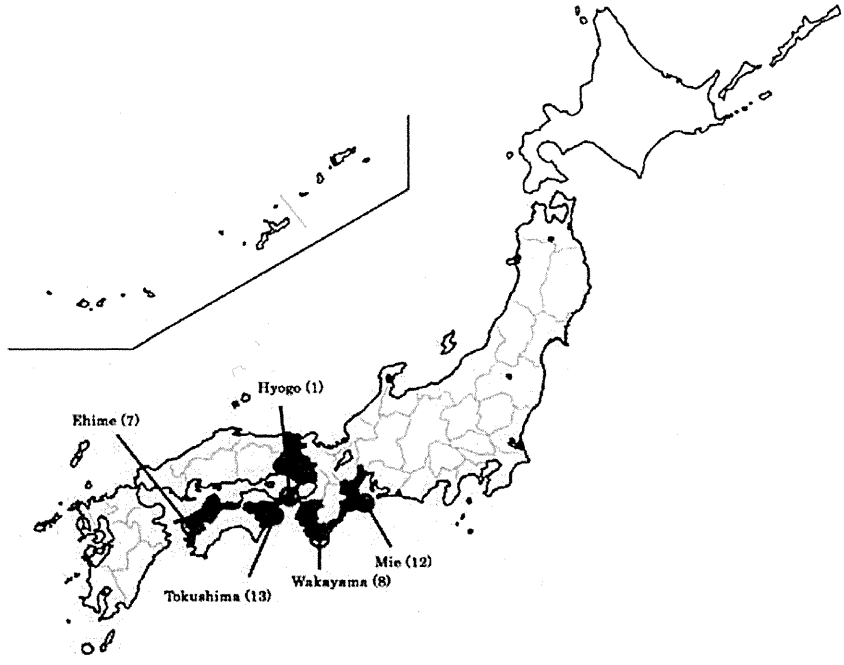

FIG. 1. Distribution of JSF in the present study ($n = 41$). Cases of JSF are seen in the south-western part of Japan, facing the Ocean. Blue shows the involved prefectures, and red circles indicate the endemic spots.

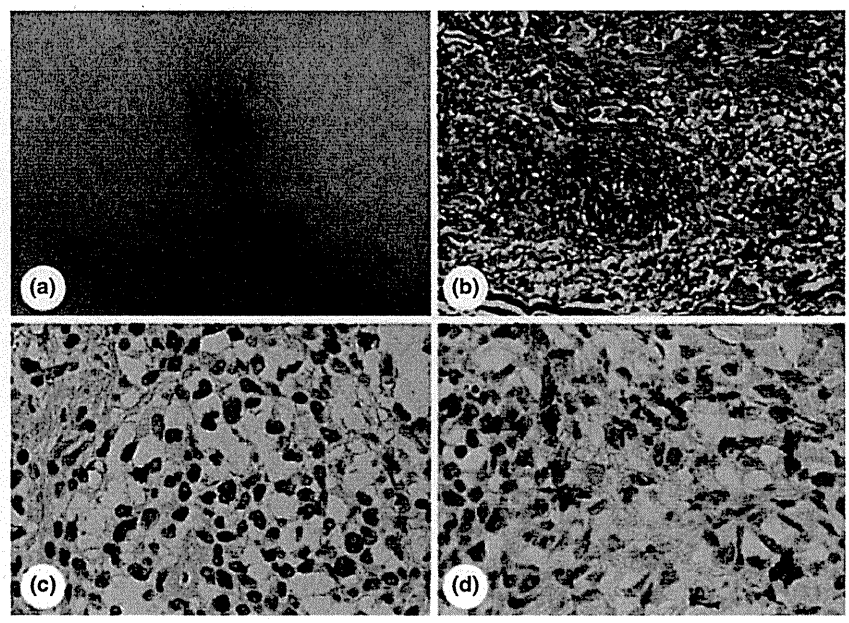

FIG. 2. Immunohistochemical diagnosis of JSF in a representative case (A18). (a) Gross appearance of eschar, (b) histopathology of eschar, haematoxylin and eosin staining, (c, d) immunostaining for SFG rickettsial antigens using monoclonal antibodies S3 (c) and X1 (d). Eschar is covered with scab, and associated with haloed redness. Small eruptions are scattered in the surrounding skin (a). Histologically, perivascular infiltration of lymphocytes and macrophages is evident in the dermis (b). Immunohistochemically, the cytoplasm of endothelial cells and macrophages shows coarse dotted positivity (coloured brown) with both monoclonal antibodies (c, d).

Cell blocks of *Rj*-infected L929 cells served as positive controls for both IHC and RT-PCR. Positive signals were also obtained from *R. conorii*-infected monkey Vero cells with both methods. No positivity of IHC and RT-PCR was seen in uninfected or *Ot*-infected cells and in normal skin. Human/monkey or mouse β2m DNA was consistently amplified from all DNA samples. Cycle threshold values varied from sample to sample (Tables 1 and 2). The mean cycle values were 26.4 for *Rj*-infected L929 cells, 36.8 for regularly fixed eschar ($n = 15$; median, 35.6; range, 30.5–48.0), and 42.6 ($n = 3$; range, 40.5–44.7) for regularly fixed eruptions and scabs. Eschar fixed in 100% formalin required 39.4 cycles ($n = 4$; range, 37.1–41.3).

Sequencing analysis

An exactly homologous sequence of the 114 bp stretch in the *Rj* 17k genus common antigen gene was confirmed in the positive control cells and five skin biopsy specimens. As

TABLE 2. Influence of formalin concentration on JSF-positive rates by IHC and real-time PCR

Formalin concentration		10%				100%			
		Group A (seropositive)[a]		Group B (seronegative)		Group A (seropositive)		Group B (seronegative)	
Site of biopsy	Specimen	IHC	Real-time PCR	IHC	Real-time PCR	IHC	Real-time PCR	IHC	Real-time PCR
Eschar	34	94% (17/18)	83% (15/18)	75% (3/4)	25% (1/4)	100% (9/9)	44% (4/9)	100% (3/3)	33% (1/3)
Mean CN (range)			39.1 (30.5–48.0)		33.6		39.4 (37.1–41.3)		40.0
Eruptions	10	100% (3/3)	67% (2/3)	100% (2/2)	50% (1/2)	75% (3/4)	0% (0/4)	0% (0/1)	0% (0/1)
Mean CN (range)			42.6 (40.5–44.7)		38.7				
Scabs	6	50% (2/4)	25% (1/4)	0% (0/1)	100% (1/1)	–	–	0% (0/1)	100% (1/1)
Mean CN (range)			42.7		40.2				43.6
Total	50	88% (22/25)	72% (18/25)	71% (5/7)	43% (3/7)	92% (12/13)	31% (4/13)	60% (3/5)	40% (2/5)

CN, cycle number representing cycle threshold values in real-time PCR.
[a]Result of non-skin lesions in one autopsy case tentatively included in the eschar.

expected (GenBank M28480), the DNA sequence of *R. conorii* showed three bases difference in the amplified fragment.

Discussion

Registered JSF cases are gradually increasing in number, and endemic areas are spreading in southwest Japan [4,5,20]. *Rj* has also been isolated in Korea and Thailand [21,22]. The mortality rate of JSF is calculated to be 1.9%, but this figure may be underestimated due to early antibiotic treatment. The reported mortality rates of other SFG rickettsioses are 2.5%, 7% and 30% for Mediterranean, Rocky Mountain and Brazilian spotted fevers, respectively [5,23–25].

We evaluated diagnostic methodology, IHC and RT-PCR, for detecting *Rj* with monoclonal antibodies and primers directed toward SFG rickettsiae in FFPE specimens. JSF is the only SFG rickettsiosis in southwestern Japan. This is the first study where *Rj* was detected in FFPE skin biopsies and the detectability was compared between both methods. Sequencing analysis using five representative specimens of eschar and scab confirmed the specificity of our approach. Handling and transfer of rickettsia-infected skin tissue may be biohazardous. This is one of the reasons why we chose FFPE samples as the target of study. Once established, our methodology is applicable to histopathological diagnosis.

PCR detection of SFG rickettsiae such as *R. conorii* and *R. prowazekii* using skin biopsy specimens has been described [26,27]. Mahara documented that all of 53 JSF cases showed skin eruptions and 94% eschar [5]. In the present study, biopsy from eschar was most suitable for diagnostic testing of JSF.

Of 31 seropositive JSF cases plus one autopsy case (group A) and nine seronegative JSF cases (group B), all but three (93%) were positive by IHC and/or RT-PCR. Under our present conditions using FFPE sections, IHC was more effective than RT-PCR in diagnosing JSF. In two of three false-negative cases, only scab samples were submitted. Scabs were insufficient for IHC. Two other scab samples were IHC negative and RT-PCR positive. Low detectability by RT-PCR was partly due to improper fixation in this series. Eighteen samples happened to be fixed in 100% formalin, and high formaldehyde concentration might have caused alteration of DNA structure, as reported previously [28], while the antigenicity was tolerant of such conditions. In fact, cycle threshold values were larger for 100% formalin-fixed specimens than for regularly fixed specimens. Cycle threshold values, smaller for eschar than for eruptions or scabs, may reflect the number of pathogens within the lesion.

Because of DNA fragmentation by formalin fixation and/or paraffin embedding, short PCR products are needed to increase detection sensitivity [29]. In the present study, primers to yield short products (114 bp length) were designed, but obstacles to formalin fixation were still inevitable. β2m DNA fragments of 86 and 152 bp length were reproducibly amplified from human/monkey and mouse samples, respectively.

Among 31 seropositive patients, the diagnosis of JSF was made earlier in 26 (84%) patients by IHC and/or RT-PCR than serology. Manifesting trias (eschar, rash and high fever >38°C) empirically lead clinicians in endemic areas to Minocyclin treatment. The trias are common to JSF and Tsutsugamushi disease [4,5]. New quinolones can be added to Minocyclin in JSF, but are ineffective for Tsutsugamushi disease [5]. In fulminant cases, early diagnosis indicated change in therapeutic regimen to save the patient's life.

Serology is widely used for diagnosing rickettsioses [30]. Seronegativity was observed in nine biopsy cases and one autopsy case (representing 24% of 41 JSF patients), and this might be related to therapeutic eradication of pathogens in the early stages of infection. Long-term serological follow-up is needed.

Acknowledgements

The excellent technical assistance given by Ms Mika Maeshima and Ms Hisayo Ban, Department of Pathology, Fujita Health University School of Medicine, Toyoake, is cordially appreciated. The authors also deeply thank Dr Hiromi Fujita, Ohara Research Laboratory, Fukushima, for providing us with strains of *R. japonica* and *O. tsutsugamushi* and also for performing serological assays. Dr Yosaburo Oikawa, Department of Parasitology, Kanazawa Medical University, Kanazawa, kindly supplied us with mouse monoclonal antibodies S3 and X1. Professor Shigeru Akimoto, Department of Microbiology, Wakayama Medical University, Wakayama, allowed us to use the biosafety level 3 facility. Professor Yasuo Chinzei, Faculty of Medical Engineering, Suzuka University of Medical Science, Suzuka, Mie, Dr Yuji Morita, Myojin Clinic, Kozagawa, Wakayama, and Dr Naoki Yakushiji, Department of Dermatology, Uwajima Municipal Hospital, Uwajima, Ehime, kindly sent us clinical samples. Dr Hidehisa Horiguchi, Pathology Division, Hyogo Prefectural Awaji Hospital, Sumoto, Hyogo, provided us with an autopsy case. Dr Toshio Kishimoto, Okayama Prefectural Institute for Environmental Science and Public Health, Okayama, cooperatively gave us valuable advice and suggestions. Dr Olivier Aoun, Bégin Military Hospital, Saint-Mandé, France, critically reviewed the manuscript. This work was supported by the Research Grant (#19590460 to F.M.) and Open Research Center Project (#30131 to Y.T.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and also by the Research Grant for Emerging and Re-emerging Infections from the Ministry of Health and Welfare, Japan (#H18-Shinko-14, H21-Shinko-6 to Y.T.). The Research Grant from Fujita Health University (2007–2010 to K.I.) also in part supported this work.

