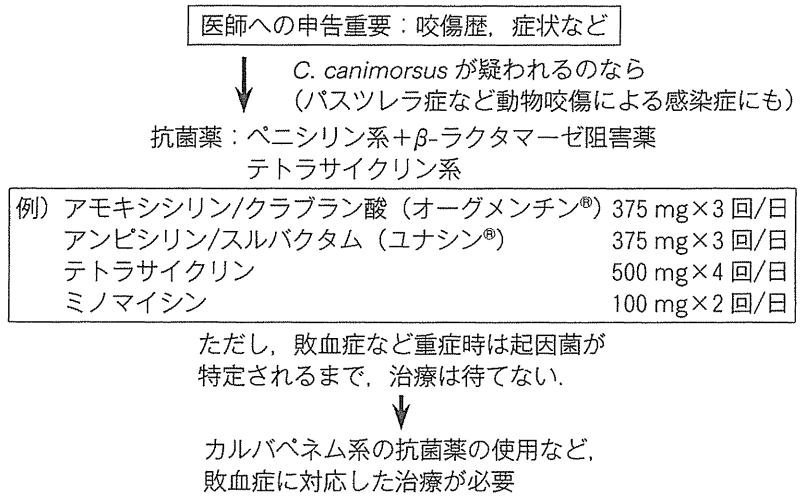

図　*C. canimorsus* 感染症への対応・治療

ずに，抗菌薬の投与のほか，敗血症に対するさまざまな対症療法が実施される（**図**）．ペニシリン系の抗菌薬が効果を示すが，β-ラクタマーゼを産生することもあるので，β-ラクタマーゼ阻害薬との合剤であるアモキシシリン/クラブラン酸（オーグメンチン®）やアンピシリン/スルバクタム（ユナシン®）が第1選択となる．これらは *C. canimorsus* 感染症に限らず，イヌ咬傷時の第1選択薬でもある[8)28)29)]．臨床分離株，イヌ・ネコ分離株を用いた筆者らの調査では，テトラサイクリン系，第3世代セフェム系，クロラムフェニコール系，カルバペネム系，ニューキノロン系に感性である一方，アミノグリコシド系には耐性であり，マクロライド系，リンコマイシン系，ST 合剤には耐性を示す株もあった（**表4**）[13)18)]．

予防については，イヌ・ネコの口腔内常在菌で除菌法も確立しておらず，かつワクチンもないので，感染予防のための一般的な心得として，動物取り扱い時に感染（咬掻傷）リスクを減少させる注意，自身の健康を維持する努力が大切になる．本感染症では，高齢者，免疫抑制状態にある者は易感染者であり，とくに注意が必要である．また，咬掻傷後に体調に異常を感じたらすぐに医療機関を受診し，咬掻傷歴を申告することが重要となる．予防投薬は，深い貫通性の傷，静脈やリンパ管叢のあるところ，骨や関節に近接もしくは達する傷，外科的処置を必要とする傷，免疫抑制者などが適応となる[8)28)29)]．

おわりに

C. canimorsus 感染症については依然として未知の部分が多い．死亡率は30%にのぼるが，これはあくまでも症例報告に値すると考えられた重症化したケースにおけるものである．また，病因にかかわらず中高齢者では重症敗血症に進展するリスクが高く，その場合には約30%程度が死亡するとされている[30)]．ゆえに，*C. canimorsus* 感染症は死亡率が30%におよぶ重症敗血症に進展することがあるということにほかならず，さらに，報告されていない軽症の患者もかなりの数いると考えられることから，実際には感染・発症時の死亡率は見かけほど高くないと考えられる．事実，オランダで2003～2005年に確認された患者26名のうち，ICU は9名，死亡3名にとどまっている．また，オランダやデンマークでは推定患者数について，それぞれ年間，人口100万人対0.67，0.5人との報告がある[16)31)]．したがって，それを日本の人口1.27億人に当てはめると，年間60～80人前後の報告されうる症状を示す患者がいると推定される．社会はますます高齢化が進むと考えら

表4　*C. canimorsus* の薬剤感受性

分　類	一般名	感受性
ペニシリン系	ペニシリン	△
	アンピシリン	△
	アモキシシリン	△
	ピペラシリン	○
	オーグメンチン	○
セフェム系	セファゾリン	△
	セフォタキシム	○
	セフトリアキソン	△
	モクサラクタム	△
カルバペネム系	イミペネム	○
	メロペネム	○
アミノグリコシド系	ストレプトマイシン	×
	ゲンタマイシン	×
マクロライド系	アジスロマイシン	△
	エリスロマイシン	△
リンコマイシン系	クリンダマイシン	△
テトラサイクリン系	テトラサイクリン	○
	ドキシサイクリン	○
	ミノサイクリン	○
キノロン系	ナリジクス酸	△
ニューキノロン系	オフロキサシン	○
	シプロフロキサシン	○
クロラムフェニコール系	クロラムフェニコール	○
ポリペプチド系	ポリミキシンB	×
抗結核薬	リファンピシン	○
サルファ剤系	ST合剤	△

※ディスク拡散法による　○：全株感性
△：一部の株が耐性
×：全株耐性

れるが，アンケート調査から得られた咬掻傷事故の多さや，それに反した疾患の認知度の低さを考えると，今後，本感染症の患者数の増加も懸念される．

厚生労働省は2010年5月21日，情報提供として「カプノサイトファーガ・カニモルサス感染症に関するQ＆A」を各自治体（管内医療関係者への周知を含む）や関係各局に通知するとともに，厚生労働省ウェブサイトへの掲載を行っている[32]．

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REVIEW

Prevalence of leptospirosis in farm animals

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Leptospirosis is a worldwide zoonosis caused by infection with pathogenic spirochetes that belong to the genus *Leptospira*. *Leptospira* spp. colonize the proximal renal tubules of various mammals and are excreted in the urine of carrier animals. Transmission of leptospirosis in humans and animals occurs by exposure to water or soil contaminated by the urine of infected animals or by direct contact with infected animals [4]. The genus *Leptospira* consists of both pathogenic and saprophytic (non-pathogenic) species. *Leptospira* species are defined according to DNA relatedness. Twenty species, 8 pathogenic, 5 intermediate, and 7 non-pathogenic groups, have been described [2]. Leptospires are divided into numerous serovars. Approximately 250 serovars and over 60 serovars were recognized among the pathogenic *Leptospira* spp. and non-pathogenic leptospires, respectively [2,4]. Antigenically related serovars are grouped into serogroups, 26 of which have been described for pathogenic strains [2,7].

