#### Materials and Methods

Clinical specimens-Respiratory secretions. Brisbane cohort. A total of 1,245 specimens (predominantly NPAs) were collected between January 1, 2003, and December 22, 2003, from patients presenting to the Royal Children's Hospital in Brisbane, Queensland, Australia, with symptoms consistent with acute lower respiratory tract infection.

St. Louis cohort #1. A total of 480 BAL specimens were tested. These included samples from a retrospective and a prospective collection. The retrospective specimens were from a sequential collection of BAL specimens submitted routinely to the Virology Laboratory at St. Louis Children's Hospital between December 2002 and August 2003 [33]. For the present study, an effort was made to select specimens from this collection from patients with acute respiratory illness, and to exclude specimens collected as routine post-lung transplant surveillance. The prospective specimens were from an ongoing study of the etiology of severe acute respiratory iliness and were collected between October 2005 and October 2006. Both collections included specimens from patients of all ages, although the large majority were from adults.

St. Louis cohort #2. This collection was made up of respiratory specimens, mostly nasopharyngeal swabs, submitted for routine virologic testing to the Virology Laboratory at St. Louis Children's Hospital between September 2005 and June 2006. The majority of these specimens were from children. Of the 410 specimens in this collection, 200 were selected because they had been found to be positive by fluorescent antibody staining or culture for influenzavirus A or B, respiratory syncytial virus, parainfluenza virus, rhinovirus, or

adenovirus.

Clinical specimens-Urine, Brisbane cohort, Urine specimens (226) that were submitted during 2003 to the diagnostic laboratory for routine investigation were collected. These represented a diverse mixture of donors, including those from (i) sexual health clinic (n = 50), (ii) pediatric clinic (n = 52), (iii) antenatal clinic (n = 33), (iv) indigenous health clinic (n = 36), and (v) bone marrow transplant patients (n = 55).

The St. Louis urine specimens were from a study of polyomaviruses in adult renal transplant recipients [24]. A total of 200 individuals were enrolled in the study between December 2000 and October 2002. From each patient, up to three specimens were tested, including a specimen obtained before the transplant and specimens

obtained at 1 and 4 mo after transplantation.

Diagnostic testing of clinical specimens for known respiratory viruses. Brisbane cohort, Nucleic acids were extracted from 0.2 ml of each specimen using the High Pure Viral Nucleic Acid kit (Roche Diagnostics Australia, http://www.rochediagnostics.com.au) according to the manufacturer's instructions. PCR assays for 17 known

respiratory viruses were performed as described [20].

St. Louis cohort. All respiratory specimens were tested originally by fluorescent antibody staining using a panel of monclonal antibodies directed against influenza A and B, respiratory syncytial, parainfluenza 1-3, and adenoviruses (Simulfluor Respiratory Screen; Chemicon, http://www.chemicon.com). Specimens that were negative were also cultured using cell culture systems that could detect the same group of viruses plus rhinoviruses, cytomegalovirus, and herpes simplex virus. Total nucleic acid extracts were purified using a Qiagen M48 instrument (http://www.qiagen.com). Nucleic acid extracts were tested for a panel of respiratory viruses using the EraGen MultiCode-PLx respiratory virus panel (EraGen Biosciences, http://www.eragen.com), a multiplex PCR assay that detects the following viruses: influenza A and B, respiratory syncytial virus A and B, parainfluenza 1-4, human meatpneumovirus, adenovirus subgroups B, C, and E, rhinoviruses, and coronaviruses OC43, 229E, and NL63.

Library construction and shotgun sequencing. Samples were prepared in the following manner for high throughput sequencing analysis. A total of 200 ul of neat NPA sample was thawed and directly treated with DNase I (Fermentas, http://www.fermentas.com) for 60 min at 37 °C. Total nucleic acid was extracted using the Masterpure Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, http://www.epibio.com). Then, 100 ng of total nucleic acid was randomly amplified using the RdAB protocol exactly as described [9]. RNA in the total nucleic acid preparation was converted to cDNA by reverse transcription with primer-A (5' GTTTCCCAGTCACGA-TANNNNNNNNNN). Two rounds of random priming with primer-A and extension with Sequenase (United States Biochemical, http:// www.usbweb.com) enabled second strand cDNA synthesis as well as random priming of DNA originally present in the total nucleic acid sample. Amplicons were then generated via 40 cycles of PCR using primer-B (5' GTTTCCCAGTCACGATA) with a cycling profile of: 94

°C 30 s; 40 °C 30 s; 50 °C 30 s; 72 °C 60 s. The primer-B-amplified material was TOPO cloned into pCR4.0 (Invitrogen, http://www. invitrogen.com) and transformed into bacteria, and white colonies were picked into 384-well plates. DNA was purified by magnetic bead isolation and sequenced using standard Big Dye terminator (v3.1) sequencing chemistry. Reaction products were ethanol precipitated, resuspended in 25 ul of water, and loaded onto the ABI 3730xl sequencer.