Transparency Declaration

Nothing to declare.

References

1. Mahara F. Three Weil-Felix reaction (OX2)-positive cases with skin eruptions and high fever. *J Anan Med Assoc* 1984; 68: 4–7 (in Japanese).
2. Mahara F, Koga K, Sawada S *et al.* The first report of the rickettsial infectious of spoted fever group in Japan: three clinical cases. *J Jpn Assoc Infect Dis* 1985; 59: 1165–1172 (in Japanese).
3. Brouqui P, Raoult D. Clinical aspect of human SFG rickettsiae infection in the era of molecular biology. In: Kazar J, Toman R, eds, *Proceeding of the 5th International symposium on rickettsiae and rickettsial diseases*. Bratislava, Slovak: Slovak Academy of Science, 1996; 195–210.
4. Mahara F. Japanese spotted fever: report of 31 cases and review of the literature [CDC Web site]. *Emerg Infect Dis* 1997; 3: 105–111. Available at: http://www.cdc.gov/ncidod/eid/vol3no2/mahara.htm.
5. Mahara F. Rickettsia in Japan and the far east. *Ann NY Acad Sci* 2006; 1078: 60–73.
6. Kodama K, Senba T, Yamauchi H *et al.* Fulminant Japanese spotted fever definitively diagnosed by the polymerase chain reaction method. *J Infect Chemother* 2002; 8: 266–268.
7. Nomura T, Fujimoto T, Ebistani C, Horiguchi H, Ando S. The first fatal case of Japanese spotted fever confirmed by serological and microbiological tests in Awaji island, Japan. *Jpn J Infect Dis* 2007; 60: 241–243.
8. Suto T. A ten-years experience on diagnosis of rickettsial diseases using the indirect immunoperoxidase method. *Acta Virol* 1991; 35: 580–586.
9. Furuya Y, Katayama T, Yoshida Y, Kaiho I. Specific amplification of *Rickettsia japonica* DNA from clinical specimens by PCR. *J Clin Microbiol* 1995; 33: 487–489.
10. Tsutsumi Y, Mahara F. Prompt diagnosis of Japanese spotted fever. Usefulness of immunostaining using skin biopsy specimens. *Infect Agents Surveil Rep* 2006; 27: 38–40 (in Japanese).
11. Tsutsumi Y, Suzuki M, Shiogama K, Horiguchi H, Sano T, Mahara F. Pathology of Japanese spotted fever. In: SADI, ed., *Acaridae and emerging and re-emerging infections*, Zennokyo, Tokyo: 2007; 119–127 (in Japanese).
12. Fujita H, Watanabe Y, Takada N, Tsuboi Y, Mahara F. Isolation and serology identification of causative rickettsiae from Japanese spotted fever patients. *Asian Med J* 1993; 36: 660–665.
13. Sano J, Yoshimoto N, Mizoguchi Y, Saito M. Utility of sodium alginate cell block method. *Nippon Rinsho Saibo Gakkai Zasshi* 2005; 44: 291–297 (in Japanese).
14. Oikawa Y, Takada N, Fujita H, Yano Y, Ikeda T. Properties and utilizations of some monoclonal antibodies against Japanese spotted fever rickettsiae. *Ann. Rep Ohara Hosp* 1993; 36: 9–14 (in Japanese).
15. Oikawa Y, Takada N, Fujita H, Yano Y, Ikeda T. Identity of pathogenic strains of spotted fever rickettsiae isolated in Shikoku district based on reactivities to monoclonal antibodies. *Jpn J Med Sci Biol* 1993; 46: 45–49.
16. Burt EA, Theodore T. Comparative sequence analysis of a genus-common rickettsial antigen gene. *J Bacteriol* 1989; 171: 5199–5201.
17. Jiang J, Chan T-C, Temenak JJ, Dasch GA, Ching W-M, Richards AL. Development of a quantitative real-time PCR polymerase chain reaction assay specific for *Orientia tsutsugamushi*. *Am J Trop Med Hyg* 2004; 70: 351–356.
18. Vandesompele J, Preter K-D, Pattyn F *et al.* Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002; 3: 34.
19. Prober JM, Trainor GL, Dam RJ *et al.* A system for rapid DNA sequencing with fluorescent chain-terminating dideoxynucleotides. *Science* 1987; 238: 336–341.
20. Fukuta Y, Mahara F, Nakatsu T, Yoshida T, Nishimura M. A case of Japanese spotted fever complicated with acute myocarditis. *Jpn J Infect Dis* 2007; 60: 59–61.
21. Chung M-H, Lee S-H, Kim M-J *et al.* Japanese spotted fever, South Korea. *Emerg Infect Dis* 2006; 12: 1122–1124.
22. Gaywee J, Sunyakumthorm P, Rodkvamtook W, Ruang-areerate T, Mason CJ, Sirisopana N. Human infection with Rickettsia sp. related to *R. japonica*, Thailand. *Emerg Infect Dis* 2007; 13: 671–673.
23. Dalton MJ, Clarke MJ, Holman RC *et al.* National serveillance for Rocky Mountain spotted fever 1981–1992: epidemiologic summary

and evaluation of risk factors for fatal outcome. *Am J Trop Med Hyg* 1995; 52: 405–413.
24. Treadwell TA, Holman RC, Clarke MJ, Krebs JW, Paddock CD, Childs JE. Rockey Mountain spotted fever in United States, 1993–1996. *Am J Trop Med Hyg* 2000; 63: 21–26.
25. Mendes do Nascimento EM, Colombo S, Nagasse-Sugahara TK *et al.* Evaluation of PCR-based assay in human serum samples for diagnosis of fatal cases of spotted fever group rickettsiosis. *Clin Microbiol Infect* 2009; 15 (suppl 2): 232–234.
26. Sfar N, Kaabia N, Letaief A *et al.* First molecular detection of *R. conorri* subsp. *conorii* 99 years after the Conor description of Mediterranean spotted fever in Tunisia. *Clin Microbiol Infect* 2009; 15: 309–310.
27. Fournier P-E, Raoult D. Suicide PCR on skin biopsy specimens for diagnosis of rickettsioses. *J Clin Microbiol* 2004; 42: 3428–3434.
28. Zimmermann J, Hajibabaei M, Blackburn CD *et al.* DNA damage in preserved specimens and tissue samples: a molecular assessment. *Front Zool* 2008; 5: 1–13.
29. Shiogama K, Teramoto H, Morita Y *et al.* Hepatitis C virus infection in a Japanese leprosy sanatorium for the past 67 years. *J Med Virol* 2010; 82: 556–561.
30. Kantsø B, Svendsen CB, Jørgensen CS, Krogfelt KA. Evaluation of serological tests for the diagnosis of rickettsiosis in Denmark. *J Microbiol Methods* 2009; 76: 285–288.

藤田学園医学会誌 2011
Vol. 35, No. 1, pp.25～27

短　　報

リアルタイムPCRによるホルマリン固定パラフィン包埋標本からの*Rickettsia japonica* DNAの検出：基礎的検討

玉熊桂子・堤　　寛
（藤田保健衛生大学医学部・病理学教室）

1．緒　　言

日本紅斑熱は，1984年に馬原文彦医師によって発見され，第四類届出感染症に指定されている新興感染症であり，ダニ媒介性の紅斑熱群リケッチア症に分類される[1-3]。日本紅斑熱の原因リケッチアである*Rickettsia japonica*（*R. japonica*）を保有するマダニに刺咬されることで感染が生じる。適切な診断・治療が遅延すると重症化するため，これまでに毎年死亡例が報告されている。このリケッチアはバイオテロリストに指定されており，公衆衛生学上の適切な対策における早期診断，早期治療の重要性が高い[4,5]。

われわれは，2006年，日本紅斑熱リケッチア患者の刺し口や紅斑部からの皮膚生検のパラフィン包埋ホルマリン固定標本から*R. japonica*を検出する免疫染色法を確立し，早期診断に応用してきている[6]。しかし，生検組織が微小だったり，痂皮のみが提出された場合，免疫染色による診断はしばしば困難だった。

本短報では，ホルマリン固定パラフィン包埋リケッチア感染細胞から日本紅斑熱リケッチアDNAをリアルタイムPCR法によって効率的に検出する方法に関して，基礎的な検討を加えた。

2．実験方法

A）リケッチア株と細胞ブロック作製

R. japonica（Aoki株，Katayama株）は，大原綜合研究所（福島市）から分与された。和歌山県立医科大学医学部微生物教室のバイオセイフティーレベル3施設において，C3H／Anマウス由来の線維芽細胞L929細胞にリケッチア株を接種した。接種後，32℃の環境下で5％仔ウシ血清（Hyclone, Logan, UT, USA）含有ダルベッコ・イーグル培地（ニッスイ）にて5～7日間培養された。一晩，10％ホルマリン固定したのち，剥離細胞をゲラチン法で細胞ブロック化した。非感染細胞の細胞ブロックを陰性対照とした。

B）DNA抽出法

*R. japonica*感染L929細胞の細胞ブロックからミクロトームで4～5 μmに薄切した切片数枚を脱パラフィンし，QIAamp DNA FFPE Tissue kit（#56404，キアゲン社）を用いてDNAを抽出した。パラフィン包埋しない固定後感染細胞からも同様にDNAを抽出した。

C）プライマー

マウス由来のβ2-ミクログロブリン（β2M）を内在性コントロールとした。プライマーは，Primer3ソフトウェアで設計した[7]。Forward［CAGTGTGAGCCAGGATATAG］，reverse［GAAGCCGAACATACTGAACTGCTAC］で，PCR産物の大きさは152bpである。

*R. japonica*ゲノムを検出するプライマーとして，FuruyaらのRj5/Rj10とStenosらの*gltA*プライマーを用いた[8,9]。それぞれのPCR増幅産物の大きさは，357bpと74bpである。

D）Sybr-greenリアルタイムPCRの条件

Sybr-green法によるリアルタイムPCRには，DNA Engine Opticom® System（Biorad Japan，東京）を使用した。反応条件は，活性：95℃ 10分，変性：94℃ 15秒，アニーリング：*gltA*とRj5/Rj10は55℃，β2Mは61.8℃で各30秒，伸張：72℃ 30秒とした。融解曲線は，50～90℃ 30秒で40サイクルを描出した。反応溶液は，マスターミックス12.5 μl（Biorad社），各プライマー10pmol，DNAサンプル5 μl，総

Real-time PCR Detection of *Rickettsia japonica* DNA in Formalin-fixed, Paraffin-embedded Samples：Basic Analysis.
Tamakuma, K. and Tsutsumi, Y.
（Department of Pathology, Fujita Health University School of Medicine）

量 25 μℓだった。

3．実験結果

R. japonica 感染 L929 細胞は，免疫染色（10mM クエン酸緩衝液，pH7.0 での圧力鍋による加熱処理後のアミノ酸ポリマー法：一次抗体にはマウスモノクローナル抗体 X1，S3 を利用）[6] によって，大部分の細胞における病原体の濃厚感染が確認された（図 1）。非感染細胞に陽性所見は得られなかった。

