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Pastern dermatitis outbreak associated with toxigenic and non-toxigenic *Corynebacterium diphtheriae* and non-toxigenic *Corynebacterium ulcerans* at a horse stable in Finland, 2021

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Abstract

Aims: *Corynebacterium diphtheriae* and *Corynebacterium ulcerans*, when producing toxin, are the cause of diphtheria, a potentially life-threatening illness in humans. Horses (*Equus ferus caballus*) are known to be susceptible to infection that may manifest clinically on rare occasions. In late 2021 and early 2022, specimens from five horses suffering from pastern dermatitis were cultured at the Laboratory of Clinical Microbiology at the Faculty of Veterinary Medicine, University of Helsinki, Finland. *C. diphtheriae* and/or *C. ulcerans* were recovered from all of these. This study aimed to (1) analyse the bacterial isolates and (2) describe the outbreak and identify possible sources of the infection and infection routes in the stable.

Methods and Results: Susceptibility testing, PCR for the *tox* gene, and Elek test for toxin production in PCR-positive isolates were performed. Whole genome sequencing was also conducted to achieve high-resolution strain typing. An epidemiological survey was done by means of a semi-structured interview of horses' caretaker, and contact tracing was done among people at the stable. Two *tox* gene-positive, toxin-producing *C. diphtheriae* belonged to sequence type (ST) 822. Other *C. diphtheriae* ($n=2$, ST828) and *C. ulcerans* ($n=2$, ST325 and ST838) isolates did not carry the *tox* gene. The epidemiological investigation explored numerous possible routes of transmission, but the definite source of infection was not identified. All established human contacts tested negative for diphtheriae. All horses recovered after antimicrobial treatment.

Conclusions: Our study shows that *C. diphtheriae* and *C. ulcerans* may readily spread among horses at the same stable and complicate pastern dermatitis infections. These potentially zoonotic bacteria can cause outbreaks even in a country with a very low prevalence. Caretakers should be encouraged to wear gloves and practice good hand hygiene when treating infected skin lesions in horses.

KEYWORDS
diphtheria, diphtheria toxin, epidemiology, horses, zoonosis

1 | INTRODUCTION

Corynebacteria are Gram-positive, catalase-positive, aerobic or facultatively anaerobic non-motile rods. Strains of *Corynebacterium diphtheriae* and some strains of *C. ulcerans* and *Corynebacterium pseudotuberculosis* may secrete exotoxin, which can cause diphtheria or diphtheria-like illness in humans (König et al., 2014; Sharma et al., 2019). It was known already in the early 1900s that horses (*Equus ferus caballus*) may be a possible source of diphtheria infection in humans (Cobbett, 1900) and that humans may infect horses (Minett, 1920). Early reports described afflicted horses with nasal discharge, lymphadenopathy, and laryngeal obstruction with breathing difficulty (Cobbett, 1900). More recently, equine cases of respiratory illness (Mathewson et al., 2018) and wound infections (Henricson et al., 2000; Leggett et al., 2010) caused by these bacteria have been described. Other diphtheria-like organisms identified in animals include biovar *C. diphtheriae* subsp. *belfanti*, *C. ulcerans*, *C. rouxii*, *C. uterequi*, and *C. pseudotuberculosis* (Baraúna et al., 2017; Corboz et al., 1996; Oliveira et al., 2014; Rückert et al., 2015; Schlez et al., 2021; Sing et al., 2016; Zendri et al., 2021). However, classic diphtheria is rare in animals.

Diphtheria in humans is an acute bacterial disease that affects primarily the mucous membranes of the upper respiratory tract. Diphtheria may be life-threatening and, in many countries, including Finland, it is classified as a generally hazardous communicable disease (Communicable Diseases Act 1227/2016 and decree 146/2017). Finland has a high vaccine coverage (91%–98%) (Muscat et al., 2022) and the most recent domestic cases ($n = 2$) were noted in 2001 in the Finnish Infectious Disease Registry. Further, one case of diphtheria was reported in a refugee in 2015 (THL, 2021). Based on epidemiologic studies and sequence homology, zoonotic transmission of *C. ulcerans*-type diphtheria between pigs and humans (Berger et al., 2013; Schuhegger et al., 2009) and between wild animals, dogs, and humans (Katsukawa et al., 2016) seems likely. Sporadically, *C. ulcerans* findings among game, hunting and pet dogs, cats and horses have been reported (Abbott et al., 2020; Carfora et al., 2018; Eisenberg et al., 2014; Katsukawa et al., 2016; Museux et al., 2023; Saeki et al., 2015; Zendri et al., 2021). During 2002–2008 most of the human *C. ulcerans* cases in France had had animal contact (dogs or cats) before infection, but animal-human transmission could not always be confirmed due to negative animal test results or lack of testing of animals (Bonmarin et al., 2009).

