日向市の新設温泉施設を感染源とするレジオネラ症集団発生

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要　旨

宮崎県日向市の日向サンパーク温泉「お舟出の湯」は，平成14年6月20，21日に各200人ずつの体験入浴を実施後，7月1日に正式開業したが，レジオネラ症集団発生のため7月24日に営業停止となった．6月20日から7月23日までの入浴者19,773名のうち295名が発症し，そのうち34名が，喀痰培養陽性，尿中レジオネラ特異抗原陽性，*Legionella pneumophila* SG1に対する血清抗体価有意上昇の何れか1つまたは2つにより確定診断され，うち7名が死亡した．*L. pneumophila* SG 1が患者喀痰と浴槽水から検出され，SfiⅠによる患者株と浴槽水株のDNA切断像が一致したことから，「お舟出の湯」が感染源と確定した．

日向市設置の「日向サンパーク温泉施設レジオネラ菌原因等究明委員会」は集団感染の原因として：宮崎県の委員会が推定した8項目の他に(1)施設関係者のレジオネラ症に対する知識と認識の不足，(2)施設完成時，試運転後の配管内の水の滞留とレジオネラ属菌増殖の可能性，および(3)体験入浴前の循環系統全体の消毒・清掃が不十分の3項目を加え，全11項目を指摘した．改善対策として：(1)維持管理マニュアルの整備，(2)浴槽水の溢水の確保，(3)ろ過装置の消毒の徹底，(4)浴槽水の残留塩素濃度の測定と維持，及び(5)ろ材を多孔質セラミックから砂に変更，など全24項目を指示した．

〔感染症誌　78：90～98，2004〕

序　文

欧米では渦流浴（Whirlpool spa）を利用した団体客にポンティアック熱が集団発生した報告[1)~4)]，循環風呂展示場でレジオネラ肺炎の集団発生[5)]，およびアメリカの外洋クルーザーの砂ろ過式循環風呂でもレジオネラ肺炎が発生し注目された[6)]．日本では1993年に温泉浴槽中で転倒し浴槽水を誤嚥した後に発症した症例が，温泉関連レジオネラ肺炎の第一報告例であった[7)]．このことから1993年に北海道～熊本にわたる1道12県の40温泉の浴槽水調査で，17温泉（42.5%）からレジオネラ属菌が検出された[8)]．平成14年9月20日付け厚生労働省健康局生活衛生課長からの健衛発第0920001号で指示された「入浴施設におけるレジオネラ症防止対策の実施状況の緊急一斉点検」[9)]に対応して全国31,826施設が点検対象となった．これは温泉だけでなく公衆浴場なども含めた浴場総数である．このうち行政検査または自主検査でレジオネラ検査を実施していたのは計17,614施設で，レジオネラを検出したのは2,946施設（17.3%）であったというが，菌数は公表されていない[10)]．

温泉とレジオネラ肺炎との因果関係に気付かず追跡調査を実施しなかった症例[11]以後，浴場および温泉水関連のレジオネラ肺炎事例は計17に達した．特に2000年と2002年には，かねて危惧されていた大規模集団感染が，計4カ所の新設温泉施設で発生した．その中で死者7人を出した日向サンパーク温泉「お舟出の湯」でのレジオネラ集団感染に関わる，日向市が設置した「日向サンパーク温泉施設レジオネラ菌原因等究明委員会」に，藪内と縣が平成14年10月21日から平成15年1月24日まで参画し，1月29日に調査報告書[12]を山本孫春日向市長に提出した．

宮崎県日向市の日向サンパーク温泉「お舟出の湯」は，平成14年6月20日に竣工式を挙行し，同日および翌21日に近隣住民200人ずつを招待して体験入浴を実施した後，7月1日に正式開業した．7月18日，市内の医療機関から「お舟出の湯」入浴者3名がレジオネラ肺炎様症状で入院しているとの通報を受けた日向保健所は，翌19日「お舟出の湯」の浴槽など7カ所から採水し，衛生管理状況などを聴き取り営業自粛を要請した．施設側は諸般の事情から23日まで営業を続け，24日から休業する旨を回答した．その後 *Legionella pneumophila* SG 1が患者喀痰と浴槽水から検出され，SfiⅠによる両菌株DNAの切断像が一致したことから，「お舟出の湯」が感染源と確定し7月30日に営業停止命令が出された．6月20日から7月23日までの入浴者19,773名のうち295名が発症し，そのうち34名が，喀痰培養陽性，尿中レジオネラ特異抗原陽性，*L. pneumophila* SG1に対する血清抗体価上昇の何れか一つまたは二つにより確定診断された．

日向サンパーク温泉「お舟出の湯」でのレジオネラ集団感染に関連して，宮崎県は「宮崎県福祉保健部レジオネラ症対策本部」及び「レジオネラ属菌汚染原因究明対策委員会」を，厚生労働省は「レジオネラ症集団感染事例の疫学調査」研究班を設置した．また，日向市議会は「日向サンパーク温泉施設レジオネラ症集団感染問題対策特別委員会」を設置し，それぞれ調査研究・対策の検討を行っている．

ここでは我々が参画した「日向サンパーク温泉施設レジオネラ菌原因等究明委員会」の活動とその報告書および日向サンパーク温泉「お舟出の湯」に係る改善計画書とを中心に，入手し得た他委員会の報告書も参考にして，複数の要因が複雑に絡み合って起きたこの不幸な事例についてまとめた．

対象と方法

1．温泉施設の設営

日向灘に面した宮崎県日向市大字幸脇（サイワキ）で，平成8年11月に温泉法による土地掘削が許可され，平成9年12月に温泉掘削に成功した．平成10年3月に源泉タンクとそれに近接する温泉スタンドを設置し，温泉水を希望する者に無料で提供することとした．

平成11年6月に施設の設計を開始し，平成13年1月には施設の建築に着工した．平成13年6月には，一般公募により施設名を神武天皇東征に際してのお舟出の地の故事に因んだ日向サンパーク温泉「お舟出の湯」と決定した．施設は平成14年3月29日に工事が完了し，その後水道水注入による昇温試験，配管内清掃，水位調整，外構工事などを実施した．6月4日に日向保健所に営業許可を申請し，同月6日に日向保健所の立ち入り検査を受けた．その後，社員の研修，検査のための湯張り・湯抜きを繰り返し，維持すべき次亜塩素酸濃度を設備業者が設定した．6月20日には日向保健所から公衆浴場としての営業を許可され，同日竣工式を挙行し，同日および翌21日に近隣住民200人ずつを体験入浴に招待した．その後社員による浴槽清掃，湯張り，湯抜きを繰り返し，6月30日に全浴槽に湯張りし，7月1日に正式開業した．

2．集団感染事例の概要と行政の対応

開業以来，毎週月曜の休館日をはさんで，7月17日（レジオネラ症集団発生が発覚する前日）までの入浴者は合計15,085名，1日平均1,006名であった．

7月18日，日向市内の医療機関から「レジオネラ肺炎を疑わせる患者3名が入院しており，その何れもが「お舟出の湯」の入浴者である」との通

平成16年2月20日

報を受けた日向保健所は,日向市商業観光課に「明日（19日）温泉の水質検査を実施する．浴槽水などに消毒剤を入れたり浴槽などを洗浄したりせず，現状のままにしておくように」と電話連絡した．

7月19日(金)，日向保健所員5名が水質検査のため来所し，浴槽6カ所及び温泉スタンドの計7カ所から採水した．同日，サンパーク温泉で，施設担当者が，衛生管理状況，残留塩素測定方法，諸測定結果の記録方法について保健所の事情聴取を受けた．そののち施設担当者が保健所に赴き，所長・次長・課長から徹底洗浄・消毒の実施と一時営業休止の要請を受けた．この時点で日向市側は,既に24日の休業と清掃・消毒実施を決めていたことを保健所に通告し，営業を続行した．この日（19日）の業者の点検で塩素剤注入装置のパイプにガス溜まりが出来て塩素が注入されない状態になっていることが判明し，すべての浴槽で塩素濃度はゼロであったことが判明した．

20日には業者によるろ過器の点検,22日には浴槽等の清掃・消毒を行っていたが，25日に日向市内在住の50歳代男性の喀痰培養と,「お舟出の湯」の浴槽水培養で *Legionella pneumophila* 血清群（serogroup，SG）1が検出され，30日には制限酵素SfiⅠを用いた患者株と浴槽由来株のDNAの遺伝子多型解析（パルスフィールドゲル電気泳動法，宮崎県衛生環境研究所実施）で両者が一致したことから,「お舟出の湯」が感染源であることが確定し，施設名が公表された．7月19日採取の浴槽水中のレジオネラ属菌の菌数は，最大で 1.5×10^6 CFU/100mlであった．*L. pneumophila* SG 1の他にSG8，*L. dumoffii*，*Legionella* sp.が検出された．宮崎県はこのことを宮崎県医師会を通じて県内医療機関に通報して診療に当たっての注意を喚起し，県民に対しても注意を促すとともに，報道機関に公表した．