ホルマリン固定細胞から抽出した DNA を用いた場合，*R. japonica* DNA（*gltA* と Rj5/Rj10 ともに）の増幅が確認された。しかし，ホルマリン固定パラフィン包埋切片から抽出された DNA からは，*gltA* のみが検出された（図 2 A，B）。

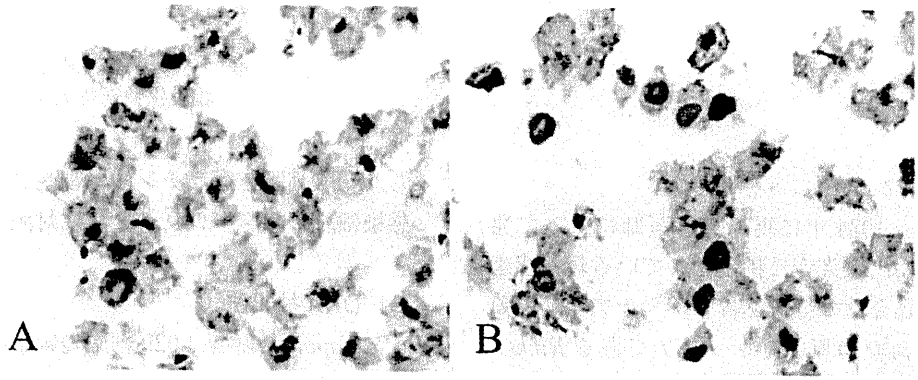

図 1　Immunohistochemical identification of *R. japonica* in formalin-fixed, paraffin-embedded sections of infected L929 cells.
Granular positivity is observed in the cytoplasm of L929 cells infected with *R. japonica* (Katayama strain). Amino acid polymer method after heat-induced epitope retrieval, using mouse monoclonal antibodies X1 (A) and S3 (B).

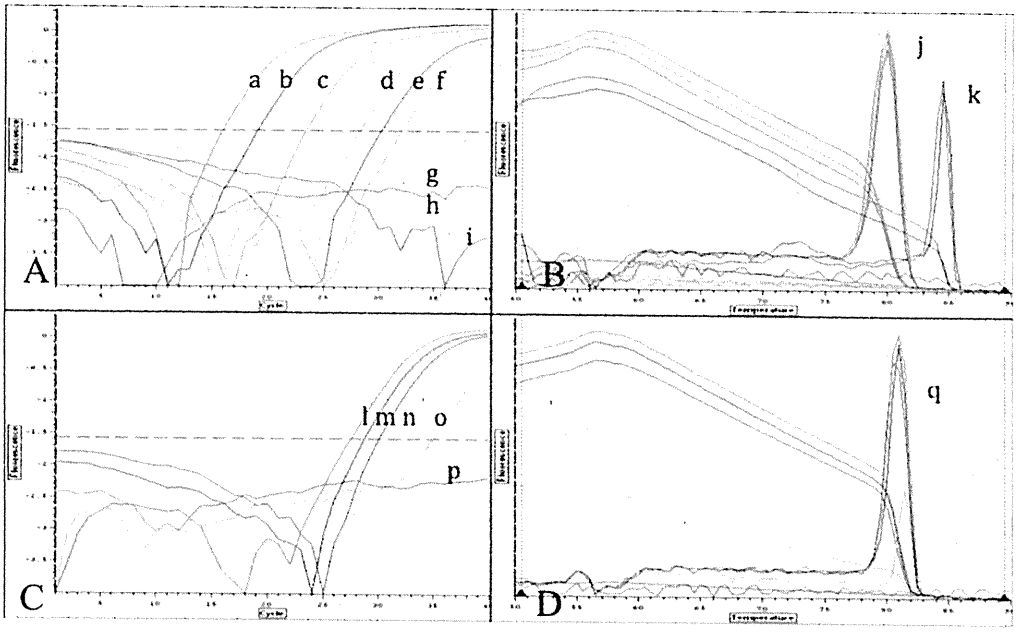

図 2　Sybr-green real-time PCR detection of *gltA*, Rj5/Rj10 and β2 microglobulin (β2M) from DNA of L929 cells infected with two kinds of strains (Aoki and Katayama) of *R. japonica*

(A) Detection of *gltA* and Rj5/Rj10 DNA：
a) formalin-fixed (FF) Aoki strain of *R. japonica* for *gltA*, b) FF Katayama strain of *R. japonica* for *gltA*, c) formalin-fixed paraffin-embedded (FFPE) Aoki strain of *R. japonica* for *gltA*, d) FFPE Katayama strain of *R. japonica* for *gltA*, e) FF Aoki strain of *R. japonica* for Rj5/Rj10, f) FF Katayama strain of *R. japonica* for Rj5/Rj10, g) FFPE Aoki strain of *R. japonica* for Rj5/Rj10, h) FFPE Katayama strain of *R. japonica* for Rj5/Rj10, and i) blank.

(B) Melting curves of (A)：
j) *gltA* and k) Rj5/Rj10

(C) Detection of mouse β2M of L929 cell origin：
l) FF cells infected with Aoki strain of *R. japonica*, m) FF cells infected with Katayama strain of *R. japonica*, n) FFPE cells infected with Aoki strain of *R. japonica*, o) FFPE cells infected with Katayama strain of *R. japonica*, and p) blank

(D) Melting curves of (C)：
q) β2M

内在性コントールのマウス由来β2Mについては，ホルマリン固定標本，ホルマリン固定パラフィン包埋切片ともに増幅が確認された（図2C，D）。

4．考　　察

ホルマリン固定は，バイオハザードの暴露を防止する。また，免疫染色による病原体の証明には欠かせないステップである。一方，PCRアッセイにおいてホルマリン固定は，DNAを断片化させ，検出感度を低下させる。

Lewisらは，ホルマリン固定されたサンプルに対するPCRプライマーの設計は，PCR産物のサイズが200bp以下となることを推奨している[10]。われわれも，ホルマリン固定パラフィン切片を用いたC型肝炎ウイルスRNAのreverse transcriptase-PCR検出において，同様の経験を発表してきた[11]。ここでは，nested PCRが用いられたが，C型肝炎ウイルス亜型（1b，2a，2b）の検出に成功したPCR産物のサイズは123～174bpだった。ホルマリン固定パラフィン包埋標本から，*R. japonica* 由来Rj5/Rj10が検出できなかった原因は，PCR産物の大きさ，357bpが関与していると思われた。事実，PCR産物が74bpに設計した *gltA* およびPCR産物が152bpのβ2Mでは，この条件下でもPCR増幅が可能だった。

5．結　　論

ホルマリン固定パラフィン包埋サンプルから，日本紅斑熱の原因菌，*R. japonica* のDNAを効率よく検出するためには，PCR産物のサイズを200bp以下に設計することが必要だった。この検討は，臨床検体を用いた *R. japonica* 検出の基礎となる。

謝　　辞

本研究を進めるにあたり，日本紅斑熱リケッチア研究の指導と応援をしていただいた馬原文彦博士（馬原医院，徳島県阿南市），病原体の提供などに多大な協力をしていただいた藤田博己博士（大原綜合研究所，福島市），バイオテロリストである病原体の感染実験を支援いただいた宮本和明博士（和歌山県立医科大学医学部微生物教室），さまざまなアドバイスと励ましの言葉をいただいた藤田保健衛生大学医学部病理学教室のスタッフのみなさまに感謝いたします。

文　　献

1）馬原文彦（1984）発疹と高熱を主徴としWeil-Felix（OX2）反応陽性を示した3症例について．阿南医報68(9月号)．4－7.

2）馬原文彦，古賀敬一，沢田敬三，谷口哲三，重見文雄，須藤恒久，坪井義昌，大谷　明，小山一，内山恒夫，内田孝宏（1985）わが国初の紅斑熱リケッチア感染症．感染症誌59. 1165－1172.

3）馬原文彦（2008）リケッチア感染症．最新医学63(増)．680－702.

4）Nomura, T., Fujimoto, T., Ebisutani, C., Horiguchi, H., and Ando, S. (2007) The first fatal case of Japanese spotted fever confirmed by serological and microbiological tests in Awaji, Japan. *Jpn. J. Infect. Dis.* 60. 241－243.

5）和田桂代子，栄枝弘司，青野　礼，千屋誠造(2008)重症化した日本紅斑熱の1例．本邦2例目の死亡例．感染症誌82. 77－81.

6）堤　寛，馬原文彦(2006)日本紅斑熱の早期診断：皮膚生検を利用した免疫染色の実用性．病原微生物検出情報27(2)．38－40.

7）Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., and Spelemen, F. (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 3. 1－11. http://genomebiology.com/2002/3/7/research/0034

8）Furuya, Y., Katayama, T., Yoshida, Y., and Kaiho, I. (1995) Specific amplification of *Rickettsia japonica* DNA from clinical specimens by PCR. *J. Clin. Microbiol.* 33. 487－489.

9）Stenos, J., Graves, S. R., and Unsworth, N. B. (2005) A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group rickettsiae. *Am. J. Trop. Med. Hyg.* 73. 1083－1085.

10）Lewis, F., Maughan, N. J., Smith, V., Hillan, K., and Quirke, P. (2001) Unlocking the archive gene expression in paraffin-embedded tissue. *J. Pathol.* 195. 66－71.

11）Shiogama, K., Teramoto, H., Morita, Y., Mizutani, Y., Shimomura, R., Inada, K., Kamahora, T., Makino, M., and Tsutsumi, Y. (2010) Hepatitis C virus infection in a Japanese leprosy sanatorium for the past 67 years. *J. Med. Virol.* 82. 556－561.

（平成23年1月28日受理）

野生動物と動物由来感染症 ①

つつが虫病と日本紅斑熱

馬原 文彦[1)] 藤田 博己[2)]

はじめに

わが国に存在するリケッチア感染症としてはつつが虫病と日本紅斑熱が主となる．本稿では再興感染症としてのつつが虫病，新興感染症としての日本紅斑熱について詳述した．2007年の感染症法改正では，新たにロッキー山紅斑熱が第4類届出感染症とされた．人と物が大量に移動する時代にあって，日本国内のみでなく，グローバルな視点からのリケッチア感染症を視野に，節足動物を介する動物由来感染症としての認識も必要である．

疫学

古来，1日を無事に過ごすことを「つつがなく」と言われたように，つつが虫病は東北地方で平安時代から恐れられていた病気であった．しかし，時代の流れと共に発生数は徐々に減少し，過去の病気となりつつあった．ところが1980年代初め頃から患者が増え始め，全国的な拡がりとなり，死亡例が出るに及んでつつが虫病に対する注意が喚起された．近年のわが国での年間発生数は，約300～1,000例前後．韓国，中国や東南アジアで多発しているとの報告もあり，輸入感染症にもなりうる．

日本紅斑熱(Japanese Spotted Fever)は1984年に馬原により初めて報告された新興感染症である[1)]．臨床的につつが虫病に類似するが，つつが虫病よりは重症化しやすく，早期診断，早期治療が必要である．発生数は発見以来，希少感染症として研究者の間で集計されていたが[2)]，1999年の「感染症の予防及び感染症の患者に対する医療に関する法律(感染症法)」により届出義務が生じたことから，全国情報も蓄積され，第4類届出感染症の頻度では日本紅斑熱はレジオネラ，つつが虫病に次いで発生数が多い感染症である．感染症発生動向調査による日本紅斑熱の届け出数は，1999年以来，年間36～67例であったが，2008年には100例を超え，発生地域も拡がりを見せている[3)]．