Analysis of shotgun sequences. Sequences were assessed for quality using Phred [34], and reads that contained less than 50 contiguous bases with a score of phred 20 or greater were rejected. The remaining reads were analyzed in the following steps: 1) reads were aligned to the human genome using BLASTn with an e<sup>-10</sup> cutoff; 2) remaining reads were aligned to a bacterial database using BLASTn with an e<sup>10</sup> cutoff; and 3) remaining reads were aligned to the viral RefSeq protein database using BLASTx with an e<sup>-2</sup> cutoff [35].

Complete genome amplification and sequencing. The WU genome derived from the index case was sequenced to 3x coverage using six unique pairs of PCR primers for the amplification. Amplicons were cloned into pCR4.0 and sequenced using standard sequencing technology. All primers used for amplification and sequencing are listed in Table S1 and their positions depicted in Figure S5. Additional complete genomes were sequenced to at least 2× coverage using the same primers listed in Table S1. Completed genome sequences have been deposited into GenBank (see Supporting Information for accession numbers).

Phylogenetic analysis. Protein sequences associated with the following reference virus genomes were obtained from GenBank: BK virus, JC virus, bovine polyomavirus, SV40, baboon polyomavirus (simian agent 12), finch polyomavirus, crow polyomavirus, goose hemorrhagic polyomavirus, African green monkey polyomavirus, hemorrhagic polyomavirus, African green monkey polyomavirus, budgerigar fledgling polyomavirus, murine pneumotropic virus, hamster polyomavirus, and murine polyomavirus (see Supporting Information for accession numbers). For WU virus, predicted open reading frames were used. For STAg, the predicted open reading frame of 194 amino acids was used for analysis. Multiple sequence alignment was performed using ClustalX (1.83). Neighbor-joining trees were generated using 1,000 bootstrap replicates.

Nucleic acid prevalence studies. For all PCR assays, standard precautions to avoid end product contamination were taken.

precautions to avoid end product contamination were taken, including the use of PCR hoods and maintaining separate areas for PCR set up and analysis. For initial screening of WU virus, PCR primers AG0044 5' tgttacaaatagctgcaggtcaa and AG0045 5' gctgcataatggggagtacc were used with Accuprime hot start Taq (Invitrogen) to amplify 1 ul of template using the following program: 40 cycles of 94 °C 30 s; 56 °C 30 s; 72 °C 60 s. For every 88 samples tested, seven notemplate negative controls were interspersed between the actual samples. Products were visualized following electrophoresis on 1% agarose gels. The resulting 250-bp amplicon was sequenced directly in both directions using primer AG0044 and AG0045. These sequences have been deposited in GenBank (see Supporting Information for accession numbers). Secondary confirmation was performed using primers AG0048 5' TGTTTTCAAGTATGTTGCATCC and AG0049 CACCCAAAAGACACTTAAAAGAAA that generate a 244-bp amplicon in the 3' end of the LTAg coding region. The same cycling profile of 40 cycles of 94 °C 30 s; 56 °C 30 s; 72 °C 60 s was used. For detection of both BK and JC viruses, primers AG0068 5' AGTCTT-TAGGGTCTTCTACC and AG0069 5' GGTGCCAACCTATGGAA-

## Supporting Information

°C 60 s.

Figure SI. Raw Sequence Data from High Throughput Screening A) The initial six shotgun reads with homology to polyomaviruses. B) The three contigs derived from the six reads.

CAG were used with a profile of 40 cycles of 94 °C 30 s; 56 °C 30 s; 72

Found at doi:10.1371/journal.ppat.0030064.sg001 (38 KB PDF).

Figure S2. Comparison of SV40 and WU Virus Replication Origin Region

The consensus TAg binding motif is GAGGC. The known primate polyomaviruses SV40, JC, BK, and baboon polyomavirus all have four copies of the copies of the binding site oriented as shown above for SV40 (NC\_001669). The first nucleotide of the third copy of the consensus TAg binding site is defined as nucleotide 1 for WU and SV40. Differences between SV40 and WU Virus are 1) one of the TAg binding sites in WU virus appears to be a non-canonical TAGGC; 2) the second and third consensus TAg binding sites in WU virus



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overlap; and 3) the nucletoide spacing between the TAg binding sites in WU virus varies from the prototype SV40 as shown. Shown in blue is the polyAsT tract that is commonly found to the late side of the origin in polyomaviruses

Found at doi:10.1371/journal.ppat.0030064.sg002 (462 KB PDF).

