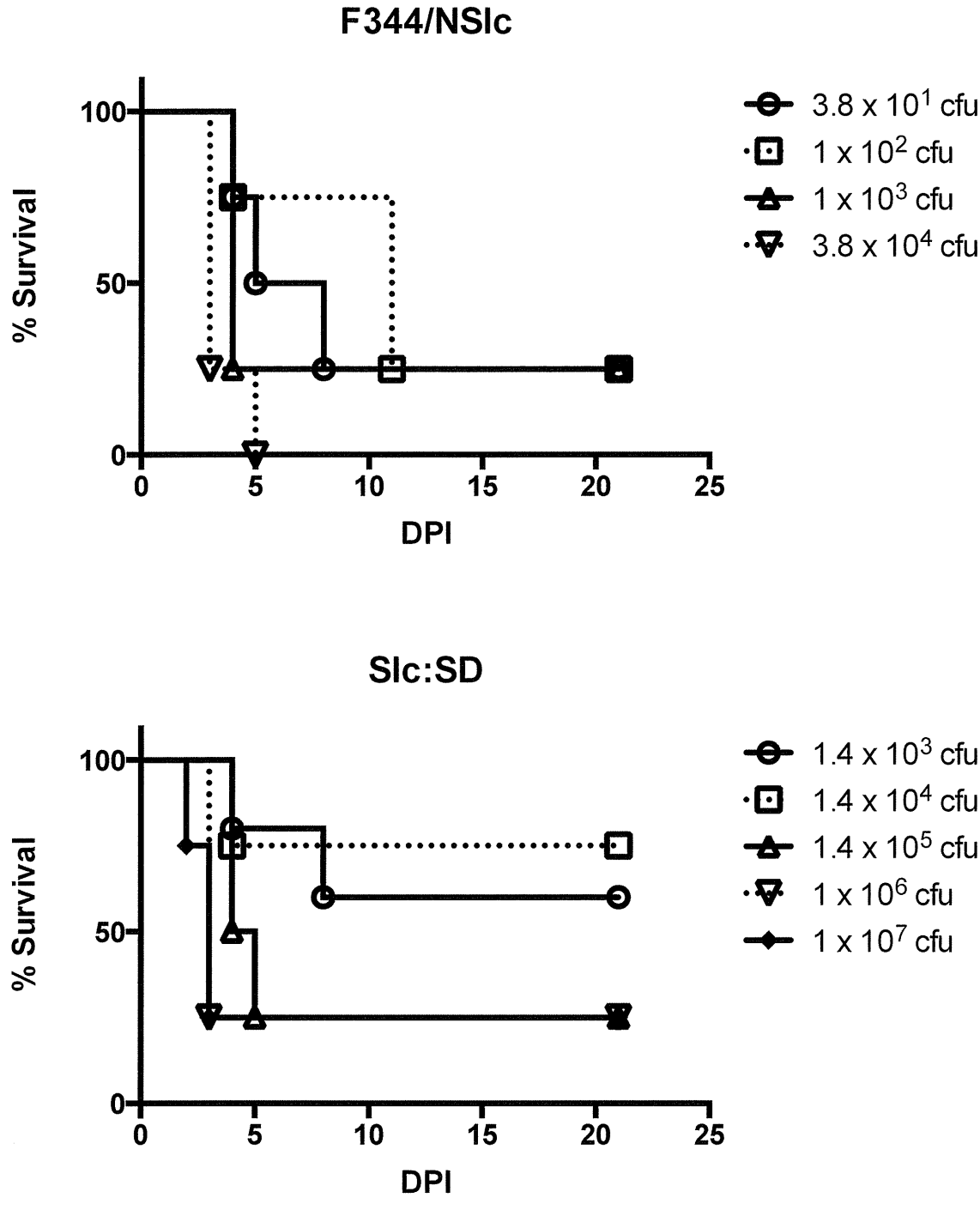

図4　NVF1株腹腔内接種ラットの生残率

Slc:SD(n=4 or 5)およびF344/Nslcラット(n=4)にNVF1株を腹腔接種し、21日間観察した。

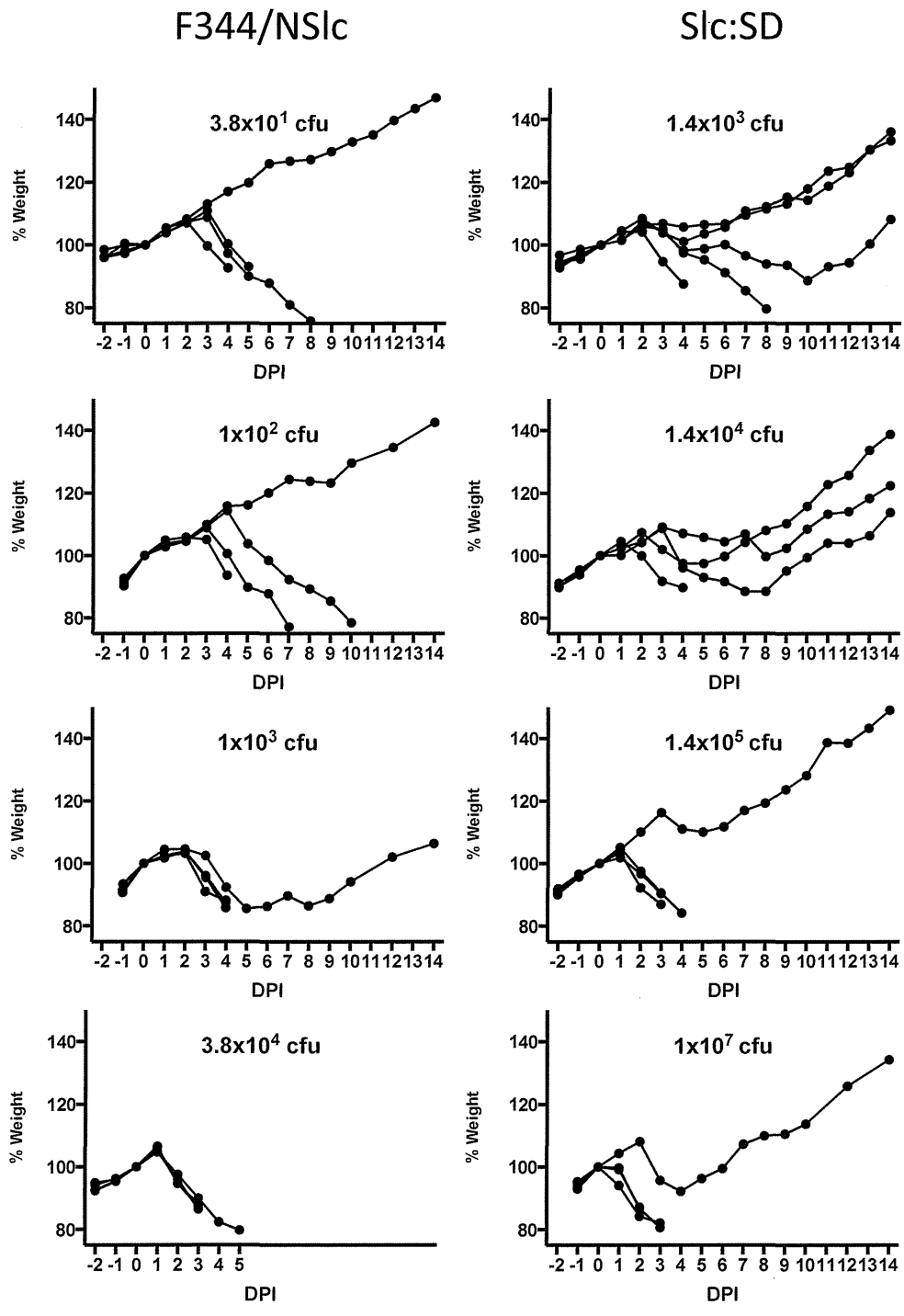

図5　腹腔内接種ラットの体重変化

F344/NSlcは10^4cfu接種にて全匹が5日以内に斃死したが、Slc:SDはいずれの接種群においても生残個体が認められた。

