発症 5 日目に 3 次医療機関へ転送された。再度実施された頭部 MRI では異常を認めなかった。転院初日の血清および髄液の中和抗体はそれぞれ 102 倍、47 倍であった。その後両者ともに上昇を認め、1,183 倍、1,300 倍となった。皮膚、項部、唾液のいずれからもウイルスは検出されなかった。人工呼吸管理となり、ケタミン、ミダゾラムを投与され、てんかん波抑制のためフェノバルビタールが加えられた。また、リバビリン、アマンタジンを経腸的に投与された。後遺症を残したが改善を認め退院した。退院後もさらに神経症状の改善を認められた。2007 年には、歩行障害、構語障害などが残存するものの、高校生活を卒業し、大学進学試験にて平均以上の点数を得たことが報告された 21)。本例は、発症前にも発症後にも狂犬病ワクチン接種を受けずに救命された初めての症例であった。

上記の救命例の治療をもとに、後述する MRR Milwaukee rabies protocol (MRP) が試みられ、また、2008 年に 2 例の狂犬病発症例で、MRB に準じた治療を受け救命された症例が報告されたが、その詳細は不明である 37)。

2009 年に米国より髄膜炎から集中治療を要さずに回復した症例で、後に狂犬病が判明した症例が報告された。41)
また、2011 年に MRP に準じた治療により回復した 1 例が報告されている。42)

上記 2 症例は、これまでの救命例と同様に、各種検体より狂犬病ウイルスは検出されず、狂犬病抗体の上昇が確認され、狂犬病と診断されたものであった。これは、症状出現時に狂犬病ウイルスが排除されていることが、発症後の転帰に影響する可能性を示唆すると思われる。狂犬病ウイルスの早期排除に、ウイルスの曝露量、受傷の程度、宿主の免疫応答などが影響する可能性がある。狂犬病ウイルスの早期排除に関する因子を今後検討する必要があると思われる。

4：狂犬病の合併症とその治療

これまでの症例の蓄積により、狂犬病の臨床経過が明らかになってきている。集中治療により生存期間の延長した症例が増加し、経過中に代謝系、循環器系、呼吸器系などの多彩な合併症が出現することが確認された 25)。

6 例目の救命例の治療を行ったウイスコンシン大学により、狂犬病患者対する治療指針が示され、Milwaukee rabies protocol (MRP) として公開されている 28)31)38)。MRP には、これまでの症例報告から、合併症とその発病日、推奨される治療が示されている。これらの治療は、特異的な治療により治癒を目指すことが前提であり、気管内挿管、人工呼吸管理、心臓ペースメーカーなどの侵襲的処置が含まれている。積極的治療を目指すのか、緩和的治療を目指すのか、統一された見解がない現状では、合併症治療をどこまで行うかについても議論が分かれる。(表 2)

5：狂犬病に対する特異的治療

これまで、狂犬病に対する特異的治療が検討されているが、実験室レベルでの有効性を示した薬剤はあるが、ヒト狂犬病の治療において効果の再現性が示されたものはない。前述したウイスコンシン大学を中心としたグループが特異的治療を積極的に行

っており、MRP に特異的治療が述べられている 30)。
以下にその概略を記す。

＜ケタミン＞

ケタミンは、非競合性 N-methyl-d-aspartate(NMDA)受容体阻害剤であり、一般的に臨床では、解離性麻酔薬として、麻酔科領域で使用されている薬剤である。

感染ラット神経細胞において、ケタミンを作用させることにより、狂犬病ウイルスの核タンパクおよび糖タンパクの合成抑制が確認したとの報告がみられた 8)。

しかし、同様の感染神経細胞を用いた実験において、ケタミンに神経保護作用は認めなかったとの報告もあり、結果は一定していない。マウスの脳内に狂犬病ウイルスに接種した実験系では、感染した神経細胞数、発現した狂犬病ウイルス量、及び死亡率に有意差を認めなかった 9)。

＜リバビリン＞

リバビリンは、1970 年代に合成されたプリンヌクレオシドアナログであり、これまでに、C 型肝炎ウイルスをはじめとして、広範なウイルス活性を有することが知られている 27)。

狂犬病にも応用され、単独で投与された例がみられたが、効果は認めなかった 11)。

MRP では、免疫応答を抑制する可能性から、その使用が禁忌とされた 38)。

＜インターフェロン・アルファ＞

狂犬病患者の体液および脳組織のインターフェロンが低値であることから、高容量のインターフェロンが治療に有望との報告 12) や、感染したサルにおいて、インターフェロン投与群で生存率上昇を認めた報告がある 10)。しかし、Warrell らは、ヒト狂犬病 3 例にインターフェロンを投与したが、いずれも 2 週間以内に死亡し、有効性を見出し得なかったことを報告している 11)。

＜抗狂犬病免疫グロブリン＞

狂犬病ウイルスの早期の排除を目的に、抗狂犬病免疫グロブリン（RIG）を試みられている。しかし、免疫グロブリンは通常血液脳関門を通過できないため、14)
狂犬病患者の中枢神経系にどの程度移行し、効果を発揮するのか不明である。また、髄腔内投与の安全性、有効性も分かっていない 13)。

ウマ抗狂犬病免疫グロブリン（ERIG）髄腔内投与を実施した症例では、生存期間延長を認めたが、救命には至らなかった 1)。

曝露前・曝露後免疫を行わず脳炎型狂犬病を発症し、ヒト抗狂犬病免疫グロブリン（HRIG）が投与された症例では、急速に四肢麻痺出現し、発症 15 日目に死亡したことが報告された 14)。

このことは、発症後の Immunoglobulin 投与が狂犬病をむしろ悪化させる可能性を示唆している。

＜狂犬病ワクチン＞

液性免疫および細胞性免疫の誘導を目的として、筋肉注射による狂犬病ワクチン接種が行われているが、明らかな効果は認められていない 2)。

通常の接種では、抗体検出までに 1 週間あるいはそれ以上の期間を要するため、複数個所の皮下接種を考慮すべきとの意見がみられる。しかし、このような強化したワ

クチン接種の有効性について、一致した見解は得られていない 4)。

＜副腎皮質ステロイド＞

狂犬病を感染させたマウスに対してステロイドを使用したところ、死亡率の上昇と潜伏期の短縮が認められている。このため、ヒト狂犬病への使用は、副腎不全を合併している例などを除いて原則禁忌と考えられている 20)。

＜Milwaukee rabies protocol (MRP)＞

これまでに、人工呼吸管理を含めた全身管理に加え、上記の薬剤を含んだ様々な治療が試みられているが、多くは救命に至っていない 26)。

2003 年米国での救命例では、人工呼吸管理に加え、ケタミン、ミダゾラム、フェノバルビタールにより、神経保護、けいれん波抑制や、自律神経系の抑制を図り、抗ウイルス剤としてリバビリン、アマンタジンを経腸投与された。これを基に、MRP が作成された。MRP の概要は、支持療法、神経保護と治療的昏睡、特異的抗ウイルス療法、免疫調整、治療期間の項目よりなっており、各項目に関する治療についての見解及び実施の可否が示されている。

