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	機構処理欄
				2007. 8. 23	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Comeau P. CMAJ. 2007 Jul 31;177(3):242.	公表国	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)				カナダ	

研究報告の概要	使用上の注意記載状況・その他参考事項等
○カナダ血液サービス、シャーガス病スクリーニング導入へ カナダ血液サービスは、2008年後半の血液製剤製造プロセス見直しの際に北緯49度以北では稀にしか見られないシャーガス病のスクリーニングを開始する予定である。 Dana Devine副社長によると、2種類のシャーガス病検査法がカナダ保健省の認可を待っている状態である。供血血液の検査実施は、血小板製剤の製造を「バフィーコート」法に切替え、細菌検査を導入する時期以降になる予定である。 メキシコや中南米の住民800万人～1,100万人がシャーガス病の保因者であり、毎年45,000人以上の人が死亡している。原虫*Trypanosoma cruzi*が引き起こす感染症で、主に農村部の貧しい地域で昆虫が媒介する。一部の感染者には症状がないが、発症した場合は発熱、むくみ、脾臓や肝臓、リンパ節の肥大、心臓の炎症などを、感染直後や、時には感染数十年後に引き起こす。 媒介昆虫は、睡眠中の人の顔を噛む。原虫は媒介昆虫の排泄物から、人の粘膜や皮膚の傷口を通って感染する。汚染された生の食物による感染、母子感染、臓器移植や実験室暴露による感染もある。 カナダでは、これまでに輸血による感染が2例マニトバ州で発生した(1986年及び2000年)。患者は原疾患で死亡している。ワシントン州及びカリフォルニア州の高リスク集団を対象にした研究では、0.2%が感染しており、そのうち20%が他人に感染させる危険があることが判明した。Devineによると、カナダの潜在的シャーガス病患者は感染地域の訪問歴や居住歴を尋ねる問診によって供血者から排除されてきた。	新鮮凍結血漿「日赤」 新鮮凍結血漿-LR「日赤」 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

報告企業の意見	今後の対応
カナダ血液サービスは、2008年後半にシャーガス病のスクリーニングを開始する予定であるとの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。今後も引き続き情報の収集に努める。

JRC2007T-056

Lancet publisher to sell arms business

Responding to pressure from physicians, readers and leading medical journals, the company that publishes *The Lancet* will stop hosting trade shows that promote arms and the defence industry (*CMAJ* 2007;176[10]:1265).

Reed Elsevier will begin withdrawing from its exhibitions, conferences, congresses and meetings in the second half of this year, CEO Sir Crispin Davis said in a June 1 news release.

"Our defence shows are quality businesses which have performed well in recent years," Davis said. "Nonetheless, it has become increasingly clear that growing numbers of important customers and authors have very real concerns about our involvement in the defence exhibitions business."

In March, more than 50 editorial consultants at *The Lancet* posted a letter at cmaj.ca arguing that Reed Elsevier's involvement in the arms trade was incompatible with the journal's commitment to health. That was followed by an online petition which contained nearly 1000 signatures, and calls by *The Lancet*, *BMJ*, *the Journal of the Royal Society of Medicine*, the London School of Hygiene and Tropical Medicine, and by *CMAJ* Editor-in-Chief Paul Hébert asking Reed Elsevier to stop participating in the arms' trade.

"We have listened closely to these concerns and this has led us to conclude that the defence shows are no longer compatible with Reed Elsevier's position as a leading publisher of scientific, medical, legal and business content," Davis said in the news release.

Pressure from *The Lancet* and *BMJ*, and the public Campaign Against Arms Trade, was critical in influencing Reed Elsevier, says Anna Jones, the campaign coordinator.

"It was the concerted efforts of Reed's stakeholders, and particularly healthcare professionals, which brought the pressure of that concern to bear," she wrote to *CMAJ* in an email. "The voices of readers and contributors to Reed's publications ... were extremely important in persuading Reed Elsevier that continuing to take part in the arms trade was compromising their credibility within the scientific, medical, legal and business communities."