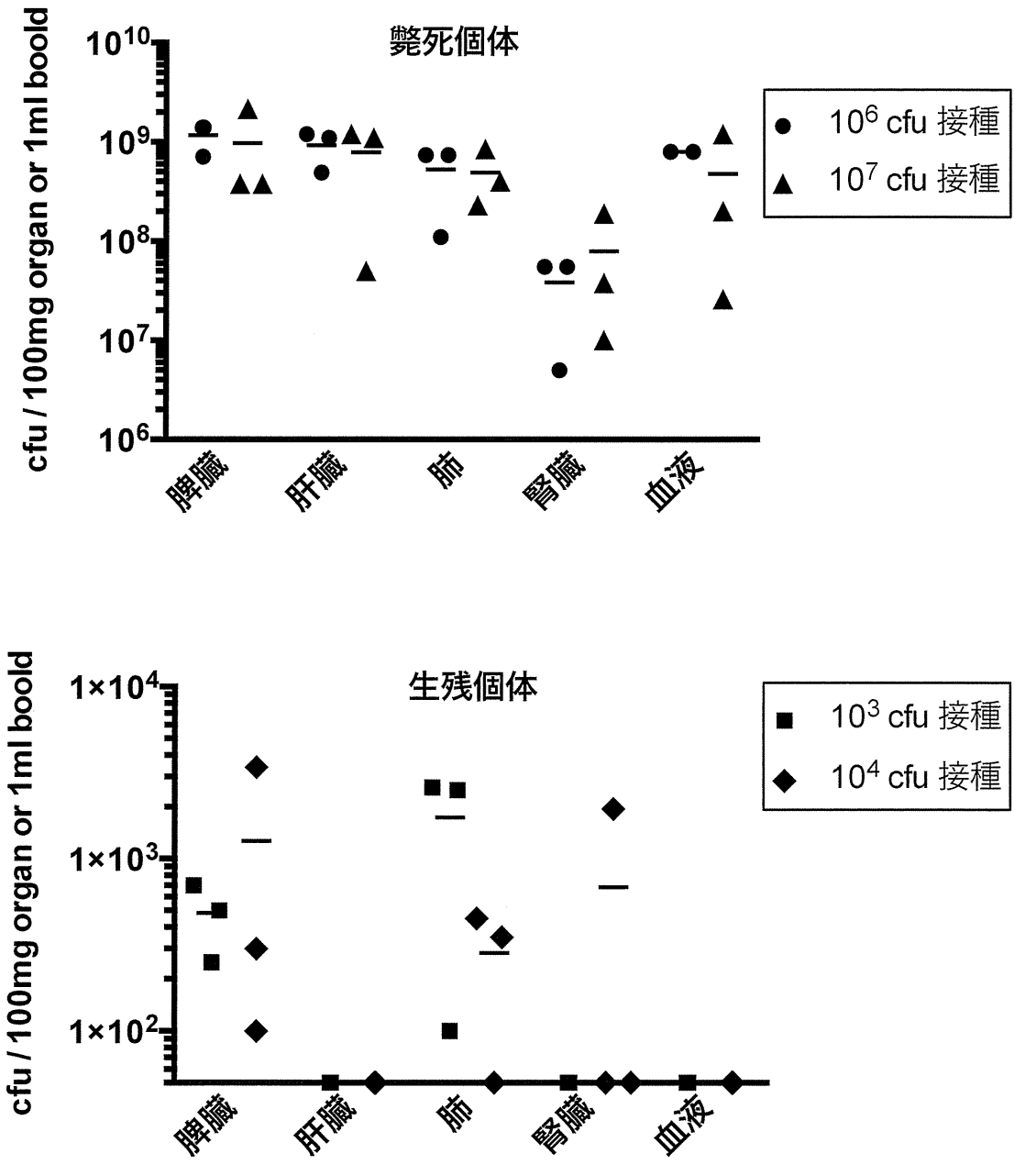

図6　斃死および回復ラット個体の臓器中菌量

Slc:SDラットの10^7および10^6cfu接種群の斃死個体6匹(各群3匹、接種後2-3日に斃死)および10^1および10^2cfu接種群の接種後21日の回復生残個体6匹(各群3匹)について脾臓、肝臓、肺、腎臓および血液中の菌量を測定した(10^6 cfu 接種群の血液のみn=2)。

