OPEN ACCESS

Case Report

Actinobacillus equuli ssp. haemolyticus in a semi-occlusively treated horse bite wound in a 2-year-old girl

Nachweis von Actinobacillus equuli ssp. haemolyticus in einer semi-occlusiv versorgten Pferdebisswunde eines 2-jährigen Mädchens

Abstract

We report on the isolation of *Actinobacillus equuli ssp. haemolyticus* from wound smears of a 2-year-old girl who was admitted to the hospital due to partial amputation of the distal phalanx of her right middle finger caused by a horse bite. *A. equuli* typically causes diseases in horses and only very few reports describing human infections (mostly associated with wounds) are available in the literature. Interestingly, although the bacteria could be found in consecutive samples taken at different points in time, there were no signs of advancing infection or inflammation. Moreover, the fingertip regenerated after 74 days under semi-occlusive dressings with very pleasant results. For strain identification two automated systems were employed producing discrepant results: VITEK 2 described the pathogens as *Pasteurella pneumotropica* while MALDI-TOF MS analysis revealed *A. equuli*. Sequence analysis of 16S rDNA gene finally confirmed *A. equuli ssp. haemolyticus* as the isolated strain. The antimicrobial susceptibility testing was performed according to the CLSI criteria for *Pasteurella spp.* Additionally we conducted a test according to the EUCAST criteria.

Keywords: Actinobacillus equuli, Pasteurella, MALDI-TOF MS, VITEK 2, semi-occlusive dressing, finger amputation

Zusammenfassung

Wir berichten über den Nachweis von *Actinobacillus equuli ssp. haemolyticus* aus Wundabstrichen eines 2 Jahre alten Mädchens, welches sich wegen eines Pferdebisses in der Klinik vorstellte. Diese Verletzung führte zu einer teilweisen Amputation der distalen Phalanx ihres rechten Mittelfingers. Bei *A. equuli handelt* es sich um Bakterien, welche typischerweise Krankheiten bei Pferden verursachen. Bei Menschen findet *A. equulii* zumeist als Erreger von Wundinfektionen Erwähnung. Die Erreger konnten zu unterschiedlichen Zeitpunkten nachgewiesen werden. Während der gesamten Behandlungsdauer waren keine Zeichen einer fortschreitenden Infektion oder Entzündung zu beobachten. Die Fingerkuppe regenerierte nach 74 Tagen unter semi-occlusiven Verbänden mit einem sehr schönen Ergebnis. Die Erreger wurden mit Hilfe der beiden automatisierten Systeme VITEK 2 und MALDI-TOF MS identifiziert. Die biochemische Untersuchung mittels VITEK 2 identifizierte den Erreger als *Pasteurella pneumotropica*, wohingegen die massenspektrometrische Analyse *A. equuli* als Resultat lieferte. Die anschließende Sequenzierung des 16S rDNA Gens erbrachte das Ergebnis *A. equuli ssp. haemolyticus* und bestätigte somit die Identifikation der MALDI-TOF MS-Analyse. Wir erstellten für den Erreger Antibiogramme nach den Kriterien der amerikanischen Norm (CLSI) für *Pasteurella spp.* und stellten einen Vergleich zur europäischen Norm (EUCAST) an.

Percy Schröttner[1]
Jurek Schultz[2]
Wolfram Rudolph[3]
Florian Gunzer[1]
Alexander Thürmer[1]
Guido Fitze[2]
Enno Jacobs[1]

1 Institute of Medical Microbiology and Hygiene, Faculty of Medicine, Universitätsklinikum Carl-Gustav-Carus, Dresden, Germany