In a related issue, members of Physicians for Global Survival have written the CMA asking MD Management to create an ethical investment portfolio for physicians who do not wish their money to support the arms and defence industries.

Despite Reed Elsevier's decision, MD Management is not planning further restrictions on its funds, says Guy Bélanger, president and CEO of MD Funds Management. MD funds currently do not hold any tobacco company stock.

"We haven't really received any significant requests from clients generally to increase the number of restrictions on our portfolio," Bélanger told *CMAJ*, adding that if individual physicians do request ethical investments, the fund managers can find them.

Although MD Management has received "the odd letter" requesting that it divest from arms manufacturers or the defence industry, "we also receive the [odd] letter on the other side of the equation, which makes it difficult to manage within a fund," Bélanger said. — Laura Eggertson, Ottawa

DOI:10.1503/cmaj.070884

Canadian Blood Services to screen for Chagas disease

Canadian Blood Services will commence testing for a tropical disease rarely found north of the 49th parallel when the blood donation production process is overhauled in mid-to-late 2008.

Dana Devine, Canadian Blood Services vice-president of medical, scientific and research affairs, says 2 tests for Chagas disease (also called American trypanosomiasis) are in line for Health Canada approval. But testing donated blood for Chagas will be delayed until the agency switches to the "Buffy Coat" method of blood platelet production. (Used in Europe for 2 decades, the method produces a more consistent product and yields more platelets. Buffy Coat refers to the layer of white blood cells left atop the mass of red cells when whole blood is centrifuged). "This is a production method that allows us to prepare a pool platelet product for transmission that we can test for bacteria," says Devine.

Between 8 and 11 million people in Mexico, Central America, and South America are believed to have Chagas, with upwards of 45 000 deaths a year. Caused by *Trypanosoma cruzi* parasites, it is transmitted by insects found mainly in rural, poverty-stricken areas. While some victims are symptom free, others suffer fever, swelling, an enlarged spleen, liver and lymph nodes, and heart inflammation immediately after infection or sometimes decades later (*CMAJ* 2006; 174[8]:1096).

Insects bite sleeping victims, usually on the face (earning them the moniker "kissing bugs"). The parasites, carried in feces, infect through mucous membranes or breaks in skin. Infection also occurs through consumption of contaminated uncooked food, congenital transmission, organ transplants and accidental laboratory exposure.

Two cases are known to have been transmitted via blood transfusions in Canada, both in Manitoba (1986 and 2000). The patients died of underlying conditions. Studies of high-risk populations in Washington and California showed 1/500 may have been infected, with 20% of those able to transmit the infection. Devine says potential Chagas sufferers in Canada have been screened out for years via an agency questionnaire that asks if potential donors have visited or lived in affected areas.

Is waiting until 2008 to test too long?

No, says Dr. Jay Keystone, Toronto General Hospital's Tropical Disease Unit staff physician. "There has always been a low but definite risk to the blood supply from people from the Chagas areas of the world." But while it's prudent to test, suggesting Chagas is a medical emergency "takes it all out of perspective," he adds. — Pauline Comeau, Ottawa

DOI:10.1503/cmaj.070882

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 2007年9月10日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	乾燥 pH4 処理人免疫グロブリン	研究報告の公表状況	Analysis of two human parvovirus PARV4 genotypes identified in human plasma for fractionation Journal of general virology (England) Aug 2007, 88 (Pt 8) p2162-7	公表国 英国	
販売名（企業名）	サングロポール（CSL ベーリング株式会社）				

