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化粧品

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販売名 （企業名）	①献血ヴェノグロブリン-IH ヨシトミ（ベネシス） ②グロブリン-Wf（ベネシス）					

研究報告の概要

米国における新しいパルボウイルス B19 遺伝子型 3 の発見と分析
背景：パルボウイルス B19（B19V）は、核酸検出でヒト血漿においてしばしば同定される病原体である。3 つの B19V 遺伝子型は、全体の DNA 配列で 10%を超える相違のある分離株に基づいて定義された。B19V 遺伝子型 3 は、主にガーナで、散発的にブラジルおよびフランスから報告されてきたが、過去に北アメリカでの報告はなかった。
研究設計と方法：B19V を広い特異性で検出するための PCR 測定系が開発された。この測定法の性能は、81,000 人以上のドナー由来の約 440,000 の臨床的サンプルを検査することによって評価された。B19V タイター、DNA 配列と抗体濃度の判定は、重要なサンプルで実施された。
結果：この評価の結果、DNA 配列分析によって B19 遺伝子型 3 に感染していると確認された米国の 1 人のドナーから 28 日の間に 8 回の血漿ドネーションを行っていることが確認された。この一連のドネーションの B19V タイターは、ピークにおいて 10^{11}IU/mL を超えるウイルス力価に達した。ウイルス力価は、免疫グロブリン M（IgM）抗体価の増加に合わせてその後数回のドネーションにおいて減少した。IgG 濃度も増加したが、IgM 濃度より約 7 日遅れた。
結論：これは、米国における血漿ドナーから検出された B19V 遺伝子型 3 の最初の報告である。我々のデータはこの遺伝子型の低い発生率を示唆している最近の報告と合致しているにも関わらず、それは血液及び血漿ドナーとの関連性が増していることを示している。

報告企業の意見	今後の対応
米国において血漿ドナーから B19V 遺伝子型 3 が検出された最初の報告である。 献血ヴェノグロブリン-IH については、万一本剤の原料血漿に B19 が混入したとしても、CPV をモデルウイルスとしたウイルスバリデーション試験成績及び B19 を用いた不活化・除去試験の結果から、本剤の製造工程において十分に不活化・除去されると考えている。 グロブリン-Wf については、血漿分画製剤の製造工程での十分な不活化・除去が困難であり、本ウイルスの伝播リスクを完全に否定することはできないため、1996年11月より、使用上の注意にパルボウイルスB19についての記載を行い注意喚起を図っている。なお、一変承認後に製造されたロットについては、献血ヴェノグロブリン-IH と同様に、製造工程において不活化/除去されると考えている。	本報告は本剤の安全性に影響を与えるものではないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
1. 慎重投与
(5) 溶血性・失血性貧血の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
(6) 免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕
2. 重要な基本的注意
(1) 略
1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
6. 妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。〕

Discovery and analysis of a novel parvovirus B19 Genotype 3 isolate in the United States

Lori A. Rinckel, Brett R. Buno, Todd M. Gierman, and Douglas C. Lee

BACKGROUND: Parvovirus B19 (B19V) is a pathogen frequently identified in human plasma donations through the detection of nucleic acids. Three B19V genotypes have been defined based on isolates having greater than 10% divergence in overall DNA sequence. B19V Genotype 3 is a rarely occurring genotype that has been detected primarily in Ghana with sporadic reports in Brazil and France but has not been previously reported in North America.

STUDY DESIGN AND METHODS: A polymerase chain reaction assay was developed with broad specificity for B19V detection. The performance of this assay was assessed by testing approximately 440,000 clinical samples representing more than 81,000 individual donors. Determinations of B19V titer, DNA sequence, and antibody concentrations were performed on samples of interest.

RESULTS: This assessment identified a series of eight plasma donations spanning 28 days from a single donor in the United States infected with B19V Genotype 3 as confirmed by DNA sequence analysis. The B19V titer of this series of donations showed virus titers that peaked at greater than 10^{11} IU/mL. The virus titer decreased significantly over the next several donations coinciding with an increase in immunoglobulin M (IgM) levels. The immunoglobulin G levels also increased but lagged approximately 7 days behind the IgM levels.

CONCLUSION: This is the first report of a B19V Genotype 3 detected from a plasma donor located in the United States. Although our data are consistent with recent reports suggesting low incidence for this genotype, they indicate its increasing relevance among blood and plasma donors.

Human parvovirus B19 (B19V) is a common human pathogen that possesses a mutation rate that is uncharacteristically high for a DNA virus.[1,2] B19V DNA sequence homology is used to classify B19V into three genotypes.[3] The genotypes are defined as having approximately 10% divergence in overall DNA sequence. Genotype 1 is the most prevalent B19V currently circulating in North America and Europe and is represented by the prototype strain Au (GenBank Accession Number M13178).[4] Genotype 2 also circulates in North America and Europe but with lower frequency than Genotype 1 and is represented by the prototype strain A6 (GenBank Accession Number AY064475).[5] Genotype 3 is endemic to Ghana but has also been found in Brazil and France.[6] Genotype 3 is represented by two prototype strains, V9 and D91.1 (GenBank Accession Numbers AX003421 and AY083234, respectively).[3] Numerous genetic variants of B19V exist within each of these three genotypes. By definition, these variants differ by less than 10% in their DNA sequence when compared to the genotypic prototype.

These three genotypes were shown to constitute one single serotype through various functional, structural, and immunologic studies;[7] current evidence suggests that all variants of B19V induce similar pathologies.[8] A primary interest and concern with the wide assortment of B19V genotypes and variants is diagnostic. Polymerase chain reaction (PCR) assays developed around specific reference standards frequently fail to detect accurately specific B19V genotypes and variants.[5,9-11] Therefore, developers of molecular diagnostics and the biologics industry have an

ABBREVIATION: B19V = parvovirus B19.

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increased interest in the prevalence of emerging B19V variants.

Here, we report a unique isolate of B19V. This isolate was discovered in a series of human plasma donations from a single donor located in the United States. Analysis indicated that this isolate exhibits strong DNA sequence homology to B19V Genotype 3. Analysis of the series of donations from this donor demonstrated the expected clinical pattern of antibody response to this B19V Genotype 3 infection. This is the first report of the discovery and characterization of B19V Genotype 3 among US plasma donors.

MATERIALS AND METHODS

A quantitative real-time PCR assay was developed with a target region within the NS1 coding sequence of the B19V genome utilizing oligonucleotide primers and probes purchased from Integrated DNA Technologies (IDT, Coralville, IA). The assay used two detection probes for the B19V target. One detection probe contained the DNA sequence of the B19V Au Genotype 1 prototype strain; the second probe contained a DNA sequence that is a consensus derived from the B19V A6 Genotype 2 prototype strain and the V9 and D91.1 Genotype 3 prototype strains. Both probes were labeled with the same fluorophore. The assay also incorporated a third detection probe for a competitive internal control that was labeled with a different fluorophore than that of the two B19V target probes. Test results indicating a PCR signal for the internal control, B19V target, or both were deemed valid; results indicating no PCR signal for both the internal control and the B19V target were deemed invalid. The quantitation standards used in the real-time PCR assay were dilutions of plasmid pYT104-C, which contains a B19V Genotype 1 strain (Au) genome.[12] A quantitative standard curve was used to assign values (copies/mL) to test samples. The results expressed as copies/mL were converted to IU/mL using a correlation factor of 2.9 copies/IU, determined by comparing the potency of the First WHO International Standard for B19V DNA nucleic acid test assays (99/800) to the potency of the dilution of pYT104-C used to create the quantitation standards.[13]

The performance of the B19V assay was assessed against a qualitative B19V assay that served as the test of record using a study sample of approximately 440,000 donor samples corresponding to roughly 81,000 individual donors. Both assays were designed to detect all three B19V genotypes; neither assay was designed to discriminate among the three genotypes. Donation samples were tested initially in pools of 384 or 480 samples to increase testing efficiency. Additional testing of B19V-reactive samples was performed using a B19V PCR assay (artus RealArt, Parvo B19 PCR assay, Qiagen, Hilden, Germany). Antibody detection was performed on test samples in duplicate using the B19V immunoglobulin M (IgM) and immunoglobulin G (IgG) enzyme immunoassay (EIA) kits (Biotrin, Dublin, Ireland).