2 Department of Pediatric Surgery, Faculty of Medicine, Universitätsklinikum Carl-Gustav-Carus, Dresden, Germany

3 Institute of Virology, Faculty of Medicine, Universitätsklinikum Carl-Gustav-Carus, Dresden, Germany

Introduction

Animal bites are an important issue in health care management. According to Thomas and Brook in the USA approximately 800,000 people seek medical aid due to animal bites each year [1]. Although there are no reliable data available, a rate of 30,000 to 50,000 cases of animal bite wounds per year are estimated in Germany [2]. Infections with both, aerobic and anaerobic organisms can lead to edema, erythema, lymphangitis, sepsis, necrosis but could also be the cause of osteomyelitis [1], [2], [3]. In recent years zoonotic infections such as rabies, tularemia, brucellosis or tetanus have become a rare incidence in Germany [2], [3]. The relevant organisms are mostly part of the oral cavity and differ depending on the animal species. While most bite injuries are caused by dogs and cats, horse bites are relatively uncommon. Bite wounds are normally characterized by a mix of animal oral flora, the victims skin flora and environmental organisms [1], [4], [5], [6]. The most common pathogens found in bite wounds belong to the family *Pasteurellaecae, Staphylococcus spp.* and *Streptococcus spp.* [1], [4], [5], [6].

Actinobacillus spp. are Gram-negative bacteria that are commensals of the oropharyngeal cavity of horses and sheep and are closely related to *Pasteurellaceae* [7], [8]. *A. equulii* leads to systemic infections in horses such as the sleepy foal disease, a mostly fatal septicemia of newborn horses. In addition to that, meningitis, mastitis, arthritis and endocarditis could be associated with *A. equuli* [9].

Recent epidemiological investigations suggested the species *A. equuli* to be divided into the two subspecies *Actinobacillus equuli ssp. equuli ssp. nov*. and *Actinobacillus equuli ssp. hemolyticus ssp. nov.* [10]. Moreover, *Actinobacillus equuli ssp. equuli ssp. nov*. is CAMP-Test negative whereas *Actinobacillus equuli ssp. haemolyticus* is positive [10]. In contrast to *Actinobacillus equuli ssp. equuli ssp. nov*. *Actinobacillus equuli ssp. haemolyticus* seems to be exclusively associated with horses [10]. The few reports affecting humans deal with wound infections and septicemia [7], [8].

Here we report the case of a 2-year-old girl who was bitten by a horse and had to undergo surgical treatment. The wound fluid was analysed at defined time points and bacterial growth could be detected revealing *A. equuli ssp. haemolyticus*, *Bacillus cereus* and mixed skin flora. In this case report we focus on the diagnostic procedures applied for identification of the causative pathogen and furthermore propose options for antimicrobial susceptibility testing of *A. equuli*.

Case description

The 2-year-old, otherwise healthy, presumably immunocompetent and orderly vaccinated girl presented to our emergency department immediately (within around 35 minutes) after a horse had bitten off 30–40% of the distal phalanx of her right middle finger at the level of the lunula (Figure 1a), i.e. classified as Type III according to Allen [11]. No amputate was found at the site of accident. The amputation was treated with a semi-occlusive dressing [12] using a custom made silicone finger cap that forms a wet chamber around the wound while allowing for aseptic fine needle aspiration of wound fluid from a small reservoir that is connected to this chamber by a capillary (patent pending). A regular gauze dressing surrounded this finger cap for additional fixation. Since the actual horse bite was initially unknown and became clear only after questioning adult witnesses later on, the wound was cleaned and antiseptically irrigated with Octenisept® (Schülke & Mayr, Norderstedt, Germany) containing 0.1% octenidine dihydrochloride and 2% phenoxyethanol. An antibiotic prophylaxis was not administered. During the course of the treatment samples of the fluid were taken on a weekly basis while the fingercap was completely removed every two weeks to assess the regeneration progress. In total six samples were sent to the microbiology department for bacteriological analysis. Despite the infection with *A. equuli ssp. haemolyticus, B. cereus* and mixed skin flora which led to the formation of creamy yellow pus, we noted no advancing inflammation, no lymphangitis and no fever. Apart from an odor that increased with time and necessitated weekly changes of the outer gauze dressing, neither the girl nor her parents reported any unusual discomfort or pain and the hand was quickly used in all daily activities despite the injured finger and its dressing. After 74 days the fingertip had almost fully regenerated with complete scarless epithelialization, full function and very pleasant cosmetics with minimal shortening of the fingertip and with little clubbing of the nail (Figure 1a–e).