研究報告の概要	使用上の注意記載状況・その他参考事項等
問題点（PARV4 の PARV5 の DNA 配列を解析比較） パルボウイルスは小型でエンベロープを持たない直鎖一本鎖 DNA ウイルスである。PARV4 は薬物使用者の血漿から PCR 法で最近同定された変異型のヒトパルボウイルスである。また血液製剤や血漿分画製剤の製造に用いるプール血漿中で発見されている。しかしヒトの疾病への関与は判明してない。 筆者らは製造用のプール血漿から分離された 4 種の PARV4 ゲノムと 2 種の PARV5 ゲノムを、GenBank のオリジナル PARV4 と DNA 配列を解析して比較した。 PARV4 の ORF は、オリジナル PARV4 との相同性は約 98-100%で、PARV5 の ORF の相同性は 91-94%であった。 このことから PARV4 には 2 つのサブグループが存在することを示唆している。 筆者らはジェノタイプ 1 とジェノタイプ 2（従来は PARV5 と命名されていた）から構成される PARV4 の単独のウイルス名を使用することを提案している。	

報告企業の意見	今後の対応
現在はPARV4について詳細が不明であり、検査方法が確立されていないので、今後も情報を収集することに努める。	今後とも新しい感染症に関する情報収集に努める所存である。

(26)

ABJ2007Z03

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Short Communication

Analysis of two human parvovirus PARV4 genotypes identified in human plasma for fractionation

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The presence of the novel parvovirus PARV4 and a related variant, PARV5, was recently demonstrated in pooled plasma used in the manufacture of blood and plasma-derived medicinal products. DNA sequence analysis of nearly full-length genomes of four PARV4 and two PARV5 strains from manufacturing plasma pools is now presented. Like PARV4, PARV5 encodes two non-overlapping open reading frames (ORF1 and ORF2), homologous to the non-structural and capsid proteins of other parvoviruses, respectively. A highly conserved region in ORF2 contains phospholipase A_2 motifs involved in parvovirus infectivity. Hybridization of strand-specific probes to DNA extracted from high-titre, PARV4-positive plasma revealed that the positive and negative strands are packaged into PARV4 virions in similar quantities. This extended analysis of nearly full-length PARV4 and PARV5 sequences suggests that they are closely related genotypes and the use of a single virus name, PARV4, comprising genotypes 1 and 2 (previously termed PARV5) is proposed.

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Parvoviruses are small, non-enveloped viruses containing linear, single-stranded DNA genomes. They infect a diverse range of vertebrate and invertebrate hosts. PARV4 is a novel human parvovirus recently identified in plasma from an intravenous drug user (IVDU) by using a sequence-independent PCR amplification method (Jones *et al.*, 2005). This individual presented with symptoms of acute viral infection, including fatigue, vomiting, diarrhoea, sore throat, neck stiffness and joint pains, and was also co-infected with hepatitis B virus. Nothing is yet known about the role of PARV4 in human disease. DNA sequence analysis of the PARV4 genome identified two open reading frames, ORF1 and ORF2, with significant similarity to the non-structural and capsid proteins of parvoviruses, respectively. Phylogenetic analysis showed that PARV4 was distinct from other known animal or human parvoviruses (Jones *et al.*, 2005).

The prototype human parvovirus B19 (B19V) is a frequent contaminant of plasma pools used in the manufacture of plasma-derived medicinal products (reviewed by Brown *et al.*, 2001; Laub & Strengers, 2002). PARV4, and a related variant virus termed PARV5, have been identified in approximately 4–5 % of these manufacturing pools (Fryer *et al.*, 2006, 2007b). PARV4 and PARV5 were found to share 92 % nucleotide identity over a 178 bp region of ORF1. In the present study, more extensive sequence analysis has been performed on PARV4 and PARV5 strains identified in recent and older manufacturing plasma pool samples (Fryer *et al.*, 2006, 2007b). Strand-specific probes were used to analyse the packaging of the PARV4 genome.

Nucleic acid was extracted from 1 ml volumes of manufacturing plasma pools, sourced from Europe and North America, as described previously (Baylis *et al.*, 2004).

The GenBank/EMBL/DDBJ accession numbers for the PARV4 and PARV5 sequences determined in this study are DQ873386–DQ873391.