DNA sequencing was performed on PCR amplicons generated using primers containing B19V consensus DNA sequences. The purified PCR amplicons were sequenced by primer walking performed at Lark Technologies (Houston, TX). The contiguous DNA sequences were assembled using sequence analysis cloning software (Vector NTI, Invitrogen Corp., Carlsbad, CA). DNA sequence alignments were performed with Vector NTI and with the GenBank database using BLAST.[14]

RESULTS

The performance of a new B19V assay was assessed using a study sample set consisting of approximately 440,000 donor samples, representing roughly 81,000 individual donors. The performance of the B19V assay was benchmarked against results obtained using an earlier version assay. During the course of the study, 1 in 2400 donor samples tested reactive for B19V. Review of results discordant between the two assays identified several samples for follow-up analysis. This investigation identified two reactive donations that were ultimately linked to a series originating from a single donor resident in the United States. Using a lookback process coupled with follow-up testing, a series comprising eight donations, designated P0 through P7, was identified and these units were pulled from the inventory for continued research. The two plasma samples with the highest titer from this series, P1 and P2, were used to characterize the B19V isolate. Additional testing using the Qiagen artus RealArt Parvo B19 PCR assay yielded negative results for neat and diluted samples (neat and 1:480).

DNA sequencing of B19V amplicons generated from P1 and P2 using our assay showed that both donations contained identical DNA target sequences. This preliminary sequence information also suggested that nucleic acid isolates from P1 and P2 have higher DNA sequence homology to B19V Genotype 3 than to Genotypes 1 and 2. The preliminary DNA sequence information was used to design and synthesize a new detection probe (P1 probe) containing 100% DNA sequence homology to the P1 and P2 isolates. This new detection probe was used to quantitate sample viral loads at 8×10^{11} IU/mL for P1 and 3×10^{10} IU/mL for P2 (Fig. 1).

The DNA sequence was determined for 4846 nucleotides of the P1 B19V genome (GenBank Accession Number FJ265736). Analysis of the DNA sequence from P1 (Fig. 2) shows that this B19V isolate has the highest DNA sequence homology to representative isolates of B19V Genotype 3. This sequence exhibited 97% homology to B19V strain V9 and 96% homology to B19V strain D91.1, suggesting that the P1 isolate belongs to Genotype 3

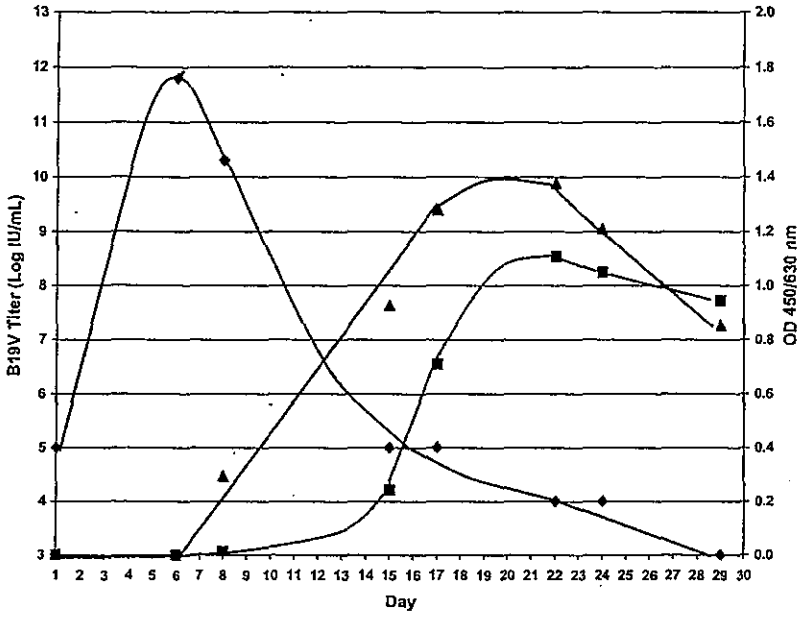
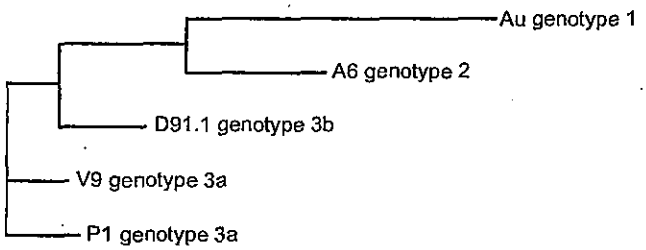


Fig. 1. B19V titer (◆) and IgM (▲) and IgG (■) levels for the series of Genotype 3 donations. The B19V titer is expressed in IU/mL.

A



B

	Genotype 1 Au	Genotype 2 A6	Genotype 3b D91.1	Genotype 3a V9	P1
Genotype 1 Au	100	91	90	89	89
Genotype 2 A6		100	93	93	92
Genotype 3b D91.1			100	96	96
Genotype 3a V9				100	97
P1					100

Fig. 2. Global DNA sequence alignment of B19V strain P1 with the prototype strains for B19V Genotype 1, Au; Genotype 2, A6; Genotype 3b, D91.1; and Genotype 3a, V9. (A) Phylogenetic tree. (B) Numerical comparison of DNA sequence homologies. This analysis shows that P1 is a member of B19V Genotype 3a.

Subtype B19/3a.[15] The P1 isolate is significantly less homologous to B19V Genotypes 1 and 2 with 89% homology to B19V Genotype 1 prototype strain Au and 92% homology to B19V Genotype 2 prototype strain A6.

When tested using the P1 probe, the B19V titers of this series of donations show the expected pattern for a B19V infection. B19V titers increased rapidly, peaking within several days after infection and then decreased gradually for several weeks (Fig. 1).

In addition, IgM and IgG B19V antibody levels were detected in this series of plasma donations using EIA methods (Fig. 1). For reference, the day of the P0 donation is referred to as Day 1. The IgM response was first detected on Day 8 (P3), peaked on Day 22 (P5), and decreased thereafter. The IgG antibody response was first detected on Day 15 (P3), peaked on Day 22 (P5), and gradually decreased through Day 29 (P7). The B19V antibody levels detected in these plasma donations displayed an increase in IgM level concurrent with the decrease in B19V titer (Fig. 1).

DISCUSSION

This is the first report of a B19V Genotype 3 detected in a blood or plasma donation in the United States. Previously, B19V Genotype 3 had been reported to occur primarily in the African country of Ghana, with less frequent reports in Brazil and France. The frequency of B19V Genotype 3 in Ghana was reported to be approximately 100% of the strains identified.[16] The frequency of B19V Genotype 3 in Brazil was approximately 50% and in France was approximately 11% of the strains identified.[3,6] Not only is the identification of a B19V Genotype 3 in the United States noteworthy, but also our characterization of this B19V Genotype 3 infection in this donation series has demonstrated that isolates of this genotype can achieve the high virus titers typically associated with acute B19V Genotype 1 infections. In contrast, previous reports concerning B19V Genotype 3 have suggested that high-titer infections involving this genotype occur infrequently.[3,16,17] The titers of the isolates described in these prior reports, however, may reflect late or persistent infections which would exhibit lower titers than an initial infection. The B19V titers of the series of donations in this report show the expected pattern for an acute infection where virus titers increase rapidly, peak within several days after infection, and then decrease gradually over a period of several weeks.