病原体

1. つつが虫病の病原体：*Orientia tsutsugamushi*

つつが虫病の病原体は*Rickettsia tsutsugamushi*とされてきたが，遺伝子解析，細胞壁の分析の結果，リケッチア属とは異なっていることが証明され，1995年，多村らにより*Orientia tsutsugamushi*とすることが提唱され，リケッチア科オリエンティア属に分類された[4)]．古典的つつが虫病の原因病原体はKato株，新型つつが虫病の原因病原体はGilliam株，Karp株，Kawasaki株，Kurosaki株，Shimokoshi株である．

2. 日本紅斑熱の病原体：*Rickettsia japonica*

日本紅斑熱の病原体は1992年国際規約に基づき*Rickettsia japonica*とされた[5)]．グラム陰性の

1) まはら ふみひこ：馬原医院院長 連絡先：〒779-1510 徳島県阿南市新野町信里6-1
2) ふじた ひろみ：大原研究所

特集

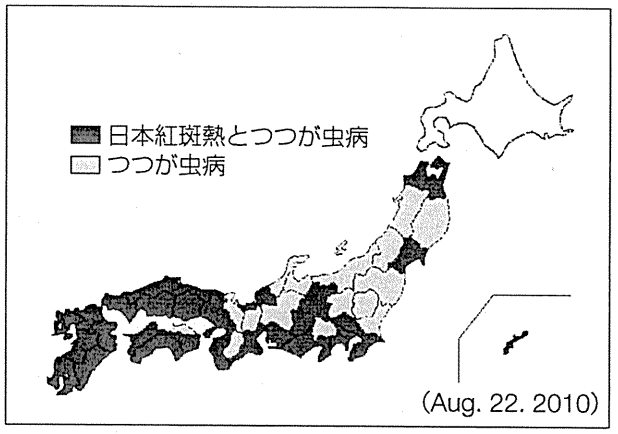

図1　日本紅斑熱とつつが虫病の発生地分布

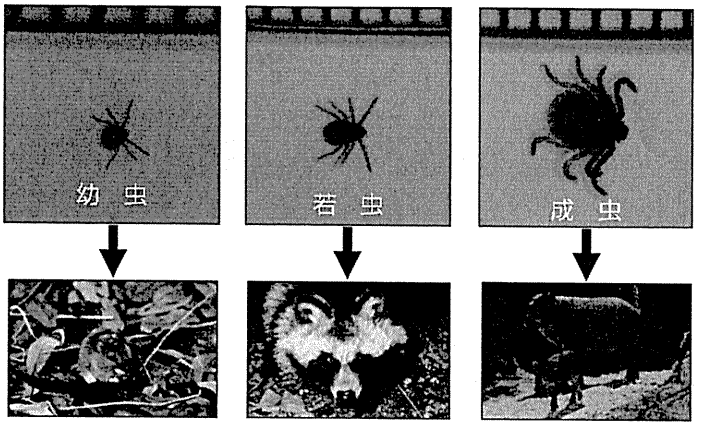

図2　マダニの生活史

桿状ないし短桿状菌で，細胞質内のみでなく核内でも見られる(偏性細胞内寄生性)．リケッチア科，リケッチア属，紅斑熱群に分類されている．近年遺伝子解析などの発達に伴い，日本紅斑熱とされた症例の中に *R. japonica* の他に極めて稀であるが *R. helvetica* や *R. Heilongjiangensis* などの混在も指摘されている[6]．

流行地域

古典的つつが虫病は秋田，山形，新潟の風土病とされていたが，新型つつが虫病の出現と共に，北海道を除く全域に発生が確認されている(**図1**)．発生時期は北陸，東北地方で4～6月と10～11月．本州の関東南部から西方，四国，九州では，10～1月が主となる．国外ではアジアを中心として幅広く分布しており，ツツガムシトライアングル(西はパキスタン，南はオーストラリア北部，東は極東ロシア)と言われている．近年，特に韓国，中国や東南アジアで多発している．

日本紅斑熱は関東以西の比較的温暖な太平洋岸沿いに多く報告されていたが，福井など日本海側，さらに東北地方でも発生が報告された(**図1**)．近年，韓国など国外からも発生が報告されている．発生時期は春先から晩秋．好発時期はダニの植生や人とダニとの接触の機会などの地域特性により異なる(徳島県では春と秋，高知県では夏に多い)．

ヒトへの感染経路

自然界では，病原リケッチアは代々経卵垂直伝播によりダニ類の体内で受け継がれている．ダニ類は，卵，幼虫，若虫，成虫と4期の発育期からなり，自然環境で生態系を保持している(**図2**)．

ヒトへの感染は病原リケッチアを保有したダニ類が皮膚を刺咬した際にリケッチアが皮内に侵入する．次いで，リンパ流や血流中に入り感染が成立する．ヒトからヒトへは感染しない．

1. 媒介者

つつが虫病はツツガムシにより媒介される．ツツガムシは一生に一度，幼虫の短い時期に地上に現れ小動物に吸着する．ヒトは偶発的な犠牲者となる．したがって，その地域のツツガムシの種類と幼虫の発生時期によって，つつが虫病の好発時期は異なる．国内のツツガムシは約120種が報告されているが，*O. tsutsugamushi* を伝搬する主力は，アカツツガムシ(*Leptotrombidium akamushi*)，タテツツガムシ(*L. scutellare*)，フトゲツツガムシ(*L. pallidum*)の3種である．

日本紅斑熱はマダニにより媒介される．日本産マダニは約50種が存在している．そのうち，ヒトへの感染はキチマダニ(*Haemaphysalis flava*)，フタトゲチマダニ(*H. longicornis*)，ヤマアラシチマダニ(*H. hystricis*)の3種が媒介種とされている．

表　つつが虫病と日本紅斑熱の臨床比較

		日本紅斑熱	つつが虫病
媒介動物		マダニ(キチマダニ，フタトゲチマダニ，ヤマアラシチマダニ)	ツツガムシ(アカ，フトゲ，タテ)
発生地		九州，四国，本州では中国地方から関東まで比較的温暖な太平洋岸沿いに多い	北海道を除く日本全域
発生時期		4～11月 夏秋に多い	春と秋(東北，北陸，山陰) 秋～冬(房総，東海，九州)
潜伏期		2～10日	10日～2週間
刺し口	形状	5～10 mmの発赤と中心部の2～3 mmの黒色痂皮	10 mm内外の中心部の黒色痂皮と周囲の発赤
発疹	分布	体幹部よりは手足末梢部に多い 手掌部の紅斑は特徴的	体幹部，顔面に多く見られる 手掌部にはない
	性状	米粒大から小豆大の不整形の紅斑，3～5日で出血性になる	斑紋状，麻疹様，時に丘疹状 やや淡い紅斑 出血性となるのは少ない
その他		紅斑熱様顔貌，リンパ節腫脹，肝脾腫は見られないことが多い	リンパ節腫脹，肝脾腫は大多数で見られる

＊治療上の留意点については，治療法の項参照のこと

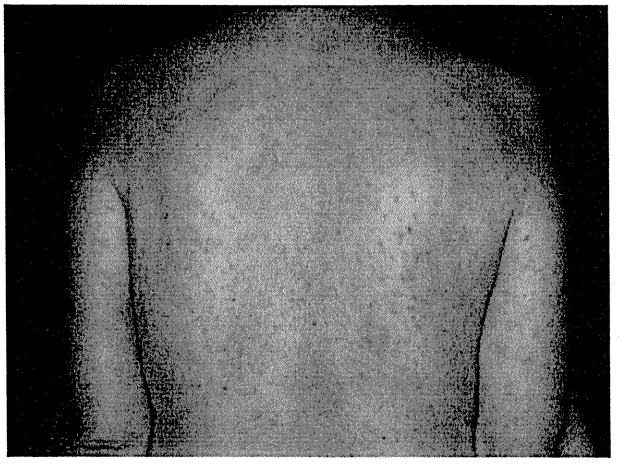

図3　日本紅斑熱に見られる定型的な発疹

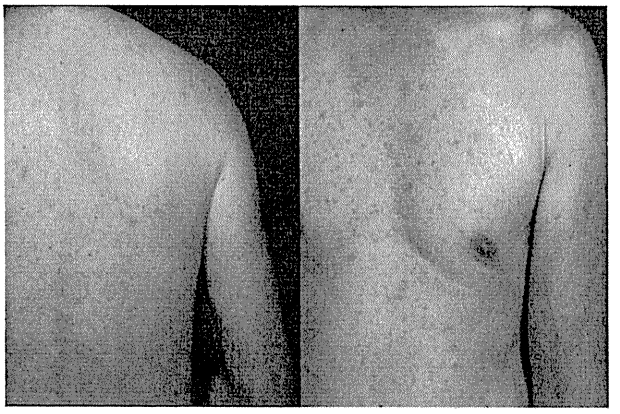

図4　つつが虫病の定型的な発疹

臨床症状

つつが虫病はツツガムシに，日本紅斑熱はマダニに刺咬後，潜伏期を経て2～3日不明熱が続いた後，頭痛，発熱，悪寒戦慄をもって急激に発症する．関節痛，筋肉痛，手足のしびれ感を訴えることもある．他覚所見は高熱，発疹，刺し口が3徴候(臨床所見比較，**表**)．急性期には39～40℃以上の弛張熱が多く，悪寒戦慄を伴う．日本紅斑熱の日中最高体温は，38.7～40.8℃，平均39.5℃であり，これはつつが虫病の最高体温38.5～39.3℃と比較して，やや高く重症感がある．

発疹(**図3，4**)は掻痒感，疼痛がないのが特徴的であり，速やかに全身に拡がる．両疾患とも重症化した症例では，次第に出血性となる．手掌紅斑は日本紅斑熱に特徴的な所見である(**図5**)．

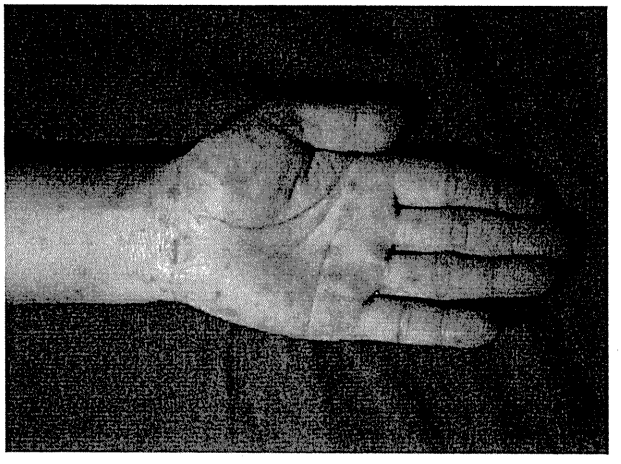

図5　日本紅斑熱に見られる手掌部紅斑

刺し口(**図6**)は，ほとんどの症例で認められる．刺し口を見つけると臨床的な決め手になるので，下着で覆われたところや毛髪部位も注意深く観察する必要がある．つつが虫病の刺し口は日本