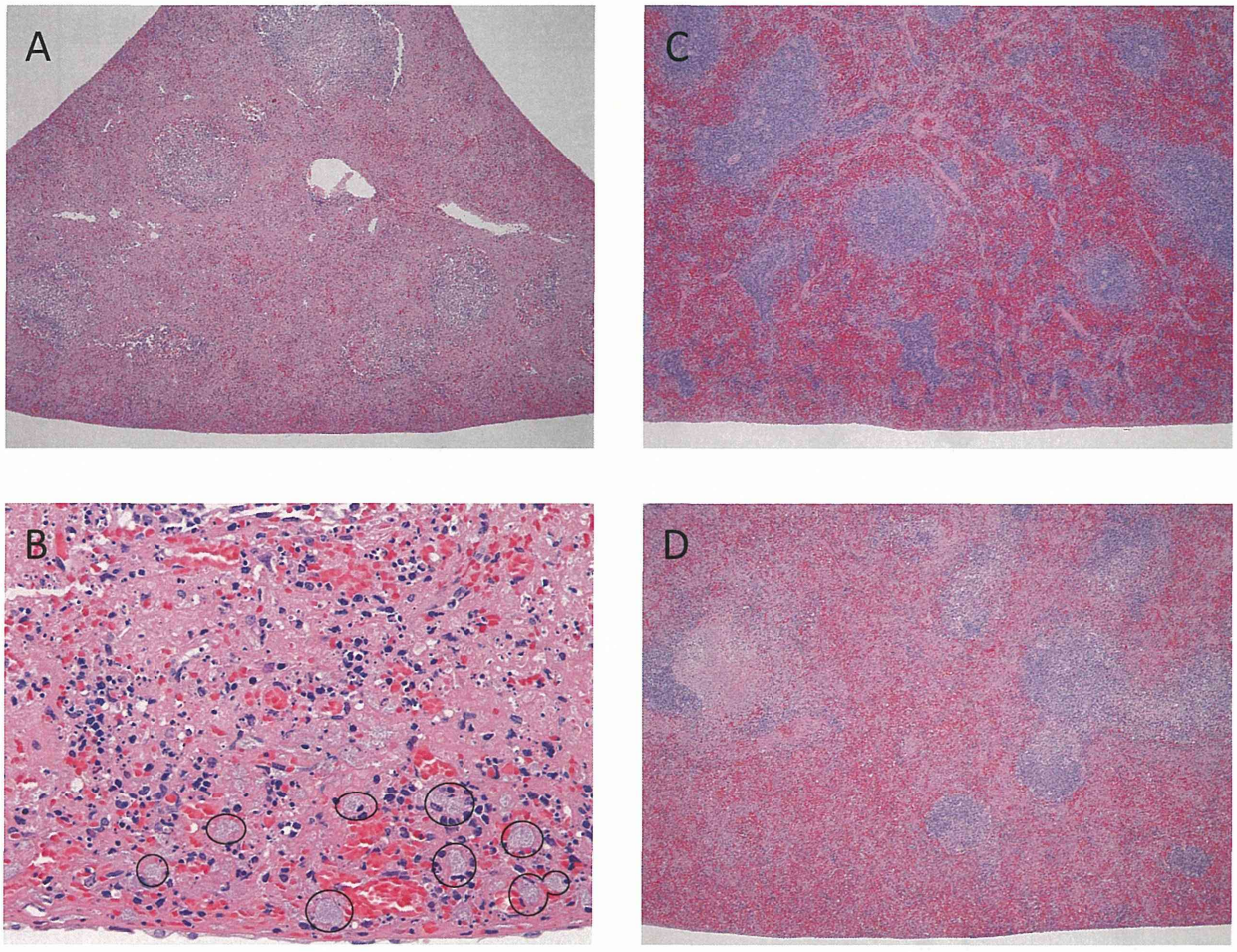

図7　野兎病菌腹腔内接種ラットの脾臓病変

NVF1株10^7cfu腹腔内接種後2日で斃死したSlc:SDラットの脾臓のHE染色像（A: x 28, B: x 280）。重度のリンパ球の減少および壊死、辺縁帯の萎縮、赤および白脾随の巣状壊死が観察された。また高倍観察像で多数の菌塊（B、黒円）が認められた。10^7cfu腹腔内接種後21日生残したSlc:SDラット回復個体の脾臓HE染色像（C: x 28）。辺縁帯の萎縮のみが認められた。10^1cfu腹腔内接種後4日で斃死したF344/NsSlcラットの脾臓のHE染色像（D: x 28 ）。中等度のリンパ球減少および壊死、重度の辺縁帯の萎縮および赤脾随の巣状壊死、中等度の白脾随の巣状壊死が認められた。

Slc:SD
10^7cfu接種2日後に斃死

F344/NsSlc
10^1cfu接種4日後に斃死

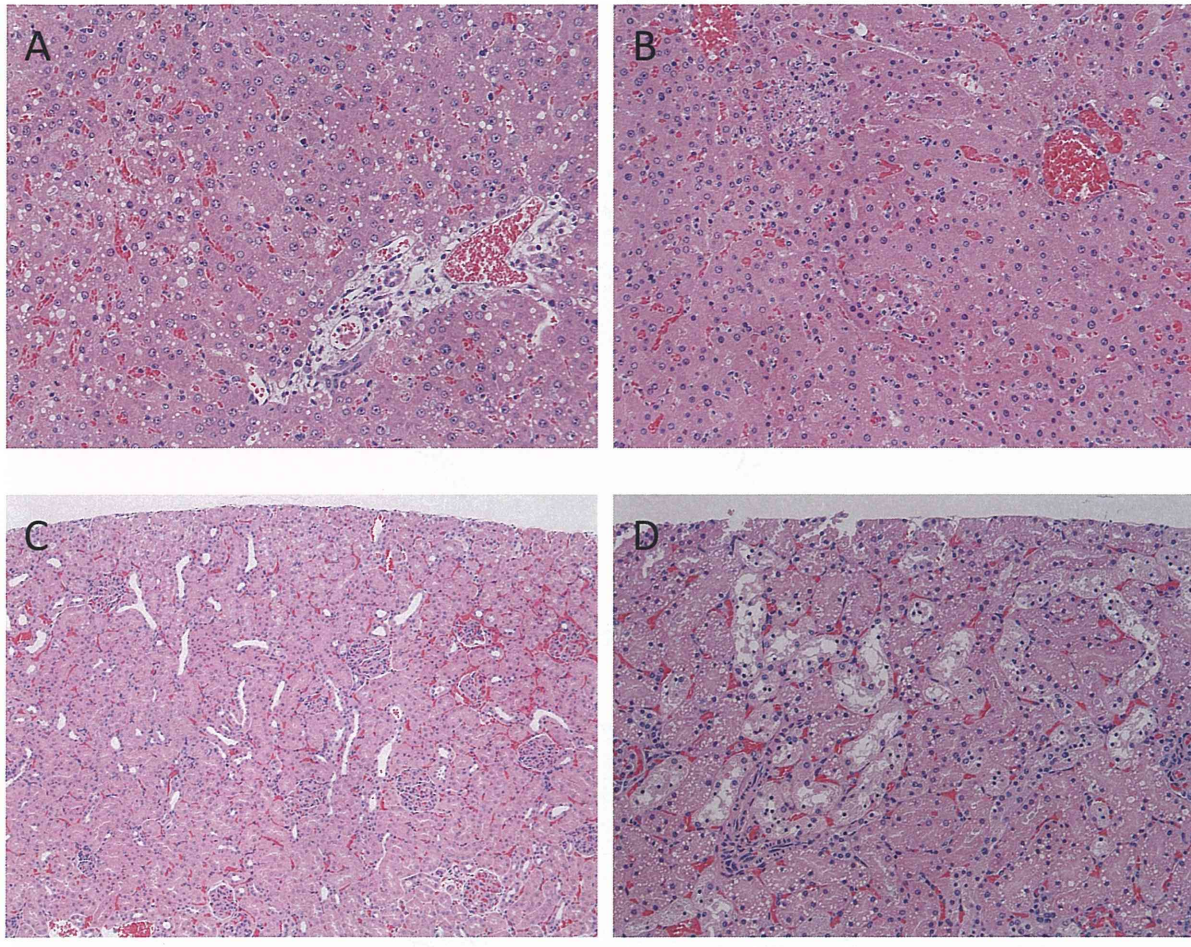

図8　野兎病菌腹腔内接種ラットの病変

NVF1接種後斃死したラットの肝臓(A,B)および腎臓(C,D)の組織像を観察した。Slc:SDの肝臓(A)では肝細胞の壊死、肝細胞内空胞形成が認められた、F344/NsSlcの肝臓(B)では肝細胞の壊死が認められた。。Slc:SDの腎臓(C)では尿細管の拡張が認められ、F344/NsSlcの腎臓(D)では尿細管上皮内の空胞形成 、尿細管の壊死が認められた。