The B19V isolate described in this report, designated P1, was found to exhibit strong DNA sequence homology with B19V Genotype 3. Alignment to V9 and D91.1, both Genotype 3 isolates, demonstrated significantly higher DNA sequence homology (at least 94%) than to representative isolates for Genotypes 1 and 2. More specifically, P1

appears to belong to Genotype 3 Subtype B19/3a.[15] Subtype B19/3a was reported to be prevalent in Ghana whereas Subtype B19/3b appears to be more prevalent in Western Europe and Brazil.[15]

The B19V titers in these donations increased rapidly and peaked at a titer of approximately 8×10^{11} IU/mL. Our results also show that the decrease in B19V titer was concurrent with an increase in IgM antibodies. The increase in IgM antibodies was followed by an increase in the levels of IgG antibodies. These results concur with published works that suggest that the Genotype 1 antigens present in the Biotrin EIA kit are effective for the detection of Genotype 3 antibodies.[18] These results are also consistent with the suggestion that a single serotype may exist for the different B19V genotypes.[7]

Recent discussion concerning the incidence of the B19V Genotype 3 infection among blood and source plasma donors has suggested that the prevalence of this genotype in the United States is low or absent.[2] The comparison of the performance of two B19V assays in this limited, high-throughput sample set (approx. 440,000 donations) identified B19V at a frequency of 1:2400 donations. This detection frequency is typical for the time of year at which the study was conducted (based on data from nearly 8 years of high-throughput testing). When the study results were analyzed by donor, B19V-reactive donations were associated with 117 individual donors among 81,000 total donors (approx. 1:700). In contrast, the putative detection frequencies for samples and donors reactive for B19V Genotype 3 appear significantly lower. Samples containing high-titer B19V Genotype 3 (i.e., $>10^6$ IU/mL) were detected at the rate of 1:220,000 and were contributed by a single donor among the 81,000 donors comprising the sample set (1:81,000). Whether these frequencies accurately reflect the incidence of Genotype 3 within the source plasma donor population remains unclear, because the assays used in this study were not designed to differentiate among the three genotypes. Moreover, this study was designed to evaluate assay performance, rather than B19V epidemiology. Nevertheless, the fact that this study resulted in the identification and interdiction of 8 plasma units from a single donor, 2 of which contained sufficient B19V to exceed the prescribed limits for plasma fractionation pools, underscores the increasing relevance of assays that can detect B19V Genotype 3.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009. 7. 21	該当なし	

一般的名称	人血清アルブミン	研究報告の公表状況		公表国
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)		FDA, CBER. Available from: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm071592.htm.	米国

研究報告の概要

○血漿分画製剤によりヒトパルボウイルスB19が伝播する危険性を低減するための核酸検査(NAT)にかかる業界向けガイダンス

米国食品医薬品局(FDA)は、血漿分画製剤製造に使用される原料血漿および回収血漿の工程内検査として、ヒトパルボウイルスB19核酸増幅検査(NAT)の実施を製造業者に対し勧告するガイダンスを発表した。これは2008年7月付ガイダンス案の最終決定版である。

B19は脂質膜を持たない一本鎖DNAウイルスで、熱処理やS/D処理等の一般的な不活化法に抵抗性があり、小型であるため除去も困難である。輸血用血液製剤や血漿分画製剤の投与によりウイルス感染が起こり、妊婦や血液疾患、免疫不全の患者等、影響を受けやすい患者が感染すると死亡(または胎児水腫、流産、死産等)する可能性も否定できない。輸血用血液による感染はまれだが、B19急性感染期で無症候の献血者から採血した最高10^{13}IU/mL程度のウイルスを含む血液が血漿分画製剤の原料として用いられた場合には、多数の血漿をプールして製造することから感染リスクが高くなる。しかし、ウイルス血症期の献血者血液中には、長期にわたり低濃度のウイルスと中和抗体としてのB19抗体が共存しており、低濃度のウイルスを保有する献血者を排除することは、プール血漿中のB19抗体レベルを下げることになるため、望ましくない。

FDAは、血漿分画製剤の製造業者に対し、B19 DNAを検出するため、以下の手順を導入することを勧告する。

1. 全ての血漿分画製剤の製造に際し、製造工程中の品質管理としてB19 DNAのNATを実施し、製造原料となる血漿プール中のB19 DNA濃度が10^4IU/mLを超えないことを保証する。
2. 血漿分画製剤の製造原料となる血漿製剤に対し、B19のミニプールNATを実施する。B19のNATに用いるプライマーやプローブは、現在判明している全ての遺伝子型を検出できるものであること。
3. 血漿製剤がB19陽性であることが判明し、使用により血漿プール中のウイルス力価が10^4IU/mLを超える可能性がある場合には、その血漿を血漿分画製剤の製造原料として用いてはならない。

製造業者は、原料血漿及び回収血漿中のB19 DNA検出に用いるNATのバリデーションを行い、血漿プール中のB19 DNAが10^4IU/mLを超えないことを実証すること。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
米国食品医薬品局が、血漿分画製剤製造に使用される原料血漿および回収血漿の工程内検査として、ヒトパルボウイルスB19核酸増幅検査(NAT)の実施を製造業者に対し勧告するガイダンスの最終版を発表したとの報告である。 ヒトパルボウイルスB19(B19V)は脂質膜のないDNAウイルスである。これまで、本剤によるB19V感染の報告はない。B19は耐熱性とされていたが最近、液状加熱で容易に不活化できることが明らかにされた。本製剤の製造工程には、当該工程が含まれている。また最終製品についてB19-NAT陰性であることを確認していることから、本製剤の安全性は確保されている。	念のため今後も情報収集に努める。なお、日本赤十字社では、以前よりRHA法によるB19抗原検査を導入しウイルス量の多い血液を排除してきた。2008年からさらに感度の高い化学発光酵素免疫測定法(CLEIA)を導入し、10^6IU/mL以上のB19を含む血液を陽性と判定し排除するものであることから、現在は原料血漿プール中のウイルス濃度が10^4IU/mL以下となっている。今後も輸血用血液及び血漿分画製剤の安全性向上のために努力する。

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Guidance for Industry

Nucleic Acid Testing (NAT) to Reduce the Possible Risk of Human Parvovirus B19 Transmission by Plasma-Derived Products

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD) (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
July 2009

Contains Nonbinding Recommendations

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Contains Nonbinding Recommendations

Guidance for Industry

Nucleic Acid Testing (NAT) to Reduce the Possible Risk of Human Parvovirus B19 Transmission by Plasma-Derived Products

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

We, FDA, are issuing this guidance to provide you, manufacturers of plasma-derived products, with recommendations for performing nucleic acid testing (NAT) for human parvovirus B19 as an in-process test for Source Plasma and recovered plasma used in the further manufacturing of plasma-derived products. Such testing will identify and help to prevent the use of plasma units containing high levels of parvovirus B19. This guidance also recommends how to report to FDA implementation of parvovirus B19 NAT.

We recognize that in the current business practice for parvovirus B19 NAT in-process testing, several weeks can elapse between collection of the units of Source Plasma or recovered plasma and identification of B19 NAT-positive pools or units. We encourage manufacturers of plasma-derived products to employ practices that will reduce the time between product collection and in-process testing to allow for the meaningful notification of blood and plasma collection establishments of positive test results within the dating period of any blood components intended for use in transfusion.