Figure S3. Predicted Splice Sites for LTAg and STAg '-

A consensus LTAg donor site was detected. Splicing to the consensus downstream acceptor would generate a LTAg of 648 amino acids. For STAg, an unspliced open reading frame of 194 amino acids was identified. A predicted slice donor site was also detected that would result in excision of a 70-nucleotide intron and production of a 217amino acid open reading frame.

Found at doi:10.1371/journal.ppat.0030064.sg003 (542 KB PDF).

Figure S4. WU Virus Lacks a Carboxyl Terminus Extension of the the LTAg

Multiple sequence alignment of WU virus LTAg with 13 other reference sequences reveal the presence of carboxyl terminus extensions in baboon polyoma, BK, JC, and SV40. WU virus does not appear to encode such a region.

Found at doi:10.1371/journal.ppat.0030064.sg004 (5.4 MB PDF).

Figure S5. Map of Primers and Sequence Reads

Locations of original shotgun reads are depicted as indicated. Locations of all sequencing primers are mapped to the complete genome. Primers used for amplification are shown in red.

Found at doi:10.1371/journal.ppat.0030064.sg005 (551 KB PDF).

Table S1. Primers Used for Amplification and Sequencing of WU

Found at doi:10.1371/journal.ppat.0030064.st001 (35 KB PDF).

#### Accession Numbers

The GenBank (http://www.ncbi.nlm.nih.gov/Genbank) protein sequen-

ces used in this paper are as follows:

ces used in this paper are as follows:

LTAg: African green monkey (NP\_848008); baboon polyomavirus

(YP\_406555); BK (YP\_717940); bovine (NP\_040788); budgerigar

(NP\_848014); crow (YP\_529828); finch (YP\_529834); goose

(NP\_849170); hamster (NP\_056730); JC (NP\_043512); KI Stockholm 60 (ABN09921); murine (NP\_041264); murine pneumotropic

(NP\_041232); SV40 (NP\_043127).

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STAg: African green monkey (NP\_848009); baboon polyomavirus i (YP\_406556); BK (YP\_717941); bovine (NP\_040789); budgerigar (NP\_848015); crow (YP\_529829); finch (YP\_529835); goose (NP\_849171); hamster (NP\_056732); JC (NP\_043513); KI Stockholm 60 (ABN09920); murine (NP\_041266); murine pneumotropic (NP\_041233); SV40 (NP\_043128).

VP1: African green monkey (NP\_848007); baboon polyomavirus 1 (YP\_406554); BK (YP\_717939); bovine (NP\_040787); budgerigar (NP\_848013); crow (YP\_529827); finch (YP\_529833); goose (NP\_849169); hamster (NP\_056733); JC (NP\_043511); KI Stockholm 60 (ABN09917); murine (NP\_041267); murine pneumotropic (NP\_041234); SV40 (NP\_043126).

VP2: African green monkey (NP\_848005); baboon polyomavirus 1 (YP\_406552); BK (YP\_717987); bovine (NP\_040785); budgerigar (NP\_848011); crow (YP\_529825); finch (YP\_529831); goose (NP\_849167); hamster (NP\_056734); JC (NP\_048509); KI Stockholm 60 (ABN09918); murine (NP\_041268); murine pneumotropic (NP\_041235); SV40 (NP\_043124).

WU complete genome sequences have been deposited under accession numbers EF444549-EF444554. VP2 partial sequences have been deposited under accession numbers EF444555-EF444593.

### Acknowledgments

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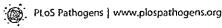
Author contributions. MDN, TPS, and DW conceived and designed the experiments. AMG, DMW, IMM, and GW performed the experiments. AMG, MDN, SBL, GAS, TPS, and DW analyzed the data. MDN, DCB, GAS, and TPS contributed reagents/materials/analysis tools. DW wrote the paper.

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Competing interests. AMG, MDN, GW, TPS, and DW are listed on a patent application to the United States Patent Office entitled "Novel Human Polyomavirus."