In December 2021, the Clinical Microbiology Laboratory (YESLAB) at the Faculty of Veterinary Medicine, University of Helsinki informed the Finnish Institute of Health and Welfare (THL) of findings of *C. diphtheriae* in specimens from pastern dermatitis lesions in three horses. Epidemiological and microbiological investigations were initiated to prevent further transmission of the infection and to characterize the outbreak.

Impacts

- Toxin-producing (toxigenic) *Corynebacterium diphtheriae*, a cause of diphtheria in humans, may spread among horses suffering from pastern dermatitis.
- Humans treating horses with such bacteria are at a risk of contracting the potentially fatal disease.
- Toxigenic and non-toxigenic *C. diphtheriae* and *Corynebacterium ulcerans* may be difficult to isolate from the mixed growth often seen in equine wound infections.

2 | MATERIALS AND METHODS

2.1 | Specimen collection and culture

In December 2021, YESLAB received bacteriological specimens from three horses with dermal lesions consistent with pastern dermatitis. The specimens, collected into transport tubes (M40, Copan Diagnostics, Italy) were cultured onto tryptic soy agar (TSA) with 5% sheep's blood (Thermo Fisher Scientific, USA). The media were incubated at +35 ± 2°C in 5% CO_2 for up to 4 days. Later, in February 2022, two further specimens from similar lesions arrived at the laboratory. These were investigated using identical methods.

Suspected corynebacterial colonies were identified using matrix-assisted laser desorption ionization mass spectroscopy time-of-flight (MALDI-TOF) (Bruker microflex LT, Bruker GmbH, Germany) by the direct smearing method using the BDAL 9607 library. Isolates identified as *C. diphtheriae* or *C. ulcerans* were sent to the laboratory of the Helsinki and Uusimaa Hospital District (HUS Diagnostic Center) for diphtheria toxin PCR. PCR primers and probes were previously described by Mothershed et al. (2002). PCR was performed with a 20-μL reaction mixture containing 1 μL of template DNA (boiled bacteria in Tris-EDTA buffer, pH 8.0), 10 μL of Amplidiag master mix (Mobidiag, Finland), and 0.3 μM concentration of primers and 0.25 μM concentration of probes (Mothershed et al., 2002). The thermocycling conditions with the MxPro 3005P system (Stratagene) were as follows: 95°C for 5 min, then 45 cycles of 95°C for 15 s and 60°C for 30 s.

2.2 | Elek toxigenicity testing

For detection of toxin production (Elek), bacterial isolates were sent to the Finnish National Reference Laboratory for Diphtheria and Pertussis, Institute of Biomedicine, University of Turku. Testing was performed with the conventional Elek test, as described previously

(Colman et al., 1992; Engler et al., 1997). Briefly, 3.5 mL of foetal bovine serum (Cytiva, USA) was added to 17.5 mL of molten Elek base, and 18 mL of this solution was transferred to a petri dish. After the agar had solidified, control (Strong+/Weak+/Negative) and newly isolated clinical strains were inoculated on the plate in horizontal lines. Finally, a strip containing 500 IU/mL of antitoxin was placed vertically in the middle of the plate. Plates were incubated at 37°C and lines of precipitation were monitored after 24 h and 48 h. Strains producing toxin will form precipitation with the antitoxin, whereas strains not producing toxin show no precipitation.