Farm animals such as cattle and swine are not only infected as incidental hosts but also are maintenance hosts of specific *Leptospira* serovar strains and serve as reservoir animals for the same animal species and/or humans. Incidental host infections of cattle with serovars such as Grippotyphosa, Icterohaemorrhagiae and Pomona cause acute, severe clinical disease [4]. Clinical signs of acute bovine leptospirosis include high fever, haemolytic anaemia, haemoglobiuria and jaundice. Infection in pregnant cattle can result in abortion. In lactating cows, infections are often associated with agalactia. Cattle also serve as a maintenance host of serovar Hardjo, which consists of two different genotypes, Hardjobovis and Hardjoprajitno. Antibodies against this serovar were detected in 25 to 65% of cattle in the US, Europe, South America and Australia and *L. borgpetersenii* serovar Hardjo type Hardjobovis (Lb Hardjobovis) is the most common in cattle worldwide [6]. Recent completion of genomic sequencing of two strains of Lb Hardjobovis revealed that Lb Hardjobovis has been evolving toward a host-adapted bacterium [1]. The Hardjobovis genome is decaying through a process of insertion sequence-mediated genome reduction. Loss of gene function is centered on impairment of environmental sensing and metabolite transport and utilization, which affects survival of Lb Hardjobovis in its environment and results in a strict host-to-host transmission cycle. Acute infection with Hardjobovis results in asymptomatic or mild cases, but chronic infection is associated with infertility and reproductive failures such as abortion, stillbirth and weak calves. Abortion and the other effects usually occur 1 to 6 weeks (serovar Pomona infection) or 4 to 12 weeks (serovar Hardjo infection) after the acute phase of infection [3]. With serovar Pomona infection,

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abortion usually occurs in the last trimester of gestation. With serovar Hardjo infection, abortion has been diagnosed at all stages and early embryonic death may occur [3]. Infertility has commonly been observed in Hardjo-infected herds [5]. The abortion rate after Lb Hardjobovis infection is 3 to 10% whereas the rate increases up to 30% for *L. interrogans* serovar Hardjo type Hardjoprajitno (Li Hardjoprajitno) infection [3,6]. Li Hardjoprajitno is often associated with acute infection in dairy cows leading to milk drop syndrome. Leptospirosis in cattle is a notifiable disease in Japan but there are almost no reports on the disease in recent years, and the actual prevalence of bovine leptospirosis remains unknown. Therefore, this study attempts to reveal the current situation of leptospiral infection and the relationship between abortion and leptospirosis in cattle in Japan.

We carried out testing for anti-leptospiral antibody among 343 healthy dairy cattle from 19 farms in Hokkaido by microscopic agglutination test (MAT). Anti-leptospiral antibody (reciprocal MAT titer $\geq$ 100) was detected from 44 cattle on 9 farms (12.8%, Table 1). The predominant reacting serogroup was Sejroe, to which serovar Hardjo belongs (42/44, 95.5%), followed by Hebdomadis (6/44, 13.6%) and Autumnalis (1/44, 2.3%). The leptospiral *flaB* gene was amplified by nested polymerase chain reaction (PCR), and then the nucleotide sequences of the amplicons were determined. Leptospiral *flaB* was detected in 2 of the 39 urine samples from 3 seropositive farms. Both PCR-positive cattle had histories of abortion. The nucleotide sequences of the two amplicons

Table 1. Prevalence of anti-leptospiral antibody in cattle in Hokkaido[a)]

Farm	No. of cattle tested	No. of positive cattle	Reacting serogroups (no. of positive cattle)
RK-1	5	0	
RK-2	5	1	Sejroe (1)
RK-3	5	0	
RK-4	10	5[b)]	Hebdomadis (2), Sejroe (5)
RK-5	5	0	
RK-6	5	0	
RK-7	10	4	Hebdomadis (1), Sejroe (3)
RK-8	5	0	
RK-9	5	0	
RK-10	10	3	Sejroe (3)
HU-1	22	0	
HU-2	22	5	Sejroe (5)
HU-3	22	0	
HU-4	22	0	
DY-1	36	15[b)]	Hebdomadis (2), Sejroe (15)
DY-2	44	2	Autumnalis (1), Sejroe (1)
DY-3	44	1	Sejroe (1)
DY-4	26	8[b)]	Hebdomadis (1), Sejroe (8)
DY-5	40	0	
19	343	44 (12.8 %)	Autumnalis (1), Hebdomadis (6), Sejroe (42)

a) Reciprocal MAT titer $\geq$ 100

b) There were 2 cattle, 2 cattle and 1 cow which reacted equally to both Hebdomadis and Sejroe in farms RK-4, DY-1 and DY-4, respectively.

Table 2. Prevalence of anti-leptospiral antibody in cattle with and cattle without histories of abortion[a)]

Farm	Seropositive rate (no. of positive cattle / no. of cattle tested)	Seropositive rate in cattle with histories of abortion (no. of positive cattle / no. of abortion-experienced cattle)	Seropositive rate in cattle without histories of abortion (no. of positive cattle / no. of abortion-naïve cattle)
DY-1	38.9% (14/36)	47.4% (9/19)	35.3% (6/17)
DY-2	4.5% (2/44)	9.1% (2/22)	0 % (0/22)
DY-3	2.3% (1/44)	0 % (0/22)	4.5% (1/22)
DY-4	30.7% (8/26)	23.1% (3/13)	38.5% (5/13)
DY-5	0 % (0/40)	0 % (0/20)	0 % (0/20)

a) Reciprocal MAT titer ≥ 100

were identical to each other and those of the Lb Hardjobovis strains mentioned above. *L. borgpetersenii* has been isolated from rodents and shrews in Hokkaido (Data not shown). However, the sequence obtained in this study was not identical to any of those obtained from small mammals in Hokkaido. These serological and DNA analyses strongly suggest wide spread of Lb Hardjobovis among cattle in Hokkaido, although attempts to isolate leptospires failed.

Next, we examined the relationship between history of abortion and presence of anti-leptospiral antibody on 5 farms where the history of abortion for each cow was recorded. The seroprevalence was high on 2 of the 5 farms (30.7% and 38.9%), but there was no difference between cattle with and cattle without histories of abortion (Table 2). On 3 other farms, low seroprevalence was detected in both abortion-experienced and abortion-naive cattle. On the other hand, comparison of the embryonic age at the time of abortion between seropositive and seronegative cattle revealed that abortion occurred earlier in seropositive cattle than in seronegative cattle (positive; 51.5 days (median, N = 8), negative; 82 days (N = 77), p = 0.038). The presence of anti-leptospiral antibody in abortion-experienced cattle and the differences in the fetal age at the time of abortion between seropositive and seronegative cattle suggested a positive correlation between abortion and leptospiral infection in Hokkaido, although further verification with a larger sample size is needed.