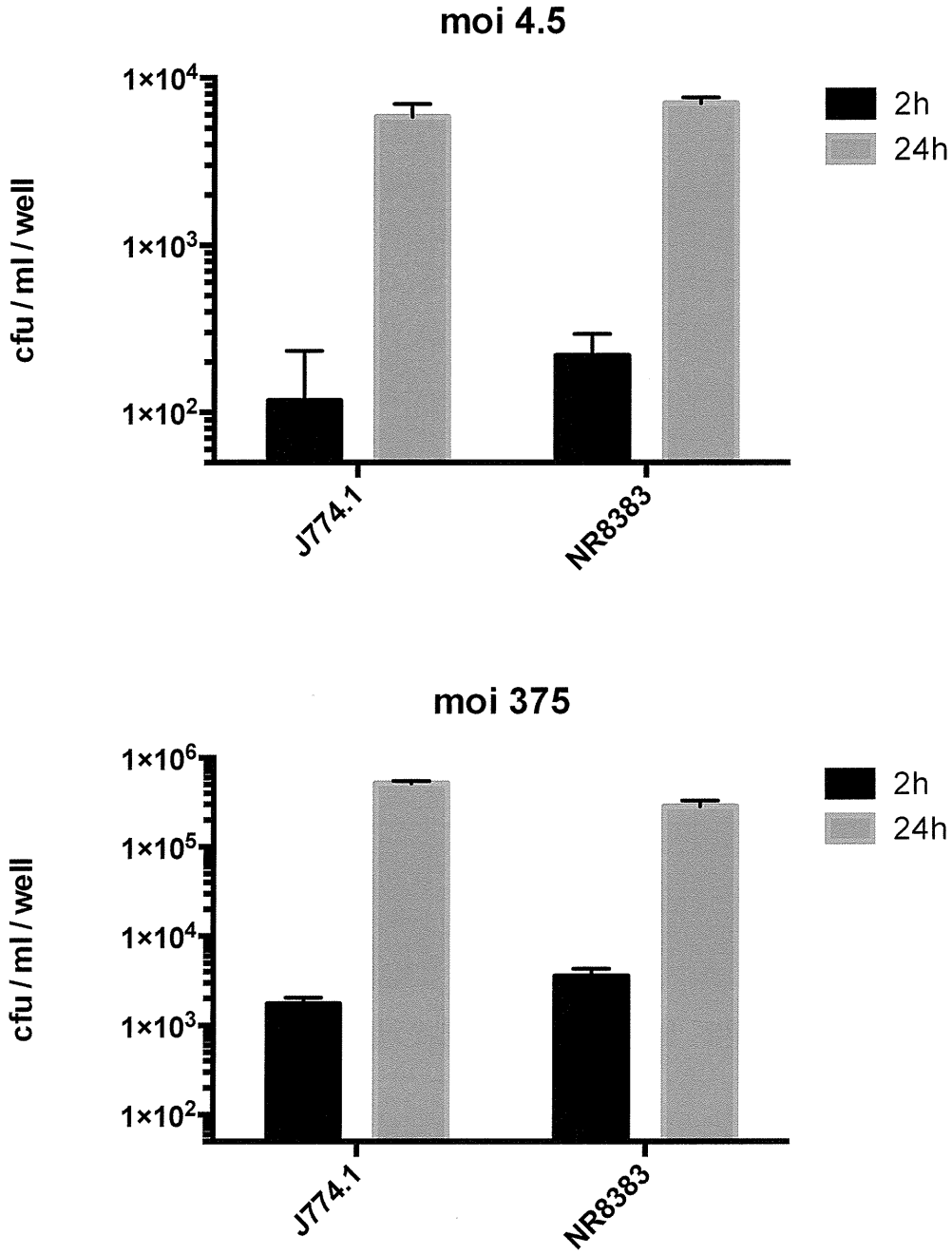

図9　マウスとラット由来細胞内におけるNVF1株の増殖性

NVF1株をJ774.1およびNR8383細胞に接種したところ、両細胞にて接種後2から24時間の間に10倍以上に菌が増えた(A)。この増殖率はmoiに関わらなかった。

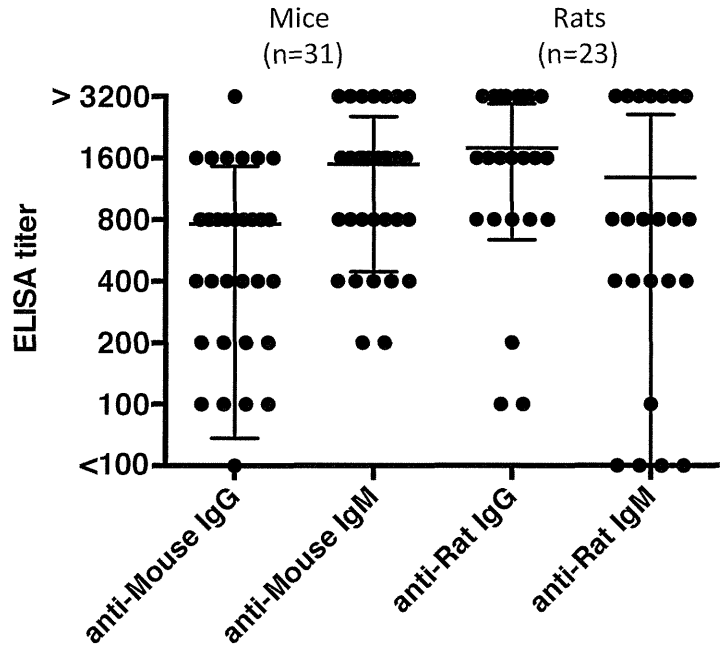

図10　マウスおよびラット感染回復個体由来血清抗体価

野兎病菌接種後21-25日に採取したマウス（n=31）およびラット（n=23）血清の抗体価をELISAにて測定した。マウスはIgM、ラットはIgGの抗体価が高い傾向が認められた。

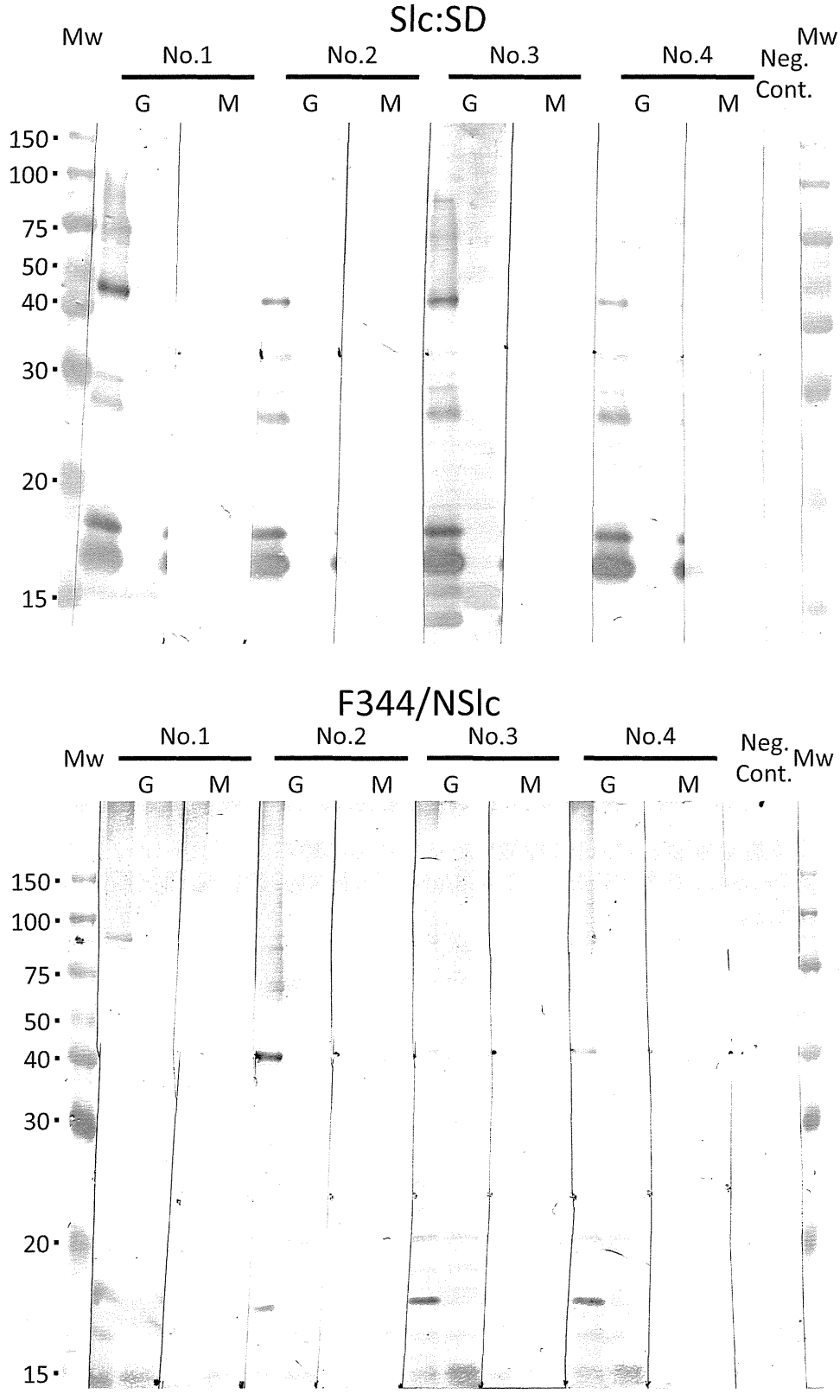

図１１　NVF1株皮内接種ラット由来血清のW.B像（1）

各シート、全菌体抗原（左）、プロテナーゼK処理抗原（右）をSDS-PAGE後PVDF膜に転写し、500倍希釈した皮内接種25日後の各ラット個体の血清（No.1-4）と反応させた。2次抗体には抗ラットIgG(G)、抗ラットIgM(M)を使用した。分子量マーカー(Mw)にはWIDE-VIEW TM Prestained Protein Size Marker（和光純薬）を使用した。F344/NsSlcの反応は他のラット系統と比較して弱かった。