2.3 | Susceptibility testing

Susceptibility testing for isolates was performed by disc diffusion on Müller-Hinton agar supplemented with 5% sheep blood (Thermo Fisher Scientific, USA). Susceptibility testing was performed according to CLSI guidelines (CLSI, 2018, 2020). Tested antimicrobials included amikacin, ampicillin, chloramphenicol, clindamycin, doxycycline, enrofloxacin, erythromycin, fucidic acid, gentamicin, moxifloxacin, sulfamethoxazole-trimethoprim, and tetracycline. Applied breakpoints are presented in Appendix S1. *Streptococcus pneumoniae* ATCC 49619 was used as a control.

2.4 | Whole genome sequencing

For whole genome sequencing (WGS), the isolates were plated onto blood agar and the DNA was extracted after an overnight culture using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The extract quality was measured using a Qubit 4 fluorometer (Thermo Fisher Scientific, USA). The Illumina DNA Prep kit (Illumina, USA) was used according to the manufacturer's instructions to prepare the sequencing library. The WGS was performed on an Illumina MiSeq device, generating paired-end 250 bp reads. Species identification was confirmed by the KmerFinder software package (version 3.2) (Clausen et al., 2018; Hasman et al., 2014; Larsen et al., 2014).

Quality assessment, trimming, and de novo assembly were done with Velvet (version 1.1.04) (Zerbino & Birney, 2008) in Ridom SeqSphere+ (version 8.3.5, Ridom GmbH, Germany) (Jünemann et al., 2013). For strain comparison, an ad hoc core genome multilocus sequence typing (cgMLST) scheme was created using the whole genome of *C. diphtheriae* NCTC 11397 (NZ_LN831026.1) as the seed genome. All whole genome sequences of *C. diphtheriae* available in the NCBI database were used as penetration genomes for cgMLST creation. The *C. ulcerans* isolates were analysed similarly, but with *C. ulcerans* NCTC 7910 (NZ_LT906443.1) as the seed genome and the corresponding penetration genomes. A cgMLST-based minimum spanning tree was created separately for *C. diphtheriae* and *C. ulcerans* isolates using Ridom SeqSphere+. The genome of *C. ulcerans* CP054583, an equine isolate published by Zendri et al. (2021), was included in the *C. ulcerans* minimum spanning tree. *C. diphtheriae* sequence types (ST) were determined using the Center for Genomic Epidemiology MLST 2.0 service (Larsen et al., 2012). STs for *C. ulcerans* isolates were determined using the MLST scheme for *C. diphtheriae*. WGS data of isolates for which an ST could not be assigned based on previous submissions were uploaded to the Pasteur Institute for allele designation (https://bigsdb.pasteur.fr/diphtheria/). The data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB61304 (https://www.ebi.ac.uk/ena/browser/view/PRJEB61304).

2.5 | Epidemiological investigation

Contact tracing was performed by regional and municipal health authorities. Persons who had potentially been exposed to horses' infected dermatitis lesions without wearing protective gloves were identified, and their diphtheria vaccination status as well as their possible symptoms were recorded. A semi-structured interview (Appendix S2) was conducted with the horses' caretaker at the stable where the toxigenic bacteria were detected. This covered topics such as horses' care, feed, use, travel, contacts, and living conditions during the last 6 months before the diphtheria findings.

2.6 | Ethical aspects

The horses' caretaker gave written consent to publish the investigation. The study was approved by the Viikki Campus Research Ethics Committee (statement 13/2022) in accordance with legislation concerning animal welfare and research.

3 | RESULTS

3.1 | Epidemiological investigation

On 30 December 2021, the duty officer at THL was informed about *C. diphtheriae* findings in pastern dermatitis lesions in three horses living in the same stable in Southern Finland. THL notified the regional and municipal health authorities about the potential zoonotic infection and recommended contact tracing of persons potentially exposed to the horses' wounds without wearing protective gloves. Six contacts were identified and were given penicillin prophylaxis and a tetanus-diphtheria-pertussis vaccine booster. All tested negative for diphtheria by throat culture and developed no diphtheria-like symptoms within 10 days of the exposure. Hand hygiene guidelines to control the spread of the infection in humans were given to the horses' caretakers.