特集

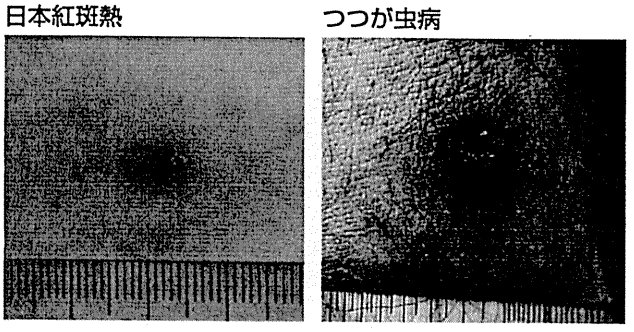

図6　日本紅斑熱とつつが虫病の典型的な刺し口

紅斑熱のそれに比して大きい傾向にあるが，刺し口の形状や大きさのみで鑑別するのは困難である．

一般検査では，つつが虫病では白血球数減少，核の左方移動，異型リンパ球の出現，日本紅斑熱で白血球数は軽度増多傾向，血小板減少，CRP強陽性，肝機能障害などが見られる．重症化すると播種性血管内凝固症候群(DIC)，多臓器不全(MOF)などを呈する．

確定診断は特異的血清診断として間接蛍光抗体法(IFA)，間接免疫ペルオキシダーゼ法(IP)を行い，抗体価の上昇を証明する．また，患者血液や刺し口のカサブタを用いたPCR法，刺し口の皮膚生検を検査材料とした免疫染色法も可能となっている[7]．

治療法

熱性疾患に一般的に使用される抗生物質であるペニシリン系，セフェム系，アミノグリコシド系薬剤などは全く無効である．しかし，テトラサイクリン系抗生剤，ドキシサイクリンやミノサイクリンは著効を示す[8]．投与方法は初期であれば経口でも十分有効である．ミノサイクリン200～300 mg/日を経口投与，解熱後も半量を1週間予防投与する．重症例では，高熱による脱水の治療も兼ねて，補液500 mlにミノサイクリン100 mgを加え，1日2～3回投与する．また，ニューキノロン剤はつつが虫病リケッチアは感受性がないが，日本紅斑熱リケッチアは感受性を有している．このエビデンスから，日本紅斑熱の重症例では「本症と臨床的に診断した場合，テトラサイクリンを第一選択薬とするが，1日の最高体温39℃以上の症例では，直ちにテトラサイクリン剤とニューキノロン剤による併用療法を行う」ことが推奨されている[9]．本症は病状が急速に進展するので，リケッチア症を疑った時点で，直ちに有効治療を開始することが必要である．

予防

野山に入る際にはダニ類が付着しないよう長袖，長ズボンを着用し，衣服の上からダニ忌避剤のスプレーを使用するなど，防護処置をとる．帰宅後はお風呂などで身体を調べ，ダニ類が付いていないかを確認する．もし，付いていれば無理に取らずに医療機関で取ってもらう．予防のためにその地域のダニを薬剤散布などで駆除することは実際的ではない．

最近の発生状況

ここ5年の発生数を見ると，つつが虫病は年間325～455例が報告され，全国的に発生が見られる．近年，福島や千葉，岐阜，鹿児島県からの発生報告が多い．日本紅斑熱は年間45～132例発生が見られ，近年増加傾向にある．発生地域も広がりを見せ，島根，福井など日本海側，さらに青森や宮城など東北地方でも発生が報告された．近年，三重や和歌山，広島，熊本県からの発生報告が多い．

近年の話題

近年の遺伝子解析などの発達に伴い，日本紅斑熱とされた症例の中に*R. japonica*の他に，極めて稀であるが*R. helvetica*や*R. Heilongjiangensis*などの混在も指摘されている．今後の日本における侵淫状況や媒介マダニの調査研究が必要となってきた．

日本紅斑熱の媒介動物の研究は，マダニ類を中心として展開されてきた．しかし，マダニを巡る共通感染者もしくは自然界におけるリザーバーの研究は少ない．

2004年8月，日本紅斑熱患者が入院中に飼犬が急死するという事例が発生した．このイヌの剖検の結果，脾臓，腎臓，消化管組織内に免疫染色法で日本紅斑熱リケッチア抗原が証明された(**図7**)．さらに，日本紅斑熱患者の飼犬や猟犬の血清抗体検査(IP)で，陽性であることが示された[10]．人獣共通感染症としてのペットや家畜の関わりに関する研究は，今後重要な課題である．

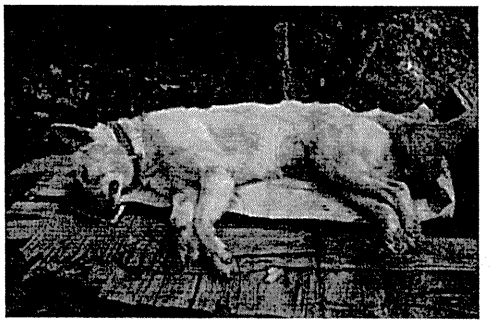

図7　日本紅斑熱患者の死亡した飼育犬

2004年入院中の日本紅斑熱患者の飼育しているイヌが突然死亡した．感染環におけるイヌの役割について，今後の研究が待たれる．

出典：Mahara F：Rickettsioses in Japan and the Far East；4th International Conferenceon Rickettsiae and Rickettsial Diseases Logrono (La Rioja). Spain, June 18-21, 2005

おわりに

つつが虫病，日本紅斑熱は，感染症法により第4類届出感染症に指定されている．

第4類感染症のうち，近年ではそれぞれ2位，3位に多い疾患であることを認識する必要がある(2008～2009年)．しかも熱性疾患に対して一般的に汎用されている抗菌薬のほとんどは無効である．リケッチア感染症は治療が遅れると重症化する．原因不明の高熱と発疹のある症例では，本症の存在を念頭におき，適切な抗菌薬を早期に投与することが肝要である．

文　献

1）馬原文彦，他：わが国初の紅斑熱リケッチア感染症．感染症学雑誌 **59**：1165-1172, 1985
2）Mahara F：Synopses, Japanese spotted fever；Report of 31 cases and review of the literature Emerg. Infect Dis **3**：105-111, 1997
3）馬原文彦：リケッチア感染症．最新医学 **63**：192-214, 2008
4）Tamura A, et al：Classification of *Rickettsia tsutsugamushi* in a new genus, Orientia gen. nov. as *Orientia tsutsugamushi* comb. Nov Int J syst Bacteriol **45**：589-591, 1995
5）Uchida T, et al：*Rickettsia japonica* sp. nov., the etiological agent of spotted fever group rickettsiosis in Japan. Int J Syst Bacteriol **42**：303-305, 1992
6）藤田博己，他：マダニ類から検出されるリケッチアの多様性．SADI組織委員会(編)：ダニと新興再興感染症，pp 129-139，全国農村教育協会，2007
7）堤寛，他：日本紅斑熱の早期診断；皮膚生検を利用した免疫染色の実用性．病原微生物検出情報 **27**(2)：38-40, 2006
8）Mahara F：Rickettsioses in Japan and the far east. Ann N Y Acad Sci **1078**：60-73, 2006
9）馬原文彦：リケッチア感染症(ツツガムシ病，日本紅斑熱)．山口徹，他(編)：今日の治療指針2008年版，pp 146-147，医学書院，2008
10）馬原文彦：日本紅斑熱の臨床と疫学．獣医畜産新報 JVM **60**(5)：365-368, 2007

Journal of Medical Virology 82:556–561 (2010)

Hepatitis C Virus Infection in a Japanese Leprosy Sanatorium for the Past 67 Years

Kazuya Shiogama,[1] Hidemi Teramoto,[2]* Yukiko Morita,[3] Yasuyoshi Mizutani,[1] Ryoichi Shimomura,[1] Ken-ichi Inada,[1] Toshio Kamahora,[4] Masanao Makino,[5] and Yutaka Tsutsumi[1]

[1]*Department of Pathology, Fujita Health University School of Medicine, Toyoake, Japan*
[2]*Department of Internal Medicine, Kojin Hospital, Nagoya, Japan*
[3]*Department of Dermatology, Kojin Hospital, Nagoya, Japan*
[4]*Faculty of Medicine, Division of Virology, Microbiology and Pathology, Tottori University, Yonago, Japan*
[5]*The National Sanatorium Oku-Komyo-En, Setouchi, Japan*

Oku-Komyo-En is one of the national leprosy sanatoria, located on a small island in Setouchi city, Okayama prefecture of Japan since 1938. Since autopsies were carried out routinely on almost all patients who had died in the sanatorium up to 1980, approximately 1,000 formalin-fixed autopsy tissue samples were available for analysis. When these samples were reviewed, the pathological data indicated a sharp rise in the death rate caused by cirrhosis of the liver and hepatocellular carcinoma (HCC) since 1960 and 1970, respectively. Hepatitis C virus (HCV) infection is a common cause of HCC in Japan. The presence of HCV RNA was demonstrated in paraffin sections prepared from the autopsied liver tissue fixed in formalin for a prolonged period of time, by employing nested RT-PCR using type-specific primers. The data showed that HCV RNA was detectable in samples of the liver archived as early as 1940, representing the liver tissues kept in formalin for up to 67 years. HCV genotypes 1b and 2a were found by RT-PCR at 85.7% and 14.3%, respectively, in patients with leprosy. ***J. Med. Virol. 82:556–561, 2010.***

KEY WORDS: leprosy; hepatitis C virus; genotyping; nosocomial infection

INTRODUCTION

Leprosy caused by *Mycobacterium leprae* is a chronic infectious disease which primarily affects exposed surfaces such as the skin (particularly the face and extremities), superficial peripheral nerves, upper respiratory tract, testis, and ciliary bodies of the eyes [Sasaki et al., 2001]. Leprosy was regarded as a divine punishment for the skin in the Old Testament and as karma in Buddhism. Since ancient times, historical records indicated that many patients with leprosy lacked permanent housing, leaving them to wander in both towns and rural areas. In Japan, Law No. 11, "The Act on Leprosy Prevention" was passed in 1907. Subsequently, in 1931, the first national leprosarium opened, and at that time, Law No. 11 was revised to the Leprosy Prevention Law, which forced all patients, regardless of whether they could be cared for at home or not, to be admitted to hospital without any financial burden levied on their families [Sato and Frantz, 2005].

This Leprosy Prevention Law remained in place until the end of 1996, and in the process depriving many patients of their basic human rights for decades. With 13 national and 2 private leprosy sanatoria in Japan, patients from the western prefectures (Osaka, Kyoto, Hyogo, Nara, Wakayama, Mie, Shiga, Gifu, Fukui, Ishikawa, Toyama, Tottori) were forced to be detained in the National Sanatorium Oku-Komyo-En. As autopsies were performed routinely on most patients who died in the sanatorium from 1940 to 1980, formalin-fixed tissues from approximately 1,000 autopsy cadavers were archived and remained available. When these specimens were newly processed again for histology during 2006–2007 and, subsequently reviewed in parallel with the clinical data, there were considerable differences in the prevalence rate of lethal conditions in each of the 10-year periods during 1940–1999. For instance, analysis of the records identified an increasing incidence of cirrhosis of the liver (1960–1969) and hepatocellular carcinoma (HCC; 1970–1979). The main

Grant sponsor: Japanese Ministry of Education, Culture, Sports, Science and Technology, [partial support, Open Research Center Project #030131 (2003–2007); Grant-in Aid for Scientific Research (C) Project #21591303 (2009–2011)].

Ryoichi Shimomura's present address is Pathology Division, Masuda Red Cross Hospital, Masuda, Japan.