In this study, of two highly seroprevalent farms, one bought cattle from the other, and the other entrusted their cattle during the summer season to another operation, suggesting that contact with carrier cows may have caused infection. Thus, it is important to identify and eradicate carrier animals introduced from outside. Carrier animals can be identified using urine samples by isolation, fluorescent antibody staining or detection of leptospiral DNA using PCR [6,10]. However, it is difficult to carry out these methods in the field. More recently, a loop-mediated isothermal amplification (LAMP) method has been developed for detecting pathogenic leptospires [8,9]. Unlike PCR, the LAMP method amplifies a target DNA sequence under isothermal conditions for about an hour with high specificity and efficiency, and the results can be assessed with the naked eye, promising lower expenses for equipment. We have developed a LAMP method for the detection of leptospiral DNA in urine with a more simple procedure, which is applicable to point-of-care testing. We will report results of evaluation of this LAMP method using field animal urine samples.

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Population Genetic Structures of Staphylococcus aureus Isolates from Cats and Dogs in Japan

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Population Genetic Structures of *Staphylococcus aureus* Isolates from Cats and Dogs in Japan

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We determined the population genetic structures of feline and canine *Staphylococcus aureus* strains in Japan by multilocus sequence typing (MLST). Ecological analyses suggested that multiple feline-related *S. aureus* clones, including ST133, naturally occur as commensals and can cause endogenous infections in felines. In contrast, *S. aureus* populations do not likely include any clone that exhibits tropism in domestic dogs. Even if *S. aureus* infections occur in dogs, the pathologies are likely exogenous infections.

Staphylococcus aureus is a coagulase-positive staphylococcus (CoPS) and is present in normal skin and nasal flora but opportunistically causes a wide range of infections in humans and animals. According to multilocus sequence typing (MLST) data, there are four major clonal complexes (CCs), CC97, CC126, CC133, and CC151, among bovine *S. aureus* isolates worldwide (5, 8, 13). Pig-associated strains exhibited sequence type 9 (ST9), ST398, and ST433 (1). These specific clones are not always common in natural populations of human *S. aureus* (4, 7, 9, 10, 12), suggesting that *S. aureus* clones have evolved host specifically.

Methicillin-resistant *S. aureus* (MRSA), which is one of the most conspicuously nosocomial pathogens in humans, is also now increasingly common in veterinary medicine. ST398 and ST9 MRSA clones have been a matter of zoonotic concern in many countries; these clones were generated from within swine-related methicillin-susceptible *S. aureus* (MSSA) clones in pig hosts (1, 15). Thus, to trace the original infectious source of MRSA zoonotic transmission, we need to understand the population structures of *S. aureus* clones in various animal species. There have been many reports involving domestic dogs and cats in outbreaks of human MRSA infections in countries where the clones are endemic (15). However, in canine and feline hosts, there has been no report on the population genetic structures of MSSA (not MRSA) strains, which reflect the natural habitation of *S. aureus* clones in the host species.

Here, we characterize feline and canine *S. aureus* strains by molecular methods and compare the strains from various host animal species. To obtain feline and canine *S. aureus* strains, we conducted the detection of *S. aureus* strains for 402 carriage specimens (dogs, $n = 232$; cats, $n = 170$) and 580 cases diagnosed as staphylococcal infection (dogs, $n = 459$; cats, $n = 121$) in eastern Japan from 2002 to 2010. We used 93 *S. aureus* strains isolated from 74 cats and 19 dogs (see Table S1 in the supplemental material), with each representing an independent individual. The bacteria were identified as *S. aureus* using a PCR method (11) and were characterized using MLST (3). Toxin typing, detection of *mecA*, and staphylococcal cassette chromosome *mec* (SCC*mec*) typing were also performed. All strains were tested for resistance to macrolides, aminoglycosides, and fluoroquinolones by the disk diffusion method based on CLSI guidelines (1a). The diversity and evenness of ST distribution in each host were calculated using Simpson's diversity index ($1 - \lambda$) and Pielou's evenness index (J'). Both values range from 0 (no diversity or evenness) to 1 (extreme diversity or evenness) and are more insusceptible to the difference of sample size than Shannon-Wiener's index (H'). These parameters have generally been used for the comparison of biodiversity between geographically separated environments. The values for feline and canine strains were compared with those previously reported for strains from humans, pigs, cows, and goats (1, 4, 5, 7–10, 12, 13). To visualize differences of diversity among host species, phylogenetic trees based on concatenate sequences of the seven genes used in MLST were constructed by MEGA version 5.05 (14).

Twenty-four unique STs and two nontypeable strains were identified among the 74 feline *S. aureus* strains: 14 unique STs were identified among the 19 canine strains (see Table S1 in the supplemental material), and 10 new STs, ST1250, ST1251, ST1252, ST1253, ST1332, ST1333, ST1408, ST1412, ST1441, and ST1837, were found and described over the course of this study.

Among the 74 *S. aureus* isolates of feline origin, 20 MRSA and 54 MSSA strains were obtained. All feline MRSA strains belonged to one of two lineages, CC5 ($n = 15$) or CC8 ($n = 5$). Sixty percent (9 of 15) of the CC5 MRSA strains exhibited the Japanese hospital-associated MRSA (HA-MRSA) genotype (ST5 SCC*mec* type II *tst*, *sec*, *seg*, and *sei* positive). Three strains with the New York clone genotype (USA100; *tst*-negative ST5 SCC*mec* type II) were also obtained. The CC8 MRSA strains showed significant genetic heterogeneity in MLST alleles, SCC*mec* types, and toxin profiles. No

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TABLE 1 Diversity and evenness indexes of *S. aureus* isolates in various populations

Host	Country	Clinical status (human population)	No. of isolates	No. of STs (CC)	Simpson's index	Pielou's index	Predominant ST(s) among MSSA isolates[a]	Source or reference
Dog	Japan	Carriage and infections	19	14 (9)	0.912	0.808	ST5	This study
Cat	Japan	Carriage and infections	74	26 (15)	0.908	0.639	ST133	This study
Human	Switzerland	Nasal carriage (adults)	132	37 (21)	0.918	0.603	ST45, ST30	10
	China	Nasal carriage (children)	147	25 (17)	0.875	0.515	ST121, ST59	4
	China	Infections (children)	51	20 (12)	0.931	0.681	ST88, ST121, ST398	4
	United Kingdom	Intravenous drug users lesion	28	12 (11)	0.910	0.680	ST59, ST5, ST12, ST30, ST45	7
	Mali	Nasal carriage (emergency patients)	88	20 (15)	0.858	0.522	ST15, ST152	9
	Gabon	Nasal carriage	34	10	0.891	0.605	ST30, ST15, ST72, ST80, ST88	12
Pig	France	Infections	14	4 (4)	0.692	0.443	ST398, ST9, ST433	1
Cow	Norway	Bulk milk	101	22 (5)	0.769	0.444	ST132, ST133	5
	United States	Bulk milk	116	16 (10)	0.633	0.334	ST124, ST126	13
	United Kingdom	Bulk milk	11	2 (2)	0.336	0.198	ST151, ST9	13
	Chile	Bulk milk	20	5 (3)	0.368	0.260	ST97	13
	Brazil	Bulk milk	227	11 (6)	0.496	0.207	ST126, ST97	8
Goat	Norway	Bulk milk	38	5 (3)	0.521	0.265	ST133, ST130	5

[a] ST(s) which accounted for not less than 10% of clones in the population.

