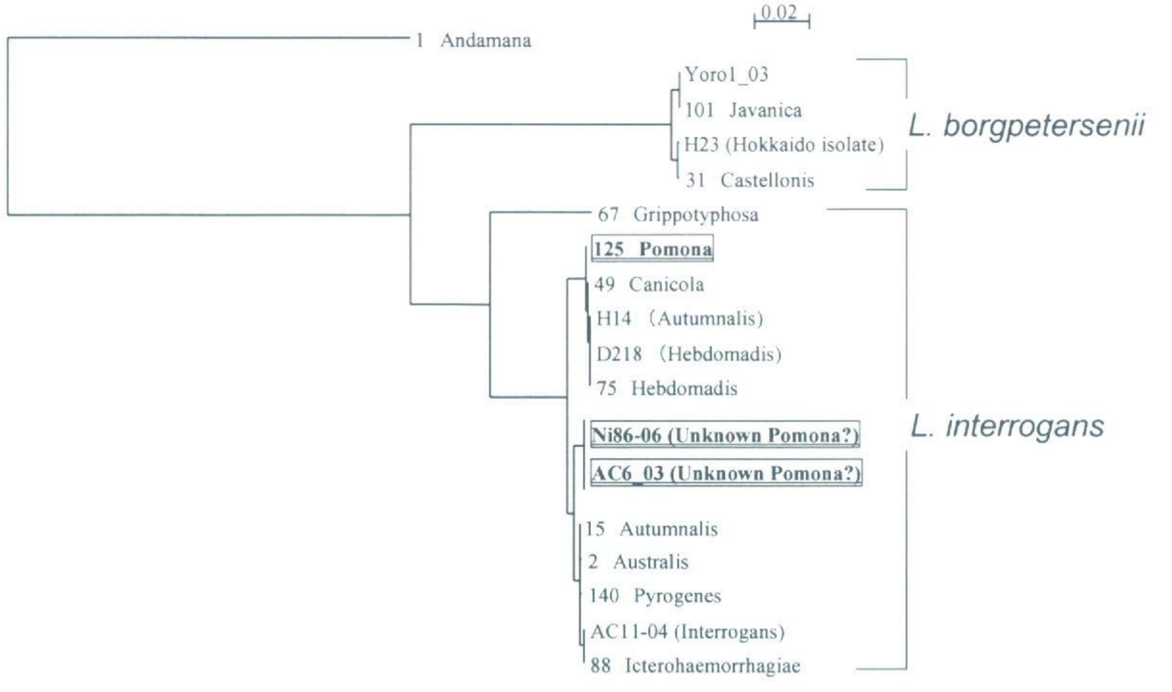

図2 未同定血清型 Ni86-06の*gyrB*配列による系統解析

厚生労働科学研究費補助金（新興・再興感染症研究事業）
分担研究報告書

18．レプトスピラ症のコントロール法に関する研究

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研究要旨

1. 2006年夏季にレプトスピラ症の多発があった宮崎県でヒトとイヌのレプトスピラ症強化サーベイランスを行った．その結果，ヒトではレプトスピラ症疑い10例中4例のレプトスピラ症が確定診断された．またイヌは20例中17例がレプトスピラ症と確定診断された．このうちイヌ9頭の血液からレプトスピラが分離され，レプトスピラの鞭毛構成遺伝子のひとつである *flaB* 遺伝子の部分塩基配列から，分離株はすべて *Leptospira interrogans* であり，また血清群は Australis, Canicola, Hebdomadis であると推定された．
2. 全国31か所の検疫港および検疫飛行場の政令区域（以下，港湾区域）で，185匹のネズミを捕獲しレプトスピラの分離を試みた結果，名古屋港で捕獲されたドブネズミ2匹からレプトスピラが分離された．分離株は，*flaB* 遺伝子の部分塩基配列から *L. interrogans* であると推定された．また新千歳空港で捕獲されたエゾアカネズミ2匹の腎臓から *L. interrogans* の *flaB* を検出した．
3. Megasort法により，*L. interrogans* の全ゲノム塩基配列決定2株とも相同性を示さない，レプトスピラ強毒株および弱毒株に特異的な遺伝子群を同定した．

研究目的

レプトスピラ症は，スピロヘータの一種である病原性レプトスピラ(*Leptospira*)の感染によりおこる人獣共通感染症である．レプトスピラ症は全国的に散発例がみられるが，集団発生は近年沖縄県でのみ報告されていた．しかしながら，2006年夏季に宮崎県北部を中心にレプトスピラ症の多発があった．昨年度は同地域でレプトスピラの保有動物調査を行った．本年度は宮崎県におけるレプトスピラ症の実態を明らかにするために，ヒトおよびイヌのレプトスピラ症強化サーベイランスを行った．

国内ではレプトスピラ症は希少感染症であると考えられているが，世界ではアジアや東南アジア，中南米で大規模な流行がおこっており，レプトスピラを保有するネズミがこれら流行地域から船舶などを介して侵入することも考えられる．そこで海外からのレプトスピラ保有ネズミの侵入監視体制の確立のため，全国の検疫所の協力により港湾区域で捕獲されたネズミからレプトスピラの分離およびレプトスピラ遺伝子の検出を試みた．

レプトスピラ症の予防にはワクチンが有効であることはすでに明らかになっているが，現行のワクチンは血清型に特異的な効果しかないため，血清型に依存しない広範囲のレプトスピラ感染に対して有効な新たなワクチン

の開発が急務となっている．本年度は病原性にかかわる因子を同定し，それをターゲットとしたワクチンあるいは診断キットの開発を目的として，レプトスピラ強毒株に特異的な遺伝子を Megasort 法により網羅的に解析した．