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# 医薬品 研究報告 調查報告書

一般的名称白血球除去人赤血球浮遊液研究報告の公表状況ProMED 20070501-1414, 2007 May 1. 情報源: Jamaica Observer, 2007 May 1.販売名(企業名)自血球除去赤血球「日赤」(日本赤十字社) 照射白血球除去赤血球「日赤」(日本赤十字社) 社)研究報告の公表状況 May 1. 情報源: Jamaica Observer, 2007 May 1.ジャマイカ	識別番号·報告回數		報告日	第一報入手日 2007. 5. 2	新医薬品等の区分 該当なし	機構処理欄
日血球除去赤血球「日赤」(日本赤十字社) 研究報告の公表状況 May 1. 情報源: Jamaica Observer,	一般的名称	白血球除去人赤血球浮遊液	· .	    ProMED_20070501-1		
	販売名(企業名)		研究報告の公表状況	May 1. 情報源: Jama	ica Observer,	

ジャマイカ保健省によると、2007年4月初めからの1ヶ月間に新規のマラリア症例11例が報告された。2例が4月15日~21日、3例 が4月8日~14日、6例が4月1日~7日に報告された。感染者の年齢は10歳~59歳だった。4月22日~27日に報告された2例は、 メスのハマダラカが媒介する熱帯熱マラリアで、デンハム・タウンとグリニッジ・タウンで報告され、それぞれの地域で初の報告と なった。発症日はそれぞれ4月4日と9日だった。

|4月30日、保健省はセントエリザベスで発生した症例は1月以降4例にとどまっていることを示し、他の地域へのマラリア感染拡大 |を抑制することができたと話した。また、2006年12月に最初の症例が報告されて以降、スーダン、インド、ハイチ、ホンジュラス、ウ |ガンダからの輸入感染症例が7例あったことを指摘した。加えて、4月1日~27日の間に実施された血液検体884の検査陽性率は|細菌、原虫等の感染 0.7%~1.8%だったことを説明し、陽性サンプルの数は減少を続けていることを示した。

一方で保健省は、最近の検査でデュアニー川周辺で捕獲されたAnopheles albimanus蚊が、媒介蚊撲滅のために使用されてい るマラチオン殺虫剤に耐性を示し始めたことが確認されたため、感染拡大を防ぐために代わりの殺虫剤を探している過程である と述べた。この検査は米国疾病予防対策センター(CDC)の指導で行われた。

|保健省は「引き続き、集中的なサーベイランス、媒介蚊の抑制、市民の教育に力を入れ、マラリア流行を収束させるために組織 横断的体制で協力していく。他地域へのマラリア感染拡大を予防するための措置が実施されている」と説明した。

# 使用上の注意記載状況 その他参考事項等

白血球除去赤血球「日赤」 照射白血球除去赤血球「日赤」

血液を介するウイルス、 vCID等の伝播のリスク

# 報告企業の意見

ジャマイカの首都キングストンでマラリアが発生しており、4月の 1ヶ月間に新規症例11例があったとの報告である。

日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の 有無を確認し、帰国後4週間は献血不適としている。また、マラリア流 行地への旅行者または居住経験者の供血を一定期間延期している (1~3年の延期を行うとともに、帰国後マラリアを思わせる症状があっ た場合は、感染が否定されるまでの間についても献血を見合わせ |る)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収 集、対応に努める。

今後の対応





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Date: Tue 1 May 2007

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BS \_\_\_NEW\_CASES\_OF\_MALARIA\_REPORTED.asp>

New cases of malaria reported

A total of 11 new cases of Malaria has been reported since the beginning of April [2007], the Ministry of Health said on 30 Apr 2007.

According to a release from the ministry, 2 cases were reported between 15-21 Apr [2007], 3 the previous week [15-21 Apr 2007] while a total of 6 cases was reported between 1-7 Apr [2007]. The ages of the affected persons range from 10 to 59.

Concerning the 2 new cases reported last week [22-27 Apr 2007], the ministry said they were found in Denham Town and Greenwich Town -- 2 of the areas in which the disease was 1st detected -- and were caused by the Plasmodium falciparum parasite which is transmitted by the female \_Anopheles\_ mosquitoe. The dates of onset were said to be 4 and 9 Apr [2007] respectively.

Yesterday [30 Apr 2007], the ministry said it had been able to limit the spread of malaria to other parishes, noting that the 4 cases detected in St. Elizabeth since January [2007] remained contained. The health ministry also pointed out that since the 1st case of malaria was reported last December [2006], there have been 7 imported cases originating from Sudan, India, Haiti, Honduras and Uganda.

Additionally, it said the number of positive samples continues to decline, explaining that the positivity rate of blood samples submitted to laboratories over the past weeks range between 0.7 and 1.8 percent from a total of 884 sample tests conducted between 1-21 Apr [2007].

Meanwhile, the ministry reiterated that it was in the process of seeking alternative insecticides to prevent further outbreaks after recent tests confirmed some resistance of the \_Anopheles albimanus\_ mosquito taken from the Duhaney River to malathion insecticide, which it was hoping to use to eliminate the parasites. The tests were

conducted by consultants from the United States-based Centers for Disease Control and Prevention (CDC).

