis or conjunctivitis, with exposure arising from use of swimming pools.

*Human adenoviruses have been shown to occur **in substantial (??) numbers in raw water sources and treated drinking-water supplies....** contaminated drinking-water represents **a likely but unconfirmed source of human adenovirus infections.** Human adenoviruses are also considered important because they are exceptionally resistant to some water treatment and disinfection processes, notably ultraviolet (UV) light irradiation.*

HAdV sind also im Wasser – Aber wozu nachweisen?

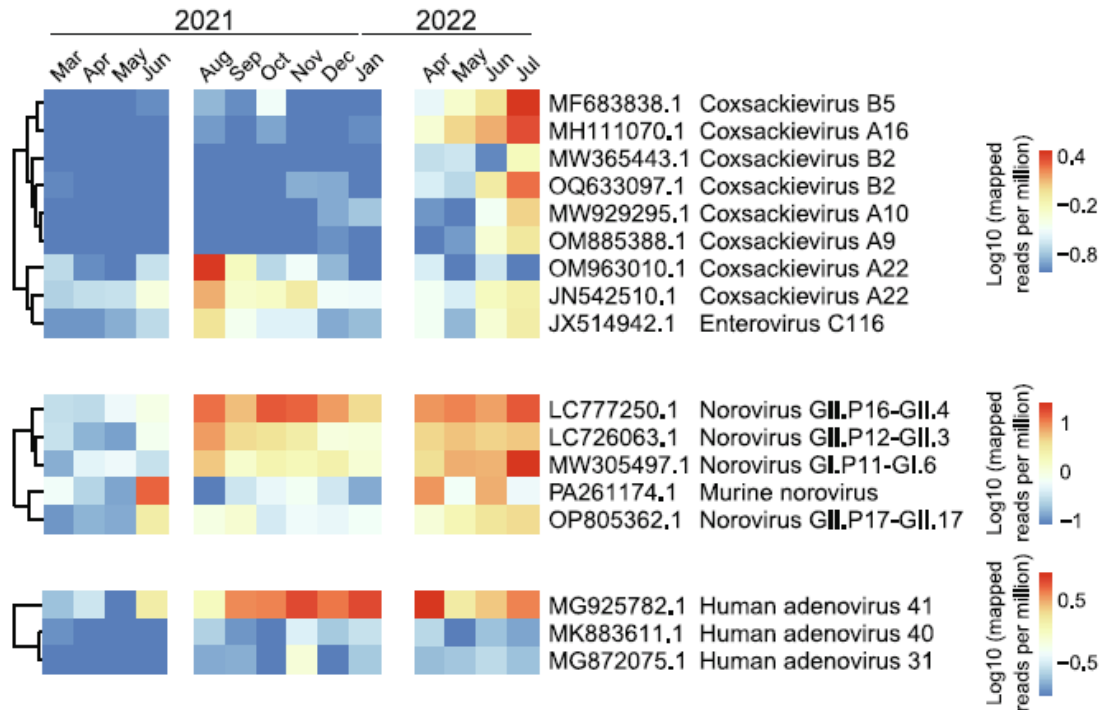
- Epidemiologische Studien (Abwasser): Prävalenz und Typenverteilung (allerdings etwas "gebiased" für Typen mit besonders hohen Stuhlviruslasten)
- Nachweis fäkaler Kontamination (als Indikator):
Oberflächenwasser/Grundwasser/Badewasser/Trinkwasser
 - nur HAdV-F Typen nachweisen? (relativ spezifisch für Stuhl)
 - oder alle Typen nachweisen (höhere Sensitivität, aber evtl. auch nur Spuren von Menschen?)
- Nachweis von Infektiosität im Trinkwasser, Badewasser o.ä.
 - CPE/Zellkultur oder ICC-PCR erforderlich! (nicht nur der DNA Nachweis!)
 - aber: "Viruslast" (quantitative PCR) für die Beurteilung beachten (hoch genug?)

Häufigste Virusfamilien im Abwasser.... (vor 2020)

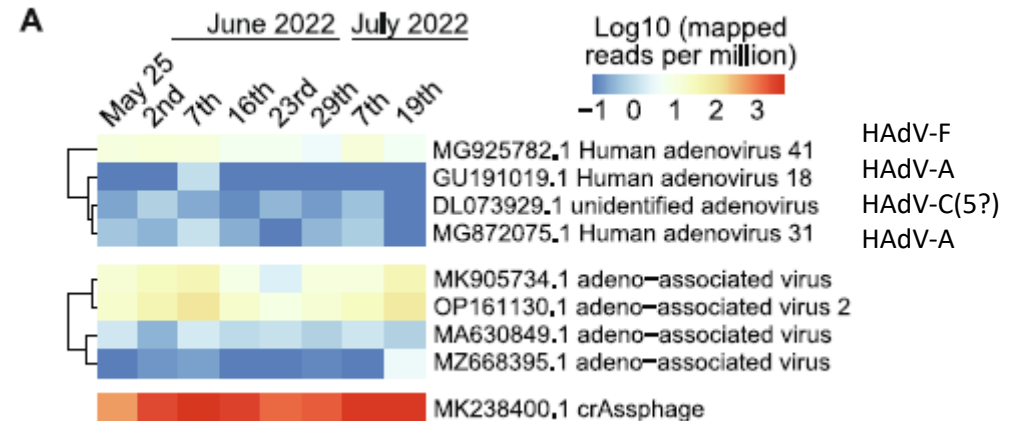
1. Picornaviridae (z.B. Enteroviren)
2. Calciviridae (z.B. Noroviren)
- 3./4. Adenoviridae / Reoviridae (z.B. Rotavirus)
- 5./6. Herpesviridae /Astroviridae