After the smears were delivered to the laboratory a Gram staining was carried out showing both Gram-negative rods and cocci and Gram-positive rods. Bacterial colonies could be detected after 24 hours of incubation at 37 °C and 5% CO_2 on Columbia Blood Agar showing phenotypical similarities to *Bacillus spp*. In the subsequent analysis this strain could be identified as *B. cereus* (positive indole reaction and MALDI-TOF MS Score: 2.13). A second strain presented with small grey-white colonies showing a discreet β-hemolysis (Figure 2). For identification two different automated systems were applied: first biochemical phenotyping by VITEK 2 (bioMérieux, Nürtingen, Germany) and second mass spectrometry MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). First, VITEK 2 identified *Pasteurella pneumotropica* (identification score 96%) second MALDI-TOF MS led to *A. equuli* (MALDI-TOF MS Score: 2.149). To clarify the true nature of these bacteria a 16S rDNA sequencing analysis was conducted using a 3130 Genetic Analyzer (Life Technologies, Darmstadt, Germany). The subsequent comparison of the DNA sequence with the NCBI nucleotide collection (nr/nt) using BLAST algorithm (http://blast.ncbi.nlm.nih.gov) revealed 100% homology to the 16S rDNA sequence of *A. equuli ssp. haemolyticus*, thereby confirming the species proposed by mass spectrometry.

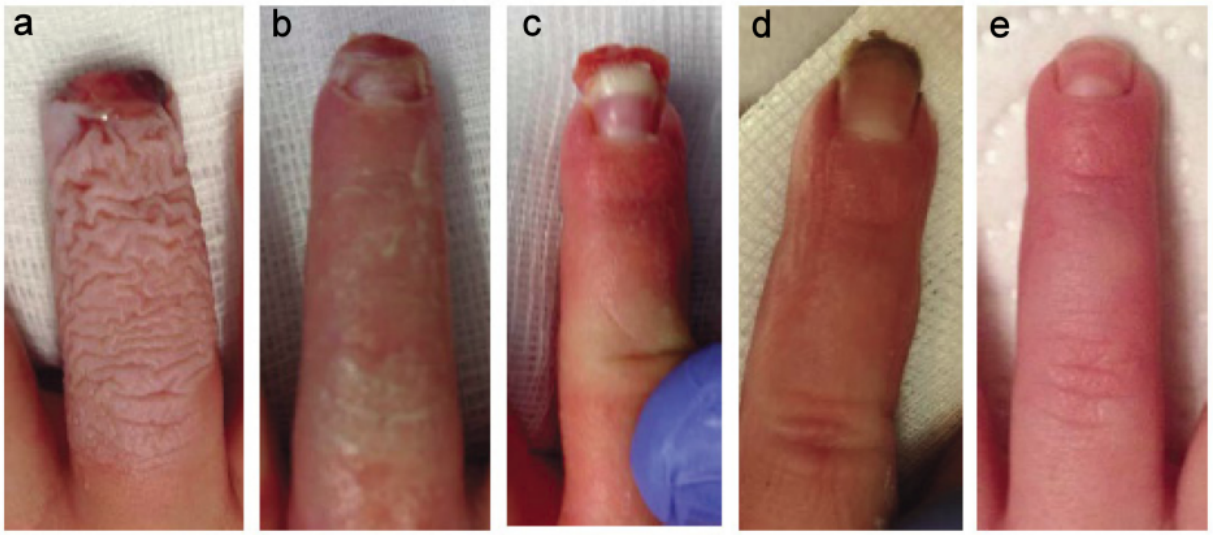

Figure 1: Photographs of the injured finger taken on days 2 (a), 28 (b), 56 (c), at the end of the treatment on day 74 (d) and finally at around 3 months after the injury (e)