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Samples were screened for the presence of PARV4 and PARV5 DNA by using primers to ORF1 and/or ORF2, as described previously (Fryer *et al.*, 2006, 2007b), and nearly full-length PARV4 and PARV5 sequences determined in positive plasma pools. To avoid sequencing on different templates, two approximately 2.5–2.8 kb overlapping cDNA fragments spanning the ORF1 and ORF2 coding regions were amplified from extracted plasma samples. The 5′ PARV4/5 fragment (nt 142–2977, GenBank accession no. AY622943) was amplified by using primers PARV4/5seq1 (5′-CGGTCCCGCGAAAATTACGTATT-3′) and PVORF2R (Fryer *et al.*, 2007b), whilst the 3′ fragment (nt 2710–5247 of GenBank accession no. AY622943) was amplified by using primers PVORF2F (Fryer *et al.*, 2007b) and PARV4/5seq21 (5′-CGCGAAAATTGCGTATTTCCG-CT-3′). The reaction mixture consisted of 1 × Phusion HF Buffer (Finnzymes OY), 200 μmol each dNTP l^{-1}, 10 pmol each primer, 1 U proofreading Phusion Hot Start DNA Polymerase (Finnzymes OY) and 5 μl extracted DNA in a final volume of 50 μl. Thermal cycling was performed as follows: 98 °C for 10 s, followed by 45 cycles of 98 °C for 10 s, 63 °C for 30 s and 72 °C for 3 min. PCR products were purified, cloned into the pT7 Blue vector (Novagen) and both strands were sequenced as described previously (Fryer *et al.*, 2006). Sequences were assembled and ORFs were mapped by using the GCG software package, version 10.2 (University of Wisconsin). Comparisons of deduced protein sequences were performed by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic analysis of nearly full-length PARV4 and PARV5 genomes was performed alongside full genome sequences of other members of the subfamily *Parvovirinae* (Lukashov & Goudsmit, 2001). Sequences were aligned by using CLUSTAL_W (Chenna *et al.*, 2003), and a neighbour-joining tree (nucleotide distance with Jukes–Cantor correction, pairwise gap deletion) with bootstrap resampling (100 replicates) was constructed by using MEGA3 software (Kumar *et al.*, 2004). Bootscan analysis was performed by using the SimPlot software, version 3.5.1 (Lole *et al.*, 1999).

For analysis of the encapsidation pattern of PARV4 virions, DNA was extracted from a high-titre PARV4-positive individual plasma donation and negative plasma controls as described above. This plasma sample was also positive for hepatitis C virus (HCV) RNA and was identified by a routine screening process to eliminate HCV-positive donations prior to the pooling of plasma for fractionation. Positive and negative plasmid controls were also tested. The PARV4-positive plasmid control contained a 1683 bp insert of PARV4 ORF2 (nt 1705–3387, GenBank accession no. AY622943). The PARV4-negative plasmid control contained a 1471 bp insert of PARV4 ORF1 (nt 603–2073 of AY622943). The PARV4 fragments were cloned into the vector pT7 Blue (Novagen) as described previously (Fryer *et al.*, 2006). All DNA samples were denatured with 0.25 M NaOH at 37 °C for 15 min prior to spotting onto a Hybond XL membrane (GE Healthcare). Nucleic acids were UV cross-linked. Membranes were pre-hybridized with Rapid-hyb buffer (GE Healthcare) in accordance with the manufacturer's instructions. Strand-specific probes were prepared by 5′-end labelling of positive- and negative-sense oligonucleotides in ORF2 of PARV4 (PVORF2F and PVORF2R; Fryer *et al.*, 2007b) by using [γ-^{32}P]ATP and T4 polynucleotide kinase (TaKaRa) and used in accordance with the manufacturer's instructions. Replicate filters were hybridized at 42 °C with either the positive- or negative-sense probes. Following hybridization, filters were washed at 42 °C with 0.1 × SSC, 0.1 % SDS, in accordance with the manufacturer's instructions (GE Healthcare). Blots were quantified by using the InstantImager (Perkin Elmer) electronic autoradiography system.