The horses affected in late 2021 were all standardbred geldings between the ages of 3 and 8 years from the same stable. Of the three tested horses, one was born in Finland (horse B) and two were born in other northern European countries. However, all three horses had been housed at the same stable for more than 6 months and had not travelled abroad during that period. The horses were trained at home twice a week by the trainer and the caretakers

responsible for the day-to-day life and care of the horses. In addition, the horses raced one to four times a month at various domestic racetracks. A dog and a cat also lived in the stable area, with full access to the stable.

During training the horses wore boots on hind legs, front legs, or all four legs depending on the horse. During transportation, the horses wore stable bandages on all four legs. All equipment (boots, harnesses, stable or dry rugs, bridles, and bits), except turnout rugs, was in collective use. The stable also had one washing stall common to all horses. Horses were turned out individually in sand/ground paddocks, but some horses shared a paddock and a stall. In addition to the stable, there were also outside stalls. Wood shavings from a local sawmill were used as bedding. Hay was also produced by a local farmer. Feed was provided commercially by a company based in Finland. No new lots of shavings, hay, or feed were acquired in the months leading up to the outbreak.

Horse A was the first to develop clinical signs. Alopecia and crusts were noted on the plantar pastern area of the hind legs. Within 4 days the lesions spread to the front legs and became exudative. The hind legs also became swollen and painful on palpation, so a 5-day course of procaine penicillin was started intramuscularly. This horse lived in the outside stall and shared the stall and paddock with horse C, which was also noted to have mild symptoms (alopecia and crusts on both hind legs). After a few days, two other horses were also mildly affected. At this point suspicion of the condition's contagiousness had arisen and specimens were taken. The affected horses were isolated to the outside stalls. After receiving culture and susceptibility results, antibiotic treatment was initiated. All affected horses (including horse A) were treated locally on the infected skin of the pastern with penicillin cream once or twice a day by the caretakers. Disposable gloves were used, and gloves were changed between horses. Treatment was continued until the clinical signs were markedly alleviated (no exudation, no crusts), which took 10–14 days depending on the horse. All horses were clinically completely healthy within a month.

Horse A had the most severe symptoms and was the only one to receive intramuscular antibiotics. Pastern dermatitis lesions (Figure 1) occurred on all four legs in all the horses but were more severe on the hind legs. The dermatitis did not spread dorsally or proximally to the pastern area in any of the horses. Only horse A had swelling and was markedly painful. Milder symptoms included alopecia, crusts, and exudation. None of the affected horses developed systemic signs, that is, lethargy, fever, or respiratory signs.

3.2 | Bacteriological investigation

Pastern dermatitis specimens from five different horses revealed *C. diphtheriae* and/or *C. ulcerans*. Additionally, several other significant secondary soft tissue pathogens, most notably *Streptococcus dysgalactiae* and *Staphylococcus aureus*, were found. The species detected and a summary of the characteristics of the *Corynebacterium* sp. isolates are presented in Table 1. *C. diphtheriae* and *C. ulcerans* were both detected in the specimens of horses A and B, but the *C. ulcerans* isolated from horse A was not stored for further investigation. All *Corynebacterium* sp. isolates were susceptible to nearly all tested antimicrobials. One isolate was resistant to erythromycin, and five isolates (only 5/6 isolates tested) were resistant to fucidic acid.

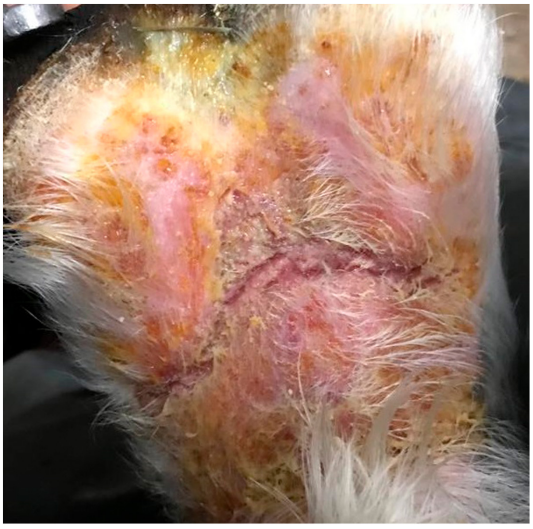

FIGURE 1 Pastern dermatitis in one of the horses from which bacterial culture specimens were obtained in December 2021 in Finland. The culture revealed a mixed growth of bacteria, including toxigenic *Corynebacterium diphtheriae*.