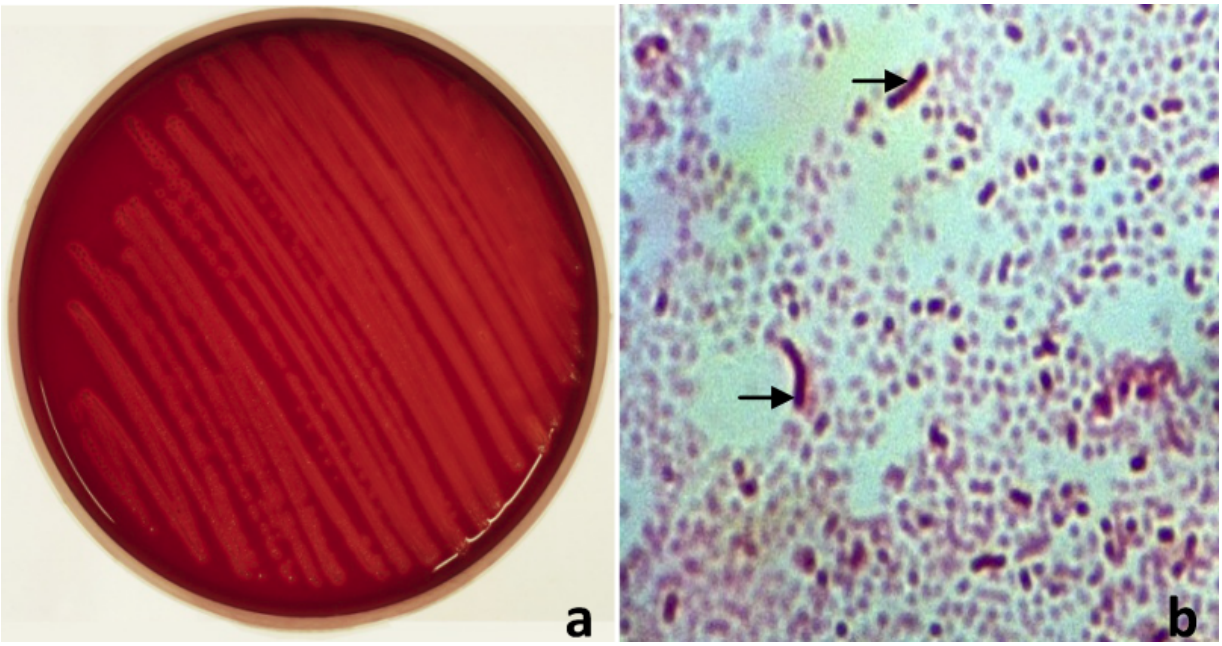

Figure 2: Growth of *Actinobacillus equulii spp. haemolyticus* on Columbia Blood Agar and Gram Staining. As demonstrated in a) the bacteria grow in small grey colonies with a sharp β-hemolysis while b) shows the typical Gram-staining showing the bacterial growth as both as gram-negative rods and cocci.

Since *Actinobacillus spp*. is a member of the family *Pasteurella* we decided to adapt the antimicrobial suspectibility testing according to the CLSI criteria for *Pasteurella spp*. The minimal inhibitory concentrations (MIC) were determined using E-Tests strips for the recommended antibiotics (bioMérieux, Nürtingen, Germany) [13], [14], [15], [16]. Since the European Committee for Antimicrobial Supectibility Testing (EUCAST) published guidelines for *Pasteurella multocida* (antibiotic panel tested by disk diffusion method on Mueller-Hinton agar with 5% sheep blood) in 2013 we decided to compare both, EUCAST and CLSI results. For evaluation, the EUCAST-breakpoints published for non-related species were applied [14]. However, since for non-species related breakpoints only MICs are available we additionally applied E-tests to detect the MICs. These results are demonstrated in Table 1 and Table 2.