Nearly full-length sequences (GenBank accession numbers indicated), comprising ORF1 and ORF2, were determined for the following strains of PARV4: BR10749-4 (DQ873386), BR11955-4 (DQ873388), A23-4 (DQ873389) and C51-4 (DQ873387); and for PARV5, BR10627-5 (DQ873390) and C25-5 (DQ873391). Strains BR10749-4 and BR10627-5 were identified in our preliminary study of manufacturing plasma pools (Fryer *et al.*, 2006), whilst the other strains were identified in further screening studies of plasma pools (Fryer *et al.*, 2007b). Strains BR10749-4, BR11955-4 and BR10627-5 were from plasma samples obtained between 2004 and 2005, whereas A23-4, C51-4 and C25-5 date from 1990–1993.

As with B19V, PARV4 and PARV5 genomes comprise two main ORFs: ORF1 (1992 nt, encoding 664 aa) and ORF2 (2745 nt, encoding 915 aa). However, unlike B19V, the two ORFs do not overlap and are separated by 103 and 106 nt for PARV4 and PARV5, respectively. By BLASTP analysis, PARV4 and PARV5 ORF1-encoded proteins are homologous to the non-structural protein of parvoviruses, NS1, showing greatest amino acid identity with NS1 of hamster (H-1) and goose parvoviruses (50 and 43 % identity, respectively). The amino acid sequence contains parvovirus-conserved motifs associated with rolling-circle replication [xuHuHuuux (aa 97–99) and uxxYuxxKxx (aa 157–161); Ding *et al.*, 2002] and ATPase [A site GxxxxGK(T/S) (aa 334–341) and B site uuuu(D/E)(D/E) (aa 374–379); Astell *et al.*, 1987; Ding *et al.*, 2002]. The ORF2-encoded proteins are homologous to the viral capsid protein of parvoviruses, VP1, sharing approximately 45 and 41 % amino acid identity with VP1 of hamster parvovirus (H-1) and B19V-Au genotype 1, respectively. The amino acid sequence contains phospholipase A_2 motifs [YxGxG (aa 224–228) and HDxxY (aa 247–251)], required for parvovirus infectivity (Zádori *et al.*, 2001). These motifs are completely conserved between PARV4 and PARV5.

Pairwise comparisons of each ORF of PARV4 and PARV5 strains indicate that ORF2 is more conserved than ORF1 (Table 1), with most nucleotide differences limited to synonymous substitutions (91 % of nucleotide substitutions in ORF2 are synonymous, compared with 89 % in ORF1). Consequently, the amino acid sequences are more conserved, particularly for ORF2, suggesting that if PARV4 and PARV5 represent two distinct genotypes, they might

Table 1. Nucleotide and amino acid identities between the original PARV4 strain (GenBank accession no. AY622943) and PARV4 and PARV5 strains from plasma pools

Virus/strain	Identity (%) (nucleotide/amino acid)	
	ORF1	ORF2
PARV4*		
BR10749-4†	99.7/99.5	99.9/100.0
C51-4‡	99.6/99.5	99.9/99.9
BR11955-4†	98.2/98.6	98.5/99.6
A23-4‡	98.2/99.4	98.5/99.6
PARV5		
BR10627-5†	90.9/96.8	93.5/98.7
C25-5‡	90.9/97.6	93.4/99.2

*Strains BR10749-4 and C51-4 fall within the same subgroup as the prototype PARV4 strain; strains BR11955-4 and A23-4 represent a separate subgroup of PARV4.
†Strains derived from recent plasma pools (2004–2005).
‡Strains derived from plasma pools from 1990–1993.