"The Ministry of Health continues its thrust in the areas of intense active surveillance, vector control, public education and inter-sectoral collaboration in a concerted effort to end this outbreak, while precautionary measures are being taken to prevent the spread of malaria to other parishes," the ministry, however, assured.

Communicated by: ProMED cpromed@promecmail.org>

[We assume that some patients have had more than one sample, and the 884 positive samples therefore represents a lower number of patients. We reported on 9 Apr 2007 that 340 people had been infected; and it would be interesting to know the number of cases and not only the number of malaria-positive blood films to know whether the outbreak is under control. [] Mod. EP]

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fsee also:
Malaria - Jamaica (Kingston) (06) 20070409.1190
Malaria - Jamaica (Kingston) (05) 20070210.0515
Malaria - Jamaica (Kingston) (04) 20070208.0500
Malaria - Jamaica (Kingston) (03) 20070127.0358
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2006
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Malaria, imported - Europe ex Dominican Rep. 20041128.3176
2001
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1999
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# 医薬品 研究報告 調査報告書

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識別番号・報告回數		·		報告日	第一報入手日 新医薬品等		等の区分	機構処理欄
d196741					2007. 4. 24	該当	なし	3
	一般的名称	人赤血珠	求濃厚液	,		,	公表国	
販	売名(企業名)	赤血球M·A·P「日示 照射赤血球M·A·P「日示 形力赤血球濃厚液-LR「E 照射赤血球濃厚液-LR	3赤」(日本赤十字社)	研究報告の公表状況	ABC Newsletter. 200	7 Apr 13.	米国	
		6年度にかけて米国		()に報告された輸血副作			* 1. 四点	使用上の注意記載状況・ その他参考事項等
研究報	荷、感染症伝播、	19例で、内訳はTRA アナフィラキシーな。 3可能性が否定できる。	ど)67例(30.6%)、約	の他の副作用(ABO不適 田菌感染20例(9.1%)、AE %)となっている。	台以外の裕皿性副1 3O不適合による溶血	作用、輸皿 <b>與</b> 性副作用15	<b>運心</b> 適負 例(6.8%)、	赤血球M·A·P「日赤」 照射赤血球M·A·P「日赤」 赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」
告の概要								血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク
					· ·			1
,		吸告企業の意見_			今後の対応		M. est - lefe her	
	年度から2006年度 血副作用による死1	にかけて米国食品医 亡症例数である。	<b>ミ薬品局に報告され</b>	日本赤十字社では、薬事を収集し、医薬品医療機 後も引き続き輸血副作用	器総合機構を通じて	て国に報告し	作用の情報ている。今	
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# **JOURNALISTIC PRODUCT DEVIATIONS: Transfusion Fatalities**

An incorrect category label in the table of transfusion recipient fatalities reported to the Food and Drug Administration, published in the March 30 issue, has caused some confusion. This category was erroneously called "Non-ABO Hemolytic Reactions (K, JKa, FYa, etc.)." It should have been titled "Other Reactions: (Non-ABC hemolytic reactions, TACO, infectious disease transmission, anaphylaxis, etc.)." Corrected tables follow.

# Transfusion Recipient Fatalities Reported to the Food and Drug Administration, FY2004 - 2006

CATEGORIES	FY04	FY05	FY06
TRALI	21	30	35
	30.9%	36.6%	50.7%
Other Reactions: (Non-ABO hemolytic reactions, TACO, infectious disease transmission, anaphylaxis, etc.)	20	25	22
	29.4%	30.5%	31.9%
Bacterial	6	9	5
Contamination	8.8%	11.0%	7.2%
ABO Hemolytic	7	5	3
Transfusion Reaction	10.3%	6.1%	4.3%
Transfusion not Ruled Out	14	13	4
	20.6%	<i>15</i> .9%	5,8%
TOTAL	, 68	82	69

### **Total Fatalities**

CATEGORIES	FY04 to FY06	Average/Year
TRALI	86 39.3%	29
Other Reactions: (Non-ABO hemolytic reactions, TACO, infectious disease transmission, anaphylaxis, etc.)	67 30.6%	22
Bacterial Contamination	20 9.1%	7
ABO Hemolytic Transfusion Reaction	15 6.8%	5 ,
Transfusion not Ruled Out	31 14.2%	10
TOTAL	219	73

Data source: Leslie Holness, MD, Office of Blood Research and Review, Food and Drug Administration, Personal Communication, 1/24/07