Panton-Valentine leukocidin (PVL)-positive strain was isolated in this study. Among the feline MSSA strains, ST133 ($n = 9$) was the most frequent ST, followed by ST5 ($n = 6$) and ST20 ($n = 5$). Multiple strains of ST188 ($n = 4$), ST508 ($n = 4$), ST25 ($n = 3$), ST1251 ($n = 3$), ST8 ($n = 2$), ST12 ($n = 2$), and ST97 ($n = 2$) were also identified. CC5 and CC8 *S. aureus* clones were not found among carriage isolates. Many of the CC5 and CC8 isolates were derived from infected wounds in inpatients or urinary tract infections and exhibited multidrug resistance. Aside from the CC5 and CC8 strains, we did not find any correlation between clinical status and genotype.

Most occurrences of *S. aureus* in dogs were cases of carriage in hospital patients. Among all cases diagnosed as staphylococcal infection in dogs, those from which *S. aureus* were isolated accounted for only 1.1% (5 of 459), and more than half of them were relevant to hospitalization and/or drug resistance (see Table S1 in the supplemental material). Of the 19 canine *S. aureus* strains, six belonged to ST5. Three of these strains exhibited the Japanese HA-MRSA genotype and three other ST5 strains were MSSA, but two had the same genotype as Japanese HA-MRSA, and one exhibited the same genotype as USA100. All of the remaining canine strains had distinct STs from one another. No correlation was found between clinical status and genotype in canine strains.

Donnio et al. reported that MSSA strains from which SCC*mec* was excised retain resistance to macrolides at a high rate, probably via a Tn*554* that is located on SCC*mec* and contains a macrolide resistance-encoding *ermA* gene (2). Such SCC*mec*-excised strains also frequently exhibited resistance to aminoglycosides and/or fluoroquinolones, resulting in the emergence and epidemic diffusion of multidrug-resistant MSSA (MR-MSSA) in hospital environments (2). In the current study, 77.8% (7 of 9) of ST5 MSSA strains exhibited erythromycin resistance and were also resistant to levofloxacin and/or gentamicin. Therefore, epidemic diffusion of ST5 MR-MSSA strains derived from the Japanese HA-MRSA clone should be expected in veterinary hospital environments. ST5 MSSA strains are also linked with antimicrobial use, suggesting that ST5 *S. aureus* clones are not naturally distributed in dogs and cats.

Populations of canine and feline *S. aureus* strains showed high diversity index values ($1 - \lambda = 0.912$ and 0.908, respectively). These high diversity index values are comparable to those of human strains (0.858 to 0.931) and distinct from greater homogeneity seen for swine (0.692), bovine (0.336 to 0.769), and caprine strains (0.521) (Table 1). As shown in Fig. 1, *S. aureus* strains of bovine origin in Brazil (8) showed relatively uneven and aggregated distribution of specific STs, ST126 and ST97, which have a strong tropism for bovine hosts. Strains from humans in Switzerland (10) and those of feline origin in the present study varied less from ST to ST than those of bovine origin. Our canine *S. aureus* strains showed an extremely high Pielou's evenness index ($J' = 0.808$) compared to those of humans (0.515 to 0.681), cats (0.639), pigs (0.443), cows (0.198 to 0.444), and goats (0.265) and did not reveal concentrated distribution of any STs other than ST5. High values of both diversity and evenness indexes in the dog strains indicate that the distribution of *S. aureus* clones in canine hosts formed a random pattern, suggesting that no *S. aureus* clone exhibits tropism in domestic dogs in Japan.

Our results show that feline hosts allow diverse *S. aureus* clones to adapt as commensals. Interestingly, ST133, which was the most frequent ST in cats in Japan, had been recognized as a host-specific clone in ruminant animals (5). The existence of substantial geographic structure has been reported in bacterial isolates from human and bovine hosts (5, 8, 13). Further studies in other geographic areas will be required to evaluate the adaptation of *S. aureus* clones in feline hosts.