aber hier gab es einen Bias durch viele Polio/Enterovirus-Surveillance Studien

Adenoviren im Berliner Abwasser



RNA Deep Sequencing Data



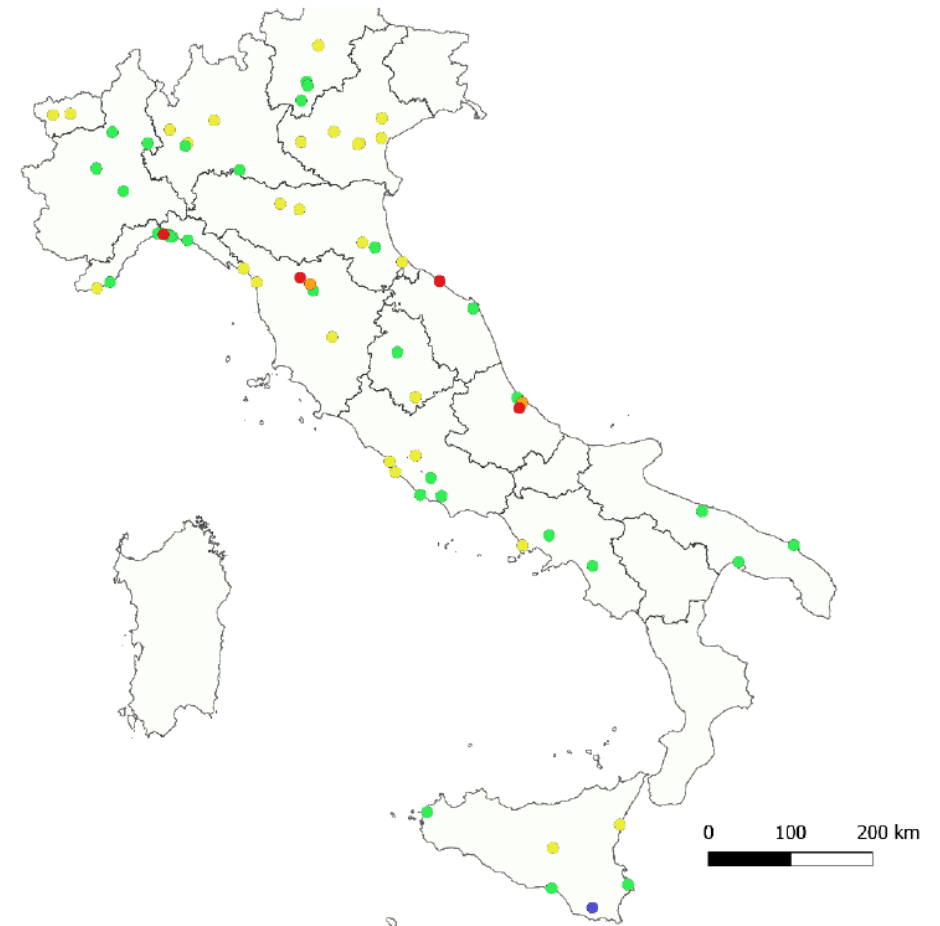
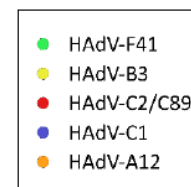
DNA Deep Sequencing Data

HAdV-F
HAdV-A

Adenoviren im Italienischen Abwasser (Januar 2023)

HAdV were detected in 157 out of 168 samples (93.5%) using dPCR, with viral concentrations ranging from 1.99×10^3 to 4.46×10^6 gc/L.