世界各地で MRP に準じた治療がおこなわれているが、その後の生存例は報告されていない。Hemachudha の報告では、33 歳の狂犬病発症者に MRP に準じた治療を行ったが、入院 8 日目に死亡した 29)。

2006 年米国で発生した 2 症例に対しても MRP による治療を行ったが、死の転帰をとった 24)。

2010 年 1 月時点において、ウイスコンシン大学の治療チームに登録された 20 症例の中で、先に示した詳細不明である狂犬病救命例の 2 例が生存例とされており、治療の再現性には疑問が残る状況である 38) 39)40)。

これらの結果から、MRP の理論的根拠が明らかでなく、実験室レベルでの研究の蓄積が必要との批判的意見も少なくない 29)。

MRP は現在も検討が加えられており、2007 年 9 月に、version2.1 示され、2009 年 6 月に Version3.1 が公開された 31) 30)。

Version3.1 の主な変更として、tetrahydrobiopterine (BH4)の適応の変更、昏睡療法に関する薬剤の推奨、脳血管痙縮に対するカルシウム拮抗薬の使用、の 3 点があげられる。Version2.1 では、神経伝達物質に関連する酵素に必須とされる BH4 の投与が推奨されていたが、Version3.1 では、低血圧などが出現する例に適応が限定された。昏睡療法は、ケタミン、ベンゾジアゼピン系薬剤、アマンタジンが推奨されている。バルビツール酸は、リンパ球減少を惹起する可能性により、必要時のみの使用となった。また、リバビリンの使用は、患者の免疫応答を抑制し、臨床症状を悪化させる可能性から禁忌となった。脳血管痙縮を認める患者に予防的に、血管拡張薬であるカルシウム拮抗薬（Nimodipine 国内未承認薬）の投与が推奨されている。

前回の変更に加えて、治療はより細部にわたり指示されている。しかし、MRP の忠実な実施には、厳重な全身管理及び合併症治療・特異的治療に相当の医療資源を要することが明らかである。また、MRP が理論のみに基づく点も多いことから、有用性の評価には、症例の蓄積が必要である。

MRP version3.1 の要点について、表 3 に示した。

6：狂犬病の院内感染対策

狂犬病の院内感染対策について、これまでに示された文献は少なく、2004 年の WHO における専門家の検討で示されたものを以下に示した 32)33)。

要点については、表 4 に記した。

狂犬病患者の治療・看護にあたり、医療スタッフだけでなく、報道機関や一般住民の不安を惹起する可能性がある。他の多くの細菌やウイルスと比べて、感染リスクは高くないが、医療スタッフには、ガウン、ゴーグル、マスク、グローブ（Personal protective equipment PPE）の着用が求められる。特に、気管内挿管、吸引の実施時に、適切な PPE 着用が重要である。ウイルスは、血液中には存在せず、唾液、髄液、尿とある組織内にのみ間欠的に出現する。

感染のリスクがあると考えられる医療スタッフに対しては、十分に調査した上で、狂犬病曝露前免疫が考慮される。他の感染症と同様に、狂犬病患者をケアする際は、適切な感染対策を徹底するように、医療スタッフの意識を高めることが重要である。狂犬病患者を診療する特別な医療機関では、狂犬病患者にかかわる医療スタッフは、狂犬病曝露前免疫を行うべきである。ある医療機関では、狂犬病曝露後免疫を、組織培養狂犬病ワクチンを用いて 0,3,7,14 日と短縮したスケジュールで行っている。

生検や剖検などにおける、脳、脊髄の不用意な取り扱い（電気のこぎり、ドリルなどを用いた生検）は感染の危険性がある。そのため、このような操作では、ゴーグルや呼吸器防護具を用いるべきである。組織や体液は、結核や肝炎などの他の感染症と同様に廃棄しなければならない。一般的に、狂犬病で死亡したヒトからの感染の危険性は低い。血液にはウイルスは含まれないが、中枢神経系、唾液線、筋肉になどの多くの組織にウイルスが存在する。また、唾液や尿にも存在しうる。遺体に防腐処置を施すことは勧められない。不注意な局所解剖（ネクロプシー）の実施は、粘膜への曝露や、エアロゾルの吸入を招く可能性があり、ガウン、フェイスマスク、ゴーグル、厚い手袋などの PPE 着用により、感染を防御しうる。使用した器具は、オートクレーブまたは煮沸消毒が必要である。遺体は、早期に火葬または埋葬されることが勧められる。

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表 1　＜2004 年までの狂犬病救命例＞　17)

感染地	年代	性別	年齢	感染経路	ワクチン接種	後遺症
米国	1970	男	6	コウモリ咬傷	曝露後免疫	なし
アルゼンチン	1972	女	45	イヌ咬傷	曝露前免疫	軽度
米国	1977	男	32	実験室内感染	曝露前免疫	あり
メキシコ	1992	男	9	イヌ咬傷	曝露後免疫	高度
インド	2000	女	6	イヌ咬傷	曝露後免疫	高度
米国	2004	女	15	コウモリ咬傷	なし	軽度～中等度

症例 4：4 年以内に死亡

症例 5：約 2 年後に死亡

表 2＜病期別にみた合併症とその治療＞38）

注）Milwaukee rabies protocol(MRP) version3.1 による記載であり、国際的な標準治療として合意を得ているものではない。また、MRP による救命例の報告はあるが、その詳細は不明であり、治療効果の再現性は確認されていない。

合併症	発病日	推奨される治療
急性期	1-6	
恐水症および痙攣による脱水	1	生理食塩水・等張液を用いた補液 Vasopressor 及び BH4 投与
SIADH（時に尿崩症へ移行）	1-5	自由水の制限、頭蓋内圧のモニタリング ナトリウム濃度の調整
発熱	1-10	39.5 度まで許容される 室温の調整
自律神経系の不安定性：頻脈、上室性頻脈	1-4	鎮静・麻酔深度の強化；短時間作用性 β 遮断薬；ヘモグロビンの維持>10mg/dl、中心静脈圧の管理、心臓超音波検査の実施
自律神経系の不安定性：徐脈、心拍停止ペースメーカーの必要、完全房室ブロック	4	鎮静・麻酔深度の強化、経静脈的ペースメーカー、アトロピン、適切な輸液負荷、動脈血酸素化及び軽度の高二酸化炭素血を目標とした人工呼吸管理
血圧変動（痙攣に関連）	1	鎮静・麻酔深度の強化 輸液管理 BH4 の補充