be expected to represent a single serotype. This would be analogous to B19V genotypes 1–3, where serological cross-reactivity has been demonstrated *in vitro* by using clinical sera against a genotype 2 virus isolate (Blümel *et al.*, 2005) and baculovirus-expressed genotype 3 virus capsid antigens (Parsyan *et al.*, 2006). PARV4 sequences share approximately 98–100 % nucleotide identity with the original PARV4 isolate (GenBank accession no. AY622943), whilst PARV5 sequences share approximately 91–94 % nucleotide identity with the original PARV4 sequence (Table 1). Sequence analysis indicates that there are two subgroups of PARV4, with a nucleotide divergence of 2 % between strains BR11955-4 and A23-4, and the prototype PARV4 sequence (GenBank accession no. AY622943). Overall, PARV4 and PARV5 strains share approximately 92 % nucleotide identity, similar to the level observed between B19V genotypes 1–3 (Servant *et al.*, 2002; Gallinella *et al.*, 2003). Comparison of recent with archived strains within both PARV4 subgroups and PARV5 shows little evidence for evolutionary change over 10–15 years (99.8 % nucleotide identity over an approx. 4800–5000 bp region between recent and archived strains). This is in contrast to current evidence suggesting that carnivore and B19V parvoviruses exhibit a high rate of genetic change (Shackelton *et al.*, 2005; Shackelton & Holmes, 2006).

Although not based on full-length genomes, the current sequence analysis of PARV4 and PARV5 strains indicates that the two ends of the viral DNA are related by inverted terminal repeats (ITRs), as is the case for many parvoviruses. In the prototype PARV4 sequence (Jones *et al.*, 2005; GenBank accession no. AY622943), nt 128–244 and 5151–5267 respectively correspond to incomplete 5′ and 3′ ITRs, possibly forming part of the stem structure of the hairpins, with nt 1–128 at the 5′ end forming part of a loop structure (an axis of symmetry lies between nt 6 and 7). Incomplete ITRs obtained in this study are distinct between PARV4 and PARV5 (97–98 % nucleotide identity) and are slightly shorter than those of the original PARV4 sequence (GenBank accession no. AY622943), reflecting the length of the sequences obtained. Further sequencing of the ITRs has proved difficult, possibly due to the presence of repeated heptanucleotide sequences (CTTCCGG) identified in the hairpin stems of the original PARV4 sequence (GenBank accession no. AY622943), which are similar to those observed in the ITRs of goose and muscovy duck parvoviruses (Zádori *et al.*, 1995).

Phylogenetic analysis of PARV4 and PARV5 with other members of the subfamily *Parvovirinae* indicates that they are equidistant from human/primate autonomous parvoviruses and the adeno-associated (AAV)/avian viruses (Fig. 1a). Based upon this nearly full-genome analysis, PARV4 and PARV5 are related most closely to porcine parvovirus 2 (PPV-2), a virus identified in swine sera from Myanmar (Hijikata *et al.*, 2001). Recombination analysis of the PARV4, PARV5 and PPV2 sequences indicates that these viruses do not appear to be recombinants of other known viruses (data not shown). Similarly, there was no evidence for recombination between PARV4 and PARV5, with the clustering pattern of all PARV4 and PARV5 strains being the same regardless of whether full-length sequences or individual ORFs were analysed. At the protein level, however, ORFs 1 and 2 of PARV4 and PARV5 showed closest amino acid identity to the non-structural and capsid proteins of hamster parvovirus H-1, a virus causing significant morbidity and mortality in neonatal hamsters (Besselsen *et al.*, 1999). Phylogenetic analysis again indicates that there are two subgroups of PARV4 (also indicated in Table 1); strains BR10749-4 and C51-4 cluster with the original PARV4 sequence (GenBank accession no. AY622943), whereas BR11955-4 and A23-4 represent a different subgroup. Phylogenetic analysis of non-synonymous substitutions also demonstrated that PARV4 and PARV5 sequences formed two separate clusters with bootstrap values of 98–100 % (data not shown). Nucleotide similarity plots along nearly full-length PARV4 and PARV5 sequences revealed a highly conserved region (>99 % nucleotide identity) between nt 2955 and 3420 (GenBank accession no. AY622943), located at the 5′ end of ORF2 (Fig. 1b). The phospholipase A_2 motifs, required for parvovirus infectivity, are located here. Based upon PARV4 and PARV5 sequence alignments of this region, we have designed a consensus real-time TaqMan PCR to quantify both PARV4 and PARV5 sequences (Fryer *et al.*, 2007b).