7月25日，日向サンパーク温泉は日向保健所の文書による営業自粛要請を受入れ,同月30日には日向保健所から60日間の営業停止命令(期間7月30日～9月27日)が出された．その後営業停止期間は順次延長され,現在は平成15年10月31日迄営業停止が続くことになっている．温泉施設は宮崎県警察本部（宮崎県警）と日向警察署によって7月30日に封鎖された．この立ち入り規制は11月28日に解除されるまで続いた．7月31日には国立感染症研究所，宮崎県衛生管理課，日向保健所，宮崎県警が立ち入り調査を行った．

宮崎県福祉保健部のレジオネラ症対策本部とは別に，日向市は10月21日，浴槽水の汚染原因の究明と再発防止対策策定のため「日向サンパーク温泉施設レジオネラ菌原因究明等委員会」を設置した．この委員会は8名の委員で構成され，藪内(委員長)と縣以外の委員は日向市助役，富島漁業組合長(日向サンパーク温泉取締役)，日向市総務課長，日向市企画課長，日向市建設課長および日向市商業観光課長の6名であった．

この委員会発足当日，現場はいまだ宮崎県警と日向警察署により封鎖されていた．委員会としては現場を視ることなしに論議を進めるのは不可能なことから,日向警察署の現場立ち入り許可を得,10月21日の第1回委員会当日，その開会に先立って，浴室・地下機械室を含む施設全体を視察した．

3. 現地視察

視察は，1）源泉水の供給系統として源泉タンク，除鉄装置，中温貯槽，高温貯槽；および2）浴槽系統として2カ所の大浴槽を始めとする各浴槽，ろ過装置，塩素剤注入装置，回収槽，給湯タンクの設置を確認し状況を調査した．調査には機器の仕様や制御方法，設備の状態および運転管理の履歴について，実設備を検証するとともに関係者から実情を聴取した．

4. 発症者の臨床

以下の3項目について「宮崎県福祉保健部レジオネラ症対策本部」の中間報告[13]から抜粋してまとめる．

1）発症者数と発症までの期間
2）臨床症状と検査結果
3）転帰

5. 学習研修会

平成14年12月20日に，藪内を講師，日向市関係者及び施設関係者等を対象として「レジオネラ

とレジオネラ症」の学習研修会を行った．供覧には *Legionella pneumophila* serogroup 1 のパイロット株 EY3492＝ATCC 33153 をマックファーランド濁度 1.0 になるよう懸濁し，その 10 倍希釈系列と各希釈段階の 100μl を BCYEα 寒天培地に塗布した培養物とを用いた．供覧後，パワーポイントを用いてレジオネラの細菌学とそれによる感染症を説明し，予防のための注意点を解説した．

結　果

1．現地視察

1）源泉タンクから中温・高温水槽

(1) 源泉タンク：容量 30m³，駐車場に設置されている．貯留時の水温は 26℃ 程度で加温装置や塩素殺菌装置は設けられていない．平成 14 年 3 月までは 2 カ月に一度清掃していたが，平成 14 年 4 月からは，源泉タンクは空であり清掃もされていなかった．源泉水を貯め始めたのは揚水ポンプの入れ替え終了後の 6 月 12 日からであり，調査時の貯留水量は 16.8m³ であった．

貯留水が常温であり，7 月 19 日採水の温泉スタンド水から，レジオネラ属菌を検出（1.2×10^4 CFU/100ml）していることから，源泉タンク水にもレジオネラ属菌が定着していた可能性が推測される．

(2) 除鉄装置：アンスラサイトおよび接触酸化式ろ材を充填したろ過器であり，源泉水に凝集剤 PAC（Poly Aluminum Chloride，ポリ塩化アルミニウム），次亜塩素酸ナトリウム，水酸化ナトリウムを注入して毎時 5m³ を処理している．開放式水槽のため，ろ材が鉄分により目詰まりすると溢水して処理水量が低下するので，1 日 3 回逆洗していた．鉄・マンガンを酸化する目的で源泉水に次亜塩素酸ナトリウムを注入していたが，除鉄装置処理水の遊離残留塩素濃度は管理されていなかった．

(3) 上水補給設備：除鉄処理水の移送ポンプから中・高温水槽までの配管に，上水（市水）配管が接続されており，水補給作業は手動であった．

(4) 中温水槽：容量 9m³，除鉄処理後の温泉水を加温せずに貯めておくための貯槽であり，遊離残留塩素濃度を維持する設備構造にはなっていない．

(5) 高温水槽：容量 31.5m³，設定温度は 58℃ である．夜間に電気式ヒートポンプで 60℃ にした温水との熱交換によって昇温する構造であり，補給水が多くなる日中の水温低下状況は不明であった．遊離残留塩素濃度を維持する設備構造にはなっていない．中温水槽と高温水槽の温泉水はミキシングバルブで混合し水温を調整していた．

2）浴槽系統

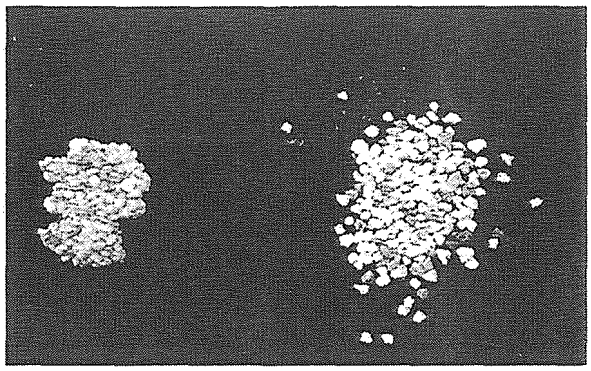
(1) 浴槽：浴槽の縁よりも低い壁面に水面を示唆する線（汚れ）が認められ，溢水が不十分であったことが推定された．目地のモルタル部からは，炭酸カルシウムが主成分のアルカリ性の「あく」が白色の「たれ」となっているのが多数認められた．この「あく」のため開業時，浴槽水の pH が高かった可能性もある．この他，浴槽壁には鉄の付着と思われる茶褐色の汚れも認められた．浴槽水の全換水は，2 つの大浴槽は 1 週間に一度，その他の浴槽は毎日行っていたという．

(2) ろ過装置：ろ過装置は支持床が砂利，ろ過層がセラミック砕粒の構造で，R-1～R-6 の 6 基が設置されており，処理能力および循環水量ともに十分と見受けられた．循環ろ過は営業時間中稼動し，夜間は停止する設定であった．逆洗時間の設定は R-1 が 12 分であるのを除いて他の 5 基では 1 分間と短く，ろ過器内にたまった汚れを十分排出出来ていなかった可能性がある．そこでろ過装置内のろ材のレジオネラ検査を実施するため，後日ろ材標品の採取・提供を依頼した．

平成 14 年 10 月 23 日に「お舟出の湯」のろ過装置 R-5 から採取したとして送付されて来たろ材は 0.6mm 前後の淡黄褐色の砕粉であった．同一社の同一製品の新品が淡灰白色でサラサラし盛り上げて置くことが困難なのに対し，使用済みろ材の表面は粘性でシャーレの中に容易に盛り上げて置くことが出来た（図 1）．このろ材 5g を滅菌精製水 5ml に懸濁・振盪したあとの上液を酸処理して培養すると 34 CFU/100μl の *L. pneumophila* SG 1 が生育した．このことから，ろ材 5g 中に少なくとも 1,700 個のレジオネラが存在したことになり，営業停止直前に強力な塩素消毒を行った筈のろ過装置

平成16年 2 月20日

Fig. 1 Filtration material used in Hiuga Sun-Park hot spring bath "OFUNADE-NO-YU". Crashed ceramics around 0.6mm in size. Left : Obtained on 23 October 2002 from one of the filtration tanks in "OFUNADE-NO-YU". Yellowish brown and sticky. 5 grams of the used material contained 1,700 CFU of *L. pneumophila* SG 1. Right : New product of material same with the left. Faint grayish-white in color, without any stickiness.