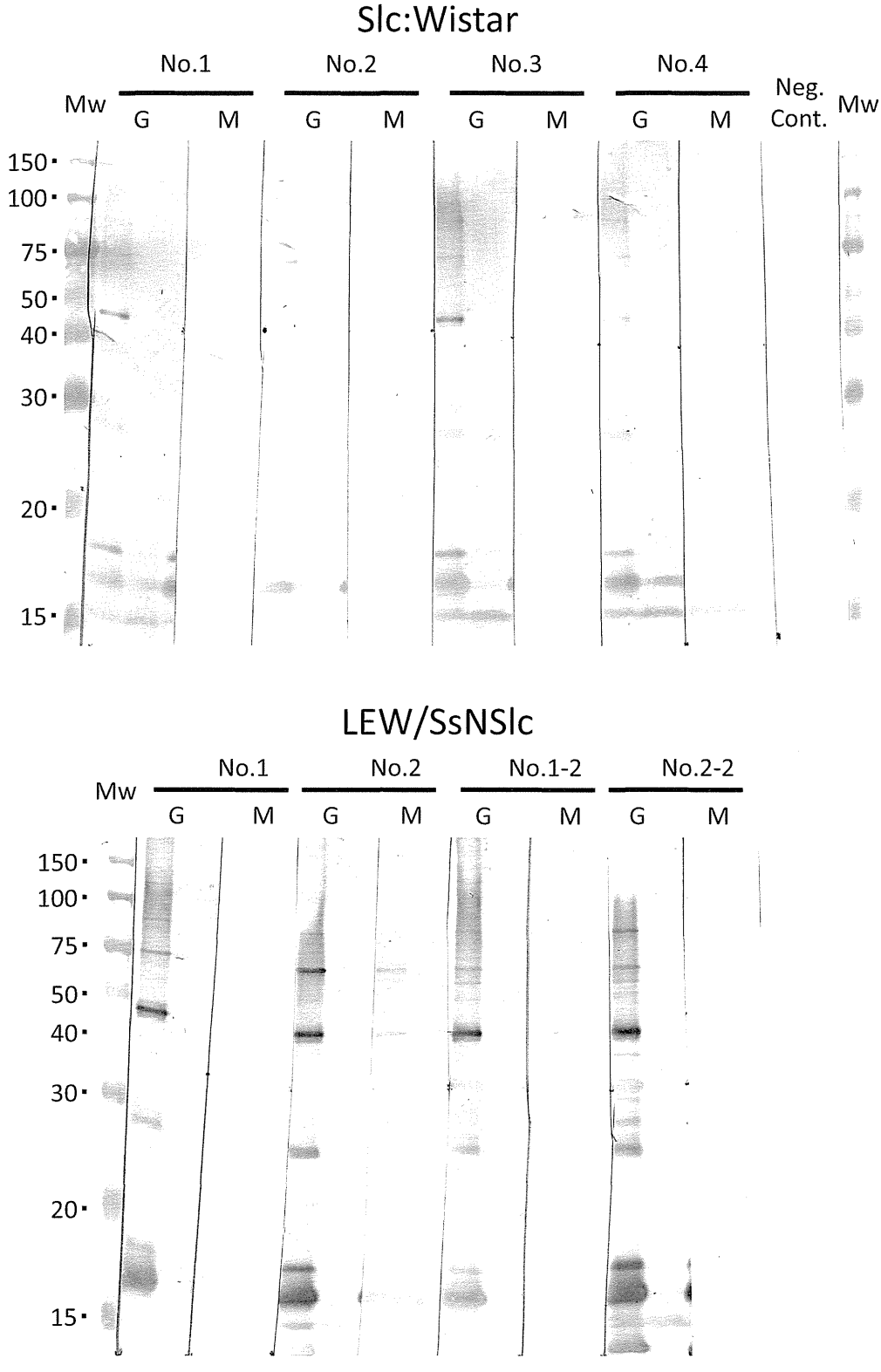

図12　NVF1株皮内接種ラット由来血清のW.B像(2)

各シート、全菌体抗原(左)、プロテナーゼK処理抗原(右)をSDS-PAGE後PVDF膜に転写し、500倍希釈した皮内接種25日後の各ラット個体の血清(No.1-4)と反応させた。LEW/SsNSlcについては菌接種後2ヶ月の血清(No.1-2およびNo.2-2)も供試した。2次抗体には抗ラットIgG(G)、抗ラットIgM(M)を使用した。分子量マーカー(Mw)にはWIDE-VIEW TM Prestained Protein Size Marker(和光純薬)を使用した。