方法

1. レプトスピラの分離培養およびネズミ腎臓からのDNA抽出

宮崎県のレプトスピラ症疑いのヒトおよびイヌの血液，また表4にある全国31か所の港湾区域で捕獲されたネズミの腎臓から，コルトフ培地およびEMJH培地（宮崎県のみ）を用いてレプトスピラの分離培養を行った．培養は30℃で3ヶ月間行い，およそ2週間ごとに暗視野顕微鏡下でレプトスピラの増殖の有無を観察した．

新千歳空港で捕獲され冷凍保管されていたネズミの腎臓35検体(エゾアカネズミ6検体，エゾヤチネズミ22検体，ドブネズミ7検体)および関西空港で捕獲，冷凍保存されていたハツカネズミ腎臓5検体から，DNeasy Tissue Kit (Qiagen)を用いてDNA抽出を行った．

2. レプトスピラ *flaB* 遺伝子の塩基配列の解析

イヌおよびネズミのレプトスピラ分離株から上記キットを用いて抽出した染色体DNAおよび凍結腎臓抽出DNAを鋳型として，特異的プライマーを用いてレプトスピラの鞭毛構成遺伝子のひとつである*flaB*遺伝子の増幅を行い(*flaB*-PCR；凍結腎臓検体についてはnested PCR)，その塩基配列の決定を行った．

3. 顕微鏡下凝集試験(MAT)

96穴マイクロタイタープレートに，PBSで希釈したヒトあるいはイヌ血清と，レプトスピラ標準株培養液をそれぞれ25 µlずつ加え，37℃，3時間インキュベートした後，暗視野顕微鏡下で観察を行った．陰性対照と比較して，凝集していないフリーの菌数が50%以下になっている場合を陽性とした．また，レプトスピラ標準抗血清とイヌ分離株培養液を上記のとおり反応を行い，分離株の血清群を決定した．

4. Megasort 法

重症患者から分離され，実験動物（ハムスター，マウス）に致死活性を示した *L. interrogans* serovar Manilae (UP-MMC-NIID：強毒株)と，軽症患者から分離され，実験動物に致死活性を示さない *L. interrogans* serovar Hebdomadis 株の(OK2：弱毒株)からゲノムを抽出し，マイクロビーズ上でそれぞれのゲノムライブラリーを作製した．作製したゲノムライブラリーを，Cy5 あるいはフルオレセインで標識したそれぞれのゲノムとハイブリダイゼーション後にソーティングを行うこと（Megasort 法）により，それぞれの株に特異的なゲノム断片を同定し，その塩基配列を決定した．

結果および考察

1. 宮崎県のレプトスピラ症強化サーベイランス

2006年8，9月に宮崎県において8例のレプトスピラ症患者が発生したため，2007年8月から11月にかけてヒトおよびイヌのレプトスピラ症強化サーベイランスを行った．ヒトでは県内の病院を対象に，表1にある症例定義を満たす患者を医師が診察した場合には，レプトスピラの分離培養・遺伝子検出および血清診断のための検体が採取された．このサーベイランスにより10例のレプトスピラ症疑い例があり，そのうち4例がペア血清を用いたMATにより抗体陽転あるいは有意な抗体上昇がみとめられたためレプトスピラ症と確定診断された(表2)．レプトスピラ症患者の血液からレプトスピラは分離できなかった

本調査によりこれまで患者の発生報告のな

かった国富町，美郷町で患者が確認され，宮崎県の広範囲でヒトのレプトスピラ症が発生していることが確認された．また感染原因は農作業3例，ため池での作業1例と，これまでと同様に労働を介した土壌や水との接触であると推測された．レプトスピラの感染予防には，レプトスピラで汚染された環境との直接的な接触を減らすことが重要である．そのためにも労働者へのレプトスピラ感染のに対する知識を普及し，注意喚起を行っていくことが重要である．

イヌについては県内12か所の動物病院を検査定点病院に選定し，レプトスピラ症を臨床診断した場合にはレプトスピラの分離培養・遺伝子検出および血清診断のための検体が採取された．その結果，レプトスピラ症臨床診断20例のうち17例が実験室診断によりレプトスピラ症であると確定した(表3)．実験室診断の内訳は，分離のみ5例，血清診断のみ8例，分離および血清診断4例であった．分離株の血清群はレプトスピラ標準抗血清との反応性から，Australis 7株(4頭)，Canicola 1株(1頭)，Hebdomadis 6株(4頭)と推定された．また分離株のレプトスピラ遺伝種は *flaB* 部分塩基配列から，すべて *L. interrogans* であると推定された．今回の調査でイヌのレプトスピラの急性感染が県内の広い範囲で起こっていることが明らかとなった．ヒト患者が発生していない地域でもイヌのレプトスピラ症が発生していることから，これらの地域でもヒトの感染リスクは存在していると考えられる．これまでの調査から，宮崎県のレプトスピラ症患者血清中には，血清群 Australis, Hebdomadis に反応する抗体が検出されている．昨年度の調査により，農作業を介してレプトスピラに感染したヒトの感染原因としてネズミが推測された．イヌがヒトの感染源となっているかを明らかにするために，今後は急性感染だけではなく，イヌのレプトスピラの保有状況を調査する必要がある．宮崎県では広範囲でイヌのレプトスピラ感染がおこっていることから，レプトスピラワクチン接種の重要性についても啓発を行う必要がある．しかしながら，表3にあるようにワクチンを接種していても，レプトスピラに感染している事例もある．現行のワクチンはレプトスピラの血清型に特異的な効果しかなく，ワクチンに含まれていない血清型には効果がないとされている．表3のワクチン接種があるイヌに十分な防御抗体が誘導されていたかは不明であるが，ワクチンの血清型特異的な効果のために，今回の感染には無効であった可能性もある．したがって，血清型に依存しない広範囲のレプトスピラ感染に有効なワクチンの開発が今後の重要な課題である．