This guidance finalizes the draft guidance of the same title, dated July 2008.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Human parvovirus B19 is a small, non-enveloped single stranded DNA virus. Virus clearance studies, using non-human parvoviruses as models for parvovirus B19, have indicated that this virus is highly resistant to all commonly used inactivation methods, including heat and

solvent/detergent (S/D) treatment, and is also difficult to remove by filtration because of its small size. More recent studies have demonstrated that human parvovirus B19 may be more readily cleared than certain model animal parvoviruses (Refs. 1, 2, 3 and 4). The parvovirus B19 can be transmitted by blood components and certain plasma derivatives, and may cause morbidity to susceptible recipients such as pregnant women (and their fetuses exposed in utero), persons with underlying hemolytic disorders, and immune compromised individuals (Refs. 5 and 6). The disease transmission by transfusion of blood components is rare. However, extremely high levels of parvovirus B19, up to 10^{12} IU/mL, in plasma of acutely infected but asymptomatic donors may present a greater risk in plasma derivatives due to pooling of large numbers of plasma units in the manufacture of these products. The virus can be detected by NAT in plasma pools when there are high levels of parvovirus B19 DNA in viremic donations. For example, the parvovirus B19 DNA can be detected in various plasma-derived products, particularly in coagulation factors (Refs. 7 and 8). There have been a few reports of parvovirus B19 infection associated with the administration of coagulation factors (Refs. 9 and 10) and S/D Treated Pooled Plasma (Refs. 5 and 11). Parvovirus B19 DNA is less frequently detected in albumin and immunoglobulin products and, when detected, the levels are usually low. There are no confirmed reports that albumin and immunoglobulin products have transmitted parvovirus B19 infection.

We have held or participated in several meetings to discuss the potential risk of parvovirus B19 infection by plasma-derived products, and the strategy for reducing such risk. The meetings included FDA-sponsored NAT workshops in 1999 and 2001 (Refs. 12 and 13), Blood Products Advisory Committee (BPAC) meetings in 1999 and 2002 (Refs. 14, 15, and 16), the National Heart, Lung, and Blood Institute-sponsored Parvovirus B19 workshop in 1999 (Ref. 5), and an ad hoc Public Health Service (PHS) panel in 2002 (discussed at the 2002 BPAC meeting (Ref. 16)). In these meetings, it was recognized that viral inactivation/removal steps that are routinely used in the manufacturing process of plasma-derived products do not alone appear to be sufficient to completely clear the virus if high viral load is present in the starting material. Therefore, in these meetings, a common recommendation for mitigating the risk of parvovirus B19 transmission by plasma derivatives has been to limit the virus load in the manufacturing plasma pool by testing the plasma donations for high titer parvovirus B19 DNA, using a minipool format. This viral load reduction strategy combined with the ability of the manufacturing process to clear the residual virus could greatly reduce the risk of parvovirus B19 infection by plasma-derived products.

The recommended limit in this guidance for viral load of parvovirus B19 DNA in the manufacturing plasma pool (i.e., not to exceed 10^4 IU/mL) was primarily derived from studies that were conducted on the transmission of parvovirus B19 associated with S/D Treated Pooled Plasma (Refs. 5, 11, and 14). In principle, testing in a minipool format to measure the viral load for parvovirus B19 DNA in a manufacturing plasma pool is acceptable in order to exclude only the high-titer plasma donations, thereby avoiding too great a loss of plasma for further manufacturing. Furthermore, during the viremic period for parvovirus B19 infected donors, which can be very lengthy, low levels of parvovirus B19 coexist with parvovirus B19 antibodies

(potentially complexing with and neutralizing the virus). Therefore, it is undesirable to remove plasma units with low levels of B19 DNA, because it would diminish the parvovirus B19 antibody levels in plasma pools and in some of the resulting plasma-derived products (Refs. 17 and 18).

III. RECOMMENDATIONS

We recommend that you implement the following procedures to detect the presence of parvovirus B19 DNA:

- For all plasma-derived products, you should perform parvovirus B19 NAT as an in-process test to ensure that the viral load of parvovirus B19 DNA in the manufacturing pools does not exceed 10^4 IU/mL.
- Use parvovirus B19 NAT on minipool samples to screen plasma units intended for further manufacturing into plasma-derived products. Primers and probes selected for parvovirus B19 NAT should detect all known genotypes of the virus (Ref. 19).
- When identified, you should not use individual plasma units, intended for further manufacturing into plasma-derived products, when such units are found to have a titer of parvovirus B19 DNA that might result in plasma manufacturing pools exceeding a parvovirus B19 DNA titer of 10^4 IU/mL.

You should assess validation data demonstrating the accuracy, sensitivity, specificity, reproducibility, and other performance characteristics of the parvovirus B19 NAT assay used for the detection of parvovirus B19 DNA in the Source Plasma and recovered plasma, and for demonstrating that the viral load of parvovirus B19 DNA in the manufacturing pool does not exceed 10^4 IU/mL.

If the above recommendations are implemented, you must inform FDA, as required under 21 CFR 601.12(a). You may submit these changes as a "Supplement-Changes Being Effected" supplement (CBE supplement), under 21 CFR 601.12(c)(5).

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医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
				2009年9月14日	該当なし	

一般的名称	トロンビン	研究報告の公表状況	Clinical Infection Deiseases 2009; 49: 852-860	公表国 マレーシア
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研究報告の概要

（背景）Plasmodium knowlesi（P. knowlesi）は東南アジアで次第にヒト・マラリアの原因と認められつつある。しかし、自然感染の詳細なプロスペクティブな臨床研究がない。

（測定法）急性P. knowlesi感染患者のpresentationと経過の系統的な研究において、臨床および検査データは2006年7月～2008年2月にKapit病院（サラワク、マレーシア）にPCRで確定された急性マラリアで入院した過去に治療経験のない非妊娠の成人から集められた。

（結果）152人の患者のうち、107人（70%）がP. knowlesiに、24人（16%）はP. falciparumに感染しており、そして、21人（14%）は三日熱マラリア原虫を持っていた。P. knowlesi感染者は、非特異的な発熱性の疾患を呈し、入院患者のベースライン中央値の寄生虫値は1387parasites/μL（四分位数間領域：6-222,570parasites/μL）を有し、そして全ての症例は入院又はその次の日に血小板減少を呈していた。
P. knowlesi感染患者のほとんど（93.5%）は、クロロキンとプリマキン治療に反応した合併症を伴わないマラリアであった。熱帯熱マラリアのWHO基準に基づくと、P. knowlesi感染の7人の患者（6.3%）は、入院時点で重症感染であった。最も頻度の高い合併症は呼吸困難であった。それは4人の患者では入院時にみられ、あとの3人の患者では入院後に発症した。入院時のP. knowlesi寄生虫血症は呼吸困難の独立した決定因子であり、入院時の血清クレアチニンレベル、血清ビリルビンそして血小板数と同様であった（各々P<0.002）。2人のP. knowlesiマラリア患者は死亡し、1.8%（95%信頼区間；0.2-6.6%）の致死率を示した。