The encapsidation pattern of PARV4 was investigated by using strand-specific oligonucleotide probes to ORF2 and nucleic acid extracted from a high-titre PARV4-positive individual plasma donation. Both positive- and negative-sense probes hybridized to the PARV4-positive plasma DNA (sample C, Fig. 2) and PARV4 ORF2 control plasmid DNA (Fig. 2, plasmid concentrations indicated), but not to

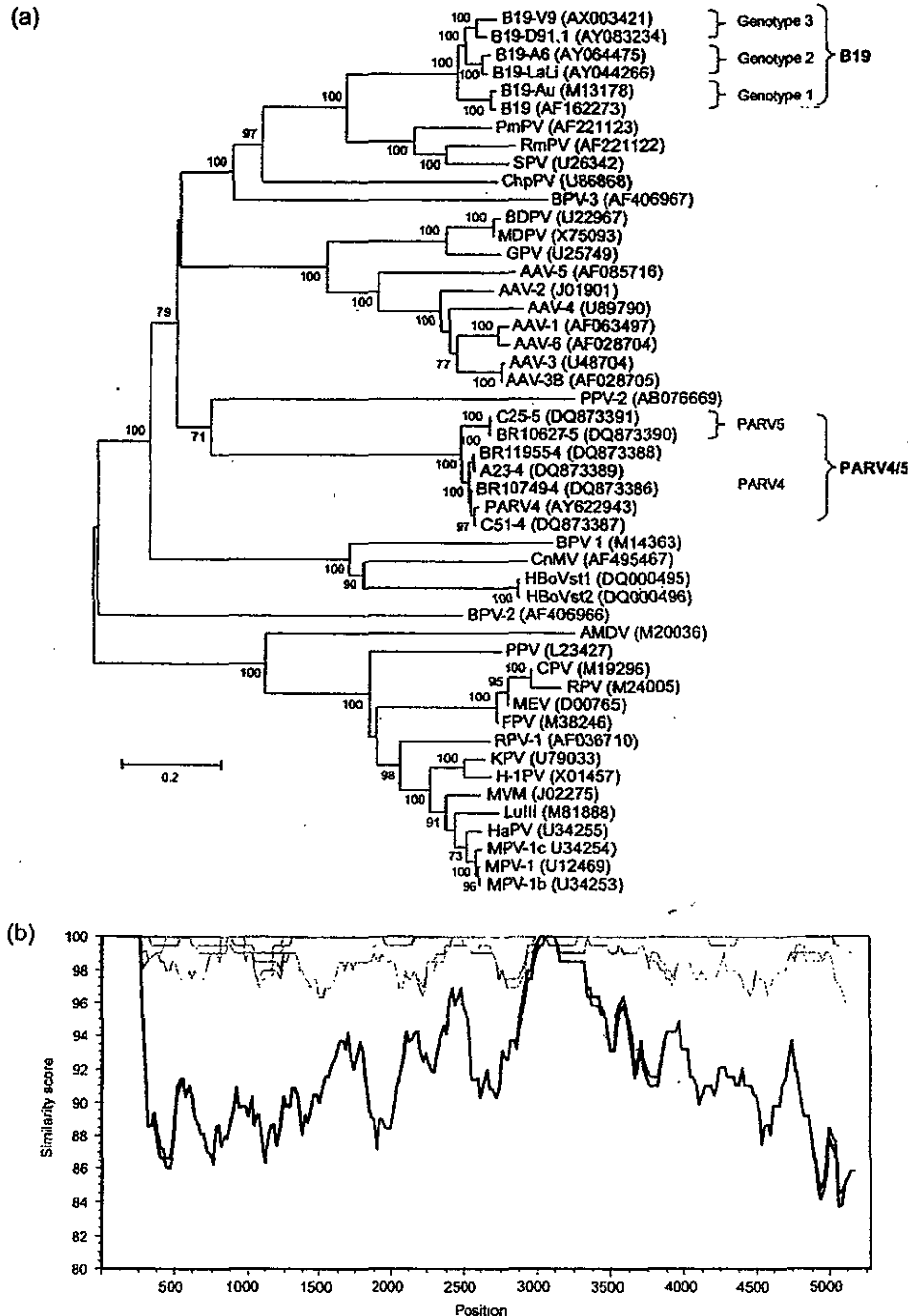

Fig. 1. (a) Phylogenetic analysis of nearly full-length PARV4 and PARV5 sequences (indicated) and other members of the subfamily *Parvovirinae*, including recently sequenced members porcine parvovirus 2 (PPV2) (Hijikata *et al.*, 2001) and human bocavirus (HBoVst1 and HBoVst2) (Allander *et al.*, 2005). B19V genotypes 1 (Shade *et al.*, 1986), 2 [A6 and LaLi (Nguyen *et al.*, 2002; Hokynar *et al.*, 2002)] and 3 [V9 and D91.1 (Nguyen *et al.*, 1999; Servant *et al.*, 2002)] are indicated. Virus names are given according to the ICTV nomenclature (Tattersall *et al.*, 2005), with GenBank accession numbers in parentheses. (b) Graphic representation of nucleotide similarity along nearly full-length sequences of PARV4 (grey lines) and PARV5 (black lines) strains, compared with the prototype PARV4 sequence (GenBank accession no. AY622943), using SimPlot. The co-ordinates for ORF1 and ORF2 are 283–2274 and 2378–5122 nt, respectively. Window, 200 bp; step, 20 bp; GapStrip, on; Kimura (two-parameter); T/t, 2.0.

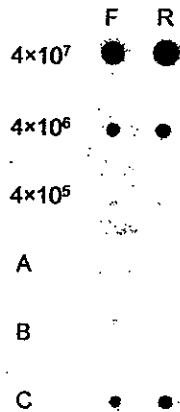

Fig. 2. Analysis of the packaging of the PARV4 genome. DNA from a high-titre PARV4-positive plasma sample was extracted, denatured, immobilized on a nylon membrane and probed with labelled, strand-specific oligonucleotide probes (F, forward; R, reverse) (C). DNA from PARV4-negative plasma (A, B) and PARV4 plasmid controls at 4×10^7, 4×10^6 and 4×10^5 copies per sample are shown.