唾液過剰分泌（最大 1.5l/日）	1-6	気管内挿管、気管切開
呼吸不全（無呼吸または非特異的肺機能障害）		気管内挿管、気管切開
腸閉塞	1-8	経鼻経腸栄養チューブ、経口薬剤、経腸栄養
排尿障害	4	尿道カテーテル留置
痙攣	1-4	鎮静、麻酔、の調整 気管内挿管を考慮 気管の刺激を避ける（リドカインを使用）
部分てんかん	1-4、15	経頭蓋骨脳血管ドップラー 発作時に脳波測定 抗てんかん薬
進行期	7-14	
尿崩症（5-15L/日）	4-14	尿管留置カテーテル 塩分喪失の除外 経頭蓋骨脳血管ドップラー・頭部 CT Vasopressor
体温変動	11－12	室温の調整
徐脈、心停止；　電気的ペーシング 完全房室ブロック	7－27	人工呼吸器の確認、系静脈的ペースメーカー 鎮静の強化、気管への刺激を避ける、 アトロピン、ヘモブロビンの維持(>10 mg%)、 適切な輸液負荷，　動脈血酸素化及び軽度の高二酸化炭素血を目標とした人工呼吸管理
中心静脈圧の上昇	5-21	
昏睡	11-12	
頭蓋内圧の上昇	6-11	脳室内ドレナージ及び近赤外線分光光度計によるモニタリング
全身性弛緩性麻痺	発症 4-8 日 症状の完成 10-14 日まで	人工呼吸管理、拘縮予防のための理学療法 じょく創予防の頻回の体位変換、予防的なヘパリン投与
副腎不全	5	
中心静脈圧上昇	5-21	BH4 補充
全般性脳血管痙縮（タイプ 1）	6-10	Minodipine 投与 経頭蓋骨脳血管ドップラー
結膜の紅潮	6-10	

脳圧上昇	6-11	脳圧のモニタリング 脳室ドレナージ 治療的腰椎穿刺
成長ホルモンの機能低下 22）	ヒトでの記載はない	
脳死に類似した症状（重度の脳症+完全な神経根症）	7-12、20	支持療法を継続
狂犬病抗体の出現	7-12	連日または隔日の血清抗体測定 連日または隔日の唾液中ウイルス測定
亜急性脳浮腫	10-33	頭部 CT・MRI ナトリウム濃度の調整
塩喪失	10-33	利尿剤投与を避ける 血清及び尿中のナトリウム及び尿酸測定 必要に応じナトリウム補充
甲状腺機能低下症	7-21	
徐脈・心停止 ペーシングの必要性 完全房室ブロック	7-27	ヘモグロビンを 10mg%以上に維持 鎮静を強化 経静脈的ペースメーカー 狂犬病抗体測定
脱神経　感覚及び運動	11-12	
体温変動	11-12	室温調整 人工呼吸菌の温度管理
昏睡	11-12	経頭蓋ドップラー超音波 緊急 CT±CT 血管造影
全般的脳血管痙縮（タイプ 2）	12-17	Minodipine 投与 脳圧管理 血清抗狂犬病抗体測定 腰椎穿刺
皮膚の紅潮、蕁麻疹様皮疹	12-25	血清狂犬病抗体価測定 皮膚生検
終末期	14-19	
心筋炎	6-21	頭部 MRI、CT などによる脳浮腫の除外
低血圧	7-24	心臓超音波検査 血管作動薬 BH4 投与

皮質活動の欠如（脳波）	14-24	経頭蓋ドップラー超音波 緊急 CT±CT 血管造影
上大静脈血栓	22	ヘパリン 弾性ストッキング

表 3　MRP Version3.1 の要点

＜支持療法＞

・カテコラミン性神経伝達物質の生成に必要な tetrahydrobiopterin（BH4）の補充は、全例には行わず、低血圧、脳血管痙縮などが認められる際に使用される。

・全身管理における麻酔深度の調整

・脳死の診断基準を当てはめることはできず、鑑別に脳生検、脳血流測定を要する

・脳血管痙縮予防のため、カルシウム拮抗薬（Nimodipine 国内未承認薬）を使用する。

＜神経保護＞

・神経保護は、主にベンゾジアゼピン、ケタミン、アマンタジンにより行われる

・フェノバルビタールは、神経障害の惹起、免疫応答を抑制する懸念があり、全例には投与せず、必要時のみに使用する。

＜抗ウイルス療法＞

・狂犬病ウイルスのクリアランスは生体の免疫反応により行われる

・発症後の抗狂犬病免疫グロブリン、狂犬病ワクチンの投与は、免疫を修飾し病勢を悪化させる可能性があり禁忌とする

・リバビリンの効果は不明であり、溶血性貧血などの副作用を有することや、免疫応答を抑制し臨床症状を悪化させる可能から、禁忌とされた。

表 4　狂犬病治療における院内感染対策　32）33）

・狂犬病患者の診療・看護に直接関わる場合には、ガウン、ゴーグル、マスク、グローブ（Personal protective equipment PPE）の着用を要する

・感染リスクのある医療スタッフに対して、曝露前免疫の実施を考慮

・剖検、生検、局所解剖を実施する際は、適切な PPE の着用を要する

・汚染された器具は、高圧滅菌消毒または煮沸消毒を要する

・遺体は防腐処置を行わず、早期に火葬または埋葬する

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻　号	ページ	出版年
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Multilocus Sequence Typing Implicates Rodents as the Main Reservoir Host of Human-Pathogenic *Borrelia garinii* in Japan▽†

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Multilocus sequence typing of *Borrelia garinii* isolates from humans and comparison with rodent and tick isolates were performed. Fifty-nine isolates were divided into two phylogenetic groups, and an association was detected between clinical and rodent isolates, suggesting that, in Japan, human-pathogenic *B. garinii* comes from rodents via ticks.

Lyme disease is a multisystemic disorder caused by infection with the tick-borne spirochetes *Borrelia burgdorferi* sensu lato (s.l.). *B. burgdorferi* sensu stricto (s.s.), *Borrelia garinii*, and *Borrelia afzelii*, which are the known pathogenic borreliae of humans. *B. burgdorferi* s.s. is geographically distributed throughout North America and Europe, whereas *B. garinii* and *B. afzelii* are distributed throughout Europe and Asia. These *Borrelia* species are transmitted by *Ixodes ricinus* in Europe and *Ixodes persulcatus* in Asia and Russia. (2, 16, 24). In Europe, serological characterization has revealed that *B. garinii* is composed of several OspA serotypes (27). At present, it is understood that one *B. garinii* serotype (*B. garinii* OspA serotype 4) is maintained by rodents (7), although other serotypes of *B. garinii* are maintained by birds (4, 5, 26). Strains classified into *B. garinii* OspA serotype 4 were found to be distinguishable from other *B. garinii* strains by multilocus sequence typing (MLST), which was recently established for Lyme disease borreliae (14, 15). In Japan, *B. garinii* is known to be the main pathogenic borrelia, and it is transmitted by *I. persulcatus* (29). However, the natural reservoir host of human pathogenic *B. garinii* remains unclear since *I. persulcatus* infests both rodents and birds in Japan (28). To resolve this question, MLST analysis was performed on clinical, tick, and rodent isolates, and phylogenetic relationships among these strains were investigated.