2. 港湾区域のネズミからのレプトスピラ分離培養およびネズミ腎臓からのレプトスピラ遺伝子検出

全国の検疫所の協力により，31か所の港湾区域で捕獲されたネズミ185匹から採取した腎臓をコルトフ培地で培養を行った結果，名古屋港で捕獲されたドブネズミ2匹からレプトスピラが分離された．分離株2株から染色体DNAを抽出し，*flaB*-PCR，つづいて増幅された *flaB* の塩基配列を決定したところ，2株の塩基配列は同一で，レプトスピラ種は *L. interrogans* であると推定された．しかしながら分離株は増殖が非常に遅く，現時点ではこれ以上の性状解析はできていない．

また新千歳空港および関西空港で捕獲されたネズミの腎臓から DNA を抽出し，*flaB*-nested PCR を行った結果，新千歳空港で捕獲されたエゾアカネズミ2匹の腎臓から *flaB* が検出された．増幅した *flaB* の塩基配列を決定したところ，2検体の配列は同一でレプトスピラ種は *L. interrogans* であると推定された．

本年度の調査では2か所の港湾区域でレプトスピラの分離およびレプトスピラ遺伝子が検出された．名古屋港ではこれまでにレプトスピラの分離の報告はあるが，新千歳空港で

のレプトスピラの検出は初めてである．名古屋港分離株および新千歳空港の*flaB*部分塩基配列は，それぞれ名古屋市街地を含む全国数か所や東北地方で捕獲されたネズミから分離されたレプトスピラと同一の配列であった．

レプトスピラ症は東南アジアや中南米では大規模な発生がみられており，これら地域から船舶などを介してレプトスピラ保有ネズミの侵入も考えられるため，今後とも港湾区域での侵入監視体制を確立していく必要がある．また港湾区域で捕獲されたレプトスピラが，土着の菌か海外から侵入した菌かを明らかにするためにも国内でのレプトスピラ保有調査を強化するとともに，レプトスピラのより詳細な性状解析方法の確立も重要であり，今後の課題として取り組んでいく必要がある．

3. Megasort 法によるレプトスピラ strain-specific 遺伝子の網羅的解析

Megasort 法により，*L. interrogans* の全ゲノム配列決定 2 株とも相同性を示さない強毒株および弱毒株に特異的な遺伝子群を同定した．弱毒株には強毒株，ゲノム株に比べて trasposase，IS，ファージ遺伝子断片が多く存在することが示唆された．また全ゲノムの制限酵素 *Not* I 切断パターンは，強毒株やゲノム株の血清型では株間で保存されているのに対し，血清型 Hebdomadis 分離株では多様であることが明らかとなり，転移因子やファージによるゲノムの再構成が起こったことが示唆された．一方強毒株では internalin と相同性を示す遺伝子断片が同定された。現在この遺伝子の解析を行っている．

本年度の研究を遂行するにあたりご協力をいただいた以下の機関の方々に深謝いたします．

小樽検疫所，仙台検疫所，成田空港検疫所，東京検疫所，横浜検疫所，名古屋検疫所，清水検疫所支所，中部空港検疫所支所，大阪検疫所，関西空港検疫所，神戸検疫所，福岡検疫所，長崎検疫所，那覇検疫所，那覇空港検疫所支所

宮崎県獣医師会，宮崎県医師会，宮崎県衛生環境研究所，中央保健所，日南保健所，都城保健所，小林保健所，高鍋保健所，日向保健所，延岡保健所，高千穂保健所，宮崎市保健所

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表 1. ヒトのレプトスピラ症の症例定義

(1) 急性発症し、発熱(≧ 38℃)かつ経過中以下の 5 項目のうち 1 つ以上の所見を有する ・黄疸（総ビリルビン値 2.0 mg/dl 以上） ・腎機能障害（血清クレアチニン値 2.0 mg/dl 以上） ・出血症状もしくは血小板値 $10.0 \times 10^4 / \mu l$ 以下 ・意識障害 ・敗血症性ショック
(2) 保菌動物の尿や、尿に汚染された水や土壌と接触する機会の多い場所での労働やレクリエーションによる感染が疑われる場合

表 2. 宮崎県レプトスピラ症強化サーベイランス陽性結果一覧　－ヒト－

検体番号	性別	年齢	居住地	推定感染血清型	推定感染原因
7003	男性	78	国富町	Hebdomadis	農作業
7005	男性	74	美郷町	Australis	農作業
7006	男性	67	国富町	Australis	農作業
7007	男性	55	宮崎市	Hebdomadis	ため池での作業