The occurrence of *S. aureus* in dogs has probably been overes-

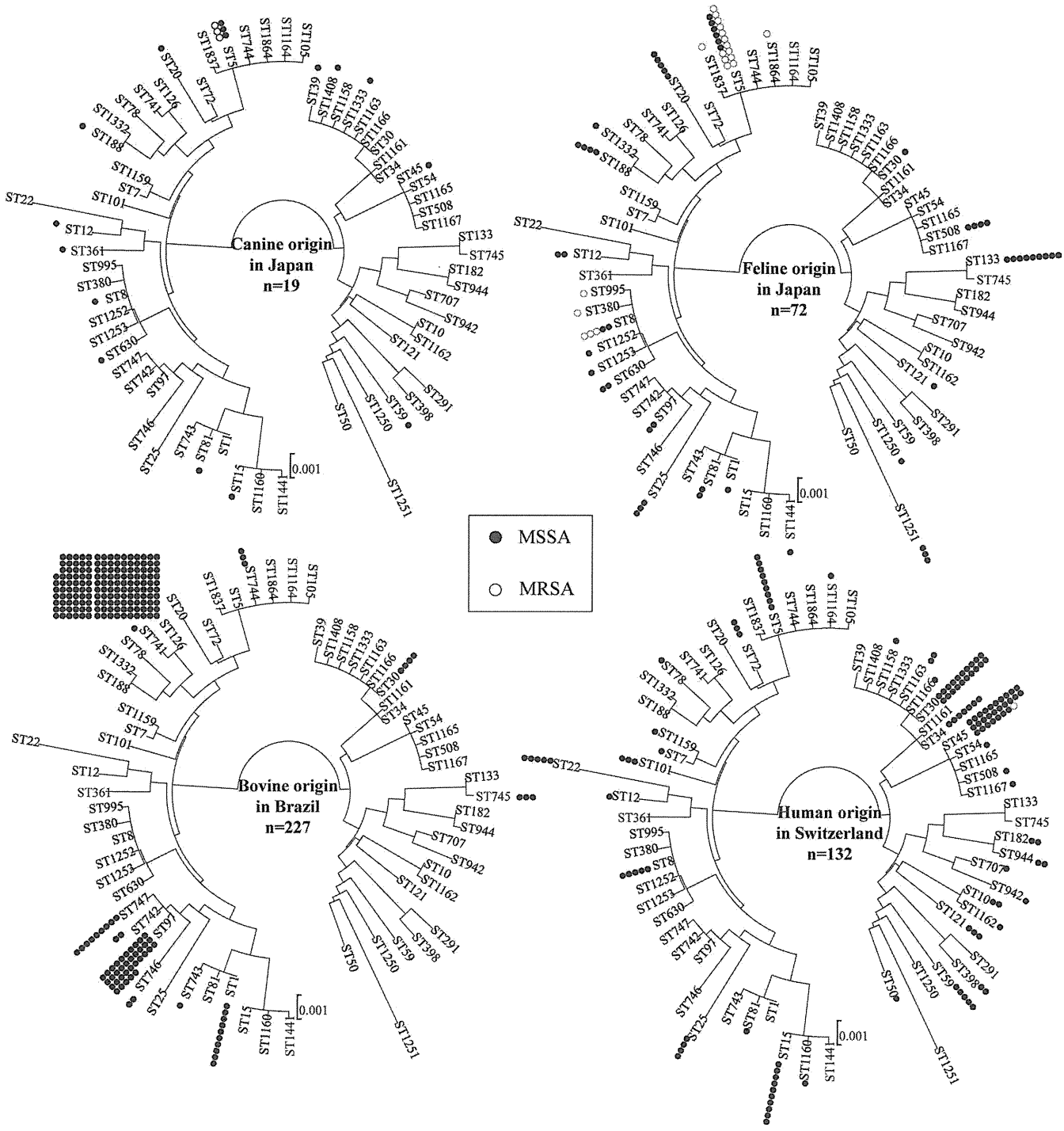

FIG 1 Phylogenetic tree based on concatenated *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL* sequences and distribution of strains from cats, dogs, humans (10), and cows (8) in population genetic structures of *S. aureus*. These trees were constructed by the neighbor-joining method using MEGA version 5.05. The numbers of MSSA and MRSA strains are indicated.

timated, because the predominant species of CoPS in dogs, *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi*, could be misidentified as *S. aureus* by conventional identification systems that use biochemical characterization (11). Recently, Kawakami et al. reported that no *S. aureus* strain was isolated from 190 cases of canine pyoderma by a molecular identification method (6, 11). Weese and van Duijkeren also speculated that *S. aureus* is not naturally a predominant commensal in dogs, based on evidence that MRSA colonization was transient in canine hosts (15). These reports support the hypothesis that the *S. aureus* population does not include any clone that has tropism for healthy domestic dogs. Even if *S. aureus* infections occur in dogs, it is likely that such

pathologies are exogenous infections caused by random or human-related clones associated with the regions where MRSA is endemic. Thus, in contrast to the case in pigs, dog-related MRSA clones will likely not be generated in canine hosts, given the lack of *S. aureus* clones adapted to domestic dogs. In the context of public health, dogs likely have low potential as a source of transmission of infectious, zoonotic MRSA.

In conclusion, multiple *S. aureus* clones naturally occur as commensals in cats and can also cause endogenous infections in felines. In contrast, domestic dogs likely acquire *S. aureus* strains from exogenous sources. These data are expected to contribute to public health and research findings on the molecular mechanisms underlying host specificity.

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Tetanus as Cause of Mass Die-off of Captive Japanese Macaques, Japan, 2008

Tomomi Nakano, Shin-ichi Nakamura, Akihiko Yamamoto, Motohide Takahashi, and Yumi Une

In 2008 in Japan, 15/60 captive Japanese macaques died. *Clostridium tetani* was isolated from 1 monkey, and 11 had tetanus-specific symptoms. We conclude the outbreak resulted from severe environmental *C. tetani* contamination. Similar outbreaks could be prevented by vaccinating all monkeys, disinfecting housing areas/play equipment, replacing highly *C. tetani*–contaminated soil, and conducting epidemiologic surveys.

Tetanus is a wound infection caused by a potent neurotoxin produced by *Clostridium tetani*. The bacterium is difficult to isolate, and no pathologically characteristic lesion is present during infection; thus, tetanus diagnosis is based on tetanus-specific clinical symptoms (*1*–*4*). Tetanus is a highly lethal zoonosis, and cases usually occur sporadically. Outbreaks among humans have occurred only after earthquakes and tsunamis (*4*). We report on an outbreak of tetanus in 2008 among a captive colony of Japanese macaques (*Macaca fuscata*) in Japan.

The Study

In 2008, deaths suddenly increased among Japanese macaques housed in a facility in the Kantou area of Japan. At that time, the facility, which had been in service for >40 years, housed ≈60 macaques, 15 (25%) of which died. This mortality rate was much higher than that during 2006 (10.9%, 7/64 monkeys), 2007 (7.1%, 4/56), 2009 (13.8%, 9/65), 2010 (5.2%, 3/58), and 2011 (5.7%, 4/70) (Figure 1). A total of 42 monkeys died during 2006–2011, and investigations at the time of death showed that 14 of the monkeys had tetanus-specific symptoms: 1 of 4 that died in 2007, 11 of 15 that died in 2008, and 2 of 9 that died in 2009). Nine of the 11 monkeys that died with characteristic symptoms of tetanus in 2008 died during the breeding season (November and December). Thus, the observed number of presumed tetanus cases during the 2008 breeding season (9/60) was 8.4× greater than the number during the 2007 breeding season (1/56).

The soil in the monkeys' enclosure was clay-like and without vegetation. In 2008 before the increase in deaths, there were no changes in maintenance procedures, such as feeding, at the facility and no evident pathogenic contamination of the monkeys' food or environment.

We performed necropsies on 3 monkeys (animal nos. 1, 2, and 3) that died 5, 2, and 3 days, respectively, after the onset of symptoms. At death, all showed a specific posture: the jaw was elevated, the back straightened, and the tail tightly stretched; the forelimbs were crossed in front of the body with the wrists bent; and the hind limbs were extended backward (Figure 2). Rigidity was abnormally severe and did not remit after death; at necropsy, the mouth was difficult to open. Congestion of the visceral organs and pulmonary edema were noted, but there were no findings to suggest poisoning, such as foreign bodies in the stomach or erosive changes in the gastrointestinal tract. No wound that might have led to infection was found in monkeys 1 or 2, but a lesion with purulent incrustation was present on a toe tip on the right hind limb of monkey 3. *C. tetani* was isolated from this lesion, and the tetanus toxin gene was detected by PCR. A mouse toxicity test confirmed tetanus toxin activity.