Discussion

In the reported case *A. equuli ssp. haemolyticus* and *B. cereus* were repeatedly detected in the wound fluid of a 2-year-old girl who underwent treatment after being bitten by a horse. Despite the positive bacterial analyses no signs of advancing infections or inflammation were detected. The overall outcome was beneficial and it was decided not to apply any antibiotics. Our observations might be seen in the context of a positive role (and the novel understanding) of the microbiome in wound healing [17].

Table 1: Antibiotics tested according to CLSI criteria. The antibiotics were tested according to the CLSI-guidelines published for *Pasteurella spp.* Ampicillin, Amoxicillin/Clavulanic Acid, Moxicillin, Trimethoprim/Sulfamethoxazole and Tetracycline were tested to be susceptible (S) and Erythromycin resistant (R).

Antibiotic	determined MIC applying E-test	Interpretation according to CLSI
Ampicillin	0.125	S
Amoxicillin/Clavulanic Acid	0.5	S
Erythromycin	2	R
Moxicillin	0.016	S
Trimethoprime/Sulfamethoxazol	0.016	S
Tetracyclin	0.5	S

Table 2: Antibiotics tested according to the EUCAST criteria. The proposed test panel for *Pasteurella multocida* was chosen. The gained MICs were interpretated according to the EUCAST non-species related breakpoints. Ampicillin, ampicillin/clavulanic acid, cefotaxim and ciprofloxacin were tested as susceptible (S). For Trimethoprim/Sulfamethoxazole and Tetracycline there is an insufficient evidence (IE) for being a good therapeutic target.

Antibiotic	determined MIC (E-test)	Interpretation according to EUCAST
Ampicillin	0.125	S
Amoxicillin/Clavulanic Acid	0.5	S
Cefotaxim	0.16	S
Ciprofloxacin	0.004	S
Trimethoprime/Sulfamethoxazol	0.002	IE
Tetracycline	0.5	IE

In this respect processes such as bacteria-bacteria antagonism, a microbial influence on the initiation of re-epithelialisation, epidermal-cell proliferation and neo-vascularisation might play a significant role [17], [18]. To date there are no reports demonstrating a role of *A. equuli ssp. haemolyticus* in wound healing but nevertheless a potential role under certain conditions (e.g., occlusion) may not be denied. On the other hand, different (possibly undetected) microorganisms might have prevented the initiation of wound infection. In contrast, when detected in wounds, *B. cereus* is seen as an environmental contaminant [19]. For that reason, further investigation and research is needed to elucidcate the conditions and the extent, to which bacteria might have contributed to such a favourable outcome and inhibition of advancing infection in the case described here.

Regarding strain identification both mass spectrometry and 16S rDNA sequencing correctly identified the species Actinobacillus whereas the biochemical phenotyping by VITEK 2 resulted in *Pasteurella pneumotropica*. This discrepancy might be explained by the fact that only four biochemical tests discriminate between *Actinobacillus spp*. and *Pasteurella spp*.: β-galactosidase, urease activity, growth on McConkey agar and indole production [7]. Therefore, it may be assumed that the strain tested here might not have possessed all properties which would have been necessary for the correct biochemical identification. Moreover, MALDI-TOF MS has been shown to reliably identify *A. equuli ssp. haemolyticus* in a previous study [20], [21].

Due to the close relationship of *Actinobacillus spp.* and *Pasteurella spp*. we decided to adapt the antimicrobial testing according to the CLSI-criteria for *Pasteurella spp*. Our strain was susceptible to ampicillin, ampicillin/clavulanic acid, moxifloxacin, trimethoprim/sulfamethoxazole and tetracycline, but showed resistance towards erythromycin (Table 1). This observation is in accordance with reports on the resistance profile of *Pasteurella multocida* which demonstrate ineffectiveness for erythromycin, while the other antibiotics mentioned here are effective therapeutical agents [22]. Since the European Committee for Antimicrobial Standardisation Testing (EUCAST) proposed an antibiotic panel for *Pasteurella multocida* in 2013 we additionally conducted an antimicrobial testing according to this panel. For evaluation we used the proposed MICs for non related species. However, since zone diameter breakpoints are only defined for *Pasteurella multocida* we performed an E-test for each antibiotic. The strain

tested here was found to be susceptible to ampicillin, ampicillin/clavulanic acid, cefotaxime and ciprofloxacin. Insufficient evidence as an appropriate therapeutic target needs to be proposed for trimethoprim/sulfamethoxazole and tetracycline (Table 2).