表 3. 宮崎県レプトスピラ症強化サーベイランス陽性結果一覧　－イヌ－

検体番号	居住地	種別	発症日	臨床症状	ワクチン接種歴	転帰	検査結果			
							分離結果	分離株血清群	分離株 *flaB*	血清抗体価
7002	木城町	狩猟犬	H19.9.4	嘔吐、黄疸	無		陽性(E)	Hebdomadis	*L. interrogans* ST1	Hebdomadis: 320
7003	門川町	ペット	19.8.1	嘔吐、粘膜の充出血、黄疸	無	死亡	未実施			Hebdomadis: 640
7004	国富町	ペット	H19.9.8	嘔吐、粘膜の充出血、黄疸、下痢	有	死亡	陰性			Australis: 80 Icterohaemorrhagiae: 80 Canicola: 80
7005	国富町	狩猟犬	H19.9.12	嘔吐、粘膜の充出血、黄疸	無		陽性(K)	Australis	*L. interrogans* ST2	陰性
7006	北郷町	ペット	H19.9.19	嘔吐	無	軽快	陽性(E, K)	Hebdomadis	*L. interrogans* ST2	Hebdomadis: 640
7007	延岡市	ペット	H19.9.19	嘔吐、粘膜の充出血、黄疸、筋肉のこわばり	無	回復	陽性(E)	Hebdomadis	*L. interrogans* ST2	陰性
7009	宮崎市	ペット	H19.9.27	発熱、嘔吐、黄疸	無		陰性			Hebdomadis: 640
7010	都城市	ペット	H19.9.27	嘔吐、黄疸、軟便	無		陽性(E)	Canicola	*L. interrogans* ST3	Canicola: 80
7011	宮崎市	狩猟犬	H19.10.4	発熱、嘔吐、粘膜の充出血、黄疸			陰性			Hebdomadis: 640
7013	延岡市	狩猟犬	H19.10.7	粘膜の充出血、黄疸	有	死亡	陽性(E, K)	Hebdomadis	*L. interrogans* ST1	陰性
7014	宮崎市	狩猟犬	H19.10.10	嘔吐、黄疸			陰性			Hebdomadis: 640
7015	野尻町	ペット	H19.10.15	黄疸、やや貧血	無		陽性(E, K)	Australis	*L. interrogans* ST2	陰性
7016	木城町	狩猟犬	H19.10.15	嘔吐、粘膜の充出血、黄疸			未実施			Autumnalis: 640 Hebdomadis: 640
7017	国富町	狩猟犬	H19.10.24	嘔吐、粘膜の充出血、黄疸	無		陰性			Australis: 160 Hebdomadis: 640
7018	宮崎市	ペット	H19.11.14	嘔吐	無		陽性(E, K)	Australis	*L. interrogans* ST2	Autumnalis: 160
7019	延岡市	ペット	H19.11.2	嘔吐、粘膜の充出血、3日前後肢疼痛	無	安楽殺	陽性(E, K)	Australis	*L. interrogans* ST2	陰性
7020	延岡市	ペット	H20.1.12	食欲廃絶, 嘔吐, 粘膜の充出血、黄疸	無	死亡	陰性			Icterohaemorrhagiae: 640

E: EMJH 培地, K: コルトフ培地
STの数字がおなじ分離株の *flaB* 部分塩基配列は同一

表 4. 日本の港湾区域で捕獲されたネズミのレプトスピラ保有状況（2007.7～2008.1）

調査機関	捕獲地	捕獲ネズミの種類および捕獲匹数					
		ドブネズミ	クマネズミ	ハツカネズミ	アカネズミ	エゾヤチネズミ	合計
小樽検疫所	旭川空港	1				1	2
	石狩港	5				1	6
	小樽港	6					6
	釧路港	3					3
	苫小牧港	8					8
	花咲港	4					4
	室蘭港	8					8
	紋別港	1					1
	留萌港	4					4
	稚内港	3					3
仙台検疫所	青森港	1	2				3
	小名浜港	2					2
	気仙沼港		1				1
	塩釜港	5					5
	八戸港	3					3
	福島空港				1		1
成田空港検疫所	成田空港			1	3		4
東京検疫所	鹿島港	3	1	2			6
	川崎港	3					3
	東京港	1					1
横浜検疫所	横浜港	1		3			4
名古屋検疫所	名古屋港	8 (2)		2			10 (2)
清水検疫所支所	清水港	4					4
中部空港検疫所支所	中部空港	7					7
大阪検疫所	大阪港			2			2
関西空港検疫所	関西空港	2		31			33
神戸検疫所	神戸港	25	1	1			27
福岡検疫所	博多港	2	2				4
長崎検疫所支所	長崎港	1					1
那覇検疫所	那覇港	13	1				14
那覇空港検疫所支所	那覇空港	3	2				5
合計		127 (2)	10	42	4	2	185 (2)

（　）内はレプトスピラが分離できたネズミの数

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Comparative Immunology, Microbiology & Infectious Diseases 30 (2007) 415–425

COMPARATIVE IMMUNOLOGY MICROBIOLOGY & INFECTIOUS DISEASES

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Tuberculosis as a zoonosis from a veterinary perspective

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Received 1 December 2006

Abstract

Tuberculosis is an important disease among many zoonoses, because both *Mycobacterium tuberculosis* and *Mycobacterium bovis*, which are the major causes of tuberculosis, are highly pathogenic, infect many animal species and thus are likely to be the source of infection in humans. In particular, monkeys are highly susceptible to these bacteria and are important spreaders. Recently, two outbreaks of *M. tuberculosis* occurred in four different kinds of monkeys and humans were also infected with the disease in Japan. In zoos, tuberculosis was reported not only in monkeys, but also in several different kinds of animals, including elephants. Pets such as dogs and cats are believed to be generally less susceptible to *M. tuberculosis*, but in this article we introduce a case of infection from man to dog by close contact. Japan is one of the few countries that have been able to control *M. bovis* infection. In other countries, however, cases of bovine tuberculosis and human *M. bovis* infection have been reported, and thus further attention is still required in the future.