内ろ材の表面および内部に，営業停止から3カ月後にもこれだけの数のレジオネラ属菌が生存していたことになる．

(3) 塩素剤注入装置：12% 次亜塩素酸ナトリウム液を注入する定量注入ポンプが浴槽ごとに設置されていた．薬注点はろ過装置の出口である．注入装置はろ過装置稼動中，浴槽毎に10～20分間隔でON-OFF運転するように設定されていたが，浴槽水の遊離残留塩素濃度の維持管理方法と測定結果の詳細は不明であった．

(4) 回収槽：回収槽へは，2つの大浴場と他3つの浴槽の溢水が入るようになっていた．回収槽は開業当初使用していたが，1週間後の7月8日から使用していない．

(5) 給湯タンク：容量38m^3，日向市水を58℃に加温して貯留し，シャワーや水栓へ供給している．加温の熱源は，電気式ヒートポンプにより作られた60℃の温水である．この温水との熱交換により市水を加温し給湯タンクに貯留する構造である．

2. 発症者の臨床

1) 発症者数と発症までの期間

平成14年9月27日現在，「お舟出の湯」の入浴者で医療機関の医師がレジオネラ症疑似患者と診断し所轄の保健所に報告した患者（発症者）は295名（男159名，女136名）に達した．発症者は6月20日の体験入浴から営業停止（7月24日）の前日までのすべての営業日で入浴していた．発症者のうちレジオネラ症と確定診断されたのは男24名，女10名の計34名であった．

患者は6月24日から8月15日にかけて発症し，特にその90%は7月7日から7月31日までの25日間に発症していたという．入浴から発症までの期間は2日から18日であり，例数が最も多かった潜伏期間は6日であった．入浴日別発症者数を調査，検討したが日によって入浴者数に増減があったので，発症割合（入浴日毎発症者数/当日入浴者数）は0.5～2.0%で，特段の傾向は認められなかった．

2) 臨床症状と検査結果

確定診断者の症状は，発熱28名(82.4%)，咳嗽10名（29.4%），胸部X線像異常陰影25名(73.5%)，肝障害5名(14.7%)で，26名が入院したという．体験入浴者のうち8名が発症したが，確定診断された患者はなかった．診断を確定した検査法は喀痰培養陽性4名，尿中レジオネラ特異抗原検出22名，*L. pneumophila* SG1に対する血清抗体価有意上昇9名であった．確定診断患者34名のうち1名は尿中特異抗原検出と血清抗体価測定の双方が陽性であった．

3) 転帰

死亡者7名のうち6名は確定診断症例であり，1名は尿中レジオネラ特異抗原陽性でレジオネラ症と確定診断されていたが，他疾患により死亡となっている．この症例の直接死因となった疾患が何であったかは明かされていない．

3. 感染源の確定

4患者の喀痰から検出された*L. pneumophila* SG 1菌株と「お舟出の湯」の浴槽由来*L. pneumophila* SG 1菌株のDNA切断像が一致したので，「お舟出の湯」が感染源と特定された．

4. レジオネラ集団感染の要因

1) 汚染原因の推定

宮崎県福祉保健部レジオネラ症対策本部と宮崎

県福祉保健部がまとめた調査結果（中間報告）では，推定される汚染原因として次の8項目が列挙された．

(1) 源泉タンクの適切な清掃，消毒などの衛生管理が不十分だったこと．

(2) 定期的な清掃，消毒などの衛生面での担保がなされていない中温タンクの温泉水を高温水と混合して浴槽水に使用していたこと．

(3) 高温タンクの温度維持能力が不十分で，設定された58℃の温度を常時維持できなかったこと．

(4) 浴槽水の残留塩素濃度の測定が適切に行われなかったため，消毒に必要な塩素濃度が維持出来ていなかったこと．同時に，塩素注入装置の操作を十分理解しておらずメンテナンスが不十分であったこと．

(5) 常時浴槽の水位を満水状態としなかったため，営業時間内での湯水の入れ替えが不十分であったこと．

(6) ろ過装置の逆洗浄時間の設定が不十分で，ろ過槽内の汚れの排出が行われず，アメーバやレジオネラ属菌の増殖場所を提供してしまったこと．

(7) ヘアキャッチャーの清掃，消毒が不十分であったこと．

(8) 適切な衛生管理を行うためのマニュアル（手順書）が作成されていなかったこと．

我々の委員会はこれまでの調査結果を踏まえ，上記8項目に以下の3項目を加え，11項目を推測される汚染原因とした．

(9) 日向市や施設関係者にレジオネラ感染症についての知識・認識が不足していた．

(10) 施設引き渡し後に行った試運転などの操作で使用した水が配管に滞留し，そこにレジオネラ属菌が増殖し，それが体験入浴時の感染源になった可能性がある．

(11) 体験入浴前に湯の循環系統全体の消毒・清掃が不十分だったこと．

2) 温泉湧出量と熱源の関係（汚染原因(3)と(5)に関連）

温泉湧出量は最大180m³/日であるが，除鉄装置で処理しているため，実際の供給量は100m³/日程度，湯温は夏季で26℃であった．浴槽の数が多く浴槽容量は合計64.4m³のため，充分な換水を行うためには，供給湯量の調整や加温が必要であった．加温の熱源に電気を充てており，夜間に温泉水の温度を上げる設定であったため，日中に高温タンク水の温度が低下した．また，浴槽の水位が常時満水状態でなかったため汚濁の排出が妨げられる結果となった．

3) レジオネラ症防止指針とパンフレット（汚染原因(9)に関連）

レジオネラ症防止指針とパンフレット「よく知ろう　レジオネラ症」は日向保健所から日向市および温泉施設の担当者に手渡されていたが，現場ではこれらが認識されていなかったことが明白となった．

4) 施設公開日が感染初日（汚染原因(10)と(11)項に関連）

静岡県掛川市の嬬恋温泉「森林の湯」，茨城県石岡市のふれあいの里石岡「ひまわりの館」，東京都目黒区の特別養護老人ホームで，施設開設以来ほぼ1カ月経過後に感染事例が発生している．これに対して「お舟出の湯」では，温泉施設公開日の6月20日と21日の体験入浴者からそれぞれ5名と3名の疑診患者が出ていた．このことは施設公開日（体験入浴日）には既に浴槽を含む循環系にかなりのレジオネラ汚染があったためと想定される．開業前に試運転や湯張り・湯抜きなどの操作で入れた水は毎回きちんと排水しており残留水はないと担当者は考えていた．しかし「お舟出の湯」の配管系には最も低い部分に排水孔が設置されておらず，体験入浴前のレジオネラ検査も循環系全体の消毒・殺菌も実施されていなかったこともこの想定を裏付ける．水が滞留した器機を未消毒・未清掃のまま使用してレジオネラ肺炎の大規模集団発生を起こした例が報告されている[14)15)]．

5. レジオネラ属菌とレジオネラ感染症についての学習研修会

汚染源の推定で我々の委員会が挙げた第9項目への対応として，平成14年12月20日，日向市勤労青少年ホームで表記研修会を行った．これによ

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り肉眼で識別できる濁りと実際の菌数とを比較し，水が透明に見えても細菌学的には清潔でないことを示すと共に菌数算定方法，発育集落の性状，発育菌の臭気などを説明した．そのあとで，汚染・感染経路，レジオネラ肺炎についてスライドを用いて解説した．この研修会には日向市長，助役，収入役，市職員，サンパーク温泉職員，保健所職員，市議会議員，市内公衆浴場職員など約100名が参加した．

6．日向サンパーク温泉「お舟出の湯」に係る改善計画書

平成15年1月31日，表記改善計画書（発日商第480号）が山本孫春日向市長から宮崎県日向保健所長に提出された．その内容は，1．施設全般に係る衛生管理　2．管理体制の整備　3．施設の改善および　4．その他必要と思われる事項の4項目から成り，その概要は以下の通りである．

1）施設全般に係る衛生管理

(1) 日常の維持管理マニュアル作成と消毒・清掃体制確立．

(2) 浴槽水の満杯維持と溢水の確保．

・条件を満たさぬ浴槽で改造可能なものは改造．溢水不可能浴槽の廃止．

・浴槽数削減も検討．

・多人数入浴後の水位復旧加速のため，原湯補給配管を新設．

(3) 溢水湯は，循環・再利用せず捨て水とする．

(4) 残留塩素濃度は午前9時から終業まで，2時間毎に測定．

(5) ろ過装置は高濃度（10mg/*l* 以上）塩素水で，ろ材の汚れが排出されるのに十分な逆洗時間で毎日逆洗．

(6) 消毒装置を毎日点検管理．

(7) 毎日営業終了後，1mg/*l* 以上の塩素水循環（1時間以上）により浴槽と配管内を消毒．

(8) 集毛器を毎日消毒・清掃する．

(9) 受託管理会社が源泉タンク及びタンクから除鉄装置までの配管を2カ月に一度消毒・清掃．

(10) レジオネラ属菌検査は，開業直前に必ず実施し，試運転時期及び開業後1カ月は毎週1回，その後は毎月1回実施．試運転時期は，模擬開業として試験入浴を複数回実施．

(11) 濁度，過マンガン酸カリウム消費量および大腸菌群数の検査は毎月1回実施．

(12) 放流水の定期的水質検査（残留塩素濃度，水温，pH，大腸菌群数，BOD）

2）管理体制の整備

(1) (株)日向サンパーク温泉に衛生管理責任者と施設責任者を配属し，日常の衛生管理業務を社員が習熟するまでの期間，管理会社と共同で実施し，衛生管理業務日誌に毎日記録する．この記録は衛生管理責任者と施設責任者が確認する．記録は3年間保管する．