Conclusion

In conclusion we could demonstrate the isolation of *A. equuli ssp. haemolyticus* in wound fluids of a 2-year-old girl. Interestingly, despite of repeated isolation of bacteria that might cause wound infections no therapeutically significant infection was seen (e.g., advancing erythema, swelling, lymphangitis, fever, lymphadenopathy, etc.). In this respect further research is needed to understand potential positive properties of the wound associated microbiome and specific conditions of *Actinobacillus equuli ssp. haemolyticus* for wound healing. Furthermore, it could be shown that MADLI-TOF MS is a reliable tool for correct identification of this pathogen. Further studies with larger numbers of bacteria will have to clarify the best diagnostic approach. Finally, we propose that an appropriate antimicrobial susceptibility testing can be performed according to both, CLSI-guidelines for *Pasteurella spp.* or EUCAST-test criteria.

Notes

Competing interests

The authors declare that they have no competing interests.

References

1. Thomas N, Brook I. Animal bite-associated infections: microbiology and treatment. Expert Rev Anti Infect Ther. 2011 Feb;9(2):215-26. DOI: 10.1586/eri.10.162
2. Gawenda M. Therapeutische Sofortmaßnahmen und Behandlungsstrategien bei Bißverletzungen. Dt Ärztebl. 1996;93:A-2776–80.
3. Kramer A, Assadian O, Frank M, Bender C, Hinz P; Working Section for Clinical Antiseptic of the German Society for Hospital Hygiene. Prevention of post-operative infections after surgical treatment of bite wounds. GMS Krankenhhyg Interdiszip. 2010;5(2):Doc12. DOI: 10.3205/dgkh000155
4. Sacks JJ, Kresnow M, Houston B. Dog bites: how big a problem? Inj Prev. 1996 Mar;2(1):52-4.
5. Dendle C, Looke D. Review article: Animal bites: an update for management with a focus on infections. Emerg Med Australas. 2008 Dec;20(6):458-67. DOI: 10.1111/j.1742-6723.2008.01130.x
6. Weber DJ, Hansen AR. Infections resulting from animal bites. Infect Dis Clin North Am. 1991 Sep;5(3):663-80.
7. Peel MM, Hornidge KA, Luppino M, Stacpoole AM, Weaver RE. Actinobacillus spp. and related bacteria in infected wounds of humans bitten by horses and sheep. J Clin Microbiol. 1991 Nov;29(11):2535-8.
8. Ashhurst-Smith C, Norton R, Thoreau W, Peel MM. Actinobacillus equuli septicemia: an unusual zoonotic infection. J Clin Microbiol. 1998 Sep;36(9):2789-90.
9. Rycroft AN, Garside LH. Actinobacillus species and their role in animal disease. Vet J. 2000 Jan;159(1):18-36. DOI: 10.1053/tvjl.1999.0403
10. Christensen H, Bisgaard M, Olsen JE. Reclassification of equine isolates previously reported as Actinobacillus equuli, variants of A. equuli, Actinobacillus suis or Bisgaard taxon 11 and proposal of A. equuli subsp. equuli subsp. nov. and A. equuli subsp. haemolyticus subsp. nov. Int J Syst Evol Microbiol. 2002 Sep;52(Pt 5):1569-76.
11. Allen MJ. Conservative management of finger tip injuries in adults. Hand. 1980 Oct;12(3):257-65.
12. de Boer P, Collinson PO. The use of silver sulphadiazine occlusive dressings for finger-tip injuries. J Bone Joint Surg Br. 1981;63B(4):545-7.