PARV4 ORF1 control plasmid DNA (data not shown) or to human genomic DNA present in the plasma pools, even when levels were of the order of 10^6 copies ml^{-1} (sample B, Fig. 2). When the number of counts binding to the PARV4-positive plasma DNA sample was determined and compared with the respective plasmid control samples, it was found that the positive and negative strands were present in similar amounts. This packaging pattern is not characteristic of all parvovirus genera (Cotmore & Tattersall, 2006).

The availability of additional, nearly full-length sequences for PARV4 and the related variant PARV5 will assist in further phylogenetic studies and in the design of consensus and discriminatory assays. The recent finding that there are three distinct genotypes of B19V has led to a review of assays available for the detection and quantification of this virus (Baylis *et al.*, 2004, 2007; Hokynar *et al.*, 2004). In some cases, assays fail to detect the newly identified genotypes 2 and 3 of B19V, with consequent issues in terms of clinical diagnosis and viral load determinations in the plasma-fractionation setting, where it is a regulatory requirement to limit levels of B19V (Baylis *et al.*, 2004). The ability to design assays for PARV4 and PARV5 will assist in future studies to determine the role of these viruses in human disease. Nothing is yet known of the pathology associated with these viruses or whether PARV4 and PARV5 cause subclinical infections. However, PARV4 and PARV5 have been identified in plasma from healthy blood donors (Fryer *et al.*, 2006, 2007b) with an increased incidence in febrile patients, including IVDUs and homosexual men (Fryer *et al.*, 2007b), and in HCV-infected individuals (including IVDUs) (Fryer *et al.*, 2007a). Phylogenetic analysis performed in this study shows that PARV4 and PARV5 cluster separately from each other, and could thus be considered as two genotypes of the same virus. We therefore propose the use of a single virus name, PARV4, comprising genotypes 1 and 2 (previously termed PARV5).

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