（結論）Knowlesiマラリアは、広範囲の疾患を起こす。大部分の症例は合併症を伴わず、速やかに治療に反応する。しかし、約10人に1人の患者が潜在的に致命的な合併症を発病する。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、陽イオン交換体処理により人トロンビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程においてリン酸トリ-n-ブチル(TNBP)/ポリソルベート80処理、ウイルス除去膜によるろ過処理、凍結乾燥の後、60℃、72時間の加熱処理を施しているが、使用に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
東南アジアでPlasmodium knowlesiが原因のマラリアはだんだん増えており、患者の多くはクロロキンとプリマキン治療に反応した合併症を伴わないマラリアであったが、約10人に1人の患者が致命的な合併症を発病する可能性があることについての報告である。 Plasmodium knowlesiは長い間、サルに感染するマラリア原虫とみなされていたが、ヒトに感染する5番目のPlasmodium属原虫と認められるようになってきている。 血漿分画製剤からのマラリア伝播の事例は報告はされていない。FDAが2000年6月に発行した”Guidance for Industry: Recommendations for Donor Questioning Regarding Possible Exposure to Malaria”においては、赤血球成分または血小板用の供血についてはマラリアに関連した供血停止条件を規定しているものの、血漿成分用の供血や血漿分画製剤の原料用の供血については規定していない。感染患者におけるマラリア原虫はメロゾイドの形で存在すると考えられ、このものは2-3μmの卵型であるとされている（最新医学大辞典第2版、医歯薬出版、1996）。万一、原料血漿にマラリア原虫が混入したとしても、除菌ろ過等の製造工程にて除去されるものと考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

MAJOR ARTICLE

Clinical and Laboratory Features of Human *Plasmodium knowlesi* Infection

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Background. *Plasmodium knowlesi* is increasingly recognized as a cause of human malaria in Southeast Asia but there are no detailed prospective clinical studies of naturally acquired infections.

Methods. In a systematic study of the presentation and course of patients with acute *P. knowlesi* infection, clinical and laboratory data were collected from previously untreated, nonpregnant adults admitted to the hospital with polymerase chain reaction–confirmed acute malaria at Kapit Hospital (Sarawak, Malaysia) from July 2006 through February 2008.

Results. Of 152 patients recruited, 107 (70%) had *P. knowlesi* infection, 24 (16%) had *Plasmodium falciparum* infection, and 21 (14%) had *Plasmodium vivax*. Patients with *P. knowlesi* infection presented with a nonspecific febrile illness, had a baseline median parasitemia value at hospital admission of 1387 parasites/μL (interquartile range, 6–222,570 parasites/μL), and all were thrombocytopenic at hospital admission or on the following day. Most (93.5%) of the patients with *P. knowlesi* infection had uncomplicated malaria that responded to chloroquine and primaquine treatment. Based on World Health Organization criteria for falciparum malaria, 7 patients with *P. knowlesi* infection (6.5%) had severe infections at hospital admission. The most frequent complication was respiratory distress, which was present at hospital admission in 4 patients and developed after admission in an additional 3 patients. *P. knowlesi* parasitemia at hospital admission was an independent determinant of respiratory distress, as were serum creatinine level, serum bilirubin, and platelet count at admission ($P < .002$ for each). Two patients with knowlesi malaria died, representing a case fatality rate of 1.8% (95% confidence interval, 0.2%–6.6%).

Conclusions. Knowlesi malaria causes a wide spectrum of disease. Most cases are uncomplicated and respond promptly to treatment, but approximately 1 in 10 patients develop potentially fatal complications.

Five species of *Plasmodium (Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale,* and *Plasmodium knowlesi)* cause naturally acquired malaria in humans. The most recently identified species is *P. knowlesi*, which we previously reported to be the most common cause of hospitalization for malaria in the Kapit Division of Sarawak in Malaysian Borneo [1]. Further studies of blood samples from patients presenting with malaria in Sarawak, Sabah, and Peninsular states confirmed a much wider distribution within Malaysia [2]. There have also been reports of locally acquired *P. knowlesi* infections from Southern Thailand, the Myanmar-China border, the Philippines, and Singapore [3–7], indicating that transmission occurs in many Southeast Asian countries.

P. knowlesi is primarily a chronic infection of the long-tailed *(Macaca fascicularis)* and pig-tailed *(Macaca nemestrina)* macaques [8]. It is easily confused with *Plasmodium malariae* on blood film microscopy in cases of human infection, because the morphologic appearances are almost identical [9, 10]. However, *P. knowlesi* is unique amongst the primate and human malarias in that it has a 24-h erythrocytic cycle [10], which is a characteristic that is likely to accelerate the development of complications [2]. Information on the characteristics of knowlesi malaria in humans, however, is restricted to single case reports [3, 5, 7]; our previous retrospective study of 94 patients with uncomplicated cases, in

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which we described available data relating to clinical features at presentation only [1]; and our report of 4 fatal cases [2]. We have, therefore, undertaken a detailed, systematic, prospective study of the presentation and clinical course of patients with a diagnosis of confirmed acute knowlesi malaria.

PATIENTS AND METHODS

Study site. This prospective study was conducted in the Kapit Division, which has a total population of 109,000 people of mostly Iban ethnicity [1]. A single World Health Organization (WHO) level 2 hospital serves the Division, together with 3 polyclinics and 22 rural health clinics. Health policy mandates that all patients with malaria are hospitalized until negative blood smear results are obtained on 2 consecutive days. Treatment for malaria is provided free of charge.

Subjects. Recruitment was consecutive and took place during 2 periods totalling 17 months from July 2006 through February 2008. All nonpregnant patients aged ≥15 years who were admitted to Kapit Hospital with a blood film result positive for any *Plasmodium* species were eligible, provided that there was no significant comorbid disease and that they had taken no antimalarial treatment within the previous 14 days. Subsequent confirmation of malaria species was determined by nested polymerase chain reaction assays [1]. All patients provided witnessed informed consent to the study procedures, which were approved by the Medical Research Ethics Subcommittee of the Malaysian Ministry of Health. In an initial 2-month pilot study, most cases of *P. vivax* and *P. falciparum* infection were among logging camp workers returning from long periods in Oceania or Equatorial Africa, respectively. Because the demographic characteristics and background immunity of these patients were significantly different from those of patients with knowlesi malaria, their clinical and laboratory data are presented but are not compared directly with data for patients with *P. knowlesi* infection.

Clinical procedures. Detailed demographic characteristics, history, and examination findings were recorded on a standard form. A baseline blood sample was obtained for routine biochemical and hematological testing, and regular monitoring of temperature, blood pressure, and pulse rate was started. Treatment was administered promptly according to the Malaysian Ministry of Health Guidelines. Because there are no current guidelines for *P. knowlesi* malaria, the guidelines for *P. malariae* were used. Patients with uncomplicated knowlesi malaria received oral chloroquine (25 mg base/kg over a 3-day period) followed by primaquine (15 mg daily for 2 days) given as a gametocidal agent. Oral and/or intravenous hydration was administered at the discretion of the treating physician. Patients presenting with or developing features of severe malaria were treated in accordance with WHO guidelines [11] except that the thresholds for hyperparasitemia and anemia were changed to >100,000 asexual forms/μL of whole blood and <7.1 g of hemoglobin/dL, respectively, to allow for the low immunity levels of the local population. If indicated clinically, patients were transferred to Sibu Hospital for intensive care.

All patients were assessed clinically and by microscopic examination of blood films on each inpatient day. Additional laboratory tests were performed as indicated by the clinical state of the patient. Parasite clearance time and fever clearance time were taken as the number of days to the first of at least 2 follow-up assessments at which the patient had negative blood film results and was afebrile, respectively. When the patient was afebrile and had negative blood film results for 2 consecutive days, additional blood samples were obtained for routine biochemical and hematological tests before discharge. Patients returned on the 28th day after hospital admission for clinical review and blood tests.

Laboratory procedures. All blood films were examined by 2 experienced microscopists. The parasite density was first determined at Kapit Hospital on the basis of the number of parasites per 500 white blood cells and the total white blood cell count for each patient. Microscopic examination was repeated in Kuching, with the second microscopist blinded to the initial result. The mean of the 2 parasite densities was used in data analysis. Parasite DNA was extracted from blood spots that had been collected on filter paper, and the *Plasmodium* species was determined by nested polymerase chain reaction for *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, as described elsewhere [1, 12].