(2) 事故，機械の故障，火災，地震等緊急時の温泉施設，日向市，管理会社等の連絡網を構築し，迅速な対応を図る．

3）施設の改善

(1) 毎日完全換水型方式とするため浴槽への供給湯量を高める．

・除鉄装置能力を $6m^3/h$ 以上とし，源泉水と市水の活用を検討．

・源泉貯留槽と市水受水槽の新設を検討．

(2) 循環湯を浴槽低層部に近い所から出す配管に変更．

(3) 原湯は，循環配管に接続せず，浴槽水面上部から浴槽に落とし込む．

(4) 高温タンクの温泉水が常時60℃を保つよう熱源にはボイラーを併用．

(5) ろ過装置内のろ材を全て廃棄，全量を砂ろ材に交換．

(6) 配管等に排水ドレンを設置．

(7) 塩素剤の注入箇所はろ過装置の直前の循環配管に設置．

(8) 中温タンクを使用する場合はタンクの前に塩素注入装置を設置．

4）その他必要と思われる事項

(1) 遊離残留塩素濃度，水温，pH，水質と細菌検査結果等を常時掲示．

(2) 社員の資質向上：社内研修の実施，社員の講習会参加，社内伝達講習会開催などにより，知識の習得と意識の徹底を共有．

考　察

日向サンパーク温泉施設「お舟出の湯」での事例は，温泉施設の衛生管理と感染防止のいずれにも無経験の集団が，恐らく無経験であるとの確たる自覚も希薄なまま，郷土の繁栄の起爆剤になろうと温泉施設の設営と運営に集中した結果，起こるべくして起こった不幸な集団感染であったと言わねばならない．明文化された「ボイラー使用規制」を確認しなかったことに見られるように，曖昧さを確認せずに済ます姿勢があったと思う．保健所から手渡されたという「レジオネラ症防止指針」とパンフレット「よく知ろう　レジオネラ症」の意義や重要性が日向市および施設関係者に認識されていなかったことも事実である．一旦，事故がおきた後には，対策委員会をはじめ様々な対応が急遽実施されるのに対して，事前の予防措置を関係者に認知・徹底させることの難しさを認識させられた．

平成14年9月3日付けで厚労省健康局の結核感染症課長と生活衛生課長の連名で出された健感発および健衛発第0903001号の「レジオネラ症患者の発生時等の対応について」には，集団感染発生時の対処法が簡潔・適正に記されている．しかし英国放送本部の屋上冷却塔が原因で発生した大規模集団感染に際し，行政ならびに関係機関が，一本化された指揮命令系統のもとで，通報を受けてから3日以内に必要な対策を立案し迅速に行動したことを，我々は学ばねばならない[16]．平成14年10月29日付けで厚生労働省健康局長から健発第1029004号として出された「条例等にレジオネラ症発生防止対策を追加する際の指針について」の中で，多孔質のろ材（多孔質のろ材砕石を「砂」と称して使用することも含む．）でろ過器に微生物を繁殖させて湯水を浄化する方式（いわゆる生物浄化方式）の循環式浴槽は，好ましくない．ことが明記された．「お舟出の湯」でもセラミックろ材と砂を混同し，セラミック砕粉を砂として使用していた．今後この点に対する業者並びに関係者の注意を喚起する必要がある．

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付記：「お舟出の湯」は平成15年10月31日まで営業停止であったが，施設・設備・管理体制の改善と試験入浴成績の好結果から，営業停止処分は10月23日に繰り上げ解除され，11月13日から営業再開した．

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An Outbreak of Legionellosis in a New Facility of Hot Spring Bath in Hiuga City

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Following cerebrating ceremony in 20 June 2002, for the completion of Hiuga Sun-Park Hot Spring Bath "Ofunade-no-Yu" facilities, Miyazaki Prefecture, Kyushu Island, 200 neighbors were invited each day to experience bathing on 20 and 21 June. The Bath "Ofunade-no-Yu" officially opened on 1 July 2002. On 18 July, Hiuga Health Center was informed that 3 suspected *Legionella* pneumonia patients in a hospital and all of them have bathing history of "Ofunade-no-Yu". Health Center officers notified Hiuga City, the main proprietor of the Bath business, that on-site inspection on sanitary managements will be done next day and requested the City to keep the bath facilities as they are. On 19 July, Health Center officers collected bath water from seven places and recommended voluntary-closing of "Ofunade-no-Yu" business. Because of various reasons, Hiuga City did not accept the recommendation and continued business up to 23 July. Because *Legionella pneumophila* serogroup 1 strains from 4 patients' sputa and several bath water specimens were determined genetically similar by Pulsed Field Gel Electrophoresis of Sfi I-cut DNA, "Ofunede-no-Yu" was regarded as the source of infection of this outbreak. On 24 July, "Ofunade-no-Yu" accepted the Command to prohibit the business. Among 19, 773 persons who took the bath during the period from 20 June to 23 July, 295 became ill, and 7 died. Among them, 34 were definitely diagnosed as *Legionella* pneumonia due to *L. pneumophila* SG 1, by either one or two tests of positive sputum culture, *Legionella*-specific urinary antigen, and significant rise of serum antibody titer against *L. pneumophila* SG 1.

In addition to the 8 items shown by Miyazaki-Prefecture Investigation Committee as the cause of infection, Hiuga City Investigation Committee pointed out following 3 items：1) Insufficient knowledge and understanding of stuffs on *Legionella* and legionellosis；2) Residual water in tubing system after trial runs might lead multiplication of legionellae in it；and 3) Inadequate disinfection and washing for whole circulation system prior the experience bathing.

The Hiuga City Committee directed 24 measures to improve the sanitary condition of the facility including following 5 items. 1) Fix the manual for maintenance and management of the bath. 2) Keep sufficient overflow of bath water. 3) Put disinfection of filters into practice. 4) Precise measurement and control of the residual chlorine concentration in bath water. 5) Replacement of filtrating material from crushed porous ceramic into natural sand.

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A 3-Year Follow-Up Study of Anti-*Legionella* Antibodies in Users of Japanese 24-Hour Hot Water Baths

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Abstract: A 3-Year Follow-Up Study of Anti-*Legionella* Antibodies in Users of Japanese 24-Hour Hot Water Baths: Masahiro IRIE, *et al.* Institute of Health Science, Kyushu University—Although it has been found that legionellae can exist in a 24-h hot water bath (24HHWB), which has been used recently in Japan, whether longer use of the 24HHWB causes legionellosis is unclear. The present longitudinal study was conducted in 2000 to investigate the 3-yr change in antibody titers in association with the continuous use, non-use, or canceling the use of the 24HHWB, and possible factors relating to the antibody changes. Ninety-two subjects (85 males and 7 females), who had had their anti-*Legionella pneumophila* (Lp) serum antibody titers measured in our initial study in 1997 and consented to blood sampling 3 yr later, were selected as subjects. There were no clinical cases who had experienced Legionnaires' disease or Pontiac fever during the 3 yr. The continuous users showed no significant changes in antibody titers within 3 yr, whereas the continuous non-users had a significant increase in antibody titers against the Lp serogroup (SG) 5 and 6. Eleven ex-users of the 24HHWB showed a significant decrease in antibody titers against Lp SG 6. The changes in the 24HHWB use, job sector, stress coping strategies, and alcohol-drinking habit were associated with the changes in antibody titers against Lp SG 1, 5 or 6. The anti-Lp antibodies were considered to be IgM dominant. In conclusion, this study indicates that 24HHWB use by healthy subjects does not tend to result in a higher onset risk of legionellosis, even if it is continuously used for 3 yr, although 24HHWB use is likely to induce production of antibodies against legionellae.
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Key words: *Legionella*, Infection, Antibody, Recycled bath water, Stress, Follow-up

Legionellae are important causative agents in the development of both nosocomial and community-acquired pneumonias[1-4]. Among various kinds of legionellae, *Legionella pneumophila* (Lp) has been considered as a representative etiological pathogen of legionellosis, Legionnaires' disease and Pontiac fever[1-4]. Legionellae are widely found in most natural water sources, but in low concentrations[1]. These bacteria readily flourish within amoebae in warm water sources, particularly in man-made apparatuses that maintain water at warm temperatures and produce aerosols[2]. When airborne water droplets contaminated with legionellae in such water sources are inhaled, they can survive and replicate inside alveolar macrophages by preventing phagosome-lysosome fusion and cause pneumonias[3]. Clinically infected patients and those who have had subclinical exposure produce humoral antibodies to legionellae[4]. Thus, the production of anti-Lp serum antibodies reflects the exposure to legionellae, and increased titers can be used to assess the risk of legionellosis retrospectively.