3.3 | Molecular epidemiology

Corynebacterium diphtheriae isolates Cdiph1 and Cdiph3 were positive for *toxA* gene in PCR and positive for toxin production in the Elek test. All other isolates were negative in PCR, and hence, the Elek test was not performed. Based on in silico multilocus sequence typing, the *C. diphtheriae* isolates belonged to two STs, 822 (Cdiph1 and Cdiph3) and 828 (Cdiph2 and Cdiph4), which are single locus (*dnaK*) variants. Of the two *C. ulcerans* isolates investigated, only Culc2 had a previously described sequence type (ST325). Isolate Culc1 showed novel alleles for *dnaE*, *leuA*, *fusA*, and *dnaK* and was assigned ST838. In cgMLST analysis, the *C. diphtheriae* isolates Cdiph1 and Cdiph3 were identical, while Cdiph2 and Cdiph4 differed by 38 alleles (Figure 2). *C. ulcerans* isolates were more diverse (Figure 3).

4 | DISCUSSION

We report, for the first time, toxin-producing *C. diphtheriae* ST822 and non-toxigenic *C. diphtheriae* ST828 in horses and an associated outbreak of pastern dermatitis at a stable. The specimens from horses A and B revealed both *C. diphtheriae* and *C. ulcerans*, while all cultured specimens revealed some degree of mixed bacterial growth. Previous studies have identified similar corynebacteria in equine infections. In a case report, Henricson et al. (2000) describe a draining chest

TABLE 1 Characteristics of *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* isolated from equine pastern dermatitis lesions in Finland in 2021/2022.

Isolate ID	Bacterial species	Horse ID	Isolation date (m/y)	ST	Sample ID	Toxin PCR	Toxin production	Other bacteria isolated from the same specimen
Cdiph1	*C. diphtheriae*	A	12/21	822	FIXT-962	+	+	*Streptococcus dysgalactiae*, *Actinobacillus equuli*, *C. ulcerans*[a]
Cdiph2	*C. diphtheriae*	B	12/21	828	FIXT-964	−	NP	*S. dysgalactiae*, *Staphylococcus aureus*, aerobic mixed growth
Cdiph3	*C. diphtheriae*	C	12/21	822	FIXT-965	+	+	*S. dysgalactiae*, aerobic mixed growth
Cdiph4	*C. diphtheriae*	D	2/22	828	FIXT-969	−	NP	*S. dysgalactiae*, *S. aureus*, *Acinetobacter baumannii*
Culc1	*C. ulcerans*	B	12/21	838[b]	FIXT-963	−	NP	Same specimen as Cdiph2
Culc2	*C. ulcerans*	E	2/22	325	FIXT-968	−	NP	*S. aureus*, *Arcanobacterium haemolyticum*

Abbreviations: +, positive; −, negative; NP, not performed; ST, sequence type.

[a]This *C. ulcerans* isolate was not stored and no further studies could be done.

[b]Novel ST.