Hematological profiles were determined on site using semiautomated methods (Sysmex model KX-21N). Serum sodium, potassium, glucose, creatinine, bilirubin, alanine aminotransferase (ALT), and albumin levels were either assayed on site (AVL 9180 and Hitachi 902; Roche/Hitachi, Roche Diagnostics) or serum samples were stored at −80°C before transfer on dry ice to the Biochemistry Department, Fremantle Hospital (Freemantle, Australia), for analysis (Cobas Integra 800; Roche Diagnostics). An additional uncuffed blood sample was collected into a chilled fluoride-oxalate tube, centrifuged immediately and separated plasma stored at −80°C before transfer on dry ice to Fremantle Hospital for plasma lactate assay (COBAS INTEGRA 800). Other laboratory investigations, including blood cultures, urine dipstick testing, microscopic examination, and chest radiography were performed as indicated clinically.

Statistical analysis. Data were analyzed using SPSS software, version 15.0 (SPSS). Normally distributed variables were compared using the Student's *t* test or analysis of variance and the Scheffé post hoc test. All other data were analyzed using nonparametric methods (the Wilcoxon rank-sum test or Friedman test). Proportions were compared with use of Fisher's exact test. Multiple logistic or linear regression analysis using forward conditional modeling was performed to determine baseline

associates of complications or markers of severity, respectively. Plausible predictive variables with a statistically significant ($P < .05$) univariate association with the specific severity outcome were selected for inclusion in the model. These variables were log-transformed prior to model entry if they were nonnormally distributed and a stepwise forward selection procedure was then performed to identify the significant independent associates in each case.

RESULTS

Baseline characteristics. The number of patients who participated in the study in relation to all malaria admissions to Kapit Hospital during the recruitment period is shown in figure 1. Their baseline demographic and clinical features are summarized in table 1. *P. knowlesi* infections were acquired locally by both sexes and across all age groups, with 93 (87%) of patients reporting recent activities in the jungle or forest-fringe in the Kapit Division. All regions along the Rejang River and its associated tributaries were represented, and there was no significant clustering of cases: Confirming our pilot study findings, most of the cases of vivax and falciparum malaria (31 cases; 69%) were imported, and the numbers were relatively small.

The overall median duration of symptoms prior to hospitalization was 5 days (interquartile range, 3–5 days), but 2 patients were unwell for >10 days before hospitalization. Symptoms were typically nonspecific. Fever and chills were present in almost all cases, and other frequent symptoms included abdominal pain, breathlessness, and productive cough. Tachypnea, pyrexia, and tachycardia were common clinical signs (table 1).

The results of baseline laboratory investigations are summarized in table 2. The level of parasitemia at hospital admission was relatively low in the *P. knowlesi* group, but there was a wide range that included 3 patients (2.8%) with parasite densities >100,000 parasites/μL and 33 patients (30.8%) with densities <500 parasites/μL. The most common abnormal laboratory finding was thrombocytopenia (<150,000 platelets/μL), which was present in 104 patients (98%), with 31 (29%) of 107 patients having a platelet count <50,000 platelets/μL. The 3 patients who did not have thrombocytopenia (155,000, 152,000, and 167,000 platelets/μL) had low parasitemias (5, 126, and 170 asexual forms/μL, respectively), and all became thrombocytopenic within 24 h (with nadir values of 90,000, 131,000, and 112,00 platelets/μL, respectively). Lymphopenia was found in 7 (6.5%) of patients at presentation, but all patients had normal values by the time of hospital discharge. Anemia was uncommon at hospital admission. Only 5 (4.6%) of the patients had a hemoglobin concentration <10 g/dL, whereas none of the patients met the criteria for severe anemia. Mild hepatic dysfunction, usually comprising an elevated serum ALT level and a low serum albumin level, was relatively common. Mild-to-moderate hyponatremia (range, 122–135 mmol/L) was evident in 29% of cases, all of which responded to rehydration and antimalarial therapy.

On the basis of WHO criteria for severe falciparum malaria [11], 8 (7.5%) of the patients with *P. knowlesi* infection had severe infections at presentation (table 3). The most frequent clinical presentations of severe infection were respiratory distress (diagnosed in 4 patients on the basis of a respiratory rate >30 breaths/min, oxygen saturation <94% by pulse oximetry, auscultatory findings, and radiographic changes), hyperparasitemia (3 patients), and jaundice (serum total bilirubin >43 μmol/L in 3 patients). There were 3 cases of renal failure (serum

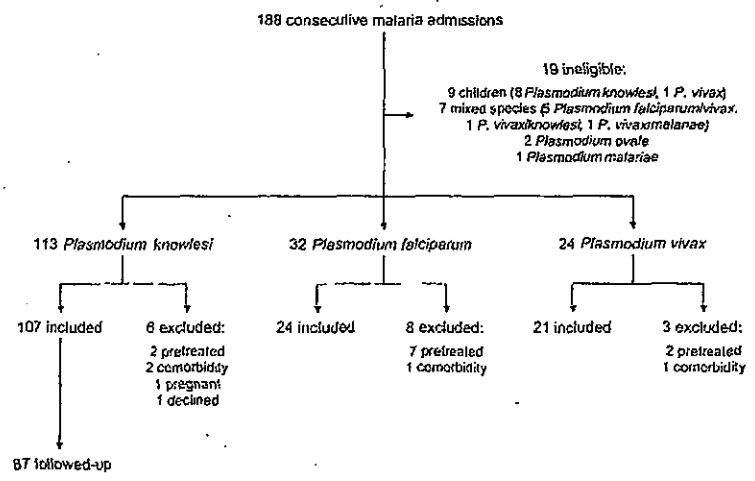

Figure 1. Flow chart showing patient recruitment, exclusion, and follow-up in a study of human *Plasmodium knowlesi* infection in Malaysia.

Table 1. Demographic and Clinical Characteristics of Patients Admitted to Kapit Hospital (Sarawak, Malaysia) with Untreated Malaria Categorized by *Plasmodium* Species

Variable	*Plasmodium knowlesi* (*n* = 107)	*Plasmodium falciparum* (*n* = 24)	*Plasmodium vivax* (*n* = 21)	*P*
Age, years				
Mean value (±SD)	44.9 ± 14.94[a]	38.7 ± 9.64	35.5 ± 10.61	.006
Range	16–79	15–63	15–51	
Male sex	56.1[b,c]	95.8	100	<.001
Iban ethnicity	91.6	95.8	76.2	.073
Occupation				<.001
Farmer	49.5	4.2	9.5	
Logging/plantation worker	27.1[b,c]	91.7	71.4	
Other	23.4	4.2	19	
Self-reported previous malaria	26.2[b,c]	75	57.1	<.001
Previous foreign travel	19.6[b,c]	91.7	71.4	<.001
Foreign travel within previous 4 weeks	0.9[b,c]	83.3	52.4	<.001
Duration of illness, median days (IQR)	5 (3–7)	2.5 (1–4.75)	3 (1–5)	<.001
Symptom				
Fever/chills	100	91.7	95.1	NA
Headache	94.4	87.5	52.4	NA
Rigors	89.7	79.2	85.7	NA
Malaise	89.7	91.7	66.7	NA
Anorexia	83.2	70.8	52.4	NA
Myalgia	87.9	79.2	90.2	NA
Cough	56.1	54.7	47.6	NA
Nausea	56.1	87.5	28.5	NA
Vomiting	33.6	41.7	19.0	NA
Abdominal pain	52.3	37.5	23.8	NA
Diarrhea	29.0	47.5	33.3	NA
Clinical findings				
Axillary temperature, median °C (IQR)	37.6 (37.0–38.5)	37.8 (37.0–38.5)	37.0 (36.8)	NA
Respiratory rate, median breaths/min (IQR)	26 (22–31)	25.5 (22.3–28.5)	27 (24.5–29.0)	NA
Pulse rate, mean beats/min (±SD)	95 ± 16	99 ± 17	97 ± 18	NA
Arterial blood pressure, mean mmHg (±SD)	89 ± 11	85 ± 9	89 ± 9	NA
Capillary refill time, median secs (IQR)	2 (2–3)	2 (2–3)	2 (2–3)	NA
Palpable liver	24.3	29.2	16.7	NA
Palpable spleen	15.0	20.8	23.8	NA

NOTE. Data are percentage of patients, unless otherwise indicated. IQR, interquartile range; NA, not assessed; SD, standard deviation.