Nineteen *B. garinii* strains were obtained for MLST analysis from Lyme disease patients with erythema migrans in Japan. Human isolates were cultured from erythema migrans lesions as previously described (22). As for tick and rodent isolates, 40 strains were examined. The sources of these strains are listed in Table 1. Eighteen strains were isolated from *I. persulcatus* ticks, which were collected from Japan (15 strains) and Russia (3 strains). Twenty-two strains were isolated from rodents. Of these, 10 were from *Myodes rufocanus* subsp. *bedfordiae* and 8 from *Apodemus speciosus* (both sets collected in Japan), and 4 were from *A. uralensis* (collected in China) (Table 1). The cultivation of borreliae was carried out at 34°C in modified Barbour-Stoenner-Kelly (BSK) medium (using minimal essential medium alpha [BioWest, Germany] as a substitute for CMRL-1066) (1). These strains were stored at −80°C until use. Cultivated bacterial cells (late-log phase) were used in DNA preparation. The genomic DNA of isolated strains was prepared by using a DNA extraction kit (DNeasy blood and tissue kit; Qiagen, Germany) according to the manufacturer's instructions. The PCR assay was performed according to Margos et al. (14, 15). After DNA amplification of eight loci (*clpA*, ATP-dependent Clp protease subunit A gene; *clpX*, ATP-dependent Clp protease subunit X gene; *nifS*, aminotransferase gene; *pepX*, dipeptidyl aminopeptidase gene; *pyrG*, CTP synthase; *recG*, DNA recombinase gene; *rplB*, 50S ribosomal protein L2 gene; and *uvrA*, excinuclease ABC subunit A gene),

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† Supplemental material for this article may be found at http://jcm.asm.org/.

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TABLE 1. *Borrelia garinii* strains used in this study

B. garinii strain (no. of isolates)	Isolation source	Location	Reference
Tick isolates (18)			
HP1, HP3, HT18, HT59, N346, HkIP1, HkIP2	*Ixodes persulcatus*	Hokkaido, Japan	3, 10, this study
NP4, NP8, NP76, NP81, NT24, NT25, NT31	*I. persulcatus*	Nagano, Japan	10, 17
FujiP2	*I. persulcatus*	Shizuoka, Japan	11
Ip90	*I. persulcatus*	Khabarovsk, Russia	23
Mp7, Np189	*I. persulcatus*	Moscow, Russia	18
Rodent isolates (22)			
Ear isolates (17)			
HkCR1, HkCR3, HkCR4, HkCR5, HkCR6, HkCR7, HkCR9, HkCR11, HkCR12,	*Myodes rufocanus* subsp. *bedfordiae*	Hokkaido, Japan	9
FsAE1, FsAE2	*Apodemus speciosus*	Fukushima, Japan	8
FiEE11	*A. speciosus*	Fukui, Japan	8
sai8E	*A. speciosus*	Aomori, Japan	This study
ChYAE2	*A. uralensis*	Yakeshi, China	13
CTA1b, CTA4a, CTA5b	*A. uralensis*	Urumqi, China	25
Spleen isolate (1)			
ASF	*A. speciosus*	Hokkaido, Japan	19
Bladder isolates (4)			
HokkaidoCRB35B	*M. rufocanus* subsp. *bedfordiae*	Hokkaido, Japan	This study
HokkaidoAS7B	*A. speciosus*	Hokkaido, Japan	This study
sai6B, sai7B	*A. speciosus*	Aomori, Japan	This study
Human skin isolates (19)			
Hiratsuka	Erythema migrans	Niigata, Japan	This study
J-14, J-15, J-16, J-17, J-18, J-20T, J-21, J-32, J-33, J-34, J-35, J-37, J-38, J-39, J-40, J-41, J-42	Erythema migrans	Hokkaido, Japan	Miyamoto et al., unpublished data
HH1	Erythema migrans	Hokkaido, Japan	Sato et al., unpublished data

PCR products were purified by using ExoSAP-IT (GE Healthcare UK, Ltd., United Kingdom) and were directly sequenced (ABI Prism 3130xl Genetic Analyzer; Life Technologies Corporation). All sequences were deposited in GenBank (see the table in the supplemental material). In addition, reference sequences of each sequence type (ST) were downloaded from the MLST website (www.mlst.net). After concatenation of the sequences, Bayesian phylogenetic inference was performed (15). The phylogenetic tree was created according to Margos et al. (15), using TreeView software (ver. 1.6.6).

Isolated *B. garinii* strains were classified into two phylogenetic groups (preliminarily designated *B. garinii* ST group A and ST group B) by analysis of the concatenated DNA sequences of 8 loci (Fig. 1 and Table 2). The results indicated that *B. garinii* ST group B contained most of the Japanese clinical isolates (16/19 [84.2%]), all of the rodent isolates from Japan (18/18 [100%]) and China (4/4 [100%]), and 5 isolates from *I. persulcatus* collected in Japan (4/15 [26.7%]) and Russia (1/3 [33.3%]). Chi-square analysis indicated a confidence level of over 99% that *B. garinii* ST group B is predominant among Japanese clinical isolates and rodent isolates.

In this study, all *B. garinii* isolates from rodents were included in ST group B. In Europe, a recent report designated STs 84 and 85 as "*Candidatus* Borrelia bavariensis," and the reservoir host was thought to be rodents (14). Since *B. garinii* STs 84 and 85 clustered with *B. garinii* ST group B, we hypothesized that rodents are the main reservoir host of this phylogenetic group. In this study, the STs of 9 clinical isolates (2 of ST128, 4 of ST131, and strains J-14, J-18, and J-41) were found among rodent isolates. Thus, it can be inferred that human-pathogenic *B. garinii* is maintained by rodents in Japan. *B. garinii* ST group B was also found among rodent isolates from China. In addition, STs 128 and 131 of *B. garinii*, which were originally recorded in the MLST database as Chinese isolates, are pathogenic to humans in Japan. These suggest that *B. garinii* ST group B may also represent a health threat of Lyme

FIG. 1. Bayesian phylogenetic inference of concatenated housekeeping gene sequences of *B. garinii*. The phylogenetic tree was constructed based on Bayesian phlylogenetic inference. The posterior probability values of the clades are provided. Bars labeled 0.05 depict 5% divergence. *B. garinii* human isolates are indicated in red, rodent isolates in green, and isolates from *I. persulcatus* in blue. The relapsing fever *Borrelia* spp. (*B. duttonii* Ly [NC_011229], *B. hermsii* DAH [NC_010673], *B. recurrentis* A1 [NC_011244], and *B. turicatae* 91E135 [NC_008710]) were used as outgroups (data not indicated). The accession numbers of alleles from *Borrelia bissettii* DN127, *Borrelia andersonii* 21123, *Borrelia sinica* CMN3, and *Borrelia spielmanii* PsigII 27/6 are listed in the table in the supplemental material.