13. Clinical and Laboratory Standards Institute. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Proposed Guideline. CLSI document M45-P. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute; 2005. Available from: http://isoforlab.com/phocadownload/csli/M45-P.pdf
14. The European Committee on Antimicrobial Susceptibility Testing. Antimicrobial wild type distributions of microorganisms. Disk Diffusion for Pasteurella multocida. Available from: http://mic.eucast.org/Eucast2/SearchController/search.jsp?action=performSearch&BeginIndex=0&Micdif=dif&NumberIndex=50&Antib=-1&Specium=18
15. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 3.1. 2013. Available from: http.//www.eucast.org/clinical_breakpoints/
16. Leclercq R, Cantón R, Brown DF, Giske CG, Heisig P, MacGowan AP, Mouton JW, Nordmann P, Rodloff AC, Rossolini GM, Soussy CJ, Steinbakk M, Winstanley TG, Kahlmeter G. EUCAST expert rules in antimicrobial susceptibility testing. Clin Microbiol Infect. 2013 Feb;19(2):141-60. DOI: 10.1111/j.1469-0691.2011.03703.x
17. Scales BS, Huffnagle GB. The microbiome in wound repair and tissue fibrosis. J Pathol. 2013 Jan;229(2):323-31. DOI: 10.1002/path.4118
18. Hentzer M, Eberl I, Givskov M. Transcriptome analysis of Pseudomonas aeruginosa biofilm development: anaerobic respiration and iron limitation. Biofilms. 2005;2(1):537–61. DOI: 10.1017/S1479050505001699
19. Bottone EJ. Bacillus cereus, a volatile human pathogen. Clin Microbiol Rev. 2010 Apr;23(2):382-98. DOI: 10.1128/CMR.00073-09
20. El-Bouri K, Johnston S, Rees E, Thomas I, Bome-Mannathoko N, Jones C, Reid M, Ben-Ismaeil B, Davies AR, Harris LG, Mack D. Comparison of bacterial identification by MALDI-TOF mass spectrometry and conventional diagnostic microbiology methods: agreement, speed and cost implications. Br J Biomed Sci. 2012;69(2):47-55.
21. Kuhnert P, Bisgaard M, Korczak BM, Schwendener S, Christensen H, Frey J. Identification of animal Pasteurellaceae by MALDI-TOF mass spectrometry. J Microbiol Methods. 2012 Apr;89(1):1-7. DOI: 10.1016/j.mimet.2012.02.001
22. Lion C, Conroy MC, Carpentier AM, Lozniewski A. Antimicrobial susceptibilities of Pasteurella strains isolated from humans. Int J Antimicrob Agents. 2006 Apr;27(4):290-3. DOI: 10.1016/j.ijantimicag.2006.02.004

Corresponding author:
Enno Jacobs
Institute of Medical Microbiology and Hygiene, Faculty of Medicine, Universitätsklinikum Carl-Gustav-Carus, Fetscherstr. 74, 01307 Dresden, Germany, Phone: +49-(0)351-458-6605, Fax: +49-(0)351-458-6310
enno.jacobs@tu-dresden.de

Please cite as
Schröttner P, Schultz J, Rudolph W, Gunzer F, Thürmer A, Fitze G, Jacobs E. Actinobacillus equuli ssp. haemolyticus in a semi-occlusively treated horse bite wound in a 2-year-old girl. GMS Ger Med Sci. 2013;11:Doc14.
DOI: 10.3205/000182, URN: urn:nbn:de:0183-0001825

This article is freely available from
http://www.egms.de/en/journals/gms/2013-11/000182.shtml

Received: *2013-06-26*
Revised: *2013-09-16*
Published: *2013-09-24*

Copyright
©2013 Schröttner et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by-nc-nd/3.0/deed.en). You are free: to Share — to copy, distribute and transmit the work, provided the original author and source are credited.