We obtained samples from the soil in monkey enclosures, from wooden playground equipment, and from the soil surrounding the enclosures and tested them for *C. tetani*; 67%, 75%, and 53% of the samples, respectively, were positive for *C. tetani*, indicating marked

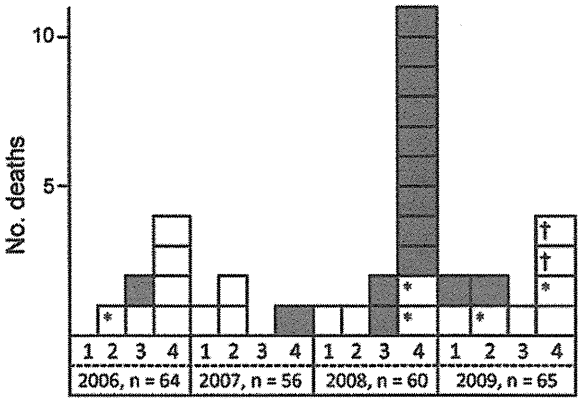

Figure 1. Number of deaths during 2006–2009 among macaques (*Macaca fuscata*) housed in an animal facility in the Kantou area of Japan. Grey boxes, monkeys with tetanus-specific clinical symptoms; white boxes, monkeys without tetanus-specific clinical symptoms. 1, January–March; 2, April–June; 3, July–September; 4, October–December; n, total number of monkeys. *Juvenile animal; †Accident at time monkeys captured for vaccination (death due to hyperthermia).

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contamination. *C. tetani* was not isolated from the monkeys' food or from soil sampled >1 km from the facility. We performed pulsed-field gel electrophoresis on isolates from the soil at the facility and from monkey number 3, and the results were identical, showing >90% homology.

On 3 occasions (October 27 and December 17, 2009, and December 8, 2010), macaques housed in the facility (total 65) were intramuscularly administered 0.5 mL of tetanus toxoid (Nisseiken Co., Ltd., Tokyo, Japan). In 1 monkey, the prevaccination serum level of tetanus toxoid antibody was higher than the level for tetanus prevention (0.1 IU/mL). At 51 days after the first vaccination, 83.3% (51/61) of the animals were antibody-positive, and 1 year after the second vaccination, 100% were antibody-positive. Since then, no tetanus symptoms have occurred in any of the monkeys. Caretakers for monkeys at the facility were examined at a community medical office and inoculated with tetanus toxoid.

Conclusions

On the basis of these findings, we diagnosed the disease as tetanus, and we concluded that it was an unprecedented, large-scale outbreak. Many animal exhibition facilities in Japan maintain Japanese macaques, and tetanus has been reported in captive macaques in other countries (*5–7*). Results of a 5-year study (July 1, 1976–June 30, 1981) among the free-ranging rhesus monkey (*Macaca mulatta*) colony on the island of Cayo Santiago, Puerto Rico, showed a high incidence of tetanus among the monkeys during the breeding seasons, but the report did not clarify the cause (*6*).

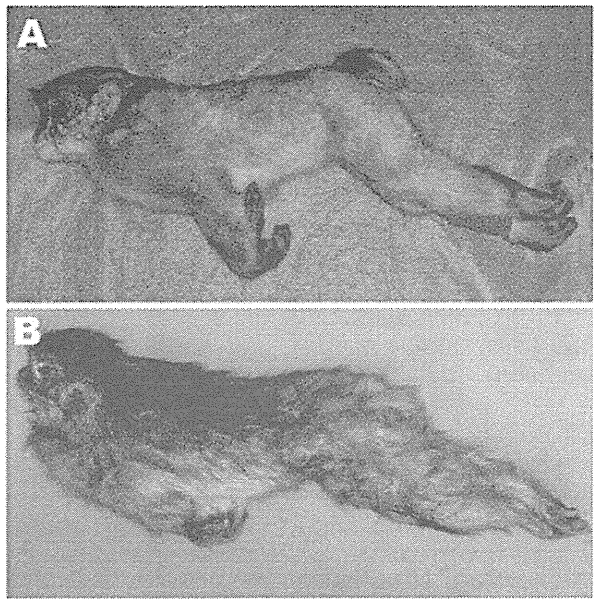

Figure 2. A) Opisthotonos as a tetanus-specific clinical symptom in a 1-year-old male Japanese macaque (*Macaca fuscata*). B) Opisthotonos with severe rigid posture in an adult male Japanese macaque.

In facilities maintaining animals, the soil is often contaminated with *C. tetani* at a relatively high rate (*1,2*). In the facility in Japan, *C. tetani* was isolated at a high rate from soil and from play structures. The genotype of these isolates was consistent with that for an isolate obtained from a monkey housed at the facility, suggesting that the soil was the source of the infection.

The facility has >40 years' experience raising monkeys, and the cause of the sudden outbreak in 2008 is unclear. The outbreak was concentrated during the breeding season, suggesting that injuries sustained through fighting during the mating season in an environment with severe *C. tetani* contamination may have led to the outbreak. *C. tetani* is present in the intestinal contents of various animal species (*1,3*). Thus, bacteria in the feces of infected monkeys may have added to the level of indigenous *C. tetani* contamination in the soil.

In Japan, tetanus is still reported in >100 persons each year: 115 cases were reported in 2005, 117 in 2006, 89 in 2007, 124 in 2008, 113 in 2009,and 106 in 2010) (*8*). It is a highly lethal zoonosis and a disease of concern with regard to public and animal health. After tetanus was diagnosed in the monkeys, we immediately administered tetanus vaccine to monkey caretakers at the facility and thoroughly enforced hygiene practices. To prevent tetanus infection in animals and animal caretakers in such facilities and in visitors, we recommend that newborn monkeys be vaccinated, housing areas and play equipment be disinfected, soil highly contaminated with *C. tetani* be replaced, and epidemiologic surveys be conducted.

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Miss Nakano, a graduate of Azabu University, Kanagawa, Japan, works as a clinical veterinarian; this paper is her graduation thesis. Her research interest is in infectious diseases of monkeys.

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etymologia

Tetanus

[tet'ə-nəs]

From the Greek *tetanos* ("tension," from *teinein*, "to stretch"), an often fatal infectious disease caused by the anaerobic bacillus *Clostridium tetani*. Tetanus was well known to the ancients; Greek physician Aretaeus wrote in the first century CE, "Tetanus in all its varieties, is a spasm of an exceedingly painful nature, very swift to prove fatal, but neither easy to be removed." Active immunization with tetanus toxoid was described in 1890, but cases continue to be reported (275 in the United States from 2001 through 2010), almost exclusively in persons who were never vaccinated or had not received a booster immunization in the previous 10 years. In developing countries, neonatal tetanus—when infants are infected through nonsterile delivery—is a major contributor to infant mortality. Worldwide, an estimated 59,000 infants died of neonatal tetanus in 2008.