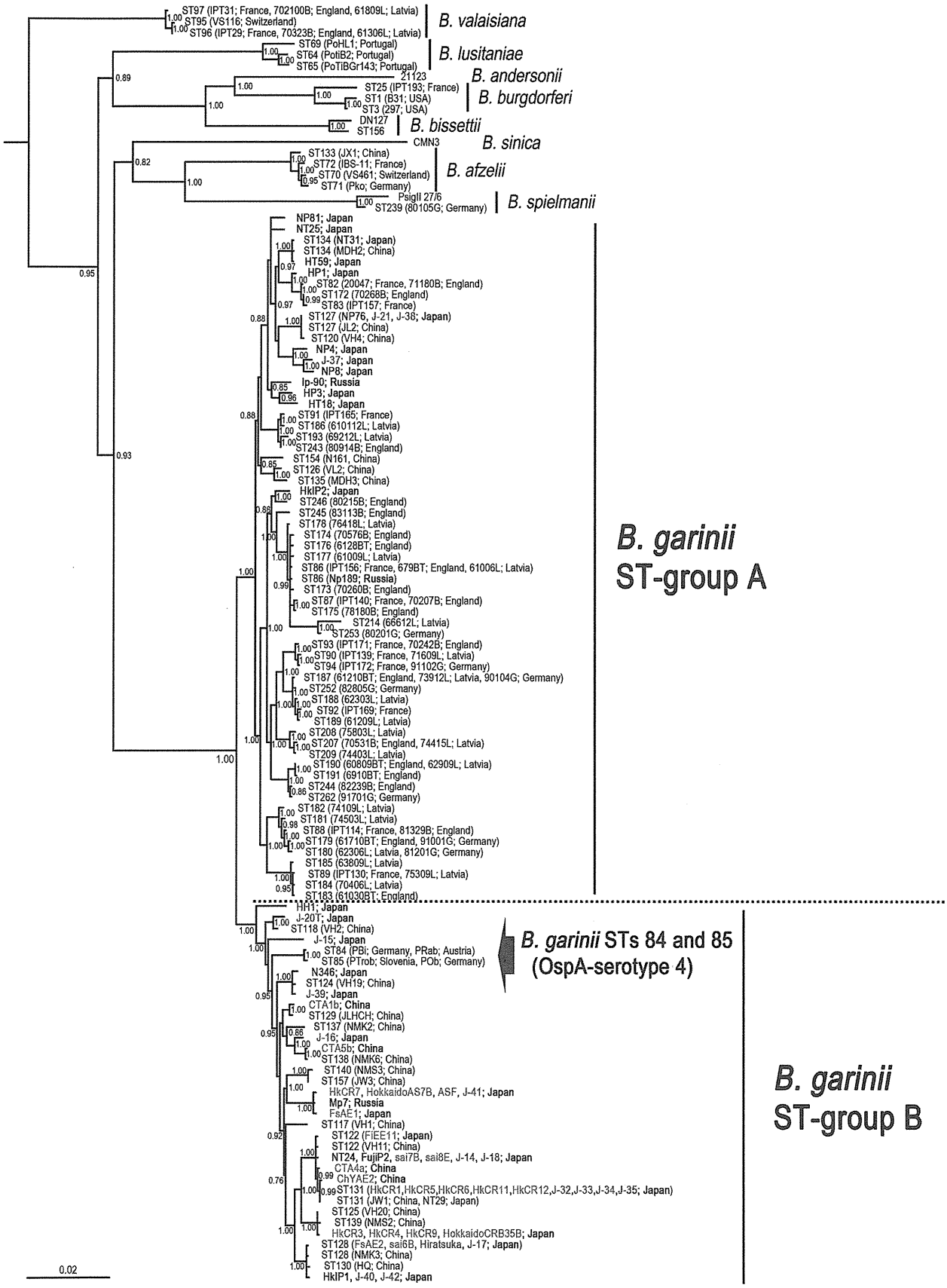
ST97 (IPT31; France, 702100B; England, 61809L; Latvia)
ST95 (VS116; Switzerland)
ST96 (IPT29; France, 70323B; England, 61306L; Latvia)
B. valaisiana
ST69 (PoHL1; Portugal)
ST64 (PotiB2; Portugal)
ST65 (PoTiBGr143; Portugal)
B. lusitaniae
21123
B. andersonii
ST25 (IPT193; France)
ST1 (B31; USA)
ST3 (297; USA)
B. burgdorferi
DN127
ST156
B. bissettii
CMN3
B. sinica
ST133 (JX1; China)
ST72 (IBS-11; France)
ST70 (VS461; Switzerland)
ST71 (Pko; Germany)
B. afzelii
PsigII 27/6
ST239 (80105G; Germany)
B. spielmanii
NP81; Japan
NT25; Japan
ST134 (NT31; Japan)
ST134 (MDH2; China)
HT59; Japan
HP1; Japan
ST82 (20047; France, 71180B; England)
ST172 (70268B; England)
ST83 (IPT157; France)
ST127 (NP76, J-21, J-38; Japan)
ST127 (JL2; China)
ST120 (VH4; China)
NP4; Japan
J-37; Japan
NP8; Japan
Ip-90; Russia
HP3; Japan
HT18; Japan
ST91 (IPT165; France)
ST186 (610112L; Latvia)
ST193 (69212L; Latvia)
ST243 (80914B; England)
ST154 (N161, China)
ST126 (VL2; China)
ST135 (MDH3; China)
HkIP2; Japan
ST246 (80215B; England)
ST245 (83113B; England)
ST178 (76418L; Latvia)
ST174 (70576B; England)
ST176 (6128BT; England)
ST177 (61009L; Latvia)
ST86 (IPT156; France, 679BT; England, 61006L; Latvia)
ST86 (Np189; Russia)
ST173 (70260B; England)
ST87 (IPT140; France, 70207B; England)
ST175 (78180B; England)
ST214 (66612L; Latvia)
ST253 (80201G; Germany)
ST93 (IPT171; France, 70242B; England)
ST90 (IPT139; France, 71609L; Latvia)
ST94 (IPT172; France, 91102G; Germany)
ST187 (61210BT; England, 73912L; Latvia, 90104G; Germany)
ST252 (82805G; Germany)
ST188 (62303L; Latvia)
ST92 (IPT169; France)
ST189 (61209L; Latvia)
ST208 (75803L; Latvia)
ST207 (70531B; England, 74415L; Latvia)
ST209 (74403L; Latvia)
ST190 (60809BT; England, 62909L; Latvia)
ST191 (6910BT; England)
ST244 (82239B; England)
ST262 (91701G; Germany)
ST182 (74109L; Latvia)
ST181 (74503L; Latvia)
ST88 (IPT114; France, 81329B; England)
ST179 (61710BT; England, 91001G; Germany)
ST180 (62306L; Latvia, 81201G; Germany)
ST185 (63809L; Latvia)
ST89 (IPT130; France, 75309L; Latvia)
ST184 (70406L; Latvia)
ST183 (61030BT; England)
B. garinii
ST-group A
HH1; Japan
J-20T; Japan
ST118 (VH2; China)
J-15; Japan
ST84 (PBi; Germany, PRab; Austria)
ST85 (PTrob; Slovenia, POb; Germany)
B. garinii STs 84 and 85
(OspA-serotype 4)
N346; Japan
ST124 (VH19; China)
J-39; Japan
CTA1b; China
ST129 (JLHCH; China)
ST137 (NMK2; China)
J-16; Japan
CTA5b; China
ST138 (NMK6; China)
ST140 (NMS3; China)
ST157 (JW3; China)
HkCR7, HokkaidoAS7B, ASF, J-41; Japan
Mp7; Russia
FsAE1; Japan
ST117 (VH1; China)
ST122 (FIEE11; Japan)
ST122 (VH11; China)
NT24, FujiP2, sai7B, sai8E, J-14, J-18; Japan
CTA4a; China
ChYAE2; China
ST131 (HkCR1,HkCR5,HkCR6,HkCR11,HkCR12,J-32,J-33,J-34,J-35; Japan)
ST131 (JW1; China, NT29; Japan)
ST125 (VH20; China)
ST139 (NMS2; China)
HkCR3, HkCR4, HkCR9, HokkaidoCRB35B; Japan
ST128 (FsAE2, sai6B, Hiratsuka, J-17; Japan)
ST128 (NMK3; China)
ST130 (HQ; China)
HkIP1, J-40, J-42; Japan
B. garinii
ST-group B
0.02

