

Zoonotic transmission of a toxigenic *Corynebacterium ulcerans* strain identified by Next Generation Sequencing

Dominik M. Meinel¹, Regina Konrad^{1,5}, Anja Berger¹, Christina König^{1,5}, Torsten Schmidt-Wieland², Michael Hogardt³, Heribert Bischoff¹, Nikolaus Ackermann¹, Stefan Hörmansdorfer¹, Stefan Krebs⁴, Helmut Blum⁴, Gabriele Margos¹, Andreas Sing^{1,5}

¹ Bavarian Health and Food Safety Authority, Oberschleißheim, Germany

² Labor Dr. Gaertner, Ravensburg, Germany

³ Institut für Medizinische Mikrobiologie und Krankenhaushygiene, Frankfurt/Main, Germany

⁴ Genecenter, LMU, Munich, Germany

⁵ National Consultative Laboratory for Diphtheria



Background

Toxigenic *C. ulcerans* can cause a diphtheria-like illness in humans. *C. ulcerans* was found in domestic animals, which could serve as reservoirs for a zoonotic infection. Here we report the case of a severe necrotizing fasciitis diagnosed in a 53-year-old man in Germany and investigate possible zoonotic transmission between the human patient and his two pet dogs.

Patient

- 53 year-old man from Baden-Wuerttemberg, Germany
- Only underlying condition: chronic venous insufficiency
- Severe necrotizing fasciitis in the calves of both legs
- Vaccination status against diphtheria unknown

Isolates

- *Staphylococcus aureus*, *Bacteroides* spp. and *Corynebacterium ulcerans*
- *C. ulcerans* was identified by API Coryne, rpoB sequencing and MALDI-TOF-MS
- *C. ulcerans* was found by real-time PCR to be toxigenic

Toxin production

- Modified Elek test yielded a negative result
- Vero cell cytotoxicity assay positive:

toxigenic titer:	isolated <i>C. ulcerans</i>	1:8
	<i>C. diphtheriae</i> NCTC	1:2000

→ Vero cell cytotoxicity assay is more sensitive than a modified Elek test

Conclusion

- Transmission of toxigenic *C. ulcerans* between a human and his pet dog was proven by NGS
- NGS based molecular epidemiology offers superior resolution compared to classical techniques such as MLST
- *C. ulcerans* isolate causing skin ulceration was toxin negative in the Elek test, but positive in cytotoxicity test using Vero Cells
 → Vero cell cytotoxicity test is more sensitive

Transmission pathway

- One of the two dogs of the patient was found to carry a toxigenic *C. ulcerans*
- Both isolates from the human and dog:
 - share the same antibiotics susceptibility (amoxicillin, benzylpenicillin, ceftriaxone, erythromycin, ciprofloxacin, vancomycin, linezolid, tetracycline and intermediate susceptibility to clindamycin)
 - rpoB and tox PCR amplicon shared 100% identity
 - share same sequence type in Multi Locus Sequence Typing (MLST: ST 326)
- Next generation Sequencing was used to unambiguously identify the transmission:
 - both isolates were grown in liquid culture, DNA was isolated
 - Nextera XT sample preparation was performed
 - DNA was sequenced on an Illumina MiSEQ using 250 bp paired end mode
 - Data analysis was carried out using GALAXY

→ both isolates were identical
 → direct zoonotic transmission of *C. ulcerans*

Zoonotic transmission:

- 4 groups of human and pet animal isolates were sequenced on Illumina MiSeq NGS device
- Neighborhood joining tree based on SNPs reveals zoonotic transmission in 4/4 cases
- the reported case is marked by the black circle