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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Outbreak of Yersiniosis in Egyptian Rousette Bats (*Rousettus aegyptiacus*) Caused by *Yersinia pseudotuberculosis* Serotype 4b

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Summary

This report describes an outbreak of yersiniosis in Egyptian rousette bats (*Rousettus aegyptiacus*) caused by *Yersinia pseudotuberculosis* serotype 4b. Twelve of 61 bats died between November and December 2008 or in May 2009. The bats often displayed multiple yellow–white nodules in the spleen and liver. Microscopically, these consisted of focal necrosis accompanied by inflammatory cell infiltration and colonies of gram-negative bacilli. The bacterial colonies were identified immunohistochemically as *Y. pseudotuberculosis* O4 and *Y. pseudotuberculosis* serotype 4b was identified by bacteriological examination. Polymerase chain reaction demonstrated that the isolate harboured the virulence genes *virF*, *inv* and *ypmA*. YPMa is as a superantigenic toxin that is associated with acute systemic infection in man and may contribute to the virulence of *Y. pseudotuberculosis* in bats.

Keywords: Egyptian rousette bat; superantigen; *Yersinia pseudotuberculosis*; zoonosis

Yersinia pseudotuberculosis is an important causal agent of zoonosis with global distribution (Fukushima *et al.*, 2001). Infection with *Y. pseudotuberculosis* is typically acquired orally via the ingestion of food and water contaminated with the faeces of carriers such as free-living rodents and birds (Schiemann, 1989; Han *et al.*, 2003). Yersiniosis due to *Y. pseudotuberculosis* is clinically manifested as enteritis, mesenteric lymphadenitis and occasionally septicaemia, and occurs in man and a wide variety of animals (Mair, 1973). Yersiniosis has caused significant mass mortality in zoological parks amongst a variety of animals (Allchurch, 2003). *Y. pseudotuberculosis* has been classified into serotypes O1–O15 based on expression of the Oantigen, and seven pathogenic serotypes are recognized (O1–O6 and O10; Nagano *et al.*, 1997). A new pathogenic serotype (O7) was isolated from a Bolivian squirrel monkey (*Saimiri boliviensis*) (Nakamura *et al.*, 2009). The pathogenicity of *Y. pseudotuberculosis* is associated with several virulence factors that are encoded on a 70 kb virulence plasmid (pYV) and include *Yersinia* adhesin A (YadA) and *Yersinia* outer membrane proteins (Yops) (Cornelis *et al.*, 1998). Additionally, a chromosomal high-pathogenicity island (HPI) encodes an iron-uptake system characterized by the siderophore yersiniabactin (Schubert *et al.*, 2004), the superantigenic toxin *Y. pseudotuberculosis*-derived mitogen (YPM) (Abe *et al.*, 1997) and invasin, which allows efficient entry into mammalian cells (Grassl *et al.*, 2003) and plays an important role in systemic infection. YPM-producing strains can be separated into three clusters based on the production of the YPMa, YPMb or YPMc variants of YPM (Carnoy *et al.*, 2002). Among these strains, those that produce YPMa display superantigenic activity and high pathogenicity (Carnoy *et al.*, 2000).

There are few reports of yersiniosis in zoo animals and little data on the serotypes and virulence

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characteristics of *Y. pseudotuberculosis* isolated from these species.

Between November 28th and December 5th 2008, nine of 61 Egyptian rousette bats housed in a zoological park in the Kanto region of Japan died consecutively without obvious clinical signs. Additionally, three bats died suddenly over 6 days in May 2009. Complete necropsy examinations were performed on the 12 bats and tissues from the last animal (number 12) were subjected to histopathological examination. Samples from this bat were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections were stained with hematoxylin and eosin (HE) and Gram's stain. Immunohistochemistry (IHC) was performed using a commercially available set of rabbit antisera specific for the *Y. pseudotuberculosis* serotypes O1, O2, O3, O4, O5 and O6 (Denka-Seiken Co., Tokyo, Japan) and a set of antisera specific for the *Yersinia enterocolitica* serotypes O1–2, O3, O5, O8 and O9 (Denka-Seiken). Bacteriological examination of the liver, spleen and lung was performed for bat 12 as described by Iwata *et al.* (2008). Additionally, polymerase chain reaction (PCR) examination of the bacterial isolates for the detection of the virulence genes *virF*, *inv*, *ypm* (*ypmA*, *ypmB* and *ypmC*) and *irp2* was performed using six sets of primers as described by Iwata *et al.* (2008). The *virF*, *inv* and *irp2* genes were used as markers for the presence of pYV, invasin and HPI, respectively (Table 1).

At necropsy examination, all of the bats displayed enlargement of the spleen and liver, which contained multiple yellow–white nodules (Fig. 1), and fine white, nodules were observed in the lung. No irregularities were seen in the intestine or lymph nodes, including the mesenteric lymph nodes. Histopathologically, the nodules observed in the liver and spleen consisted of focal necrosis accompanied by inflammatory cell infiltration, including neutrophils and macrophages, with numerous bacterial colonies (Fig. 2). The bacterial colonies were composed of gram-negative bacilli. Intracapillary bacterial colonies were detected in the lung and kidney. Additionally, small bacterial colonies associated with small foci of necrosis were seen in the femoral bone marrow. There were no microscopical changes in the intestine or mesenteric lymph nodes. The bacterial colonies were immunolabelled for *Y. pseudotuberculosis* O4 only (Fig. 3). On bacteriological examination only *Y. pseudotuberculosis* serotype 4b was isolated and PCR analysis demonstrated that the isolate had *virF*, *inv* and *ypmA* genes, but did not possess *ypmB*, *ypmC* or *irp2* (Fig. 4).

Similar gross lesions have been reported in animals infected with *Y. pseudotuberculosis* (Hubbert, 1972; Baskin *et al.*, 1977), and on the basis of the microbiological and immunohistochemical findings, the deaths of these bats were interpreted to relate to sepsis associated with *Y. pseudotuberculosis* serotype 4b infection.