Since the early 1980s, the 24-h hot water bath (24HHWB), which maintains a consistent temperature all day long with the use of recycled bath water, has become a new style of bath in Japan because of its water conservation and convenience. As shown in Fig. 1, a total of 1,550,910 units were sold in Japan from 1984 to 2003. Japan's population being approximately 127 million, roughly 1% of Japanese are assumed to own a 24HHWB. This rate could be larger in view of the fact that a bath may be used by more than one family member. Water in the 24HHWB is purified mainly by biological cleaning with microorganisms naturally occurring in the filtering section[5]. In brief, waste matter derived from the 24HHWB users is consumed as nutrients by bacteria

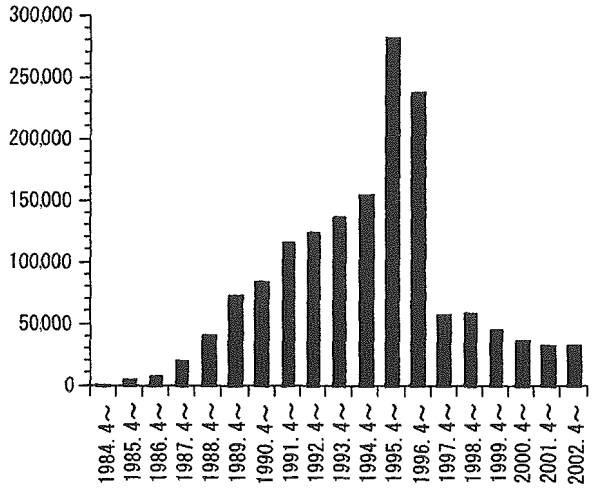

Fig. 1. Changes in the number of 24HHWB units sold in Japan. After the detection of *Legionella pneumophila* in this apparatus in 1996, sales of the 24HHWB have fallen off considerably. (Data were obtained from the 24HHWB association with permission).

for their growth, and amoebae phagocyte the bacteria. The bath water is then cleaned and reused after the use of physical disinfectants, such as heat, ultraviolet, or ozone. This biological activation of microorganisms resulted in the isolation of Lp, which can grow within amoebae, from this apparatus in 1996[5]. Nagai *et al.*[6] recently reported the first case of neonatal Legionnaires' disease associated with water birth in the 24HHWB. In comparison with the large number of 24HHWB units sold in Japan, however, the number of cases of legionellosis due to 24HHWBs is extremely small, possibly due to physical disinfectants used or other countermeasures such as chlorine use. Nevertheless, sales of the 24HHWB have fallen off remarkably since the detection of Lp in 1996[5] because of fear of *Legionella* infection (Fig. 1). As a result of such economic damage, the number of members of the 24HHWB association, consisting of the 24HHWB manufacturing and marketing corporations, has decreased from 44 to 14 due to bankruptcy or withdrawal. In addition, with respect to the occupational problems related to *Legionella* infection, Straus and coworkers[7] reported that legionellosis was associated with working more than 40 h a week. The possibility of legionellosis due to using 24HHWBs has therefore been an important problem in view of not only public health, but also occupational health.

To clarify the risk of *Legionella* infection due to the use of 24HHWBs and other factors, we initially investigated the prevalence of anti-Lp serum antibody titers in 24HHWB users and non-users, and also analyzed the possible factors causing the antibody production[8]. As a result, an increase in the antibody titers in dilutions of ≥8 against Lp serogroup (SG) 5 and Lp SG 6 was found in the 24HHWB users compared to the 24HHWB non-users, though the 24HHWB use resulted in subclinical infection. Stress-related factors, bathtub materials, and lifestyles regarding bathing also seemed to contribute to the increase in antibody titers. No significant correlations were observed retrospectively between the duration of 24HHWB use and the antibody titers. We next conducted a one year follow-up study and found that the continuous use of 24HHWBs did not cause an apparent increase in the production of anti-Lp antibodies, when compared to the 24HHWB non-users[9]. These studies suggested that immunological sensitization tended to occur when healthy subjects were exposed to legionellae not only by the 24HHWBs, but also by other bio-psycho-socio-ecological factors. It was also suggested that the risk of legionellosis was not increased with prolonged use of the 24HHWBs. Nevertheless, the interpretation of the relationship between the antibody-related factors, including the use of the 24HHWBs, and increased antibody titers and the time course of the antibody titers in the 24HHWB users, were relatively limited because of the cross-sectional nature of the study or the short time observation. It still remains unclear whether longer use of the 24HHWBs causes higher susceptibility to legionellosis. Moreover, sufficient evaluation of the effects of countermeasures to prevent the infection (chlorine-disinfection etc.) could not be made, because of the very small number of subjects investigated.

The present longitudinal study was thus carried out 3 yr after our initial study, to investigate the possibility of legionellosis due to the continuous use of the 24HHWBs, the changes in the anti-Lp serum antibody titers during 3 years, the effects on the antibody titers of the cancellation or initiation of 24HHWB use, the effects on the antibody titers of the bactericidal improvements in the 24HHWBs and the use of chlorine disinfectant, and the factors relating to the changes in the antibody titers during 3 yr.

Materials and Methods

Subjects

Participants were recruited from 134 employees of a 24HHWB manufacturing corporation (Showa Manufacturing Co., Ltd.) whose anti-Lp serum antibody titers had been measured in 1997[8]. A total of 92 subjects (85 men and 7 women) agreed to participate in the present study in 2000, and gave a written form of informed consent. They ranged in age from 23 to 60 yr (mean ± S.D., 45.1 ± 9.6). The number of participants decreased compared to that in 1997 (n=204), because one of two corporations investigated in 1997 had gone bankrupt after the 1997 study[8].

The demographic characteristics of the subjects are shown in Table 1. This group did not differ from the

Table 1. Characteristics of the subjects

Characteristics	Category	n	%
Age	<30	8	8.7
	30–39	18	19.6
	40–49	32	34.8
	≥50	34	37.0
Sex	Men	85	92.4
Types of work	White collar	65	70.7
Work position	Executive	3	3.3
	Director	9	9.8
	Section head	14	15.2
	Chief clerk	20	21.7
	Clerk	46	50.0
24-h hot water bath	Continuous users	30	32.6
(24HHWB)	Continuous non-users	46	50.0
	Past users	4	4.3
	Ex-users	11	12.0
	New users	1	1.1

group in 1997 in the demographic and lifestyle characteristics, so that the effect of the decrease in the number of subjects on the outcome was considered to be negligible. There were no subjects who had immunosuppressing conditions (eg, cancer, HIV infection/AIDS, organ or bone marrow transplantation, kidney dialysis, use of immunosuppressive medications). The subjects in the present study included: 30 subjects continuously using the 24HHWB since before the 1997 study[8] (continuous users); 46 never having used the 24HHWB (continuous non-users); 4 using the 24HHWB only before the 1997 study[8] (past users); 11 ceasing to use the 24HHWB after the 1997 study[8] (ex-users), and 1 starting to use the 24HHWB during the 3 yr interval (new user). The water filter units of the 24HHWBs consisted of activated charcoal, filter gravel, and either coral or granite porphyry, and were equipped with an ultraviolet lamp. These bactericidal systems were generally used in 24HHWBs developed by other corporations. Thirteen continuous users of the 24HHWB were provided with antibacterial material 3 months after our initial study[8]. Four continuous users changed the filter materials of the 24HHWB during 3 yr. Six continuous users began to disinfect the bath water by using chlorine (0.9 g) every other day about 3 months after the initial study[8]. Based on the previous study[10], in which viable legionellae were undetectable within 15 min in a sodium hypochloride solution with 0.4 mg/l free chlorine, all of the chlorine users had a sufficient residual chlorine concentration (≥0.5 mg/*l*) at least 15 min after the use of chlorine.

Data collection

A self-administered questionnaire was provided to these 92 subjects in May, 2000. In accordance with the 1997 study[8], the questions addressed demographic factors, present and past disease histories of the subjects and their family members, bathing-related factors, contacts with legionella-inhabiting environments, lifestyles regarding cigarette smoking and alcohol drinking, the 60-item version of the General Health Questionnaire (GHQ)[11] and its subscales (somatic symptoms, anxiety and insomnia, social dysfunction, severe depression), and the possibility of relieving considerable stress. The questions about disease histories included physician-diagnosed pneumonia (supposed to include Legionnaires' disease) and influenza-like symptoms (supposed to include Pontiac fever), and other diseases, such as bronchitis, gastroenteritis, and fever of unknown origin. The 24HHWB users were also asked about the duration of use. Besides these questions, stress coping items from the NIOSH general job stress instrument[12] were added to the questionnaire in the present study. Stress-related factors were associated with the anti-Lp antibody titers in the 1997 study[8], so that stress coping strategies, which play a significant role in stress responses, might be associated with the change in antibody titers. The stress coping items had also been taken, but not used, in the 1997 study[8]. The items measure six dimensions of stress coping strategies regarding planning, neglect, self-blame, wishful-thinking, consultation, and involvement. All of the items indicate higher distress or poorer coping patterns as their scores become higher. In addition, fasting blood samples were taken early in the morning on weekdays.