*Correspondence to: Hidemi Teramoto, MD, PhD, Department of Internal Medicine, Kojin Hospital, 1-710 Shikenya, Moriyama, Nagoya 463-8530, Japan. E-mail: hteramoto@kojin-hospital.jp

Accepted 30 June 2009
DOI 10.1002/jmv.21612
Published online in Wiley InterScience (www.interscience.wiley.com)

cause of HCC in Japan is infection with hepatitis C virus (HCV) infection in 75% of the cases [Kiyosawa et al., 2004], and it is of considerable importance that the prevalence of HCV antibody in the serum of patients with leprosy has been demonstrated to be much higher than that of the general population [Denis et al., 1994; Egawa et al., 1996]. Within the confines of the leprosy sanatoria, it was a common and routine medical practice to share needles and/or syringes for administering leprosy medication consisting of Chaulmoogra oil (a traditional medicine) and Promine (a novel synthesized anti-leprosy drug). The possibility of nosocomial infection with HCV is suspected in this cohort of patients.

In this study, the data of autopsies performed at the National Sanatorium Oku-Komyo-En between 1940 and 1999 were reviewed. As there was no facility for storing sera of leprosy patients in those days, formalin-fixed liver and HCC tissues were archived, together with the patients' records, and used to determine the prevalence of HCV infection.

MATERIALS AND METHODS

Ethical Aspects

The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was also approved by three Ethics Committees of the National Sanatorium Oku-Komyo-En, Kojin Hospital, Fujita University, School of Medicine, respectively.

Tissue Samples

Formalin-fixed tissues archived at the National Sanatorium Oku-Komyo-En after a prolonged period of fixation (12–67 years) were sampled again between 2006 and 2007 and embedded subsequently in paraffin. A total of 48 liver samples, including cirrhosis alone ($n = 34$) and HCC with cirrhosis ($n = 14$) were selected for the study. Total RNA was extracted from formalin-fixed and paraffin-embedded tissue sections. All the HCC cases were accompanied by cirrhosis of the liver, and sections containing non-neoplastic, cirrhosis of the liver were used for the analysis. Patient demographics included 36 males (mean age: 63.2 years) and 12 females (mean age: 74.7 years), with a combined age ranging from 28 to 84 years.

RNA Extraction From Tissue Sections

Five slices of 5 μm thickness were prepared from liver tissue blocks using a microtome. A new sterile blade was used for each block to avoid cross-contamination between the samples. Total RNA was extracted from dewaxed sections using a Recover All Total Nucleic Acid Isolation kit (Applied Biosystems, Austin, TX), in accordance with the manufacturer's protocol, and stored at −80°C until use. The protocol consisted of the following six steps: microtome sectioning of tissue blocks, deparaffinization, proteinase K digestion, RNA isolation, DNase incubation, and RNA purification [Ribeiro-Silva et al., 2007].

HCV Genotyping by Nested RT-PCR Using Type-Specific Primers

RT-PCR was carried out by applying 1 μl of extracted RNA (reaction mixture volume, 10 μl) to the one-step RT-PCR kit (Qiagen, Valencia, CA). The universal primer pairs at a concentration of 200 nM included No. 256 (5′-CGCGCGACTAGGAAGACTTC-3′) and No. 186 (5′-ATGTACCCCATGAGG TCGGCC-3′) [Okamoto et al., 1992]. The thermal profile for the reaction was as follows: reverse transcription at 50°C for 30 min, initial denaturation at 95°C for 15 min, and then followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 54°C for 1 min, and elongation at 72°C for 1 min. The final elongation step was done at 72°C for 10 min.

The second amplification step was carried out, by employing 1 μl of first PCR product (diluted 1:1,000), at 95°C for 5 min, then 40 cycles of denaturation at 95°C for 1 min, annealing at 54°C for 1 min, elongation at 72°C for 1 min, and final elongation at 72°C for 10 min. The nested reactions were performed using a combination of 200 nM universal sense primer No. 104 (5′-AGGAAGACTTCCGAGCGGTC-3′) and 200 nM genotype-specific anti-sense primer No. 133 (5′-GAGCCATCCTGCCCACCCCA-3′), No. 134 (5′-CCAAGAGGGACGGGAACCTC-3′), and No. 135 (5′-ACCCTCGTTTCCGTACAGAG-3′), together with the HotStar Taq Plus Master Mix kit (Qiagen).

The amplified products were analyzed by electrophoresis using 3% agarose gel and ethidium bromide.

Statistical Analysis

Statistical software package PASW Statistics 17.0 was used. The pathological data of cirrhosis of the liver, HCC, and tuberculosis in 1940–1949, 1950–1959, 1960–1969, 1970–1979, 1980–1989, and 1990–1999 were analyzed by Fisher's exact test (two-sided). $P < 0.05$ was considered to be statistically significant.

RESULTS

Autopsy Diagnosis in the National Sanatoria Oku-Komyo-En (1940–1999)

The pathological data of a total of 996 patients autopsied in Oku-Komyo-En, including 753 males and 243 females, are summarized in Table I. As indicated, review of the records found that there were large numbers of tuberculosis infections between 1940 and 1969, with 47% of these patients eventually dying of the disease between 1940 and 1949. As the number of inpatients at Oku-Komyo-En was approximately 400, it was surprising to note that 604 patients had died during 1940–1949. This suggests nosocomial infection of tuberculosis, and thus a separate line of investigation which is being pursued. Notably, during World War II continued from 1941 to 1945, which resulted in a chronic shortage of food as well as medicine at the sanatorium, and of course, drugs for treating tuberculosis was not available yet during this period. The average age at the time of death between 1940 and 1949 was 35.2 years, and a

TABLE I. Summary of Pathological Data of the National Sanatorium Oku-Komyo-En (1940–1999)

Year of death	No. patients (male/female)	Mean age at death (male/female)	Cirrhosis (male/female)	HCC + cirrhosis (male/female)	Tuberculosis (male/female)
1940–1949	604 (471/133)	35.2 (33.7/43.9)	8 (1.3%) (8/0)	0 (0%) (0/0)	284 (47.0%) (221/63)
1950–1959	62 (44/18)	48.4 (47.4/50.8)	0 (0%) (0/0)	0 (0%) (0/0)	24 (38.7%) (20/4)
1960–1969	114 (87/27)	61.4 (60.6/64)	12 (10.5%) (9/3)*	1 (0.9%) (1/0)	15 (13.2%) (11/4)*
1970–1979	46 (29/17)	66.4 (65/68.9)	3 (6.5%) (3/0)*	2 (4.3%) (1/1)*	0 (0%) (0/0)*
1980–1989	111 (77/34)	73.3 (71.6/77)	9 (8.1%) (6/3)*	9 (8.1%) (7/2)*	10 (9.0%) (7/3)*
1990–1999	59 (45/14)	76.3 (76.7/75.1)	4 (6.8%) (2/2)*	5 (8.5%) (3/2)*	1 (1.7%) (1/0)*
Total	996 (753/243)		36 (28/8)	17 (12/5)	334 (260/74)

*$P < 0.05$ in Fisher's exact test (two-sided).

surprising feature noted from the analysis was that this feature had risen every 10-year period as follows: 35.2 years in 1940–1949, 48.4 in 1950–1959, 61.4 in 1960–1969, 66.4 in 1970–1979, 73.3 in 1980–1989 and, 76.3 in 1990–1999. Conversely, the proportion of tuberculosis as a cause of death decreased significantly every 10-year period [47% in 1940–1949, 38.7% in 1950–1959, 13.2% ($P = 0.23$) in 1960–1969, 0% ($P < 0.01$) in 1970–1979, 9% ($P < 0.01$) in 1980–1989, and 1.7% ($P < 0.01$) in 1990–1999].

A noteworthy feature in the sanatorium is the dramatic increase in the incidence of cirrhosis of the liver and HCC (Fig. 1 and Table I). The incidence of cirrhosis of the liver was 1.3% (8/604 males) between 1940 and 1949 and with no record of HCC. In the following decade (1950–1959), cirrhosis of the liver or HCC was not noted. Thereafter, an increase in the incidence of cirrhosis was observed [10.5% ($P < 0.01$) in 1960–1969, 6.5% ($P = 0.04$) in 1970–1979, 8.1% ($P < 0.01$) in 1980–1989, and 6.8% ($P = 0.02$) in 1990–1999]. An increase of HCC in patients with cirrhosis of the liver was also noted [0.9% ($P = 0.16$) in 1960–1969, 4.3% ($P = 0.01$) in 1970–1979, 8.1% ($P < 0.01$) in 1980–1989, and 8.5% ($P < 0.01$) in 1990–1999]. The main cause of HCC is considered regarded to be infection with HCV [Kiyosawa et al., 2004] (Table I).

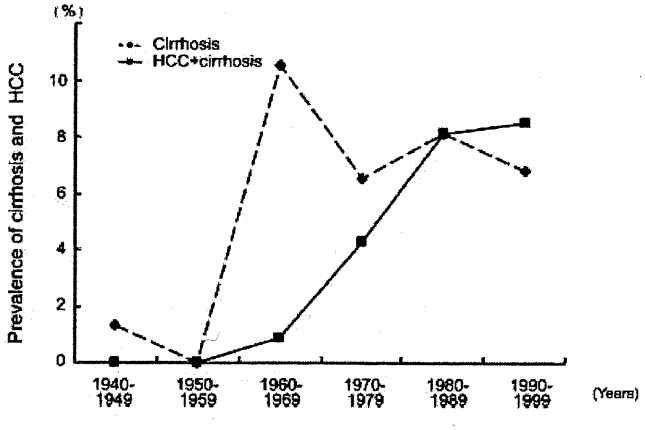

Fig. 1. Prevalence of cirrhosis of the liver and HCC in cirrhotic liver in autopsy samples archived at the National Sanatorium Oku-Komyo-En (1940–1999).

Detection of HCV by Nested RT-PCR Using Type-Specific Primers From Paraffin Sections of Cirrhosis and HCC

In patients with HCC, examined sections containing non-neoplastic, cirrhosis of the liver tissue were analyzed. Total RNA was examined by nested RT-PCR using type-specific primers, matching to the core region of the HCV sequence [Okamoto et al., 1992] (Fig. 2). Sera negative for HCV, as well as positive control sera for genotypes 1b (144 bp), 2a (174 bp), and 2b (123 bp), were initially tested by the nested reactions. As shown in Table II, the genotypes of HCV 1b and 2a were detected in the archival tissue sections of cirrhosis and HCC, while the genotype 2b or mixed type was undetectable. HCV RNA was shown in 70.6% of cirrhosis (24/34) and 78.6% of HCC (11/14) cases. The HCV genotypes were 85.7% for 1b (30/35), and 14.3% for 2a (5/35) in patients with leprosy (Fig. 3). HCV genotyping for each 10-year period is shown in Table II. The genotype 1b was predominant in each of the 10 years assessed, 85.7% (6/7) in 1940–1949, 87.5% (7/8) in 1960–1969, 100% (4/4) in 1970–1979, 72.7% (8/11) in 1980–1989, and 100% (5/5) in 1990–1999. No cirrhosis of the liver was recorded during 1950–1959.

DISCUSSION

When autopsy records at Oku-Komyo-En were reviewed, three noteworthy and critical features were recognized. One is that the age of death increased every 10-year period, as shown in Table I. In 1990–1999, the mean age of death was 76.3 years, while in 1940–1949 this was a mere 35.2 years. A similar tendency, though less drastic, was also observed outside the leprosy sanatoria throughout Japan, primarily because of a decrease in the prevalence of tuberculosis.