PCR analyses revealed that the *Y. pseudotuberculosis* isolate possessed *virF*, *inv* and *ypmA* genes. The *virF* gene is a marker for the presence of the virulence plasmid pYV, which encodes several critical pathogenic factors, including YadA, which is involved in adhesion to host cells and autoagglutination, and 11 secreted Yop proteins, which play important roles in avoiding the host immune response by affecting the function of phagocytes (Cornelis *et al.*, 1998). Invasin is involved in adhesion to host intestinal epithelial cells (Grassl *et al.*, 2003), while YPMa encoded by the *ypmA* gene functions as a superantigenic toxin. YPMa is considered to be the virulence factor associated with a variety of the clinical signs observed in human patients

Table 1
Primers for PCR detection of virulence genes from *Y. pseudotuberculosis*

Virulence factor	*Target gene*	*Sequence (5′–3′)*	*Annealing temperature (°C)*	*Size of product (base pairs)*
pYV	*virF*	TCATGGCAGAACAGCAGTCAG ACTCATCTTACCATTAAGAAG	55	590
Invasin	*inv*	TAAGGGTACTATCGCGGCGGA CGTGAAATTAACCGTCACACT	55	295
YPMa	*ypmA*	CACTTTTCTCTGGAGTAGCG GATGTTTCAGAGCTATTGTT	55	350
YPMb	*ypmB*	TTTCTGTCATTACTGACATTA CCTCTTTCCATCCATCTCTTA	52	453
YPMc	*ypmA* and *ypmC**	ACACTTTTCTCTGGAGTAGCG ACAGGACATTTCGTCA	49	418
HPI	*irp2*	AAGGATTCGCTGTTACCGGAC TCGTCGGGCAGCGTTTCTTCT	55	280

*Since *ypmC*-specific primers have not been described, both *ypmA* and *ypmC* were detected for evaluation of *ypmC*. An isolate can be presumed to be positive for *ypmC* if it is negative for *ypmA* and positive for *ypmA* and *ypmC*.

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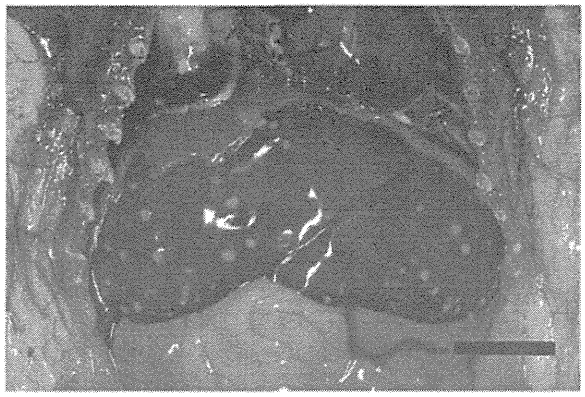

Fig. 1. Multiple yellow–white nodules in the liver. Bar, 1 cm.

including fever, scarlatiniform rash, diarrhea, vomiting and arthritis (Uchiyama *et al.*, 1993). YPMa-harbouring *Y. pseudotuberculosis* is considered to be highly virulent and capable of inducing the above clinical signs in addition to sepsis, while other *Y. pseudotuberculosis* strains have low virulence and result in signs confined to gastroenteritis (Fukushima *et al.*, 2001). HPI-positive serotypes do not typically harbour YPMa and are considered to have lower virulence than YPMa-positive strains (Fukushima *et al.*, 2001). As pointed out above, pathogenic *Yersinia* spp. have numerous virulence factors, but the relationship between these virulence factors and pathological findings have not been investigated extensively.

Y. pseudotuberculosis is able to survive for long periods of time in soil and water, even in cool environments, and the contamination of food and water can be a potential source of infection (Schiemann, 1989; Han *et al.*, 2003). Additionally, wild rodents and birds are considered to be carriers of *Y. pseudotuberculosis* (Mair, 1973; Fukushima *et al.*, 1988). In non-

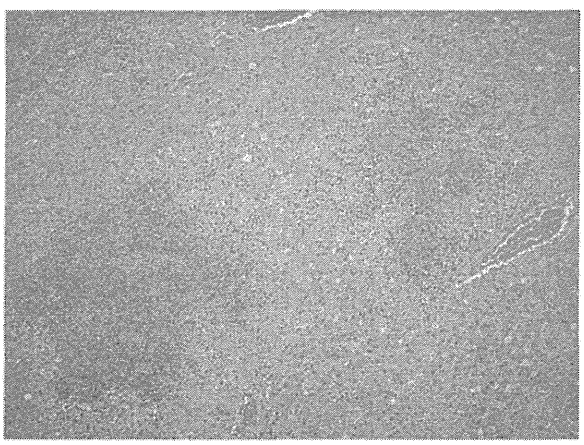

Fig. 2. Multifocal necrosis with numerous bacterial colonies in the liver. HE. ×100.

Fig. 3. Bacterial colonies in the liver immunolabelled for *Y. pseudotuberculosis* O4. IHC. ×200.

human primates, large quantities of *Yersinia* spp. are shed in faeces and can spread rapidly directly or indirectly to other animals (Baggs *et al.*, 1976). For these reasons, the possibility of faeces-mediated transmission among chiropteran species cannot be ruled out. Furthermore, it is possible that asymptomatic liver, spleen or lung carriage for long periods followed by stress may have triggered the development of yersiniosis.

To our knowledge, this is only the third report of yersiniosis in chiropteran species (Childs-Sanford *et al.*, 2009; Muhldorfer *et al.*, 2010). However, detailed investigations of the causative *Yersinia* strains by pathological and bacteriological examinations were not performed in the two

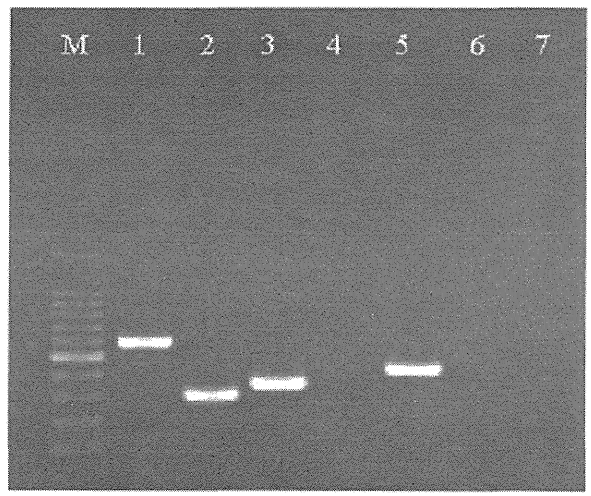

Fig. 4. PCR for detection of virulence genes of the *Y. pseudotuberculosis* 4b isolate. Lane: M, molecular weight markers (100 base pair ladder); 1, *virF*; 2, *inv*; 3, *ypmA*; 4, *ypmB*; 5, *ypmA* and *ypmC*; 6, *irp2*; 7, negative control.

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previous reports. From the public health point of view and to help resolve the mechanisms of infection by bacterial pathogens, additional studies of yersiniosis are needed in animals.

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