TABLE 2. ST groups of *Borrelia garinii* isolates from *Ixodes persulcatus* ticks, rodents, and humans

Isolation source	Country	*B. garinii* ST	
		Group A	Group B
I. persulcatus	Japan	HkIP2, HP1, HP3, HT18, HT59, NP4, NP8, NP76, NP81, NT25, NT31	HkIP1, N346, NT24, FujiP2
	Russia	Ip90, Np189	Mp7
Rodents	Japan	None	HkCR1, HkCR3, HkCR4, HkCR5, HkCR6, HkCR7, HkCR9, HkCR11, HkCR12, HokkaidoCRB35B, HokkaidoAS7B, ASF, FsAE1, FsAE2, FiEE11, sai6B, sai7B, sai8E
	China	None	ChYAE2, CTA1b, CTA4a, CTA5b
Humans	Japan	J-21, J-37, J-38	Hiratsuka, J-14, J-15, J-16, J-17, J-18, J-20T, J-32, J-33, J-34, J-35, J-39, J-40, J-41, J-42, HH1

disease in China. In contrast, *B. garinii* ST group A was not isolated from rodents in this study, yet was found to include almost all of the *B. garinii* isolates in Europe. Given that several reports claim *B. garinii* is detectable from birds in Asia (10, 20, 21), we suspect that *B. garinii* ST group A is maintained by birds, as are most of the *B. garinii* isolates in Europe.

I. persulcatus and *I. ricinus* ticks are known vectors of pathogenic *B. garinii* in Asia and Russia and Europe, respectively. In this study, *B. garinii* ST group A, which is most often isolated in Europe, was infrequent in Japan (Fig. 1). The reason remains unclear, but the inhabitant species of ticks may be associated with this geographical difference. Furthermore, Korenberg et al. recently found that *I. pavlovskyi* and *I. persulcatus* ticks differ in their abilities to transmit borrelia (12). This finding may support the notion that the resident tick species contributes to the determination of the endemic species *B. garinii*.

In this study, it was observed that the *B. garinii* strains which infect humans in Japan, are often found in rodents, but not nearly as often in ticks. In Europe, it was reported that *B. garinii* STs 84 and 85 are pathogenic to humans, although they were infrequent among tick isolations (6). These STs are not found in Japan, but they are clustered with most of the Japanese clinical isolates. Therefore, we hypothesized from our data that *B. garinii* ST group B isolates may be more pathogenic to humans than isolates of *B. garinii* ST group A.

In conclusion, *B. garinii* could be divided into two phylogenetic groups by MLST analysis, and one group (*B. garinii* ST group B) was predominant among clinical and rodent isolates in Japan. These results suggest that rodents are the reservoir host for most human-pathogenic *B. garinii* isolates in Japan. We also revealed that Japanese clinical isolates may be distinct from most European isolates. This may be due to the different vectors of *B. garinii* in Asia and Russia versus Europe. Our findings may contribute to the elucidation of *B. garinii*-caused Lyme disease epidemiology.

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Prevalence of *Corynebacterium ulcerans* in dogs in Osaka, Japan

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Diphtheria-like human illness caused by *Corynebacterium ulcerans* is an emerging threat in developed countries, with incidence sometimes higher than that of diphtheria caused by *Corynebacterium diphtheriae*. Companion animals are considered a potential source of human infections. In order to determine the prevalence of *C. ulcerans* among dogs, we performed a screening for the bacterium in 583 dogs in the custody of the Osaka Prefectural government. Forty-four dogs (7.5 %) were positive for the bacterium, although they did not show any clinical symptoms. All bacterial isolates showed resistance or decreased sensitivity to clindamycin, and some showed decreased sensitivity to levofloxacin. Comparative analysis of isolates using PFGE, toxin gene typing and antibiotic sensitivities suggests that transmission between asymptomatic dogs might have occurred.

INTRODUCTION

Some strains of *Corynebacterium ulcerans* produce diphtheria toxin and may cause human infection with diphtheria-like symptoms. In Japan, eight cases of infection by toxigenic *C. ulcerans* in humans were reported between 2001 and 2011 (Asakura *et al.*, 2006; Hagiwara *et al.*, 2006; Hatanaka *et al.*, 2003, 2011; Komiya *et al.*, 2010; Noguchi *et al.*, 2009; Nureki *et al.*, 2007; Yoshimura *et al.*, 2010). *C. ulcerans* causes purulent inflammatory diseases such as mastitis, lymphadenitis, dermatitis and respiratory infections in various domestic and wild animals. Human infections are caused by ingestion of untreated milk (Bostock *et al.*, 1984) or by close contact with a variety of animals (De Zoysa *et al.*, 2005; Hatanaka *et al.*, 2011; Noguchi *et al.*, 2009; Schuhegger *et al.*, 2009; Tiwari *et al.*, 2008; Yoshimura *et al.*, 2010). Dogs are of pronounced importance because they are sometimes linked to serious human cases (Hogg *et al.*, 2009; Lartigue *et al.*, 2005).

Abbreviations: CLDM, clindamycin; CLSI, Clinical and Laboratory Standards Institute; LVFX, levofloxacin.

The GenBank/EMBL/DDBJ accession numbers for the *tox* sequences of *C. ulcerans* Dog0803, Dog0804 and Dog0811 and *C. diphtheriae* ATCC 11049, ATCC 11051, RIMD 0343044 and CD1994-1 are AB602353, AB602354, AB602355, AB602356, AB602357, AB602358 and AB602359, respectively.

Supplementary tables are available with the online version of this paper.

To determine whether domestic dogs serve as a reservoir for human *C. ulcerans* infection, we carried out a series of initial screenings for *C. ulcerans* in 65 healthy dogs who were, for various reasons, in the custody of the Osaka Prefectural Government from December 2006 to September 2007. Toxigenic *C. ulcerans* was isolated from one dog (Katsukawa *et al.*, 2009). *C. ulcerans* was not found in any of the 218 family dogs living in the area where the infected dog originated (Takahashi, 2009).

In this study, we report the results of our continuation of the screening for *C. ulcerans* in the dogs in custody. In order to compensate for any seasonal effects on the screening, the screening period was extended to cover 13 months from November 2007 to December 2008. Upon examination of 583 dogs, 45 *C. ulcerans* isolates were obtained from 44 dogs. The characteristics of these isolates are described here.

METHODS

Dogs and sample collection. Between 27 November 2007 and 26 December 2008, 944 adult dogs were under the care of the Osaka Prefectural Government. Due to a tight schedule for euthanizing the dogs, throat swabs were collected from only 583 of the 944 dogs. The test population included 363 male and 220 female (401 mongrels and 182 purebreds) dogs. These dogs were either abandoned or had escaped from their guardians. Throughout the screening period,

sample collection was usually carried out twice a week, on Tuesdays and Fridays. Throat swabs were collected immediately after euthanasia and stored in preservation medium (SEEDSWABγ3 '*Eiken*'; Eiken Chemical) at 4 °C.