The anti-Lp antibody titers of the 92 subjects were measured by means of a microplate agglutination test

Table 2. Comparisons of the titers of each anti-*L. pneumophila* antibody between 1997 and 2000

L. pneumophila serogroups	Antibody titer								p value†	p value††
	<8	8	16	32	64	128	256	512		
L. pneumophila serogroup 1										
1997	90 (92)	0 (0)	1 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	ns	ns
2000	87 (91)	1 (0)	4 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
L. pneumophila serogroup 3										
1997	86 (92)	1 (0)	3 (0)	1 (0)	1 (0)	0 (0)	0 (0)	0 (0)	ns	ns
2000	83 (90)	5 (2)	3 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
L. pneumophila serogroup 4										
1997	91 (92)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	ns	ns
2000	90 (92)	0 (0)	2 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
L. pneumophila serogroup 5										
1997	86 (90)	4 (2)	2 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	ns	ns
2000	80 (92)	7 (0)	2 (0)	1 (0)	2 (0)	0 (0)	0 (0)	0 (0)		
L. pneumophila serogroup 6										
1997	62 (83)	19 (5)	6 (2)	1 (1)	1 (0)	2 (1)	1 (0)	0 (0)	ns	ns
2000	63 (81)	13 (4)	11 (3)	5 (4)	0 (0)	0 (0)	0 (0)	0 (0)		

Numbers in the table are subjects with total antibody titers, and in the parentheses are subjects with 2-mercaptoethanol-treated antibody titers. A Wilcoxon signed-ranks test was used to compare the distributions of total antibody titers (†) or 2-mercaptoethanol-treated antibody titers (††) between 1997 and 2000.

(MPAT), which is described in detail elsewhere[8]. The antigens were prepared from Lp SG 1 (ATCC 33152), SG 3 (ATCC 33155), SG 4 (ATCC 33156), SG 5 (ATCC 33216) and SG 6 (ATCC 33215). The agglutination reaction was observed after serial dilutions (≥8) of each serum sample. Anti-Lp IgG antibody titers were also examined after treatment of the serum with 0.2 mol/l 2-mercaptoethanol (2ME), which has been reported to destroy IgM antibodies[13].

Statistical methods

Data analysis was performed by means of SAS, version 6.11 (SAS Institute Inc, Cary, NC). Statistical analysis was conducted by comparing the distribution of anti-Lp antibody titers between 1997 and 2000 by means of the Wilcoxon signed-ranks test. To investigate the factors in the questionnaire possibly relating to the antibody titers in 1997 or 2000, Spearman's correlation coefficients between various factors and the antibody titers in each year were calculated. A total of 35 previously investigated variables (age, sex, body mass index (BMI), cigarette smoking, alcohol drinking, frequency of bathing in a week, duration of bathing time, types of methods of heating bath water, materials of bathtub, use of air-conditioners at the work place, use of humidifiers at home, use of well water at home, use of hot-water supply system at home, contacts with soil during gardening, GHQ score, possibility of relieving considerable stress), including multiple choices and the GHQ subscales in the questionnaire, and job sector, total score of stress coping strategies and the duration of the 24HHWB use were analyzed in relation to the antibody titers. Spearman's correlations between the changes in the selected factors relating to the antibody titers in each year and the antibody titers within 3 years were also estimated to analyze their longitudinal relationships. In addition, the factors related to the changes in the antibody titers in each SG from 1997 to 2000 were examined by multiple regression analyses, where model selections were done with minimum Mallow's C (p) as the criterion. Differences were considered significant if $p<0.05$.

Results

Clinical features of Legionnaires' disease or Pontiac fever from 1997 to 2000

No clinical cases diagnosed with either Legionnaires' disease or Pontiac fever were found within 3 yr, not only among the 24HHWB users, but also among the other subjects in the present study. Moreover, the 24HHWB users did not show higher rates of influenza-like symptoms (supposed to include Pontiac fever) than the others. No cases of fever of unknown origin during 3 yr were observed in the 24HHWB users in the present study.

Comparisons of anti-Legionella antibody titers between 1997 and 2000

Table 2 shows the distributions of total and 2ME-treated antibody titers in 1997 and 2000. In 2000, the

Table 3. Comparisons of the titers of each anti-*L. pneumophila* antibody between 1997 and 2000 in continuous 24HHWB users and continuous non-users

L. pneumophila serogroups	Antibody titer								p value†	p value††
	<8	8	16	32	64	128	256	512		
L. pneumophila serogroup 1										
Users (n=30)										
1997	29 (30)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	ns	ns
2000	28 (30)	0 (0)	2 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
Non-users (n=46)										
1997	45 (46)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	ns	ns
2000	44 (46)	1 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
L. pneumophila serogroup 3										
Users (n=30)										
1997	26 (30)	1 (0)	2 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	ns	ns
2000	26 (29)	2 (1)	2 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
Non-users (n=46)										
1997	44 (46)	0 (0)	1 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	ns	ns
2000	42 (45)	2 (1)	1 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
L. pneumophila serogroup 4										
Users (n=30)										
1997	30 (30)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	ns	ns
2000	29 (29)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
Non-users (n=46)										
1997	45 (46)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	ns	ns
2000	45 (45)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
L. pneumophila serogroup 5										
Users (n=30)										
1997	24 (30)	4 (0)	2 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	ns	ns
2000	26 (30)	3 (0)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)		
Non-users (n=46)										
1997	46 (46)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	$p<0.05$	ns
2000	41 (46)	2 (0)	2 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)		
L. pneumophila serogroup 6										
Users (n=30)										
1997	14 (26)	10 (2)	3 (1)	0 (1)	1 (0)	2 (0)	0 (0)	0 (0)	ns	ns
2000	20 (26)	4 (1)	3 (1)	3 (2)	0 (0)	0 (0)	0 (0)	0 (0)		
Non-users (n=46)										
1997	41 (44)	2 (0)	2 (1)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	$p<0.05$	ns
2000	31 (41)	9 (2)	5 (2)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)		

Numbers in the table are subjects with total antibody titers, and in the parentheses are subjects with 2-mercaptoethanol-treated antibody titers. A Wilcoxon signed-ranks test was used to compare the distributions of total antibody titers (†) or 2-mercaptoethanol-treated antibody titers (††) between 1997 and 2000.

investigated antibody titers were generally low and the maximum value amounted to 64-fold. No significant differences in the distributions of total or 2ME-treated antibody titers were found in any SG. When the subjects were analyzed further by division into continuous users, continuous non-users and others, the antibody titers of the continuous non-users showed a significant increase from 1997 to 2000 against Lp SG 5 and 6 (Table 3). On the other hand, the antibody titers of the continuous users showed no significant increase within these 3 yr. As a whole, the antibody titers markedly decreased after the 2ME-treatment (Tables 2 and 3). No significant differences were found in the 2ME-treated antibody titers between 1997 and 2000, irrespective of the SGs. Among the 11 ex-users, 7 subjects showed a decrease in the antibody titers against Lp SG 6 (Wilcoxon signed-ranks

Table 4. Spearman's correlation coefficients between several factors and the titers of each anti-*L. pneumophila* antibody in 1997

Variables	N	Anti-*L. pneumophila* antibody titers						
		SG 1	SG 3	SG 4	SG 5	SG 5‡	SG 6	SG 6‡
Average number of baths in a week	92	–0.14	0.00	–0.24*	–0.05	–0.15	0.09	–0.05
Frequency of use of air-conditioners	86	–0.03	–0.06	–0.02	0.11	0.33**	–0.05	0.10
Present 24HHWB use (No/Yes)	92	0.01	0.11	–0.10	0.36**	0.16	0.46**	0.20
Present or past 24HHWB use (No/Yes)	92	0.01	0.09	–0.10	0.33**	0.15	0.44**	0.17

SG=Serogroup. ‡Antibody titers after treatment with 2-mercaptoethanol. *p<0.05. **p<0.01.

Table 5. Spearman's correlation coefficients between several factors and the titers of each anti-*L. pneumophila* antibody in 2000

Variables	N	Anti-*L. pneumophila* antibody titers					
		SG 1	SG 3	SG 4	SG 5	SG 6	SG 6‡
Age (yr)	92	–0.11	–0.14	–0.19	–0.21*	–0.08	0.04
Body mass index (kg/m^2)	92	–0.18	–0.22*	–0.22*	–0.26*	–0.02	0.13
Duration of 24HHWB use (months)	43	–0.34*	–0.17	–0.15	–0.31*	0.03	0.17
Frequency of use of air-conditioners	81	–0.16	0.02	–0.24*	–0.01	–0.05	0.06
Use of humidifiers (No/Yes)	71	–0.11	0.02	0.15	–0.05	0.24*	0.31**
Inadequate stress coping strategies	84	0.09	0.17	0.13	0.23*	0.14	–0.06
Social dysfunction in the GHQ test	90	0.13	0.24*	0.07	0.14	–0.03	0.02
Use of antibacterial materials (No/Yes)	30	0.16	–0.02	0.21	0.01	0.31	0.38*

SG=Serogroup. ‡Antibody titers after treatment with 2-mercaptoethanol. *p<0.05. **p<0.01.

test, p<0.05). One new user, with all negative responses (<8) in 1997, was found to be positive for Lp SG 1 (1:16), 3 (1:8), 5 (1:32), and 6 (1:16).