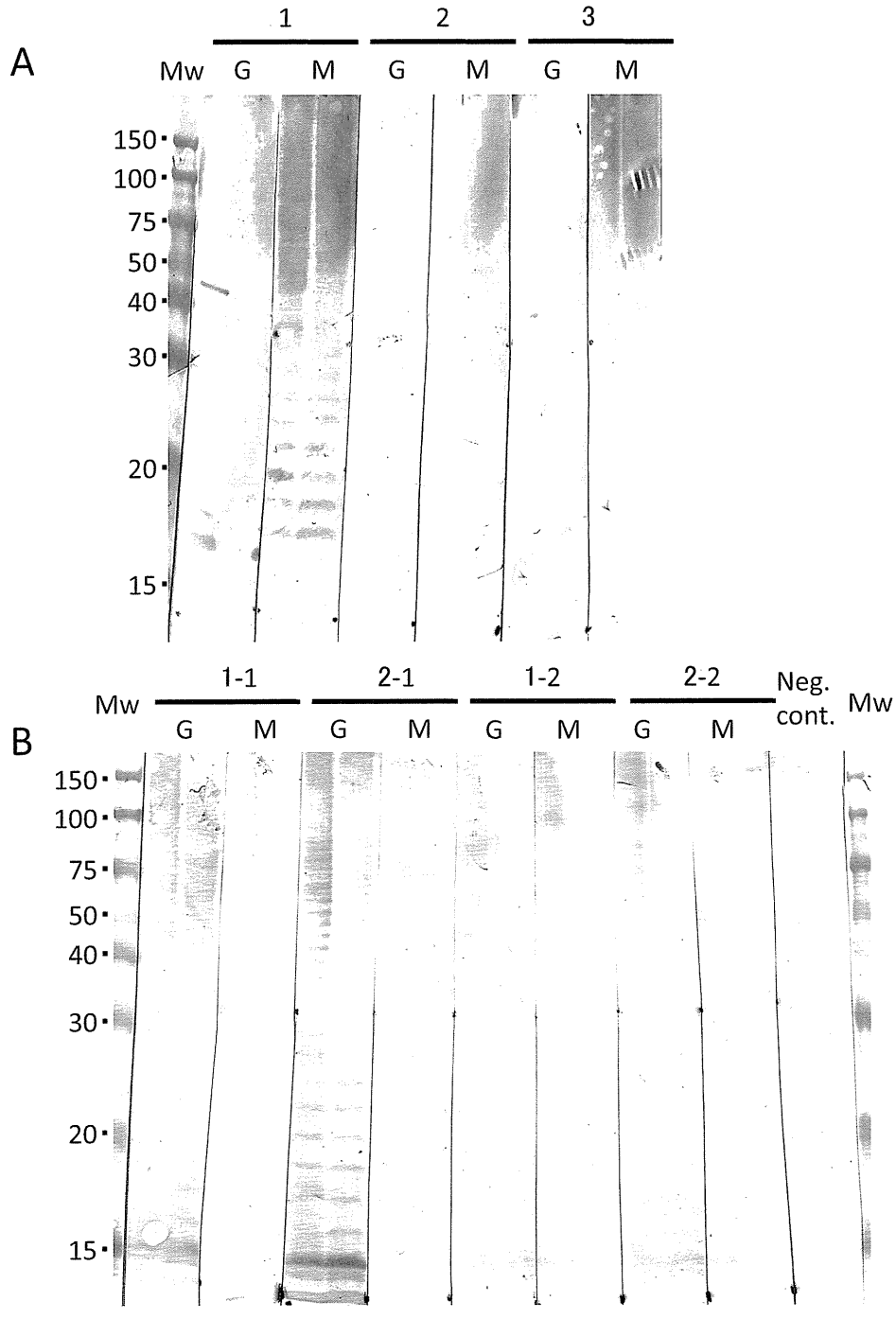

図13　野兎病菌接種マウスおよび野兎病患者血清のWB像

各シート、全菌体抗原（左）、プロテナーゼK処理抗原（右）をSDS-PAGE後PVDF膜に転写し、野兎病菌Yama 株皮内接種後21日の回復マウス個体の血清を100倍希釈で反応させた（A）。抗IgM抗体の反応は梯子状バンドとして認められたが抗IgG抗体の反応は弱かった。 野兎病患者2名の推定感染日16日後（1-1、2-1）および23日後（1-2、2-2）の血清の反応（B）。分子量マーカー(Mw)にはWIDE-VIEW TM Prestained Protein Size Marker（和光純薬）を使用した。

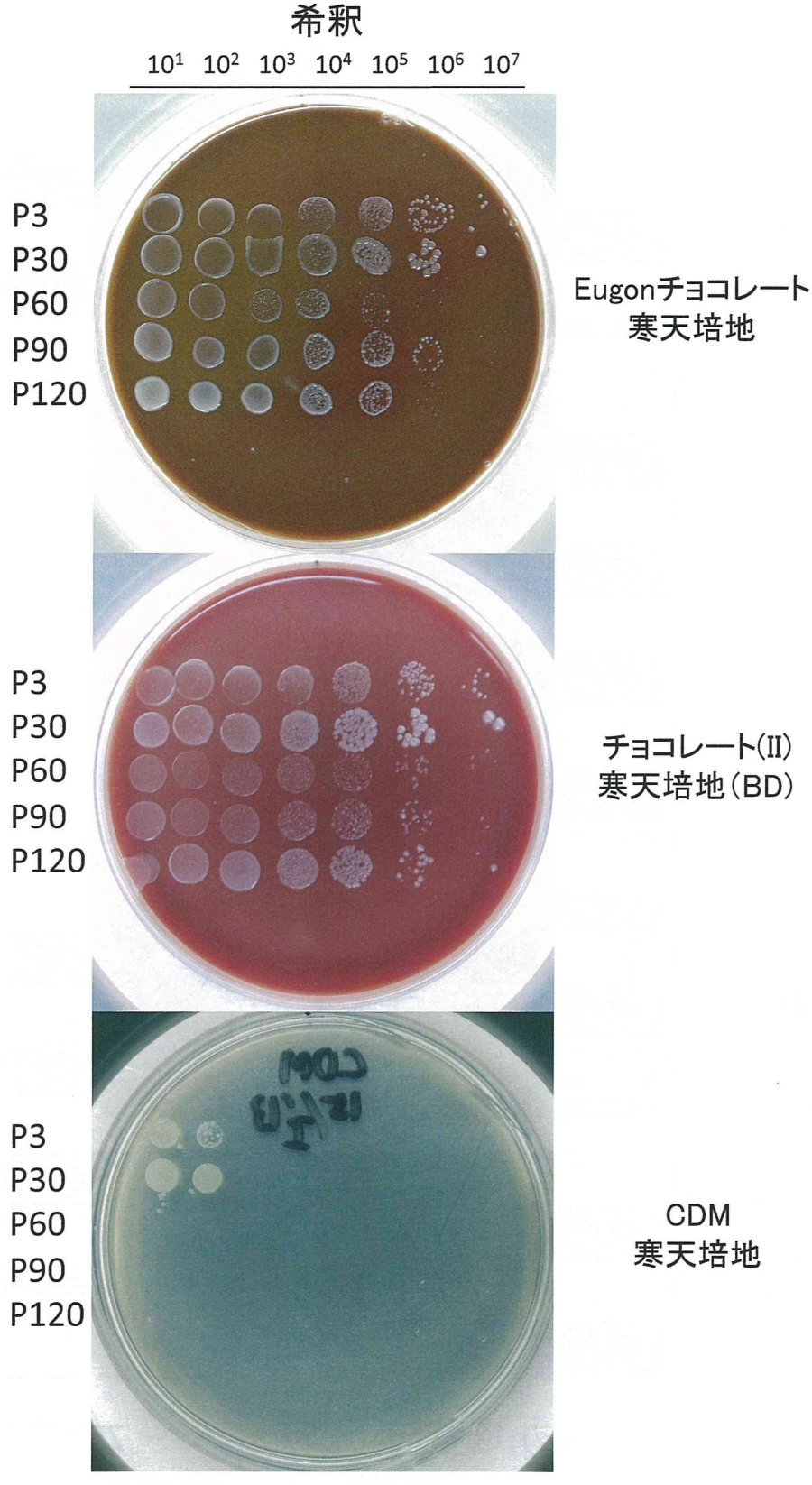

図14　NVF1株継代菌の増殖性の変化

チョコレート(II)寒天培地にて3.3-7.9x10^9cfu/mlであったNVF1株の3(P3)、30(P30)、60(P60)、90(P90)および120代継代菌(P120)菌液を10倍段階し、各希釈を5μlずつスポットした。CDM培地にてP3および30は100倍希釈液までコロニーを認めたが、他はCDM培地でコロニー発育が認められなかった。