Bacterial isolation and identification. Bacterial strains used in this study are listed in Table 1. For the isolation of bacteria, culturing was started on the day of sample collection by inoculating the swabs on sheep blood agar and selective medium (charcoal–tellurite blood agar), and incubating them at 35 °C (Katsukawa *et al.*, 2009). Charcoal–tellurite blood agar contains heart infusion agar, 0.03 % (w/v) potassium tellurite, 10 % (v/v) sheep blood and 0.05 % (w/v) activated charcoal (Katsukawa *et al.*, 2009). Colonies appearing after 18–24 h on sheep blood agar and after 24, 30 and 48 h on charcoal–tellurite blood agar were transferred to dextrose-sucrose-starch agar medium (Jacherts, 1956) to investigate glucose and sucrose fermentation. Isolates positive for glucose but negative for sucrose fermentation were then characterized by Gram staining and by the catalase and urease tests. Thus, all Gram-positive organisms that tested positive for catalase and urease production were suspected to be *C. ulcerans* and further analysed using an API Coryne (bioMérieux) kit, followed by the determination of partial RNA polymerase β-subunit (*rpoB*) sequences (Khamis *et al.*, 2004).

Toxigenicity testing and nucleotide sequencing of the *tox* gene. Toxigenicity testing was performed by modified Elek test (Engler *et al.*, 1997; Katsukawa *et al.*, 2009; Reinhardt *et al.*, 1998), Vero cell cytotoxicity and neutralization tests (Katsukawa *et al.*, 2009; Miyamura *et al.*, 1974) and PCR for the diphtheria toxin (*tox*) gene. Primers used for PCR are listed in Supplementary Table S1 in JMM Online. Primers Tox 1 and Tox 2 (Nakao & Popovic, 1997), corresponding to the A subunit of the toxin, were first used for detection of the *tox* gene. In isolates negative for the primer pair, further PCR analyses using primers Dipht 6F and Dipht 6R (Nakao *et al.*, 1996) for the B subunit and toxFw and toxRv for the entire *tox* gene (Seto *et al.*, 2008) were carried out to confirm the absence of the gene. The nucleotide sequence of the *tox* gene was determined by amplifying the entire gene fragment with primers toxFw and toxRv (Seto *et al.*, 2008) (Supplementary Table S1) and then by sequencing the fragment with primers placed at appropriate intervals. A phylogenetic tree of *tox* nucleotide sequences was constructed using the neighbour-joining method (Saitou & Nei, 1987).

Table 1. Bacterial strains and isolates used in this study

Strains used for comparison studies are listed here.

Isolate/strain	Source	Reference
Corynebacterium ulcerans		
0102	Human	Hatanaka *et al.* (2003); Komiya *et al.* (2010)
0211	Human	Komiya *et al.* (2010)
0509	Human	Asakura *et al.* (2006)
0510	Human	Nureki *et al.* (2007)
0607	Human	Hagiwara *et al.* (2006)
0902	Human	Noguchi *et al.* (2009)
Dog0708	Dog	Katsukawa *et al.* (2009)
Dog0803	Dog	This study
Dog0804	Dog	This study
Dog0807	Dog	This study
Dog0809-1	Dog	This study
Dog0809-2	Dog	This study
Dog0809-3	Dog	This study
Dog0811	Dog	This study
Corynebacterium diphtheriae		
ATCC 11049	Human	ATCC
ATCC 11051	Human	ATCC
RIMD 0343044	Human	RIMD*
CD1994-1	Human	Thailand (this study)

*Culture Collection of the Research Institute for Microbial Diseases, Osaka University, Japan.

PFGE. PFGE was performed on all 45 isolates as well as on the *C. ulcerans* strains previously isolated from dogs and humans in Japan. PFGE of *Sfi*I-digested genomic bacterial DNA (De Zoysa *et al.*, 1995) was performed using 1.5 % (w/v) agarose gels and 0.5 × Tris/borate EDTA buffer at 14 °C, 6 V cm^{-1}, 5–20 s pulse for 18 h, followed by 1–5 s pulse for 14 h, using a CHEF-DR II system (Bio-Rad). PFGE patterns were analysed with the Diversity Database software by the UPGMA algorithm.

Antibiotic susceptibility. Antibiotic susceptibility tests were performed by the broth microdilution method using a Dry Plate DP24 (Eiken Chemical). We tested the following antibiotics: benzylpenicillin, ampicillin, cefazolin, cefotiam, cefotaxime, cefaclor, cefditoren, flomoxef, imipenem, meropenem, erythromycin, clindamycin (CLDM), minocycline, vancomycin, levofloxacin (LVFX) and sulfamethoxazole–trimethoprim. Sensitivities were assessed according to the Clinical and Laboratory Standards Institute's (CLSI) standard (M45-A) for *Corynebacterium* species. To assign sensitivities to the eight drugs for which CLSI standard values are not available, standards for similar drugs were considered (sensitivity to ampicillin tested using benzylpenicillin sensitivity standard; cefazolin, cefotiam, cefaclor, cefditoren and flomoxef tested using cefotaxime standard; minocycline tested using tetracycline standard; and LVFX tested using ciprofloxacin sensitivity standard).

RESULTS

Isolation of *C. ulcerans* and toxigenicity

Fig. 1 displays the number of isolates obtained over the course of the study. Supplementary Table S2 also summarizes the study population with respect to gender and seasonality of *C. ulcerans* isolation. A more comprehensive summary of the *C. ulcerans* isolates is shown in Table 2.

The first isolate was detected in March 2008, followed by the isolation of indistinguishable isolates from three other dogs that had the same guardian. These four isolates were classified as group 1. Screening continued intermittently, with 27 *C. ulcerans*-positive dogs identified between April and the beginning of June (group 2), six in July (group 3) and none until September. Seven isolates were obtained from six dogs in September and October (group 4, subdivided into three subgroups, 4-1 to 4-3, by toxigenicity and PFGE patterns) and one isolate from one dog in November (group 5). In summary, 45 *C. ulcerans* isolates (42 toxigenic and three non-toxigenic) were obtained from 44 dogs. From one of these dogs, toxigenic and