Relationships between various factors and anti-Legionella antibody titers in 1997

Table 4 shows Spearman's correlation coefficients between the factors in the questionnaire and the antibody titers in 1997. The relationships between various factors and the 2ME-treated antibody titers could be analyzed only against Lp SG 5 and 6, since there were no or too few subjects who indicated positive titers (≥8) against Lp SG 1, 3, and 4. The average number of baths in a week was negatively related to the antibody titers against Lp SG 4. A positive correlation was observed between the frequency of the use of air-conditioners and the 2ME-treated antibody titers against Lp SG 5. Present 24HHWB use or present or past use of a 24HHWB was positively associated with the antibody titers against Lp SG 5 and 6.

Relationships between various factors and anti-Legionella antibody titers in 2000

Table 5 shows Spearman's correlations between the factors in the questionnaire and the antibody titers in 2000. The relationships between various factors and the 2ME-treated antibody titers could be analyzed only against Lp SG 6, since there were no or too few subjects who indicated positive titers (≥8) against Lp SG 1, 3, 4 and 5. The duration of 24HHWB use was negatively correlated to the antibody titers against Lp SG 1. Social dysfunction (GHQ subscale) was positively, whereas BMI was negatively, related to the antibody titers against Lp SG 3. The frequency of the use of air-conditioners and BMI were negatively correlated to the antibody titers against Lp SG 4. Age, BMI and the duration of 24HHWB use were negatively, whereas overall inadequate stress coping strategies were positively correlated to the antibody titers against Lp SG 5. There was a positive relationship between the use of humidifiers and the antibody titers against Lp SG 6. The use of humidifiers and the use of antibacterial materials were positively associated with the 2ME-treated antibody titers against Lp SG 6.

Relationships between the changes in factors and anti-Legionella antibody titers within 3 yr

Table 6 shows the Spearman's correlations between the changes in the antibody-related factors within 3 yr and the antibody titers within 3 years. Four blue collar workers changed to white collar workers after the 1997 study[8] because of the downsizing of the 24HHWB manufacturing section. The change in job sector, in which a shift from blue collar to white collar was dominant

Table 6. Spearman's correlation coefficients between the changes in the selected factors and the changes of the titers of each anti-*L. pneumophila* antibody during 3 yr

Variables	N	Anti-*L. pneumophila* antibody titers						
		SG 1	SG 3	SG 4	SG 5	SG 5‡	SG 6	SG 6‡
Change in job sector (Blue collar/White collar)	92	−0.16	0.00	−0.01	−0.21*	0.01	−0.20	−0.23*
Change in use of humidifiers (Non-use/Use)	71	−0.04	−0.14	−0.02	0.08	0.26*	0.09	0.26*
Change in the possibility of relieving considerable stress (Much/Somewhat/No)	60	−0.09	−0.06	0.00	0.14	0.30*	0.19	0.18
Change in 24HHWB use (Yes/No)	92	−0.14	−0.11	−0.02	−0.16	−0.37**	−0.32**	−0.09

SG=Serogroup. ‡Antibody titers after treatment with 2-mercaptoethanol. *$p<0.05$. **$p<0.01$.
Items in parentheses show dominant changes from 1997 (left side) to 2000 (right side), and the coefficients indicate correlations between the changes in the variables as shown in parentheses and the changes in the antibody titers.

Table 7. Variables related to the changes in the titers of each anti-*L. pneumophila* during 3 yr by multiple regression analyses

Variables	Parameter estimate	SE	Standardized estimate	*p*
Serogroup 1 (n=83)				
Change in 24HHWB use (Yes/No)	−0.07	0.03	0.22	0.04
Serogroup 5 (n=83)				
Change in 24HHWB use (Yes/No)	−0.55	0.24	−0.23	0.02
Change in job sector (Blue collar /White collar)	−0.87	0.31	−0.28	0.01
Change in stress coping strategies (adequate/inadequate)	0.08	0.03	0.26	0.02
Change in alcohol-drinking habit (No/Yes)	−0.72	0.26	−0.30	0.01
Serogroup 6 (n=91)				
Change in 24HHWB use (Yes/No)	−0.84	0.35	−0.24	0.02
Change in job sector (Blue collar /White collar)	−1.03	0.50	−0.21	0.04

Intercept terms were omitted. Dominant changes from 1997 (left side) to 2000 (right side) are shown in the parentheses.

during 3 yr, was negatively associated with the change in the antibody titers against Lp SG 5 and the 2ME-treated Lp SG 6. The change in the use of humidifiers, in which an increase in use was dominant, was positively related to the change in the antibody titers against the 2ME-treated Lp SG 5 and 6. The change in the possibility of relieving considerable stress, in which a decrease in the possibility was dominant, was positively related to the change in the antibody titers against the 2ME-treated Lp SG 5. The change in 24HHWB use, in which a decrease in use was dominant, was negatively associated with the change in the antibody titers against Lp SG 6 and the 2ME-treated Lp SG 5.

Factors related to the changes in the antibody titers of each anti-Legionella within 3 yr by multiple regression analyses

As shown in Table 7, the change in 24HHWB use, in which canceling of the use was dominant, was significantly associated with a decrease in the antibody titers against Lp SG 1, 5 and 6. The change in job sector, in which a shift from blue collar to white collar was dominant, was significantly related to a decrease in the antibody titers against Lp SG 5 and 6. The change in alcohol-drinking habit, in which an increase in the alcohol use was dominant, was significantly related to a decrease in the antibody titers against Lp SG 5. The change in stress coping strategies, in which exacerbation of the strategies was dominant, was significantly associated with an increase in the antibody titers against Lp SG 5.

Discussion

In accordance with our previous findings that most of the anti-Lp antibody titers of the 24HHWB users were generally not higher than those of healthy Japanese subjects[8], the antibody titers were generally low and did not have values high enough to be an etiologic agent of legionellosis in 2000. This result supports the clinical

observations in the present and previous study[8] that there were no cases who had had Legionnaires' disease or Pontiac fever after they started to use the 24HHWB. The anti-Lp antibodies were considered to be IgM dominant, since the antibody titers considerably decreased after the 2ME-treatment, and this was compatible with the findings of our previous study[8].

As a whole, it is recognized that the factors relating to the antibody titers in 2000 differ from those in 1997. These findings indicate that there is considerable variation in the factors relating to the anti-Lp antibodies among 24HHWB users and non-users. As to the reasons, it might have been due to the decreased number of subjects in the present study, because the factors related to the antibody titers of the 92 subjects in 1997 were somewhat different from those of the 204 subjects in 1997[8]. Another possible reason is the changes in the 24HHWB use. Indeed, change in the 24HHWB use was found to be the factor relevant to the changes in the antibody titers against Lp SG 1, 5 and 6 from 1997 to 2000. The antibody titers significantly decreased in the cases where the use of the 24HHWBs was canceled after the 1997 study[8]. The decrease in the antibody titers is not unusual, because antibodies were considered to be IgM, but not IgG, dominant. Nevertheless, it must be kept in mind that unknown factors, which were not investigated in either year, might have affected the variation.

In the present study, anti-Lp SG 5 and 6 antibody titers by correlation and regression analyses, and anti-Lp SG 1 antibody titers by regression analysis, showed a decrease in relation to the cancellation of the 24HHWB use. This seems to support our previous findings that the 24HHWB use was associated with the production of the antibodies against Lp SG 5 and 6[8]. Among other factors related to the antibody titers, the change in job section seemed to play a significant role in the changes in the antibody titers against Lp SG 5 and 6. The shift from blue collar to white collar was dominant, and this change was associated with a decrease in the antibody titers during 3 yr. It may be possible to consider that blue collar workers are likely to be susceptible to *Legionella* infection during the manufacturing process or at the time of maintenance of the 24HHWBs. The use of humidifiers was positively correlated to the antibody titers against total and 2ME-treated Lp SG 6 in 2000, and an increase in the use of humidifiers was associated with an increase in the antibody titers against 2ME-treated Lp SG 5 and 6 during 3 yr. The results suggest that the humidifier is a causative factor in legionellosis. Humidifiers have been reported to be able to be responsible for the pathogenesis of legionelosis, mainly owing to Lp SG 1[14]. Our findings seem to support such previous observations, although the humidifier-related SGs are different. The use of air-conditioners was positively related to the antibody titers against 2ME-treated Lp SG 5 in 1997, whereas it was negatively associated with the antibody titers against Lp SG 4 in 2000. Since no consistent associations could be found in these analyses, the use of air-conditioners might not be such an important factor related to the antibody production.

As for stress-related factors, no report had been published on the effect of stress in the pathogenesis of legionellosis. We initially found that depressive state assessed by the GHQ was possibly and positively related to the antibody titers against Lp SG 6, and a decrease in the possibility of relieving considerable stress was associated with the increased titers against Lp SG 6 in 1997[8]. We could not confirm the results concerning depressive state in the present longitudinal study, but a decrease in the possibility of relieving considerable stress showed a significant correlation with an increase in the antibody titers against 2ME-treated Lp SG 5, but not Lp SG 6, as a longitudinal variable, although the association was different in SGs between 1997 and 2000. Social dysfunction assessed by the GHQ tended to be positively related to the antibody titers against Lp SG 3 in 1997, although it was not significant[8]. In accordance with such previous findings, social dysfunction was positively correlated to the antibody titers against Lp SG 3 in 2000. In addition, inadequate stress coping was a significant predictor of the increase in the antibody titers against Lp SG 5 in the present study. Taking these findings into consideration, stress-associated depression of immune functions seems to be a factor related to the risk of legionellosis.