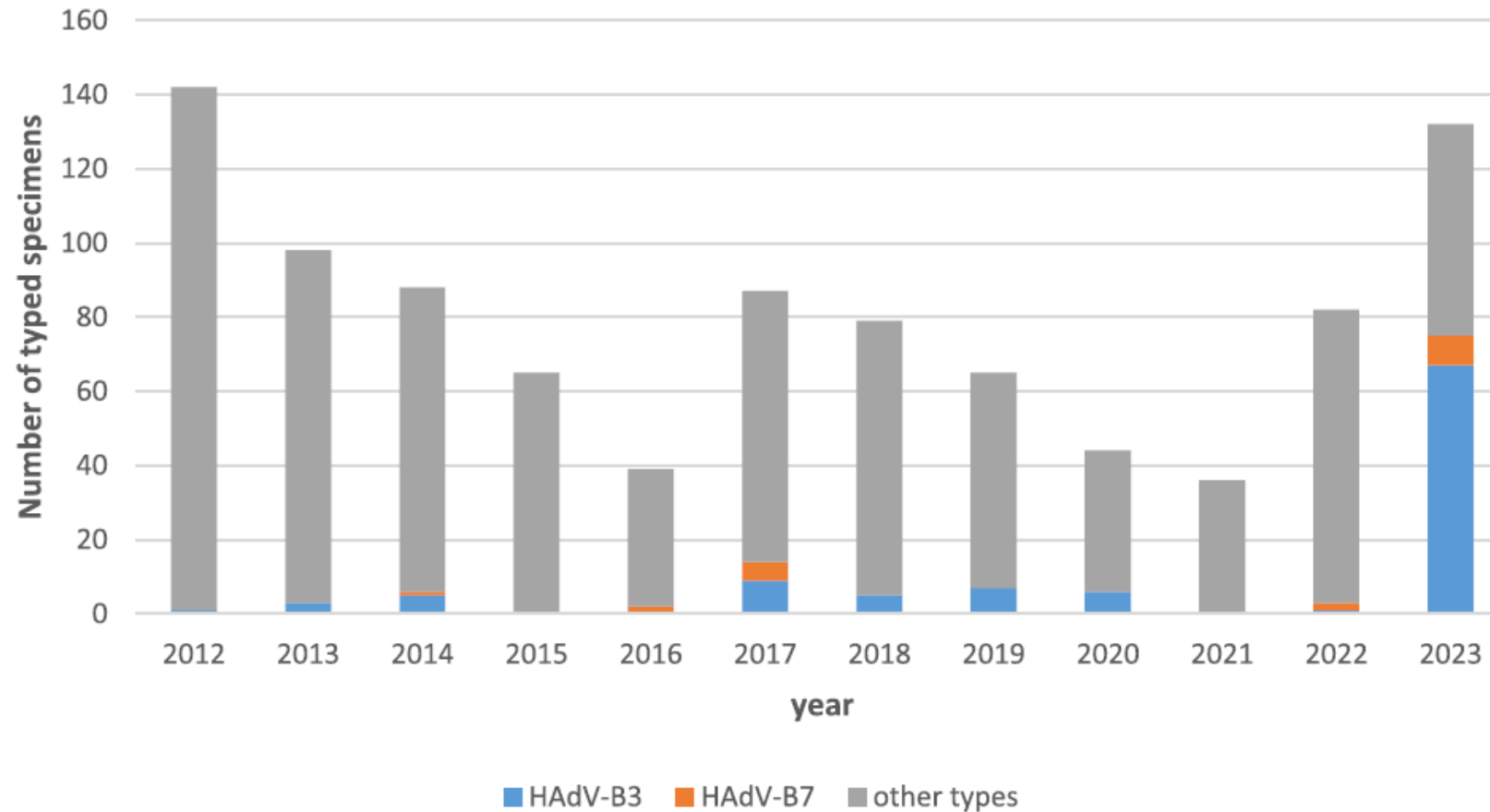
- HAdV-A12 (2 samples)
- HAdV-B3 (31 samples)
- HAdV-C1 (1 samples)
- HAdV-C2/C89: (4 samples)
- HAdV-F41 (36 samples)
- less abundant in amplicons: HAdV-B21, HAdV-C5, HAdV-D45, HAdV-D46, HAdV-D49, HAdV-D83, and HAdV-F40 (Oxford Nanopore Sequencing)



Koinzident in D:

An Epidemic of Respiratory and Ocular Infections Caused by the Reemergence of a Recombinant Human Adenovirus, the Novel Type HAdV-B114 (P7H3F3)

Tina Ganzenmueller^{1,2}  | Magnus Wolf¹ | Lasse Wolfram^{3,4}  | Charikleia Gkioule¹ | Lars Steinbrück⁵ | Albert Heim^{5,6} 



← Ursprünglich als Typ 3 diagnostiziert...
aber ein neuer Genotyp

Zusammenfassung

HAdV-DNA Nachweise sind stabile, lang nachweisbare "menschliche Fußspuren" im Wasser und in der Umwelt...

- können zur Überwachung der HAdV-Epidemiologie genutzt werden
- können als Indikatoren für fäkale Kontamination genutzt werden (insbesondere HAdV-F40 & 41)

aber leider: der Nachweis einer Übertragung/Gefährdung durch Trinkwasser ist schwierig (Nachweis von Infektiosität? Quantifizierung?)

– *und bei Lebensmitteln? (könnte es leichter sein, wenn sie nicht erhitzt werden)*