[a] $P<.05$ vs *P. vivax*.
[b] $P<.01$ vs *P. falciparum*.
[c] $P<.01$ vs *P. vivax*.

creatinine level ≥265 μmol/L despite fluid resuscitation), 2 cases of hypotension (systolic blood pressure ≤80 mmHg despite fluid resuscitation), and 1 case of hypoglycemia (venous plasma glucose level <2.2 mmol/L). There were no cases of unrousable coma. A combination of features was present at hospital admission in 3 patients.

Clinical course. Clinical and parasitological outcomes together with changes in key hematological and biochemical variables during hospitalization and at day 28 for patients with knowlesi malaria are summarized in tables 4 and 5. There was no clinical, laboratory, or radiological evidence of other infections or conditions at study entry, during hospitalization, or at follow-up that would have influenced outcome. When patients with knowlesi malaria were discharged from the hospital, platelet counts had increased, and all patients had values that were within the normal range by day 28. Most of the remaining hematological and biochemical parameters had improved by hospital discharge. Abnormal laboratory values had resolved in all 87 patients with knowlesi malaria who attended for day 28 review.

Three patients, including 2 patients without complications at hospital admission, developed respiratory distress (table 3). A total of 7 (6.5%) of the 107 patients in the knowlesi group, all of whom were female, presented with or developed respiratory distress. Of those patients with evidence of severe knowlesi malaria either at presentation or during treatment, 2 died (table 3). Patient 1 had parasitemia at presentation (parasite density, 222,570 parasites/μL), evidence of multiorgan failure,

Table 2. Laboratory Results for Patients Admitted to Kapit Hospital with Untreated Malaria Categorized by *Plasmodium* Species

Variable	Normal range	*Plasmodium knowlesi* (n = 107)	*Plasmodium falciparum* (n = 24)	*Plasmodium vivax* (n = 21)
Parasite count, parasites/μL	NA	1387 (6–222,570)	26,781 (1840–271,760)	4258 (324–32,132)
Hemoglobin level, g/dL	11.3–15.7	13.3 (12.0–14.3)	12.9 (12.3–13.6)	13.5 (12.6–13.8)
White blood cell count, × 10^3 cells/μL	3.1–10.3	5.6 (4.7–7.0)	6.3 (5.3–8.6)	6.1 (4.9–7.8)
Neutrophil count, mean neutrophils × 10^3/μL (±SD)	.2–5.3	3.7 ± 1.8	4.6 ± 2.4	4.6 ± 2.2
Lymphocyte count, × 10^3 cells/μL	0.8–2.7	1.5 (1.1–2.0)	1.0 (0.8–1.4)	1.0 (0.6–1.7)
Platelet count, mean value × 10^3 platelets/μL (±SD)	150–450	71 ± 35	108 ± 59	118 ± 51
Prothrombin time, secs	NA	13 (12–15)	15 (13–16)	12 (12–14)
Blood group O, % of patients	NA	28.0	12.5	9.5
Serum creatinine level, μmol/L	<133	86 (73–100)	89 (80–97)	89 (76–98)
Serum sodium level, mmol/L	136–152	137 (135–140)	138 (135–140)	138 (135.5–141)
Serum total bilirubin, μmol/L	<21	13 (9–18)	17 (12–22)	16 (10–21)
Serum alanine aminotransferase level, IU/L	<40	36 (25–54)	26 (20–40)	27 (13–55)
Serum albumin level, g/dL	>36	36 (33–39)	38 (35–41)	41 (39–46)
Serum glucose level, mmol/L	4–8	6.2 (5.3–6.7)	6.4 (5.7–7.2)	6.2 (5.5–7.0)
Plasma lactate level, mmol/L	<2	1.6 (1.2–2.0)	1.5 (1.2–2.0)	1.5 (1.1–2.0)

NOTE. Unless otherwise indicated, data are median value (interquartile range). NA, not applicable.

hypoglycemia, and lactic acidosis. This patient died within 6 h after hospital admission despite intensive treatment with intravenous quinine, broad spectrum antibiotics, and ionotropic and ventilatory support. Patient 8 presented with symptoms and signs of a right hemiparesis and sensory inattention and had a history of uncontrolled hypertension. The patient's parasite density at hospital admission was 214,000 parasites/μL. She was treated with intravenous quinine but developed respiratory distress that required mechanical ventilation. After showing signs of improvement, she experienced neurological deterioration on the seventh day of hospitalization and died 24 h later. No neuroimaging studies were possible.

Baseline* P. knowlesi *parasitemia, complications, and markers of severity. Patients reporting breathlessness or vomiting had greater geometric mean parasite counts than did those who did not report these symptoms (P = .025 and P = .038, respectively). In a logistic regression model, presentation with or development of respiratory distress was positively and independently associated with the admission ln(parasitemia) and inversely associated with the admission hemoglobin level (P = .004 and P = .015, respectively). In multiple linear regression, (1) ln(parasitemia) and age were independent positive associates of ln(admission serum creatinine) (P < .001 and P = .007, respectively), (2) ln(parasitemia) and ln(plasma glucose) were independent associates of ln(admission serum total serum bilirubin) (P = .003 and P = .008, respectively), and (3) ln(parasitemia) was an independent associate of the ln(admission platelet count) and absolute differences between day 28 and hospital admission platelet counts (P = .002 and P = .004, respectively). In other multivariate models, ln(parasitemia) was not an independent associate of the admission hemoglobin level (P = .49) or serum ALT level (P = .70). In receiver operating characteristic curve analysis, parasitemia was a good predictor of complications after excluding hyperparasitemia (area under the receiver operating characteristic curve, 0.90 [95% confidence interval, 0.82–0.98]; P < .001). The prespecified 100,000/μL threshold was highly specific (specificity, 100%) but had a sensitivity of 30%.

DISCUSSION

The present study provides the first detailed, prospective evaluation of *P. knowlesi* infection in an area of Malaysian Borneo in which it is the most common locally acquired human malaria. Although there were demographic differences between the 3 groups of patients with malaria, there were no presenting symptoms or signs that distinguished knowlesi malaria from either falciparum or vivax malaria. Consistent with available—albeit, incomplete—retrospective data [1, 2], most cases of knowlesi malaria were uncomplicated and responded promptly to treatment with chloroquine and primaquine, but complications developed in nearly 1 in 10 patients. Because the number of cases of severe knowlesi malaria was small, an accurate case fatality rate is difficult to ascertain, but the case fatality rate was 1.8% (95% confidence interval, 0.2%–6.6%) in our sample. Malaria may have been a contributory factor rather than the sole cause in our patient who presented with a stroke. Nevertheless, *P. knowlesi* infections occur in older as well as younger adult patients in the Kapit Division, and the vital organ dysfunction caused by this parasite may unmask underlying significant comorbidities.