Keywords: Zoonosis; *Mycobacterium tuberculosis*; *Mycobacterium bovis*; Monkey; Elephant; Dog

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0147-9571/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cimid.2007.05.002

Résumé

La tuberculose, parmi de nombreuses zoonoses, est une maladie importante, parce que ses deux causes principales, *Mycobacterium tuberculosis* et *Mycobacterium bovis*, sont toutes les deux très pathogéniques et infectent beaucoup d'espèces, ce qui les rend susceptibles d'infecter aussi les humains. Les singes, en particulier, sont facilement atteints par l'infection de ces bactéries, dont ils deviennent ainsi des propagateurs importants. Récemment, au Japon, il y a eu deux cas d'infection répandue de *M. tuberculosis*, qui se trouvait chez des singes de quatre espèces et aussi chez des humains. Dans les jardins zoologiques, l'infection a été rapportée non seulement chez les singes, mais aussi chez des animaux de plusieurs espèces, y compris les éléphants. On croyait que les chiens et les chats domestiques étaient moins susceptibles à l'infection *M. tuberculosis*, mais nous présentons ici le cas d'une infection transmise par un homme à un chien avec lequel il était en contact prochain. Le Japon est l'un des rare pays qui ont pu contrôler l'infection *M. bovis*. Dans la plupart des pays, des cas de tubercule bovine ont été rapportés de même que les cas d'infection *M. bovis* chex les humains, ce qui porte à croire que ce sujet mérite encore de l'attention future.

Mots clés: zoonose; *Mycobacterium tuberculosis*; *Mycobacterium bovis*; singe; elephant; chien

1. Introduction

Tuberculosis is a major emerging disease in humans and is now the leading cause of death in adults worldwide. According to WHO estimates, 2 billion people, about one-third of the world's population, are infected with tuberculosis. In 2003, about 8.8 million people were estimated to have developed tuberculosis (incidence rate 140 per 100,000 population), and 1.7 million people (mortality rate, 28 per 100,000 population) died of tuberculosis, with 99% of them being concentrated in developing countries, particularly Asia and Africa [1]. This situation is believed to be closely associated with the spread of HIV in developing countries, in addition to the poor sanitary and living conditions due to poverty and to delay in action against tuberculosis [1,2]. In contrast, the incidence rate of tuberculosis is low in developed western countries (7 per 100,000 population). In Japan, however, the tuberculosis incidence rate had steadily decreased until the 1970s, but the decrease slowed down and then in late 1990s showed a temporary upsurge, with the number of new tuberculous patients reaching 39,384 (incidence rate 31.0) in 2000. In 2005, with 28,319 patients (incidence rate, 22.2), Japan is still classified as a country of intermediate-level tuberculosis epidemic [3].

The pathogen that causes tuberculosis, which is a hazard to public health, is the (highly pathogenic) *Mycobacterium tuberculosis* complex (tubercle bacillus), which comprises *M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti* and *Mycobacterium canetti* [4]. Of these five species, *M. tuberculosis* and *M. bovis* are most highly pathogenic. *M. tuberculosis* is prevalent all over the world and is the cause of almost all cases of mycobacteriosis in Japan.

Humans are the only reservoir hosts for *M. tuberculosis*. The human-to-human infection cycle rotates; however, tubercle bacilli have a wide host range and *M. tuberculosis* has been detected in fish, reptiles, birds, and mammals including marine animals. Naturally, the first contamination of these animals with *M. tuberculosis* is caused by humans, and then infection occurs among animals, which become the source of infection in humans. Therefore, in this report, we describe tuberculosis, a zoonosis, particularly *M. tuberculosis* and *M. bovis* infections, from a veterinary perspective.

2. Tuberculosis in monkeys

Table 1 shows that different animal species have different degrees of susceptibility to tubercle bacilli and various frequency of open tuberculosis according to the animal species. From a public health print of view, the role of each animal differs according to its species. Animals belonging to the high-score group (Group 1), especially monkeys, are important sources of infection with tuberculosis in terms of susceptibility and transmission. Infection risks differ among different species of monkeys. Old-world monkeys are important from the viewpoint of public health, because they are by far the most susceptible to both *M. tuberculosis* and *M. bovis* and are likely to be unrestrained [5].

In Japan, tuberculosis in monkeys has been reported in zoos. Between 1960 and 1995, *M. tuberculosis* infection occurred in pig-tailed macaques, Taiwan macaques, orangutans, and chimpanzees [6,7, private communication]. In this report, we present the two outbreaks of *M. tuberculosis* infection that occurred recently. In an exhibition facility housing 17 Japanese macaques in the Kansai area, two monkeys that died in July and October 2004 were diagnosed with *M. tuberculosis* disease. The rest of the monkeys that were housed with these two monkeys were also found positive with tuberculosis skin test, and thus were euthanized [8].

The other outbreak involved three species of monkeys infected in succession (private communication) in the one facility. In this facility, two reptiles (a Malay gavial [*Tomistoma schlegelii*] and a spectacled caiman [*Caiman crocodilus*]) died in December 2000 and in February 2001 before the outbreak of tuberculosis in monkeys. Both these animals had disseminated lesions in the organs and numerous acid-fast bacteria in the lesions. In February 2001, a tuberculin-positive chimpanzee died. This animal had suppurative granulomatous inflammation with infiltration of multinucleated giant cells in the liver, and a small number of acid-fast bacteria in the lesions. In October 2003, an old Asian elephant died in the same facility. This animal had lung abscesses, and histopathological examination revealed acid-fast bacteria in the lesions. Although all these animals had acid-fast bacteria in common in their lesions, the bacterial species were not identified, because the bacteria were not cultured. PCR of paraffin sections of the lesions of the two different reptiles, however, revealed a band specific to *M. tuberculosis* complex when the IS1-2 (123 bp) primer was used, although no band was noted when the TB1-2 (320 bp) primer was used. In October 2003, when the Asian elephant died, the first of three prosimians