Secondly, the observation of the high prevalence of tuberculosis at the sanatorium when compared with the general population of Japan was unexpected. The mortality rate of tuberculosis per 100,000 across all ages in Japan was 484 (0.484%) in 1948 and 524 (0.524%) in 1960 [Zaki, 1968]. In contrast, the death caused by tuberculosis reached 47% in the leprosy sanatorium between 1940 and 1949. This is likely due to horizontal transmission of mycobacteria in the sanatorium because of the closed living environment

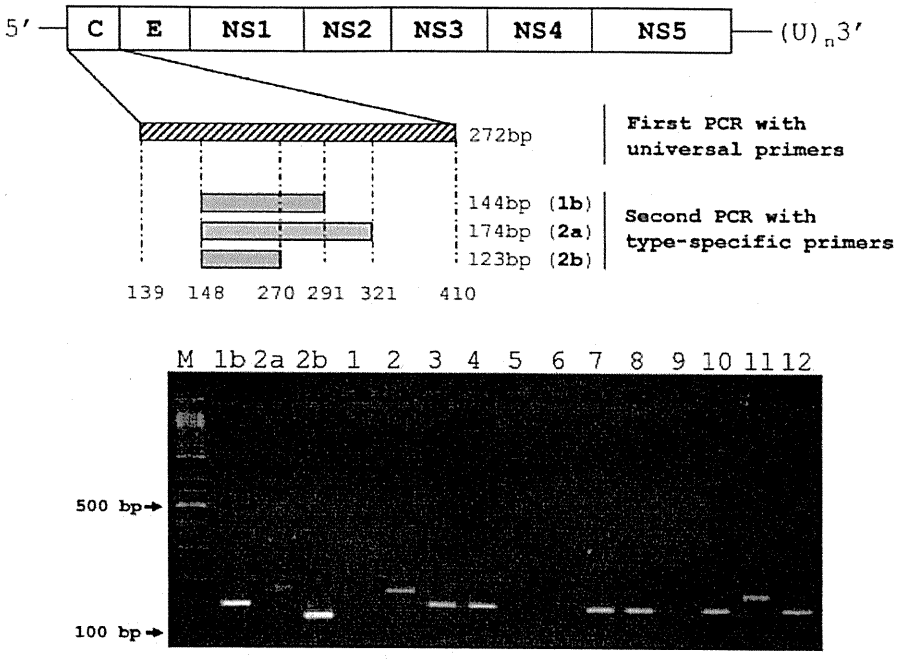

Fig. 2. Genotyping of HCV by nested RT-PCR using type-specific primers. cDNA, prepared by priming with No. 186, was amplified by the first PCR with universal primers (Nos. 256 and 186), in order to obtain a fragment of 272 bp, as indicated by the shaded block. The products were then amplified by the second PCR with a universal primer (No. 104) and a mixture of three type-specific primers (Nos. 132–134). Gray blocks indicate products of different nucleotide lengths that were specific to each of the three HCV types 1b (144 bp), 2a (174 bp), and 2b (123 bp) [Okamoto et al., 1992]. M: 100 bp ladder indicator; 1b, 2a, 2b: sera from Kojin hospital; 1: negative control (serum from Kojin hospital); 2: cirrhosis (1940); 3: cirrhosis (1942); 4: cirrhosis (1942); 5: cirrhosis (1964); 6: cirrhosis (1964); 7: HCC + cirrhosis (1970); 8: cirrhosis (1970); 9: cirrhosis (1987); 10: cirrhosis (1989); 11: HCC + cirrhosis (1989); 12: cirrhosis (1996).

during this time period. Another reason for the spread of tuberculosis in the leprosy sanatorium was that the appropriate medication to treat the disease was then not available. To this end, streptomycin was first synthesized in 1944, followed by isonicotinic acid hydrazide, and pyrazinamide (1952), ethambutol (1961), and rifampicin (1966) [Rosenblatt, 1973]. As the regular number of inpatients at this sanatorium was around 400, it is surprising that the total number of deaths between 1940 and 1949 reached 604. The possibility of nosocomial infection of tuberculosis with the leprosy sanatorium is now under investigation using molecular methods.

Thirdly, the pathological data at Oku-Komyo-En showed a sharp increase in death rates attributable to cirrhosis of the liver since 1960 and to cirrhosis-based HCC since 1970 (Table I and Fig. 1). It has been reported that HCV infection causes HCC in 75% of Japanese cases [Kiyosawa et al., 2004]. It is known that patients with leprosy are at a higher risk of HCV infection [Denis et al., 1994; Egawa et al., 1996]. Most of the patients with leprosy in the sanatoria had received subcutaneous or intravenous injections of medicine regularly for treating

TABLE II. Number of Isolates of HCV Genotypes

Genotyping	1b	2a	2b	Total
1940–1949	6	1	0	7
1950–1959	0	0	0	0
1960–1969	7	1	0	8
1970–1979	4	0	0	4
1980–1989	8	3	0	11
1990–1999	5	0	0	5
Total	30	5	0	35

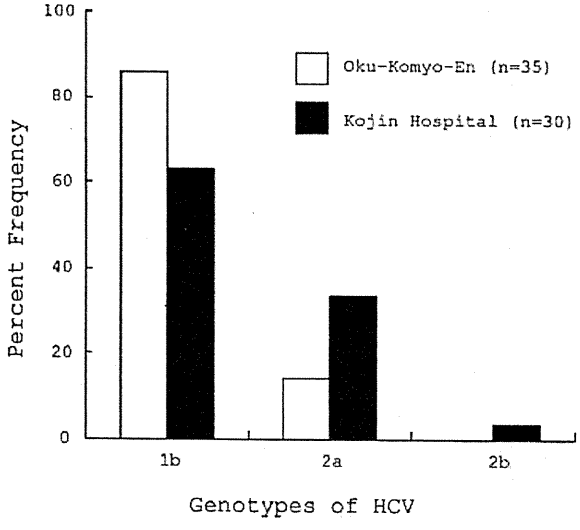

Fig. 3. HCV genotyping in leprosy patients in Oku-Komyo-En and in non-leprosy patients in Kojin Hospital. Total RNA samples extracted from formalin-fixed, paraffin-embedded liver tissues (n = 35) of HCV-infected patients with leprosy at Oku-Komyo-En and total RNA extracted from sera (n = 30) of HCV-infected patients (Kojin Hospital) were applied to the nested RT-PCR technique using type-specific primers.

pain by using non-disposable needles and/or syringes during this time period, and this practice may have contributed to the spread of HCV. The subcutaneous injection of Chaulmoogra oil was the main and traditional treatment of leprosy in the sanatoria until just after the World War II period. Promine injection, a specific anti-leprosy treatment, introduced after the war, was proven very effective in most patients. The patients and also medical staff members were completely unaware of the risk of nosocomial infection in those days, and subsequently reuse of needles and/or syringes was a common practice in most medical institutions. It follows that HCV was discovered much later in 1989 [Choo et al., 1989].

In Japan, transmission of HCV is reportedly to be linked to two distinct events [Tanaka et al., 2002]: The widespread treatment of schistosomiasis with intravenous injection of antimony sodium tartrate since 1923 [Iida et al., 1999], and the use of intravenous injection of methamphetamine during and after the World War II, often sharing repeatedly needles and/or syringes. Another important factor of note included vaccination against tuberculosis or smallpox of school children and regional inhabitants without disposing needles after the war and up to 1958 [Higuchi et al., 2002]. During this period of time, tuberculosis and *Helicobacter*-induced peptic ulcer prevailed in association with malnutrition of varying degrees, and the lesions had been treated surgically often with the need of blood transfusion. Since many blood donors were paid professionals, the "Yellow Blood" was often contaminated with hepatitis viruses. It is estimated that half of the blood recipients received contaminated blood, and that the HCV genotype 1b, introduced during the 1880–1889 period, began to spread exponentially in the 1920–1929 and the 1930–1939 time period [Mizokami et al., 2006]. Within the sanatorium, leprosy patients were accommodated in narrow spaces for a prolonged period of time, and many of them had open skin lesions, which could become a source for horizontal transmission of HCV.

It is thought that horizontal transmission of the HCV occurred during the 1940–1949 period. During 1940–1949, a total of eight male cases of cirrhosis (1.3%) were observed. It is suggested that infection with HCV would have been brought into the leprosy sanatorium from outside. The infected patients with open skin wounds and receiving repeated injections could be the source for horizontal transmission of infection with HCV in Oku-Komyo-En. There were no HCC cases in the 1940–1949 and the 1950–1959 time periods, and no cases of cirrhosis recorded in the 1950s, while a few cases (0.9%) of HCC were noted in the 1940s.

In the 1940–1949 period, two types of HCV, the genotypes 1b (6/7) and 2a (1/7) were observed from the records (Table II). The HCV genotypes 1b and 2a in Oku-Komyo-En in toto were 85.7%, and 14.3%, respectively, while the subtypes of HCV in samples from Kojin Hospital in 2008 included 1b (63.3%), 2a (33.3%), and 2b (3.4%) (Fig. 3). The distribution of the HCV genotypes in Kojin Hospital corresponded well with that of the general population of Japan [Yamada et al., 1995]. In the leprosy sanatoria, HCV genotype 1b was predominant especially in cirrhosis of the liver. The high prevalence of the HCV genotype 1b is very similar to the results from another Japanese leprosy sanatorium in which serum samples collected between 1993 and 1994 were the basis of analysis [Egawa et al., 1996]. This suggests the presence of some parallel factors in these two leprosy sanatoria. In fact, the distribution of the genotypes remained unchanged in each 10-year period.

If nosocomial infection with HCV occurred in the sanatoria, there are several questions to be addressed. One is why there was no mixed infection of genotypes 1b and 2a. Mixed infection of different strains of genotype 1b might also be encountered. Another question is why the genotype 2a was not as prevalent, even when needles and/or syringe sharing had occurred. These issues are also under investigation using molecular methods.

In conclusion, this is the first proof for the earliest (1940) occurrence of HCV infection using formalin-fixed samples stored for almost 70 years. This is also demonstrated by nested RT-PCR using type-specific primers that HCV genotypes 1b and 2a already existed in Japan during the 1940–1949. Furthermore, HCV genotypes 1b was predominant up to 85.7% in patients with leprosy. These observations suggest the occurrence of horizontal transmission of HCV during the period 1940–1980 in this leprosy sanatorium.

ACKNOWLEDGMENTS

We thank Dr. Vyomesh Patel (NIDCR, NIH, USA) for the critical reading of the manuscript.

REFERENCES

Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244:359–362.

Denis F, Aussel L, Ranger S, Martin P, Itoua NA, Frommel D, Teckle HR, Sangrae A, M'Boup S, Millan J, Al Qubati Y. 1994. Prevalence of antibodies to hepatitis C virus among patients with leprosy in several African countries and the Yemen. J Med Virol 43:1–4.

Egawa K, Yukawa T, Arakawa S, Nakao H, Inoue T, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M. 1996. Hepatitis C virus antibody, viral RNA and genotypes in leprous patients in Japan. J Hepatol 24:397–402.

Higuchi M, Tanaka E, Kiyosawa K. 2002. Epidemiology and clinical aspects on hepatitis C. Jpn J Infect Dis 55:69–77.

Iida F, Iida R, Kamijo H, Takaso K, Miyazaki Y, Funabashi W, Tsuchiya K, Matsumoto Y. 1999. Chronic Japanese schistosomiasis and hepatocellular carcinoma: Ten years of follow-up in Yamanashi Prefecture, Japan. Bull World Health Organ 77:573–581.

Kiyosawa K, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Gad A, Tanaka E. 2004. Hepatocellular carcinoma: Recent trends in Japan. Gastroenterology 127:S17–S26.

Mizokami M, Tanaka Y, Miyakawa Y. 2006. Spread times of hepatitis C virus estimated by the molecular clock differ among Japan, the United States and Egypt in reflection of their distinct socioeconomic backgrounds. Intervirology 49:28–36.

Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, Tanaka T, Sato K, Tsuda F, Miyakawa Y, Mayumi M. 1992. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: Application to clinical surveys and tracing infectious sources. J Gen Virol 73:673–679.

Ribeiro-Silva A, Zhang H, Jeffrey SS. 2007. RNA extraction from ten year old formalin-fixed paraffin-embedded breast cancer

samples: A comparison of column purification and magnetic bead-based technologies. BMC Mol Biol 8:118.

Rosenblatt MB. 1973. Pulmonary tuberculosis: Evolution of modern therapy. Bull N Y Acad Med 49:163–196.

Sasaki S, Takeshita F, Okuda K, Ishii N. 2001. *Mycobacterium leprae* and leprosy: A compendium. Microbiol Immunol 45:729–736.

Sato H, Frantz JE. 2005. Termination of the leprosy isolation policy in the US and Japan: Science, policy changes, and the garbage can model. BMC Int Health Hum Rights 5:3.

Tanaka Y, Hanada K, Mizokami M, Yeo AE, Shih JW, Gojobori T, Alter HJ. 2002. A comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades. Proc Natl Acad Sci USA 99:15584–15589.

Yamada G, Tanaka E, Miura T, Kiyosawa K, Yano M, Matsushima T, Tsubouchi H, Ishikawa K, Kohara M, Hino K. 1995. Epidemiology of genotypes of hepatitis C virus in Japanese patients with type C chronic liver diseases: A multi-institution analysis. J Gastroenterol Hepatol 10:538–545.

Zaki MH. 1968. On the epidemiology of tuberculosis in some selected countries: Highlights and prospects for control and eradication I. Am J Public Health Nations Health 58:1692–1712.

法 医 病 理 Vol. 16
No. 2, pp. 69-82, 2010

特　　集　劇症型感染症の病理

劇症型感染症の病理

堤　　寛
藤田保健衛生大学医学部病理学

(受付: 平成22年3月5日　　掲載決定: 平成22年3月5日)

Pathology of Fulminant Infectious Diseases

Yutaka TSUTSUMI
Department of Pathology, Fujita Health University School of Medicine

(*Received Mar. 5, 2010; Accepted Mar. 5, 2010*)

Abstract

Histopathological findings of a total of 20 cases of fulminant infectious diseases were presented. These include ①fulminant group A β-hemolytic streptococcus infection, ②*Vibrio vulnificus* infection, ③fulminant *Staphylococcus aureus* infection, ④fulminant pneumococcal septicemia, ⑤fulminant pneumococcal pneumonia, ⑥legionnaire's pneumonia, ⑦gas gangrene, ⑧diphtheria, ⑨fulminant meningococcal meningitis, ⑩enterohemorrhagic *E. coli* infection, ⑪fulminant necrotizing fasciitis in diabetes mellitus, ⑫lethal rhinocerebral mucormycosis in diabetes mellitus, ⑬fulminant viral myocarditis, ⑭influenza encephalopathy and Reye syndrome, ⑮swine-type influenza and diffuse alveolar damage, ⑯Japanese encephalitis, ⑰cerebral malaria, ⑱fulminant *Naegleria* meningoencephalitis, ⑲acanthoamebic encephalitis, and ⑳visceral leishmaniasis. Appropriate autopsy diagnosis of these fulminant infectious diseases leads to "kindhearted" medicine for the dead, and should be indispensable to avoid the risk of infection and eventually to keep the human society safe.

Key words: Fulminant infectious diseases, Autopsy, Pathological diagnosis, Immunostaining, Biohazard

抄　　録

予後不良の劇症型感染症の病理所見を，計20症例を提示する形で記述した．①劇症型A群β溶連菌感染症，②*Vibrio vulnificus* 感染症，③劇症型黄色ブドウ球菌感染症，④劇症型肺炎球菌感染症，⑤劇症型肺炎球菌性肺炎，⑥レジオネラ肺炎，⑦ガス壊疽，⑧ジフテリア，⑨劇症型髄膜炎菌性髄膜炎，⑩腸管出血性大腸菌感染症，⑪糖尿病に伴う劇症型壊死性筋膜炎，⑫糖尿病に続発した致死的鼻脳型ムコール症，⑬劇症型ウイルス性心筋炎，⑭インフルエンザ脳症とライ症候群，⑭新型インフルエンザとびまん性肺胞傷害，⑯日本脳炎，⑰脳性マラリア，⑱ネグレリア性劇症型髄膜脳炎，⑲アカントアメーバ脳炎，⑳内臓リーシュマニア症．これら劇症型感染症に対する正しい解剖診断は，"死者に優しい医療" につながると同時に，これら感染症からの脅威からの社会の安全維持に欠くことができない．

はじめに

劇症型感染症 (fulminant infectious diseases) は病態が急激に進行し，患者の生命を脅かす感染症で，急性感染症と慢性感染症の急性増悪の場合がある．健常人に突然発症する場合のほか，基礎疾患を有するために感染が劇症化することもある．疾患の性質上，病理解剖例ないし法医解剖例として遭遇する頻度が高い．疾患によっては，剖検者が解剖中に高いバイオハザードに曝されるリスクが無視できない．本稿では，著者がこれまでに遭遇した劇症型感染症のうち，とくに解剖医に役立つ情報を中心に，症例提示の形で紹介する．剖検時の培養検査や血清保存の重要性は言うまでもない．

感染症の病理全般に関しては，著書[1]および web site[2]を参照してほしい．

1) 劇症型 A 群 β 溶連菌感染症

図 1 に，上肢の筋肉痛と発熱で突然発症した筋肉質体型の 40 代男性例の組織所見を示す．急激な経過で壊疽性変化を随伴したため，上肢が切断されたが，対側上肢や下肢にも壊疽が進行し，敗血症性ショックで死亡した(全経過 3 日)．血液および壊死組織から A 群 β 溶連菌が培養され (図 2)，ペニシリンを含む多くの抗菌剤に高感受性の成績が得られた．組織学的には，横紋筋組織の壊死と組織間に多数分布するグラム陽性球菌が観察された．好中球反応は認められなかった．本例には，外傷の既往や扁桃炎の所見はなかった．

抗生物質感受性が高いにもかかわらず，急激な四肢壊疽をきたして劇症の経過をたどる A 群 β 溶連菌の感染症で，しばしば致死的である．抗菌剤感受性だが，壊疽に陥った組織には血液循環がないために抗菌剤が菌と接触できない．救命に外科的処置が必要となる．streptococcal toxic shock-like syndrome とも称される "人喰いバクテリア症" の一型である．本病態では，streptococcal pyrogenic exotoxin (Spe) A, B, C, F や streptococcal superantigen (SSA) といったスーパー抗原が病因として注目されている[3][4]．スーパー抗原については，第 3 項を参照されたい．

2) *Vibrio vulnificus* 敗血症

肝硬変を罹患する 50 代男性が，急速に進行する下肢壊疽を罹患した．大腿部の皮膚生検所見で，生体反応を欠く高度の細菌感染が真皮深層～皮下脂肪織の血管周囲に観察された．菌は Gram 陰性で，莢膜形成により一見大型球菌状の形態を呈していた (図 3, 4)．患者は全経過 2 日で死亡した．*Vibrio vulnificus* 敗血症の典型例であり，劇症型溶連菌感染症と並ぶ "人喰いバクテリア症" の代表例である．生カキの食歴聴取が重要である．

V. vulnificus は海産物 (近海魚，生カキ) から感染し，肝硬変患者に致死的敗血症を生じる (*Vibrio* 属細菌は海水を好む好塩菌である)．本菌は鉄付加培地のみで培養可能であり，血清鉄の高くなる肝硬変の症例に限って劇症化をきたす．高温状態で海産物の腸管内に定着しやすいので，冷蔵されなかった生カキはとくに危険である．感染経路には，経腸管性感染と創傷感染がある．前者はハチに刺されたような四肢の有痛性皮疹で発症し，"壊死性筋膜炎" の形で進行する[5]．後者では，鮮魚の調理中に傷つけた手指から感染が生じる．わが国に多い致死性疾患である[6]．

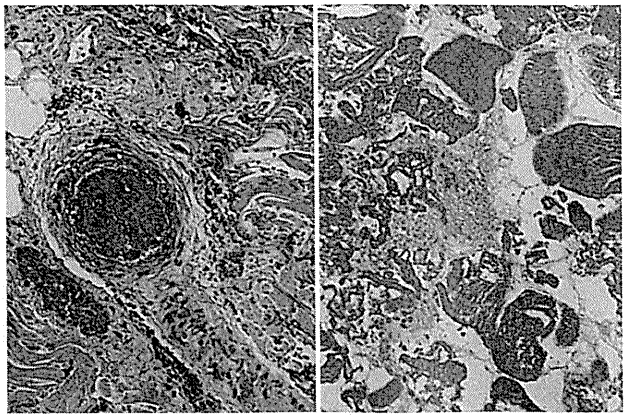

図 1. 劇症型 A 群 β 溶連菌感染症 (上肢の HE 染色)．凝固壊死に陥った軟部組織に，小動脈の血栓 (左) と横紋筋壊死 (右) が観察される．炎症細胞反応はみられない．

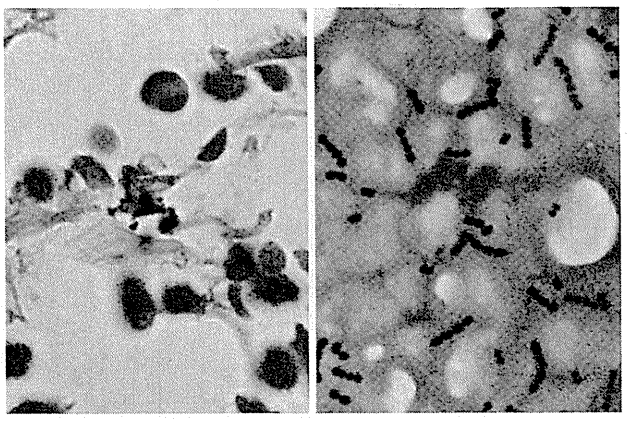

図 2. 劇症型 A 群 β 溶連菌感染症 (Gram 染色，左: 壊死組織，右: 末梢血)．グラム陽性球菌が壊死組織内および培養血液内に認められる (血液では連鎖状配列が明瞭)．

3) 劇症型黄色ブドウ球菌感染症: toxic shock syndrome (TSS)

図 5 に，劇症型黄色ブドウ球菌敗血症による肺動脈内感染性塞栓を提示する．左側腹部痛で発症した 70 代男性がショックにより死亡した．全経過は 7 日間だった．全身諸臓器に無数のグラム陽性球菌のコロニーが形成され，好中球反応は軽度だった．剖検時の培養で，市中型 methicillin-resistant *Staphylococcus aureus* (MRSA) が陽性となった．皮膚に発疹がみられたが，表皮剥離の所見はなかった．四肢の壊疽性変化もなかった．菌の進入路は不明だった．肝細胞癌を伴った C 型肝硬変が剖検時に初めて見いだされた．その病態は劇症型 A 群溶連菌感染症に類似性が求められた．

トキシックショック症候群 (TSS) は，黄色ブドウ球菌の産生する外毒素 TSST-1 (toxic shock syndrome toxin-1) によって，突然の高熱，咽頭炎，皮疹 (びまん性の斑状紅斑)，ショック，DIC，多臓器傷害 (水溶性下痢，筋痛，腎傷害，肝傷害など) を示す病態を呈する．発症後 1～2 週間で手掌・足蹠に落屑を認める．1979 年～1980