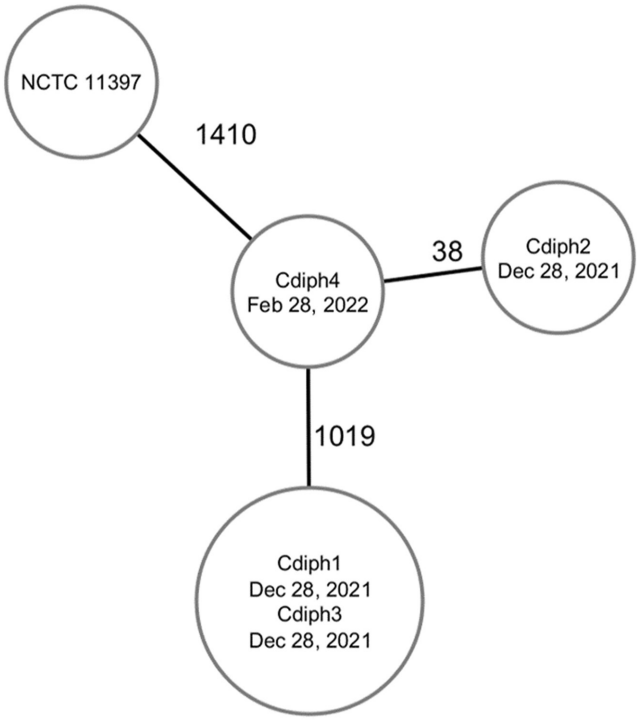

FIGURE 2 Relatedness of *Corynebacterium diphtheriae* isolates based on cgMLST analysis. Minimum spanning tree is based on comparing 1539 target genes and ignoring missing values in pairwise comparison. The numbers next to the lines connecting the strains indicate allelic differences (lines in logarithmic scale). Date of isolation is indicated below the strain. The toxin-producing isolates Cdiph1 and Cdiph3 belonged to ST822, while non-toxigenic Cdiph2 and Cdiph4 belonged to ST828. NCTC 11397 refers to NCBI database entry *C. diphtheriae* NZ_LN831026.1.

wound, where *C. diphtheriae* biotype gravis was discovered after a chest injury. Leggett et al. (2010) described another equine case as presenting with a chronic, discharging, poorly healing wound that was treated surgically. Bacteriological specimens in that study revealed *Streptococcus equi* ssp. *zooepidemicus* and *C. diphtheriae*. More recently, a publication by Zendri et al. (2021) describes an equine respiratory infection case very similar to that of human disease caused by toxigenic *C. ulcerans*. A recent review on equine pastern dermatitis by Gerber et al. (2023) also summarizes bacterial findings associated with the condition. While other corynebacteria, such as *C. pseudotuberculosis* and *Corynebacterium jeikeium* were described in standard bacteriology, diphtheria-like organisms were not.

The mixed growth noted in our study is typical of equine wounds and earlier studies have suggested one cause to be a diverse bacterial biofilm (Freeman et al., 2009; Westgate et al., 2011). *S. dysgalactiae* was a prominent finding in the specimens in our study, as it was discovered in four out of five specimens. This species is very common in equine infections (Erol et al., 2012). Another common (Freeman et al., 2009; Westgate et al., 2011) finding in equine wounds, *S. aureus*, was detected in three of the five specimens investigated. Other identified bacteria were *Acinetobacter baumannii*, *Actinobacillus equuli* (subspecies unknown), and *Arcanobacterium haemolyticum*. *A. baumannii* is viewed as a pathogen associated primarily with health care facilities, while other *Acinetobacter* sp. are ubiquitous in the environment (Antunes et al., 2014). These species have, however, also been described in equine wounds (Westgate et al., 2011). *A. equuli* is part of the normal microbiota of the horse but is regarded as an opportunistic pathogen (Layman et al., 2014), while *A. haemolyticum* has been described in human wound infections (Vu & Rajnik, 2022). With all of these potential other pathogens discovered in the pastern dermatitis specimens, any one of them might have been the primary infective agent. However, *C. diphtheriae* has been isolated from equine wound infections before (Henricson et al., 2000; Leggett et al., 2010), and thus, it may have played an important role in the infection. It is also noteworthy that an equine parapoxvirus, which may also cause outbreaks of pastern dermatitis in horses, has recently been described in Finland (Airas et al., 2016; Virtanen, Hautala, et al., 2023; Virtanen, Hautaniemi, et al., 2023) and should be considered as a differential diagnosis or as a possible co-infector. Pastern dermatitis specimens from the horses in this study were not investigated for parapoxvirus.