Table 1
Relative mycobacteria susceptibilities and spread

Group	Species 1	No. of bacilli in lesions[a]	Species 2	Susceptibility to infection with three types of tubercle bacilli[a]			Spread
				Bovine	Human	Avian	
1	Primitive humans #1	1		5	5	1	5
	Monkeys	2	Great apes	3	2	3	5
			Asian monkeys	5	5	2	
			African monkeys	4	4	2	
			South American monkeys	2	2	2	
	Guinea pigs	1		5	5	2	1
	Rabbits	2		1	5	4	1
	Mice	3		1	5	4	1
2	Modern humans #2	1		2	2	1	5
	Elephants	3		3	3	1	?
	Cattle	1		1	4	1	5
	Goats	1		1	4	2	1
	Pigs	1		2	4	2	1
3	Chickens	4		1	1	3	4
4	Horses, etc.	3		1	2	1	1
5A	Dogs	2		2	2	0	0
5B	Cats	3		1	4	2	1
	Ferrets	5		1	5	2	0
5C	Hamsters	4		5	5	1	0

The maximum value for each feature in this table is 5. The values for spread represent the degree of case with which tuberculosis spreads naturally between members of any one species. #1: aboriginal people, #2: contemporary human.

[a]The rating scale is as follows: 1, not likely; 2, rare, 3, occasional; 4, common; 5, classic [5,16].

(red ruffed lemurs [*Varecia variegata rubra*]) died of tuberculosis. The remaining two developed the disease in succession by May 2004, as a result of which one died and the other one was euthanized. In addition, four out of nine old-world monkeys (Abyssinian colobus monkeys [*Colobus guereza*]) and eight new-world monkeys (tufted capuchin monkeys [*Cebus apella*]), both of which shared part of the animal facility with the red ruffed lemurs, developed tuberculosis from January 2004 and died or had a positive tuberculosis skin test and thus were euthanized. The acid-fast bacilli isolated from each monkey were identified as *M. tuberculosis*, and were found to be of the same strain belonging to the Beijing family, which is prevalent in the Far East. Subsequently, four out of ten workers, including two veterinarians who performed necropsy on the monkeys, were found to be infected with tuberculosis

(QuantiFERON-TB® Gold positive), and one of the veterinarians developed the disease. The *M. tuberculosis* isolated from this patient was identical to the bacterium isolated from the monkeys.

The type of lesion and amount of bacteria in the lesion varied depending on the species of monkey. The prosimians, in particular, presented suppurative changes, including suppurative pneumonia, lung abscesses, cervical lymph node abscesses, and pyonephritis, with acid-fast bacteria forming a large mass in the lesion. The exudate from the lymph node abscesses on the body surface which self-destructed contained large amounts of bacteria, detected positive on smear.

To detect tuberculosis in live monkeys, a tuberculin skin test, culture for acid-fast bacteria using gastric lavage fluid and/or feces, and chest radiography examination are carried out.

Tuberculosis in monkeys probably arises from the following two situations. One is the case where imported monkeys that were already infected with tuberculosis develop the disease after being imported. In Japan, this case is represented by an orangutan imported from Indonesia [private communication] which had been taking care by Indonesian staff with tuberculosis. However, of the 10,462 laboratory monkeys from 10 consignments imported between 2000 and 2004, none were reported to be positive for tuberculin skin test [7]. In Japan, the import of pet monkeys was completely banned in June 2004, and for exhibition monkeys, tuberculosis testing is obligatory, and thus it is unlikely that imported animals will be the source of infection.

The other situation is where infection occurs within the confines of the country. In this case, animals are generally infected from a human spreader of tubercle bacilli. This manner of transmission seems to be more likely in Japan, a country of intermediate-level tuberculosis epidemic. The original source of infection could not be identified in either of the two institutions referred to, because there was no introduction of an animal that could be the source of infection, nor were there any tuberculous patients among the zoo staff.

3. Tuberculosis in elephants and other exhibition animals

In Japan, infection with *M. tuberculosis* was reported in Asian elephants and polar bears as early as 1962 [6,7] and in Malayan tapirs (*Tapirus indicus*) in 1991 [7] and 2004 (private communication).

We describe here tuberculosis in elephants, which very common, and a problem not only in the country of origin, but also in Europe and America [9–15]. Susceptibility to *M. tuberculosis* depends on the species of elephant. Asian elephants are more susceptible to *M. tuberculosis* (susceptibility score, 4) than African elephants (susceptibility score, 1) and their level of risk to humans is 4 [5,16]. In Sweden there was an outbreak of tuberculosis in Asian elephants, which became the source of infection in giraffes [10]. In the US, eight out of 379 elephants in one report died of tuberculosis [9]. There have also been cases in which handlers were infected with tuberculosis from Asian elephants [9,11]. Therefore, in the US, the culture and

PCR of trunk wash is officially carried out regularly to detect tuberculosis in elephants in captivity [15,17]. In one report 12 of 118 elephants (10.1%) were found positive for tubercle bacilli by culture of trunk wash samples [12], while another report stated that 3.3% of the elephants in captivity in North America have active disease [5]. The fact that Asian elephants have a much higher carrier rate of *M. tuberculosis* and a much higher incidence rate of the disease than African elephants is attributed to both greater susceptibility and greater risk of exposure to *M. tuberculosis* in Asian than in African elephants [5]. Asian countries originally have a high level of tuberculosis prevalence in the human population [1] and elephants are raised in close contact with humans, who are reservoirs. These factors are considered to result in the high infection rates in the elephant population. Particularly in Thailand, the number of tuberculous patients is increasing with increased incidence of HIV [1]. Therefore, a further increase in the infection rate among animals and particularly elephants is feared [18].