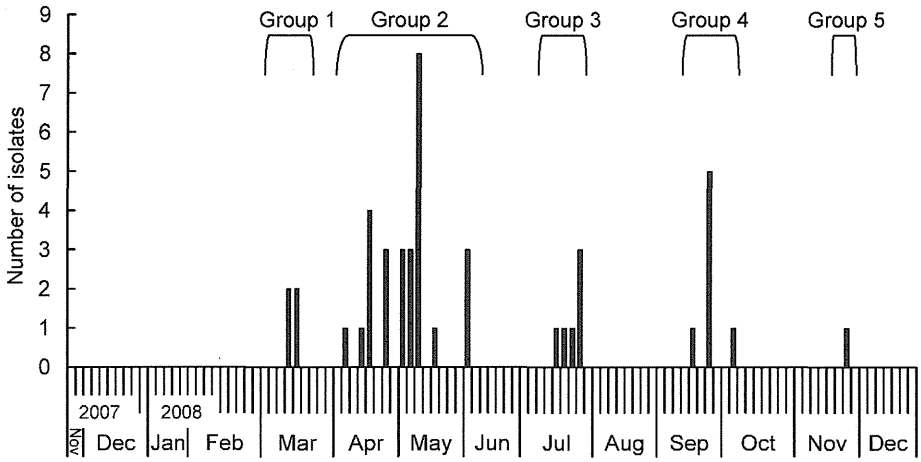

Fig. 1. Chronological representation of *C. ulcerans* isolation. Samples were collected as described in the text. Each sampling period is expressed as a small cell between the horizontal axis and month indications. Bars represent the number of isolates obtained during each sampling period.

non-toxigenic isolates were isolated simultaneously (Table 2, nos 39 and 40). For each of the other dogs, one isolate was obtained per dog (41 toxigenic and two non-toxigenic). None of the 44 dogs from which *C. ulcerans* was isolated were apparently symptomatic: nasal discharge, pharyngitis and dermatitis were not observed. There was no correlation between detection rates and the sex of the animals.

Each of the 45 isolates were obtained from the primary culture growing on charcoal–tellurite blood agar plates. Isolation from primary cultures on blood agar was not successful: only three isolates were obtained, mainly due to masking by the growth of other bacterial species present on the swab. The three isolates were later proven to be identical to the isolates obtained from the charcoal–tellurite blood agar plates of the same specimens.

All strains tested positive for catalase and urease production and glucose fermentation and negative for sucrose fermentation. They shared the same API code (0111326, % id 99.7, T=1.0). Forty-two isolates were toxigenic, as demonstrated by positive results of PCR for the *tox* gene (A subunit), modified Elek test, and Vero cell cytotoxicity and neutralization tests. Three isolates were negative for all of these tests and were thus considered to be non-toxigenic. Detailed characteristics of these isolates are summarized in Table 2.

PFGE analysis

All 45 isolates and the previous dog and human isolates were compared using PFGE. The isolates were classified into four types (A2, B, C, D) by PFGE (Table 2 and Fig. 2). PFGE classifications of isolates were as follows: PFGE type C included group 1 isolates (four toxigenic strains isolated in March); type A2 included groups 2, 3, 4-1 and 4-2 (37 toxigenic strains isolated from April to October and two of three non-toxigenic strains isolated in September); type B included group 4-3 (one non-toxigenic strain isolated in September); and type D included group 5 (one toxigenic strain isolated in November). As shown in Fig. 2, subtype A2, together with two other subtypes classified according to slight differences in PFGE patterns, composed type A. Previously isolated human strains have been classified with respect to PFGE type as follows: PFGE type A1, human isolates 0102 and 0210 (Komiya *et al.*, 2010) that were closely related to type A2, including 0509 (Asakura *et al.*, 2006) and 0902 (Noguchi *et al.*, 2009), and type A3 including 0607 (Hagiwara *et al.*, 2006). Another human isolate, 0510 (Nureki *et al.*, 2007), was classified in a comparatively distant type D.

Analysis of the diphtheria toxin (*tox*) gene

The complete *tox* gene sequence (1683 bp) was determined for 42 toxigenic isolates for which toxigenicity was confirmed. Their sequences were divided into three types as shown in Table 2 and Fig. 3. The first type (designated tox0803; GenBank accession no. AB602353) included four isolates of group 1 including Dog0803; the second type (tox0804; GenBank accession no. AB602354) included 37 toxigenic isolates of groups 2, 3 and 4-1, obtained from April through October, including Dog0804; and the last type (tox0811; GenBank accession no. AB602355) included only one isolate, Dog0811 (group 5), obtained in November. These sequences were also compared to the previously published *tox* gene sequences of *C. ulcerans* human strains 0102, 0510, A6361, A2911, X959 and KL126 (Komiya *et al.*, 2010; Schuhegger *et al.*, 2009; Seto *et al.*, 2008; Sing *et al.*, 2003, 2005; Wellinghausen *et al.*, 2002) (Table 3, Fig. 3). More than 98 % similarity was shown between any two of the *C. ulcerans* isolates and strains. However, the sequences showed reduced similarity (~95 %) to the reported *tox* gene

Table 2. Characteristics of the isolates

No.	Group	Designation as the isolate representing the group	Date of isolation*	Sex†	Isolation on‡:		API Coryne code	*tox* PCR	Elek	Vero cell cytotoxicity	PFGE type	*tox* gene type	Drug susceptibility§
					BA	C							
1	1	Dog0803	3/11	F	−	+	0111326	+	+	+	C	tox0803	CLDM: R
2	1		3/11	M	−	+	0111326	+	+	+	C	tox0803	CLDM: R
3	1		3/14	F	−	+	0111326	+	+	+	C	tox0803	CLDM: R
4	1		3/14	F	−	+	0111326	+	+	+	C	tox0803	CLDM: R
5	2	Dog0804	4/4	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
6	2		4/11	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
7	2		4/15	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
8	2		4/15	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
9	2		4/15	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
10	2		4/15	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
11	2		4/22	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
12	2		4/22	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
13	2		4/22	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
14	2		5/2	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
15	2		5/2	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
16	2		5/2	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
17	2		5/9	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
18	2		5/9	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
19	2		5/9	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
20	2		5/13	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
21	2		5/13	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
22	2		5/13	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
23	2		5/13	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
24	2		5/13	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
25	2		5/13	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
26	2		5/13	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: I
27	2		5/13	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
28	2		5/20	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
29	2		6/3	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: I
30	2		6/3	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: I
31	2		6/3	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: R
32	3	Dog0807	7/15	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: I, LVFX: I
33	3		7/18	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: I, LVFX: I
34	3		7/22	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: I, LVFX: I
35	3		7/25	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: I, LVFX: I
36	3		7/25	F	−	+	0111326	+	+	+	A2	tox0804	CLDM: I, LVFX: I
37	3		7/25	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: I, LVFX: I
38	4-1	Dog0809-1	9/16	M	+	+	0111326	+	+	+	A2	tox0804	CLDM: I
39‖	4-1		9/26	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: I
40‖	4-2	Dog0809-2	9/26	M	−	+	0111326	−	−	−	A2	−	CLDM: I
41	4-3	Dog0809-3	9/26	M	−	+	0111326	−	−	−	B	−	CLDM: I
42	4-2		9/26	M	−	+	0111326	−	−	−	A2	−	CLDM: I
43	4-1		9/26	M	+	+	0111326	+	+	+	A2	tox0804	CLDM: I
44	4-1		10/7	M	−	+	0111326	+	+	+	A2	tox0804	CLDM: I
45	5	Dog0811	11/25	M	+	+	0111326	+	+	+	D	tox0811	CLDM: R

*Month/day in 2008.
†M, Male; F, female.
‡BA, Blood agar; C, charcoal–tellurite blood agar.
§CLDM, Clindamycin; LVFX, levofloxacin; R, resistant; I, intermediate.
‖Isolated from the same dog.