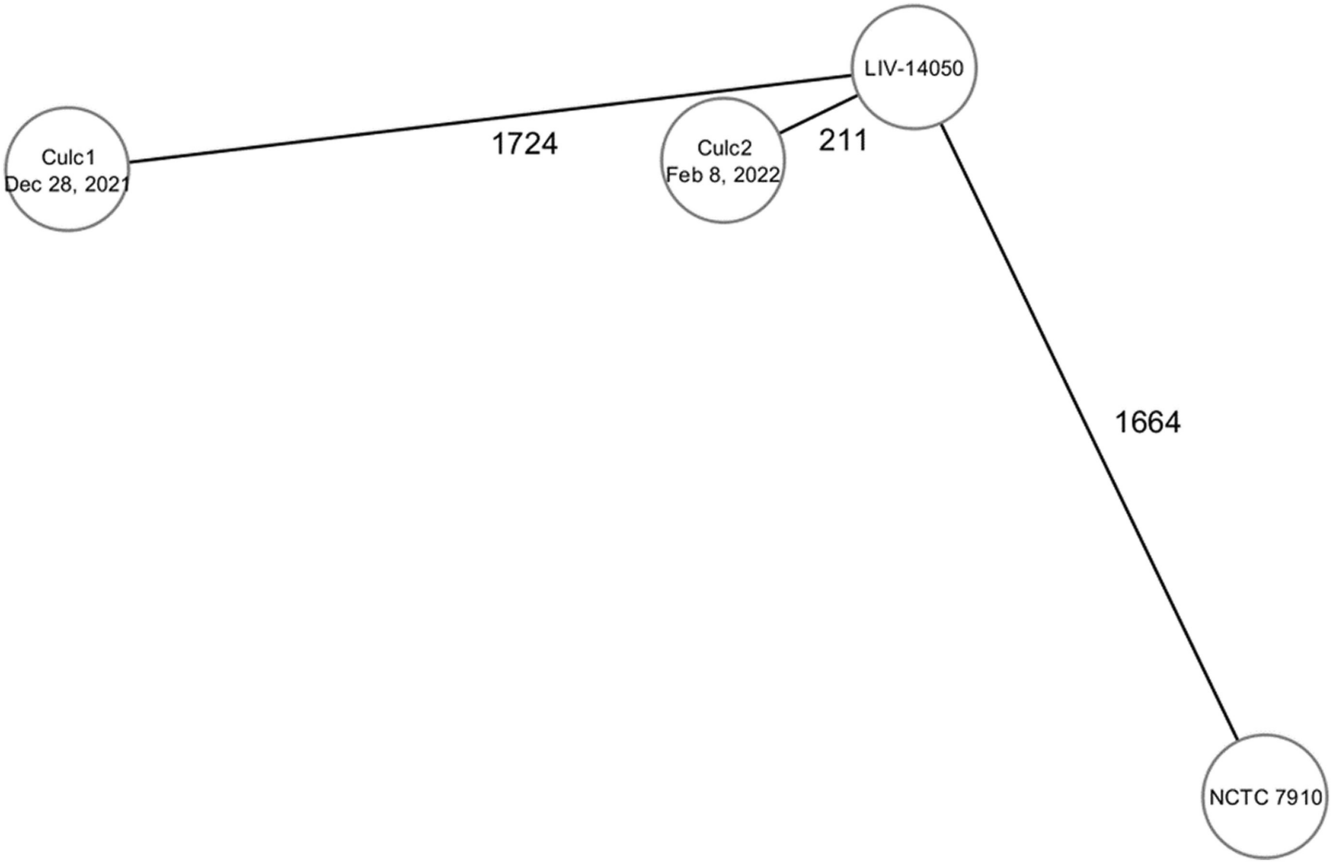

FIGURE 3 Relatedness of *Corynebacterium ulcerans* isolates based on cgMLST analysis. Minimum spanning tree is based on comparing 1818 target genes and ignoring missing values in pairwise comparison. The numbers next to the lines connecting the strains indicate allelic differences. Date of isolation is indicated below the strain. Isolates Culc1 and Culc2 are from the present study. NCTC 7910 refers to NCBI database entry *C. ulcerans* NZ_LT906443.1 and LIV-14050 to CP054583, a previously described *C. ulcerans* isolate from a diphtheria-like case in a horse in the UK (Zendri et al., 2021).