In Thailand, periodic tuberculosis screenings are performed on elephants, but positive elephants unlikely to receive treatment partly because of high medical costs [18]. In Japan, where about 120 elephants are in captivity, tuberculosis tests are not performed.

Tapirs are less susceptible to *M. tuberculosis* complex than elephants but are slightly more susceptible to *M. bovis* than to *M. tuberculosis* [1]. In Japan, *M. tuberculosis* infection occurred in four Malayan tapirs (*T. indicus*) spanning three generations within one pedigree (private communication).

4. Tuberculosis in pets

Public health risks from dogs and cats are classified as group 5, as shown in Table 1, because these animals are less susceptible to *M. tuberculosis* and, moreover, are not likely to be spreaders. Even dogs, which are more susceptible than cats, have a low incidence of tuberculosis [19]. Most cases of canine tuberculosis are transmitted by human reservoirs; dog-to-dog transmission is very rare [20]. Therefore, the incidence of canine tuberculosis is closely related to the incidence of human tuberculosis. In one study, *M. tuberculosis* was isolated from 75% of dogs with tuberculosis, and 88% or more of these dogs were known to have contact with patients with active tuberculosis [20]. The incidence is also higher in urban areas, where human patients are concentrated, than in the suburbs [19,21]. In addition, from the 1930s to the 1950s, in Europe and America, the incidence of canine tuberculosis was between 0.1% and 4.6% among dogs necropsied [20,21], but now there are hardly any cases of canine tuberculosis with a decrease in the number of human tuberculous patients. In Japan, only four cases have been reported, in 1954 [22]. In 2004, however, canine tuberculosis was reported in US [23] and we have presented a case that occurred in Japan (private communication).

The affected dog was a 3-year-and-8-month-old miniature dachshund. In April 2003, one of the owner's family developed tuberculosis, was isolated in a hospital, and was discharged in July after receiving treatment. As the dog developed a respiratory symptom (wet cough) in December, it was brought to a veterinary clinic. Since it did not respond to treatment and a family member had open tuberculosis, the dog's pharyngeal swab and bronchial lavage fluid were cultured, and *M. tuberculosis* was isolated. The dog was euthanized and necropsied in January the following year. The RFLP patterns of bacteria isolated from the owner (Fig. 1), those isolated from the dog before its death, and those isolated from its organs collected during necropsy were completely identical. Considering the time course, it was thus concluded that the disease was transmitted from human to dog.

This case shows that although dogs are only weakly susceptible to *M. tuberculosis*, they may be infected if they come in close contact with a source of infection (e.g. human). The dog did not have any findings suggesting immunosuppression.

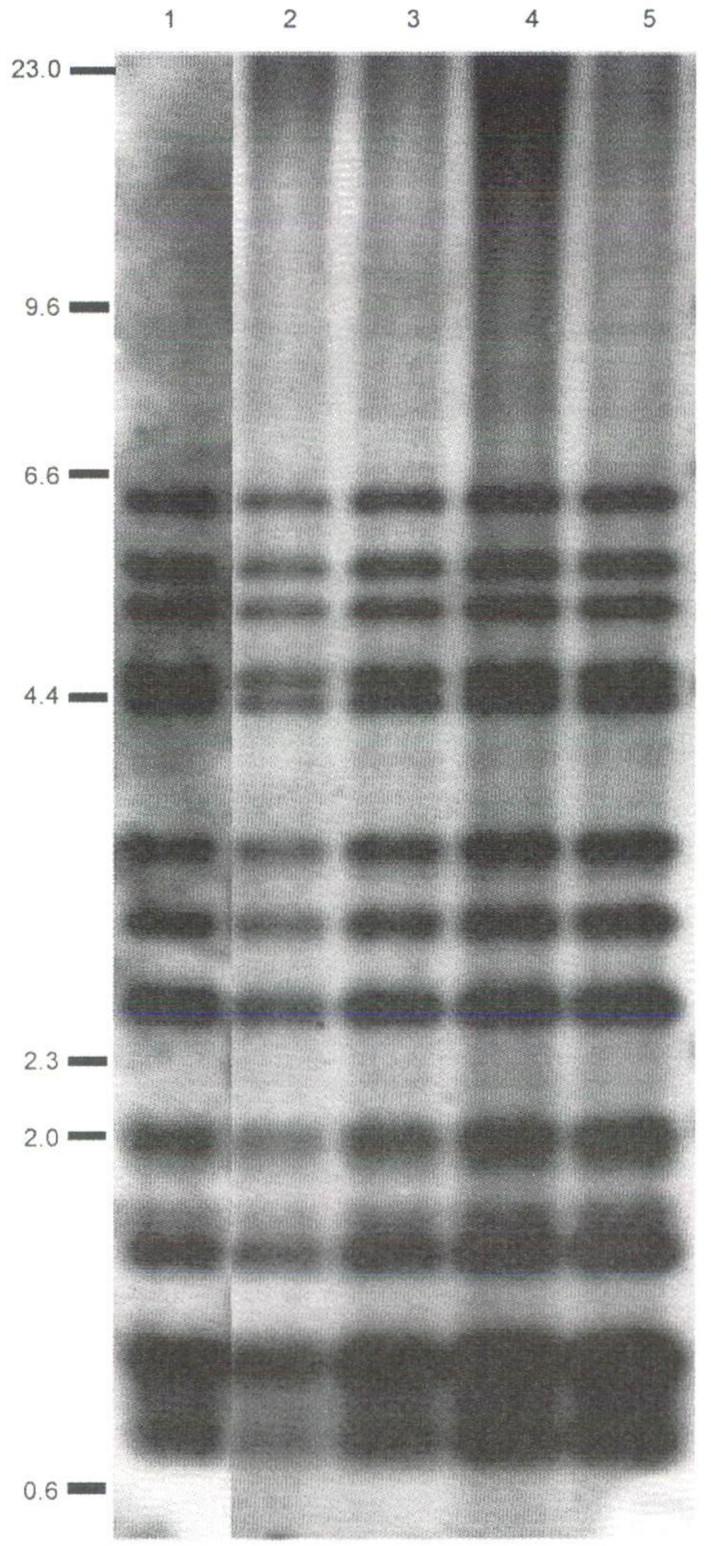
On the other hand, dogs can very rarely be the source of infection in humans. In the present case there is a possibility that the dog might have been the source of infection, because *M. tuberculosis* was also isolated from the dog's pharyngeal swab. Afterwards, the health of veterinary staff involved in the treatment or necropsy of the dog was investigated. It was found that the person who necropsied the dog had a strongly positive tuberculin reaction and a positive QuantiFERON test. These findings suggested infection with tuberculosis during necropsy. Extreme care must be exercised in the necropsy of animals infected with tuberculosis, as in the aforementioned cases of tuberculosis in monkeys.