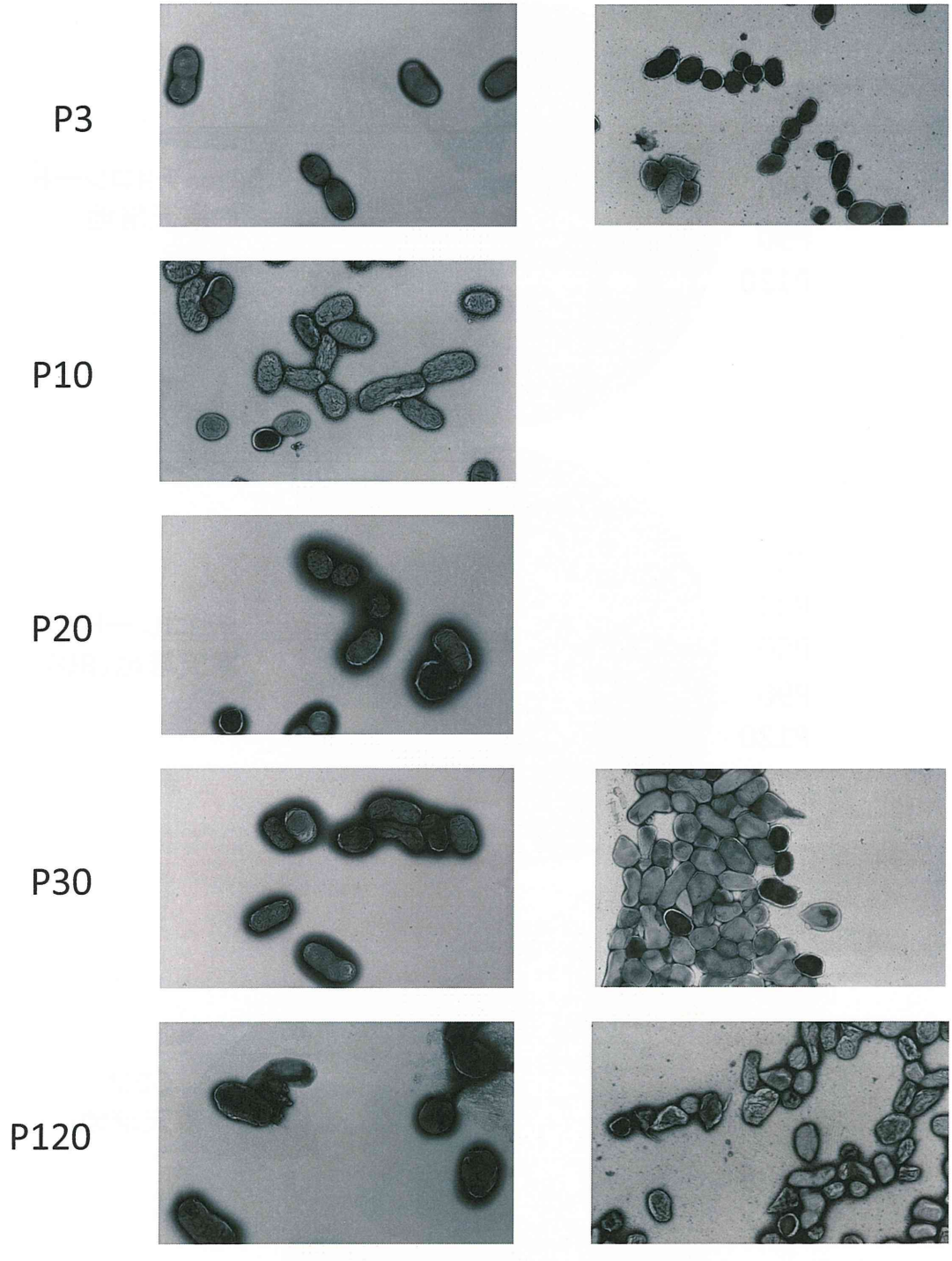

図15　NVF1株継代菌の形態学的観察（SEM像）

NVF1株のEugonチョコレート寒天培地継代菌のnegative stain 像（x10,000）を観察した。継代菌間で著しい形態的相異は観察されなかったが、75時間培養の菌では48時間培養の菌と比較し不整形の菌体が多い傾向が認められた。

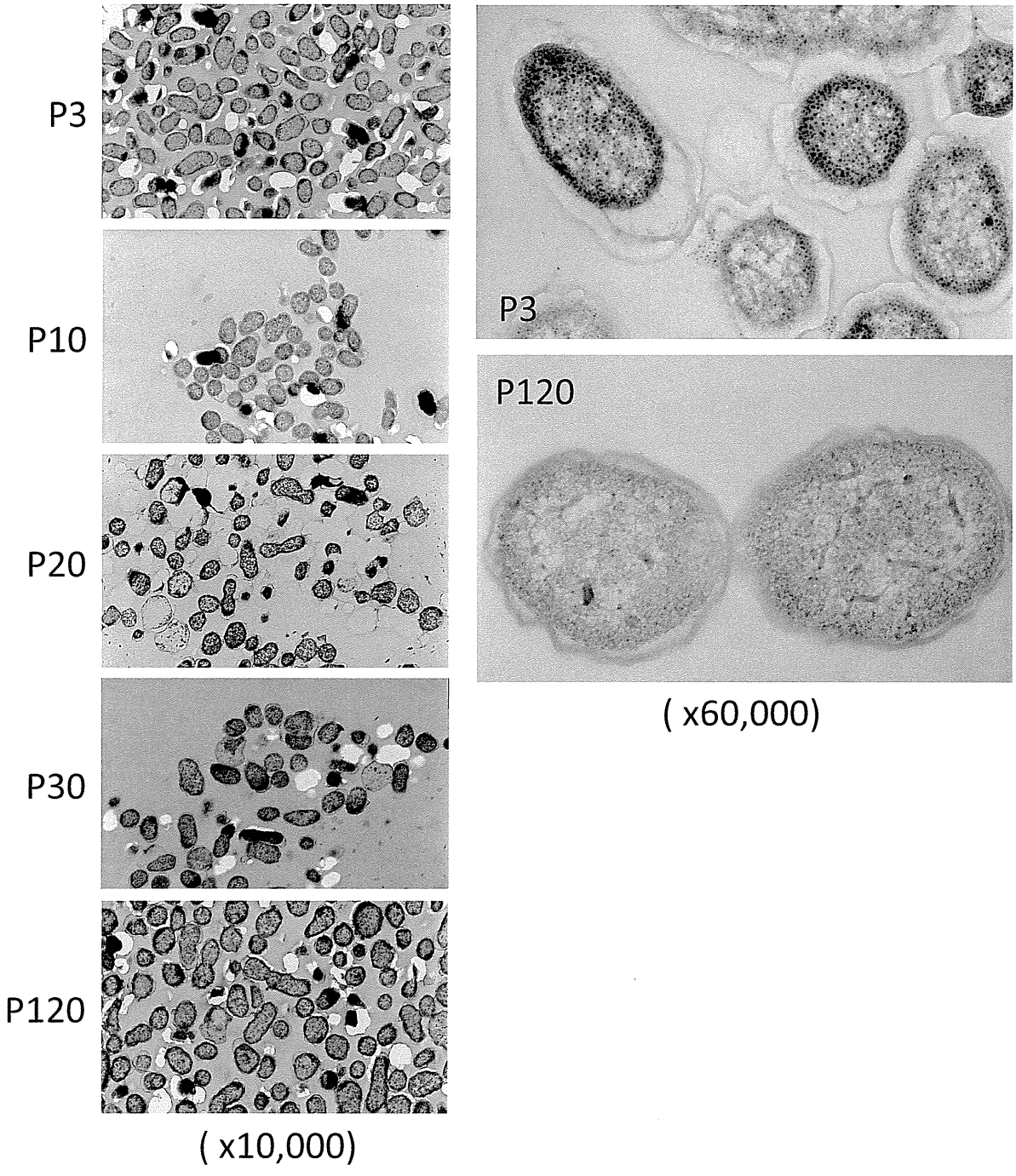

図16　NVF1株継代菌の形態学的観察（TEM像）

NVF1株の3（P3）、10（P10）、20（P20）、30（P30）および120（P120）継代菌の超薄切片を電子顕微鏡で観察した。電顕像10,000倍（左）よりP30とP120の間で変化が認められたため、両者を60,000倍（右）にて観察したところ、菌体の大きさと外膜構造が異なった。