The epidemiological investigation could not identify the source of the corynebacteria, as no new animals, feed or bedding had been introduced recently. Since diphtheria is rare in humans in Finland, it is unlikely that the horses' human contacts would have been the source of the infection. In our study, the symptoms of the persons with close contact with the horses were monitored and all tested negative for diphtheria by throat culture. This is in line with most previous research, as human contacts have tested negative in at least three equine diphtheria cases (two *C. diphtheriae* and one *C. ulcerans*) from the USA (Henricson et al., 2000), the UK (Zendri et al., 2021), and Ireland (Leggett et al., 2010). Currently, human cases of diphtheria caused by *C. ulcerans* have outnumbered those caused by *C. diphtheriae* (Bonmarin et al., 2009; Wagner et al., 2010). It also appears that cases of diphtheria-like illness in animals are more often caused by *C. ulcerans* than *C. diphtheriae* (Sing et al., 2016). Since the two toxin-negative isolates (Cdiph4 and Culc2), discovered in February 2022, were genetically very different from the ones discovered in December 2021, it is unlikely that an epidemiological connection existed between them. However, the backgrounds of the horses from which the isolates were discovered in February 2022 were not further investigated since they were toxin-negative. Furthermore, while only two out of the *C. diphtheriae* isolates had the toxin gene and produced diphtheria toxin, it is noteworthy that several human cases of non-toxigenic *C. diphtheriae* have been reported in Europe (Dangel et al., 2018; Zasada & Rzeczkowska, 2019). Non-toxigenic *C. diphtheriae* has been classified as an emerging global health concern (Sangal & Hoskisson, 2016), although it is not currently notifiable (Muscat et al., 2022). However, very little is known about the zoonotic potential.

Recently, Muscat et al. (2022) summarized diphtheria cases in the WHO European region during 2010–2019. A total of 451 cases were reported in the region, with 12 diphtheria-related deaths described in 6 countries (case fatality 3%). Of the 52 cases reported from 11 countries in 2019, altogether 26 had information on age and 15 (58%) were older than 30 years, suggesting waning immunity against diphtheria in adults. Indeed, in a recent large seroprevalence study conducted among adults aged 40–59 years in 18 European countries, the proportion of testing sera lacking the protective level (<0.1 IU/mL) of diphtheria antibodies varied between 22.8% and 82.0% (Berbers et al., 2021). Clearly, the lack of vaccine-induced seroprotection against diphtheria is of concern in middle-aged adults in Europe. It is also interesting to note that, as a recent report from the Netherlands describes (Elsinga et al., 2023), the cutaneous form of diphtheria is now more common than the respiratory form of the disease in some countries.

Another possible source of the corynebacteria was other animals with access to the horses. Infections of *C. ulcerans* have been reported in dogs and cats (Abbott et al., 2020; Carfora et al., 2018; Fuursted et al., 2015; Saeki et al., 2015), but no clinical signs were reported in the cat and dog at the stable. They were, however, not tested for asymptomatic infection, which is possible at least in dogs (Katsukawa et al., 2012). Further, rodents and other pest animals are also a potential source of infection, but no such animals were tested. It is noteworthy that the spread of clonally related *C. ulcerans* isolates has previously been described in water rats (Eisenberg et al., 2015).

This study is descriptive in nature and thus cannot prove a cause-and-effect relationship between the presence of corynebacteria and the skin lesions. It is possible that diphtheria-like bacteria are semi-ubiquitous in equine pastern dermatitis lesions but are rarely isolated due to the heavy growth of other bacteria and the growth being classified as mixed without further characterization of the species involved. It is clear, however, that the detection of toxigenic strains poses a hazard to humans in contact with these horses, as demonstrated by the measures taken by public health authorities. Caretakers should be reminded to always use protective gloves and follow good hand hygiene when handling potentially infected lesions in horses. Equipment should be thoroughly cleaned between horses if individual equipment cannot be used. Moreover, veterinary practitioners are encouraged to obtain specimens from equine pastern dermatitis lesions in situations where numerous horses are affected simultaneously.

5 | CONCLUSIONS

In this study, we show that toxigenic *C. diphtheriae* as well as non-toxigenic *C. diphtheriae* and *C. ulcerans* are potential co-infectors in equine pastern dermatitis and that these bacteria may spread between horses. Veterinary laboratories should be vigilant as these bacteria may be easily missed in non-selective bacterial cultures from these lesions since mixed growth is common. Furthermore, this study highlights the importance of proper hand and equipment hygiene to reduce the risk of inter-animal or zoonotic spread of these potentially hazardous pathogens.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to report.

DATA AVAILABILITY STATEMENT

The authors confirm that all data supporting the findings of this study are available in the article and its supplementary materials.

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