A comparison of the antibody titers between 1997 and 2000 indicated that the antibody titers of the continuous non-users showed a significant increase from 1997 to 2000, exclusively against Lp SG 5 and 6. The results appear to be partially consistent with the findings of our follow-up study carried out in 1998, which showed that anti-Lp SG 6 antibody titers increased from 1997 and this tendency was particularly remarkable in the continuous non-users[9]. Several factors that were positively associated with the changes in total or 2ME-treated antibody titers against Lp SG 5 and 6 during 3 yr, such as the use of humidifiers, a decrease in the possibility of relieving considerable stress, and inadequate stress coping strategies, might have contributed to the results, although the subjects investigated were not limited to continuous non-users because of the small number of subjects. We cannot neglect the possibility that unknown factors, which were not investigated in either year, could have affected the results, so that the precise reason for the increased antibody titers against Lp SG 5 and 6 in continuous non-users remains unknown.

Since the risk of legionellosis in relation to 24HHWB use has become a great public concern, manufacturers have tried to develop bactericidal techniques and improve the water recycling units by, for example, altering the

filter materials. Further countermeasures, such as disinfection with chlorine, have also been employed. In this longitudinal study, however, we could not find obvious evidence that the use of antibacterial substances or the alteration of filter materials decrease the antibody titers (data not shown). On the contrary, the use of antibacterial materials was positively associated with the 2ME-treated antibody titers against Lp SG 6. Rather, it was suggested that the cancellation of 24HHWB use would largely result in a decrease in the antibody titers. In this study, however, no significant increase in the antibody titers was observed in the continuous users during 3 yr, when compared to the continuous non-users who showed a significant increase in the antibody titers against Lp SG 5 and 6. That is to say, it can hardly be assumed that the risk of legionellosis is increased by prolonged use of 24HHWBs, so that we could not completely rule out the possibility that the countermeasures, such as improvements in apparatuses by the manufactures, might contribute to protecting 24HHWB users from legionellosis, although we could not draw conclusions because of the small number of subjects investigated. As for the countermeasures to legionellae, it is therefore supposed that not only short-term inhibitory effects, but also long-term disinfectant effects of the 24HHWBs on legionellae should be further investigated.

Several problems as previously described in our study[8] must also be considered in discussing our findings. First, the power of this study to demonstrate the factors related to the antibody titers was relatively limited because of the small number of samples analyzed and low levels of the titers. Second, the possibility that the MPAT method might have included cross-reactivity with other organisms could not be completely neglected. Third, as mentioned above, there is a possibility that uninvestigated unknown factors might have affected the outcome. For example, there have been some outbreaks of legionellosis in users, particularly older persons, of circulating bath water at public bath houses in recent years[15]. The cleaning mechanisms for bath water are physical filtration and chlorine-disinfection, which are different from those of 24HHWBs. Nevertheless, inadequate disinfection in the filtering system leads from the physical filtration system to the biological cleaning system, because bacteria, including legionellae, inhabit the filtering system. We could not neglect the possibility that the subjects in the present study might have visited public bath houses, and this might have influenced the outcome. Demographic factors described in Table 1, such as sex, type of work, and work position, were at least unrelated to the antibody titers in 2000. Fourth, the reason why an increase in the alcohol use was significantly related to a decrease in the antibody titers against Lp SG 5 remained unclear. Fifth, the fact that the subjects were limited to employees in a 24HHWB manufacturing corporation might have included a selection bias, because the employees might be more careful in maintaining their 24HHWBs than consumers in general. Sixth, whether our findings can be applied to other 24HHWB users using different disinfecting mechanisms needs further consideration.

Despite the limitations, this longitudinal study indicates that the longer use of 24HHWBs manufactured by a corporation does not tend to result in a higher risk of the onset of legionellosis, although the use of the 24HHWBs is likely to induce production of antibodies against legionellae. Except for our studies, there have been no reports in which the risk of legionellosis in relation to 24HHWB use is examined by the antibody titers in a number of cases. The findings obtained therefore have important implications regarding the risk of legionellosis relating to 24HHWB use and will lead to more effective and comprehensive management of legionellosis. Although numerous studies have been carried out on legionellosis, few reports have been made so far relating to the longitudinal examination of the risk of legionellosis. From this viewpoint, it is considered that our studies include extremely important findings concerning the comprehensive risk of legionellosis over a prolonged period.

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臨床疫学に基づく非定型肺炎診断の確率について

Diagnostic Probability for Atypical Pneumonia

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市中肺炎の起炎菌には種々あるが、臨床細菌学的知識に基づく的確な想定のもとに治療を開始することは実地医家においては容易ではない。ここでは臨床疫学に基づく市中肺炎の診断について考察する。

1．非定型肺炎である確率に言及する

【症例1（図1－次頁）】

27歳女性、保母。1週間ほど前より咳嗽が強く、37℃台の微熱を認める。市販薬を内服しても症状が改善しないため受診した。胸部X線では左上肺野に淡い浸潤影を認める。白血球数 8200/μL。

この患者が非定型肺炎である確率は95%以上である。

【症例2（図2－次頁）】

67歳男性、慢性C型肝炎を有する。風邪様の症状に引き続き、咳嗽が強くなり発熱が持続するため受診。胸部X線では右中肺野に浸潤影を認める。白血球数 11200/μL。

この患者が非定型肺炎である確率は50%である。

どうすれば上記のように確率を算出できるのだろう。「経験豊富な呼吸器病学の専門家であればこれ位の見当をつけることができるかも知れないが、感染の起炎菌推定や胸部写真の読影などに専門家ほど馴染みのない一般臨床医には難しい…？」と考える必要はない。ベイズ解析[1]という臨床疫学的手法を用いれば、呼吸器を専門としない実地医家においても診断確率について言及することは可能である。

2．肺炎患者の診療アプローチ

一般に、気道感染の治療では喀痰グラム染色により起炎菌の推定を行い抗菌薬を選択することがスタンダードな手順である。

本稿で述べる診療アプローチは、専門家的経験や臨床細菌学的知識のない状況で目前の患者の臨床像に基づき非定型肺炎の正診断確率を定量化し、この結果に基づき抗菌薬を選択する、というものである。以下、その解析法につき順を追って述べる。

3．非定型肺炎の診断に重要な臨床parameter（あるいは診断基準項目）の感度・特異度、および陽性尤度比・陰性尤度比について

まず市中肺炎の臨床研究などで蓄積されたデータベースを基に、各項目の非定型肺炎の診断に関する感度（Sn；sensitivity）および特異度（Sp；specificity）を決定する。佐賀医科大学附属病院および佐賀市中肺炎研究会（SPOT study；Saga Pneumonia Optimal Treatment Study）で一定期間に経験した定型肺炎患者97名、および非定型肺炎患者39名のデータベースを基に算出した診断基準項目の感度および特異度を**表**（次頁）に示す（この対象患者の診断――定型肺炎か非定型肺炎かの鑑別――は喀痰細菌培養やマイコプラズマ・ニューモニエ、クラミジア・ニューモニエの抗体価測定など標準的診断法に基づく）。

我々は日本呼吸器病学会の推奨する非定型肺炎（マイコプラズマ肺炎）の診断基準[2]を参考に、①60歳未満である、②基礎疾患がない、③頑固な咳がある、④白血球がseptic range（3000～12000/μL）にない、の4項目について、それぞれ非定型肺炎の診断に対する感度と特異度を求めた[3]。**表**に示すように各項目の感度および特異度は当然ながら異なっており、診断基準項目の陽性（あるいは陰性）数だけで当該疾患を除外（rule out）あるいは肯定（rule in）することはできない。しかし、この結果を基に尤度比を算出することにより疾患の存在確率について言及することが可能となる。

尤度比（likelihood ratio；oddsと同等に解釈）を用いた診断確率は各診断parameterの有無（陽性あるいは陰性）により陽性尤度と陰性尤度とを掛け合わせて行けば良い。掛け合わせた数値は結合尤度比（joint likelihood ratio）と呼ばれる[1]。

Oddsが1ということはある事象の発生する確率が50%であるということなので、oddsと確率との間には；

$$確率（p）= odds /（1 + odds）$$

という式が成り立つ。

ここで本稿の冒頭に紹介した2例の市中肺炎の実例と**表**を見ていただきたい。

【症例1（図1および表）】

27歳女性、保母。1週間ほど前より咳嗽が強く、37℃台の微熱を認める。市販薬を内服しても症状が改善しないため受診した。胸部X線では左上肺野に淡い浸潤影を認める。白血球数 8200/μL。

この27歳の女性患者が非定型肺炎である結合尤度は280（確率99.6%）であると考察できる。従って主治医は非定型肺炎に効果を有する抗菌薬を選択するだろ