5. *M. bovis* infection

M. bovis is an important species from the viewpoint of public health for the following reasons: it is the second most pathogenic mycobacterium, following *M. tuberculosis*; it has a wider host range and thus infects more varied animal species, including ruminants, its original host, as shown in Table 1; many of the animals it affects, which can become sources of infection, are in the human living environment [5,24].

Human infection with *M. bovis* is mostly caused by the intake of contaminated milk or dairy products. Transmission by direct contact or droplet transmission is also possible among high-risk people, such as veterinarians and animal keepers, who are in frequent contact with animals. Unlike *M. tuberculosis*, however, it is considered that *M. bovis* does not transmit easily from human to human or by air [5], expect in the case of carriers with lung lesions [25]. Therefore, the public health risks of *M. bovis* should be reduced if it is controlled sufficiently in affected animals such as cattle.

In Japan, dairy cattle receive a tuberculin skin test under a bovine tuberculosis eradication project established in 1901 and the Animal Infectious Diseases Control Law enacted in 1951, and cattle found positive are culled. The number of cases has been reduced to 0–2 per year since 2000, although there were as many as 100 or more cases per year in the 1980s, a relatively large-scale outbreak also involving deer occurred between 1992 and 1993, and an outbreak among beef cattle in 1999 (Table 2). *M. bovis* as such, however, was not detected in any of the culled



Lane 1 ; human
Lane 2 ; canine **trachea lavage fluid**
Lane 3 ; canine Lung
Lane 4 ; canine Liver
Lane 5 ; canine **trachea** swab

Fig. 1. *Mycobacterium tuberculosis* restriction fragment length polymorphism pattern using IS6110.

Table 2
Changes in population of cattle with positive TB skin test in Japan

Year	No.	Year	No.	Year	No.
1980	120	1989	35	1998	1
1981	121	1990	32	1999	37
1982	45	1991	33	2000	2
1983	35	1992	195	2001	0
1984	18	1993	203	2002	1
1985	32	1994	10	2003	1
1986	45	1995	9	2004	1
1987	89	1996	8	2005	1
1988	40	1997	2		

tuberculin-positive cattle, which are so-called reactors with no visible lesions, and thus they are very unlikely to transmit *M. bovis* to humans. There have been no reports of isolation of *M. bovis* from wild animals in Japan. Cases of *M. bovis* were reported between 1954 and 1976 in rhinoceroses, camels, giraffes, goats, and raccoon dogs in Japanese zoos [6,7], but there have been no such cases since then.

Japan, however, is one of the few countries that have been able to control *M. bovis*. Cases of human *M. bovis* infection are reported worldwide. In Asia, *M. bovis* infection has been occurring in Korea and Taiwan [1].

One of the reasons *M. bovis* is difficult to control in livestock even in developed countries is that wild animals are contaminated with this bacterial species. As described earlier, many animals are highly susceptible to *M. bovis* and thus are potential sources of infection. In fact, bovine *M. bovis* is transmitted from badgers in the UK, from wild pigs in Australia, and from opossums in New Zealand [24].

M. bovis is very well controlled in Japan. To maintain the high level of control of *M. bovis* in Japan the culling of tuberculin-positive cattle should be continued and the introduction of *M. bovis* infected animals from other countries which can contaminate wild animals with *M. bovis* should be prevented. In particular, in the case of imported animals, a certificate showing that the animal is free of *M. bovis* infection should be requested according to the Office International des Ēpizooties (OIE) guidelines.

6. Conclusions

Thus far, 700 or more different zoonoses have been identified. Among them, tuberculosis is especially important because of the large numbers of human patients and of animals susceptible to this disease. In Japan, tuberculosis in livestock is controlled by the Animal Infectious Diseases Control and has been virtually eradicated in dairy cattle. Therefore, there is a very low risk to humans. Human tuberculosis is controlled by the Tuberculosis Prevention Law, but Japan is still a country of intermediate-level tuberculosis epidemic. Under the present circumstances, incidences of tuberculosis have occurred in pet animals and monkeys, causing a public health problem, yet neither of the relevant laws applies to these animal species. In Japan at present, revision of the Infectious Diseases Control Law is under discussion for more effective control of tuberculosis transmitted from animals.

Acknowledgments

We express our gratitude to Dr. S. Haga and Dr. T. Yamazaki (National Institute of Infectious Diseases) and Dr. M. Takahashi, Ms. Y. Kazumi, Mr. K. Ootomo (Research Institute of Tuberculosis) for their bacterial identification studies. We also thank the staff of facilities who provided the materials for research.

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