Despite the significantly lower peripheral blood parasitemia, the patients with knowlesi malaria had clinical and laboratory profiles that were largely similar to those for patients with *P.*

Table 3. Details of Knowlesi Patients Presenting with (Patients 1–8) or Developing (Patients 9 and 10) Severe Malaria

Patient	Age, years	Sex	Hyperparasitemia	Hypotension	Acute renal impairment	Jaundice	Hypoglycemia	Lactic acidosis	Severe anemia	Acute pulmonary edema or respiratory distress syndrome	Outcome
1	68	F	Yes (parasite count, 222,570 parasites/μL)	Yes (systolic blood pressure, 80 mmHg)	Yes (serum creatinine level, 320 μmol/L)	Yes (total serum bilirubin, 45 μmol/L)	Yes (plasma glucose level, <1.1 mmol/L)	Yes (plasma lactate level, 17.4 mmol/L)	No	Yes	Died
2	36	M	Yes (parasite count, 178,000 parasites/μL)	No	Yes (serum creatinine level, 385 μmol/L)	No	No	No	No	No	Discharged
3	50	F	No	No	No	Yes (total serum bilirubin, 87 μmol/L)	No	No	No	Yes	Discharged
4	71	M	No	Yes (systolic blood pressure, 79 mm/Hg)	No	No	No	No	No	No	Discharged
5	66	M	No	No	No	Yes (total serum bilirubin, 66 μmol/L)	No	No	No	No	Discharged
6	61	F	No	No	No	No	No	No	No	Yes	Discharged
7	69	F	No	No	Yes (serum creatinine level, 418 μmol/L)	No	No	No	No	Yes	Discharged
8	36	F	Yes (parasite count, 214,000 parasites/μL)	No	No	Yes (total serum bilirubin, 178 μmol/L)	No	No	No	Yes	Died
9	73	F	No	No	No	No	No	No	No	Yes	Discharged
10	54	F	No	No	No	No	No	No	No	Yes	Discharged

NOTE. Severe malaria was defined on the basis of World Health Organization criteria for severe falciparum malaria [11]. Hyperparasitemia was defined as >100,000 parasites/μL. Severe anemia was defined as hemoglobin concentration <7.1 g/dL. Hypotension was defined as systolic blood pressure ≤80 mmHg; Acute renal impairment was defined as a serum creatinine level >265 μmol/L despite rehydration. Jaundice was defined as serum bilirubin level >43 μmol/L. Hypoglycemia was defined as a serum glucose level <2.2 mmol/L. Hyperlactaemia was defined as a lactate level >6.0 mmol/L. Acute pulmonary edema or respiratory distress was defined as a respiratory rate >30 breaths/min plus oxygen saturation <94% on room air and/or pulmonary infiltrates visible on a chest radiograph.

Table 4. Measures of Outcome in Patients Categorized by *Plasmodium* Species

Variable	*Plasmodium knowlesi* (*n* = 107)	*Plasmodium falciparum* (*n* = 24)	*Plasmodium vivax* (*n* = 21)
Fever clearance time, h	20 (12–31)	20 (11–37)	16 (4–28)
Parasite clearance time, days	1 (1–2)	3 (2–3.75)	3 (2–3)
Duration of hospitalization, days[a]	3 (3–4)	4 (4–5)	4 (3–4)

NOTE. Data are median value (interquartile range).

[a] Excludes 2 patients who died and 2 patients with hospital admission and day 1 data only.

falciparum and *P. vivax* infection, with a wide spectrum of illness. The most frequent complication in our cohort was respiratory distress, which affected 1 in 15 patients. It is also a relatively common sequelum of severe falciparum malaria [13]. Respiratory distress can reflect pulmonary edema, acute respiratory distress syndrome, or metabolic acidosis. In our group, a pulmonary, rather than metabolic, etiology was the main cause, because we measured blood lactate concentrations and had access to chest radiographs and pulse oximetry. The strong association between parasitemia at hospital admission and the development of respiratory distress in our patients suggests that parasite-specific effects that increase pulmonary capillary permeability rather than iatrogenic fluid overload or the syndrome of inappropriate anti-diuretic hormone secretion are responsible, as in falciparum malaria [14]. Patients with falciparum malaria who develop respiratory distress have a relatively poor prognosis [13], and both of our patients who died developed this complication. Respiratory distress has also been reported as a rare complication of vivax [15–17] and ovale [18, 19] malaria. We cannot explain the disproportionate number of female patients with this complication in the *P. knowlesi* group. Although the women in our cohort, compared with the men, had lower serum albumin concentrations at presentation (34.5 g/L vs 38.0 g/L; $P < .001$), sex association has not been reported in the case of the other human malarias and is likely to be attributable to the play of chance in the present study.

The *P. knowlesi* parasitemia at hospital admission was also strongly and independently associated with renal dysfunction, and 3 patients developed renal failure despite resuscitation and rehydration. As with respiratory distress, this is another complication of falciparum malaria that could be mediated by the parasite [20], although the microvascular sequestration that may contribute to *P. falciparum*–associated renal dysfunction [21] is not known to occur in *P. knowlesi* infection. The presence of *P. knowlesi* parasitemia at hospital admission was also independently associated with the total serum bilirubin but not serum ALT level. This could reflect relatively brisk hemolysis associated with the short (24-h) erythrocytic cycle rather than abnormal liver function, but the median parasitemia was low, and there was no inverse association with hemoglobin level at hospital admission. It is still possible that hepatic dysfunction is a relatively late vital organ complication of *P. knowlesi* malaria

Table 5. Changes in Laboratory Test Results between Hospital Admission and Discharge and Hospital Admission and Day 28 in Patients with *Plasmodium knowlesi* Infections

Variable	Change from hospital admission to discharge (*n* = 103)	Change from hospital admission to day 28 (*n* = 87)
Hemoglobin level, g/dL	−1.3 ± 1.0	0 ± 1.3[a]
White blood cell count, $\times 10^3$ cells/μL	0.1 ± 1.8	1.2 ± 2.1[a]
Neutrophil count, median value $\times 10^3$ cells/μL (IQR)	−0.6 (−1.5 to 0.6)[a]	0.5 (−0.5 to 1.45)
Lymphocyte count, median value $\times 10^3$ cells/μL (IQR)	0.7 (0.40–1.3)[a]	0.9 (0.4–1.5)[a]
Platelet count, median value $\times 10^3$ platelets/μL (IQR)	65 (31–113)[a]	184 (144–222)[a]
Serum creatinine level, median μmol/L (IQR)	−8.5 (−19 to 1)[a]	−12 (−21 to 0)[b]
Serum sodium level, median mmol/L (IQR)	2 (0.1–5)[a]	3 (0–8)[a]
Serum total bilirubin, median μmol/L (IQR)	−6 (−15 to −3)[a]	−7 (−12.9 to −4.3)[a]
Serum alanine aminotransferase level, median IU/L (IQR)	−1 (−10 to 11)	−18 (−32.9 to −4)[a]
Serum albumin level, g/dL	−1.0 ± 2.8	4.8 ± 4.1[a]
Serum glucose, median mmol/L (IQR) (*n* = 56)	−0.4 (−1.1 to 0.8)	−0.6 (−1.21 to 0.37)[b]
Plasma lactate level, median mmol/L (IQR) (*n* = 56)	0.1 (−0.5 to 0.4)	−0.1 (−0.5 to 0.5)

NOTE. Unless otherwise indicated, data are mean value ± standard deviation. IQR, interquartile range.

[a] $P < .01$.

[b] $P \leq .05$.