雑誌

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Akitoyo Hotta, Osamu Fujita, Akihiko Uda, Neekun Sharma, Kiyoshi Tanabayashi, Yoshie Yamamoto, Akio Yamada, Shigeru Morikawa	In vitro antibiotic Susceptibility of *Francisella tularensis* isolates from Japan	Japanese Journal of Infectious Diseases			

In vitro Antibiotic Susceptibility of *Francisella tularensis* isolates from Japan

Akitoyo Hotta[1*], Osamu Fujita[1], Akihiko Uda[1], Neekun Sharma[1, 2], Kiyoshi Tanabayashi[1], Yoshie Yamamoto[1], Akio Yamada[3], Shigeru Morikawa[1, 2]

1: Department of Veterinary Science, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku, Tokyo 162-8640, Japan

2: The United Graduate School of Veterinary Science Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

3: Laboratory of Veterinary Public Health, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo 113-8657, Japan

Correspondence to

Akitoyo Hotta Ph.D., DVM

Department of Veterinary Science, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku, Tokyo 162-8640, Japan

Summary

The antibiotic susceptibility of 36 isolates of Japanese *Francisella tularensis*, an etiological agent of zoonotic disease tularemia, was analyzed using E test. All isolates were susceptible to ciprofloxacin, doxycycline, erythromycin, and gentamicin but resistant to benzylpenicillin and cephalothin. The susceptibility to seven other β-lactams (aztreonam, cefotaxime, cefoxitin, ceftriaxone, cefuroxime, imipenem, and meropenem) varied among isolates. These findings suggest that the *F. tularensis* isolates susceptible to some β-lactams are distributed in Japan and that the antibiotic therapies generally recommended for tularemia are considered to be more effective for the patients in Japan.

Francisella tularensis, is an intracellular gram-negative, fastidious coccobacillus, that causes the zoonotic disease tularemia in humans and animals. It is widely distributed throughout the northern hemisphere including Japan (1). Its low infectious dose, ease of dissemination, and a history as a bioweapon have led to the inclusion of *F. tularensis* in CDC's Category A select agents (2). Humans may be infected through direct contact with infected animals, arthropod bites, ingestion of contaminated water or food, or inhalation of infectious aerosols. Predominant symptoms are high fever, lymph nodes enlargement and ulcers at the site of bacteria entry. Aminoglycosides, chloramphenicol, tetracyclines, and quinolones are recommended for antibiotic treatment (1).

Approximately 1,300 cases of human tularemia were reported in Japan between 1924 and 1985, although the incidence of tularemia and isolation of the pathogens have been extremely rare since 1990 (3). In 2008, five cases of tularemia were reported in the Infectious Diseases Weekly Report Japan database (http://idsc.nih.go.jp/idwr/ydata/report-Ea.html) and the pathogen was isolated in Japan for the first time in 19 years (4). Japanese *F. tularensis* isolates are known to differ from the North American and European isolates in terms of their biochemical (1) and genetic properties (5). However, their antibiotic susceptibility is not well characterized. Recently, the antibiotic susceptibility of *F. tularensis* strains isolated in North America (6), Austria (7), France (8), and Turkey (9) were analyzed by use of E test. Information on

the antibiotic susceptibility pattern of Japanese *F. tularensis* is necessary to ensure appropriate prescription of antibiotic for postexposure prophylaxis and therapy for tularemia patients. Therefore, in this study, the antibiotic susceptibilities of Japanese *F. tularensis* were analyzed by using the E test.

Thirty-four isolates of Japanese *F. tularensis* isolated between 1926 and 1989 were kindly provided by Dr. H. Fujita (Ohara Research Laboratories, Fukushima, Japan). Recent isolates KU-1, a generous gift from Prof. H. Sato (Kitasato University, Aomori, Japan) (4), and NVF1 were isolated from wild hare carcasses in 2008 and 2009, respectively. All the *F. tularensis* isolates were grown on 8% (w/w) sheep blood chocolatized Eugon agar plates (10) and passaged twice in Mueller-Hinton broth (BD, Sparks, MD) supplemented with 0.1% (w/v) glucose, 2% (v/v) IsoVitalX (BD), and 0.025% (w/v) ferric pyrophosphate (11). *F. tularensis* requires cysteine for growth, and hence, the subcultured bacteria were inoculated onto chocolate II agar plates (BD, Fukushima, Japan). The E test strips (AB Biomerieux, Solna, Sweden) including the 13 antibiotics listed in Table 1 were placed on these plates. After incubation at 37 °C for 2 days, the minimum inhibitory concentration (MIC) was determined as the point of intersection between the zone of bacterial growth observed and the E test strip. If bacterial growth occurred along the entire strip, i.e., no inhibition ellipse was observed, MIC was recorded to be more than the highest value on the MIC scale for each E test strip (8). *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus*

(ATCC 29513) were used as quality control strains. A P/Case test-N (Nissui Pharmaceutical Co. Ltd., Japan) was used to analyze β-lactamase production by each isolate, according to the manufacturer's instructions. Work with *F. tularensis* cultures was performed in a biosafety level 3 laboratory at the National Institute of Infectious Diseases, Tokyo, Japan.

The MICs of the quality control strains (*E. coli* and *S. aureus*) were similar to the reference MICs found in the instructions of the E test, as reported by Valade et. al. (8)(data not shown). Using this assay system, ciprofloxacin, doxycycline, erythromycin, and gentamicin effectively inhibited the growth of all isolates with the MIC ranges of 0.003–0.023, 0.094–1.5, 0.094–1.5, and 0.023–0.5 mg/L, respectively (Table 1). These results showed that the susceptibility of Japanese isolates to these four antibiotics was similar to that of the North American isolates (6) but different from that of the isolates from Turkey (9) and Austria (7), especially with regard to susceptibility to erythromycin.

The other nine β-lactams antibiotics tested (aztreonam, benzylpenicillin, cefotaxime, cefoxitin, ceftriaxone, cefuroxime, cephalothin, imipenem, and meropenem) did not effectively inhibit the growth of the isolates tested. Three isolates (including the recent isolates, KU-1 and NVF1) grew all around the chocolate II agar plate, whereas others showed inhibition ellipse around the E test strips with some of these antibiotics. The MIC_{90} results indicated that these nine β-lactams would be ineffective in the treatment of tularemia

patients while the MIC range and the MIC_{50} suggested that some Japanese isolates were susceptible to cephalosporins, cephamycin, carbapenems and monobactam. These results and the previous reports by Baker et al. (11) and Markowitz et al. (12), suggest that cephalosporins-susceptible *F. tularensis* isolates are distributed in Japan and North America. Cephalosporins are frequently used to treat patients with possible bacterial infections in clinics (13). The early clinical symptoms of human tularemia are flu-like, and hence, its diagnosis is difficult without laboratory tests. There is a possibility that some patients who are exposed to *F. tularensis* and treated by cephalosporins are abandoned without being suspected and diagnosed as tularemia.

In this study, we tried to clarify the mechanisms underlying the different β-lactams susceptibitlities of Japanese *F. tularensis* isolates, by analyzing the β-lactamase (penicillinase and cephalosporinase) production of bacteria using the P/Case test-N. The results showed that all the isolates tested produced penicillinase but not cephalosporinase (data not shown). Thus, the mechanism underlying the differences in susceptibility to β-lactams was not clarified during this analysis. It is possible that *F. tularensis* possesses an unknown antibiotic resistance mechanism such as the non-expression of porin genes and/or efflux pump-based antibiotic resistance (13).

In conclusion, our results suggest that the guidelines for antibiotic treatment of tularemia issued by WHO (WHO guideline for Tularemia;