Rev. Med. Virol. 2010; **20**: 231–244.
Published online 28 May 2010 in Wiley InterScience
(www.interscience.wiley.com)
DOI: 10.1002/rmv.648

REVIEW

The expanding range of parvoviruses which infect humans

Kevin E. Brown*
Virus Reference Department, Centre for Infection, Health Protection Agency, UK

SUMMARY

The first human parvoviruses to be described (1960s) were the adeno-associated viruses (AAVs, now classed as dependoviruses), originally identified as contaminants of cell cultures, followed by parvovirus B19 (B19V) in 1974, the first parvovirus to be definitively shown to be pathogenic. More recently two new groups of parvoviruses, the human bocaviruses (HuBoV) and the Parv4 viruses have been identified. These four groups of human viruses are all members of different genera within the Parvovirus family, and have very different biology, epidemiology and disease associations from each other. This review will provide an overview of the virological, pathogenic and clinical features of the different human paroviruses, and how these new viruses and their variants fit into the current understanding of parvovirus infection. Copyright © 2010 John Wiley & Sons, Ltd.

Received: 6 November 2009; Revised: 4 January 2010; Accepted: 12 January 2010

INTRODUCTION

Parvoviruses are, as their name suggests, small viruses (from the Latin '*parvum*' meaning small), with a single stranded DNA genome [1]. They are ubiquitous in nature and common infectious agents of animals, including birds and insects. At least four different types of parvovirus are now known to infect humans: dependoviruses, human bocaviruses (HuBoV), parvovirus B19 (B19V) and Parv4. Although these viruses look similar under the electron microscope (Figure 1) they are members of different genera within the *Parvoviridae* family, with different replication and transcription strategies (Table 1) and different cellular tropisms. Most information is known about B19V, and for many years it was the only parvovirus known to cause human disease. With the discovery of new human parvoviruses this is now under review, although for most of the new viruses there is only limited information, and their full pathogenicity has not been determined. This review will summarise the key features of each of these different virus families and the evidence for their role in human disease. There have been many excellent reviews on B19V, and the reader is advised to consult them for a more extensive review of the clinical presentation of B19V infection.

PARVOVIRUS TAXONOMY

Parvoviruses, are small (18–26 nm) non-enveloped, icosahedral viruses. They have a linear single-stranded DNA genome, with hairpin sequences at each end. The length of the DNA is between 4500 and 5500 nucleotides. The *Parvoviridae* family consists of two subfamilies, the *Densovirinae* and the *Parvovirinae*: the *Densovirinae* are all viruses of insects while the *Parvovirinae* are viruses of vertebrates [1]. The *Parvovirinae* is further subdivided into five genera based on replication pattern, transcription map and sequence homology (Table 1). Most of the parvoviruses have a relatively simple genome with two large open reading frames encoding the non-structural (NS or Rep) proteins and capsid (VP or Cap) proteins, respectively. The short parvovirus genome does not encode a DNA polymerase, so all parvoviruses are dependent on either host cell enzymes (transiting S phase) or co-infection with another virus for efficient DNA replication.

*Corresponding author: K. E. Brown, Virus Reference Department, Centre for Infection, Health Protection Agency, 61 Colindale Avenue, London, NW9 5EQ, UK.
E-mail: kevin.brown@hpa.org.uk

Abbreviations used:
AAVs, adeno-associated viruses; B19V, parvovirus B19; BPV1, bovine parvovirus; HuBoV, human bocavirus; PPGSS, papular-purpuric gloves and socks syndrome; PRCA, pure red cell aplasia; TAC, transient aplastic crisis; TEC, transient erythroblastopenia of childhood.

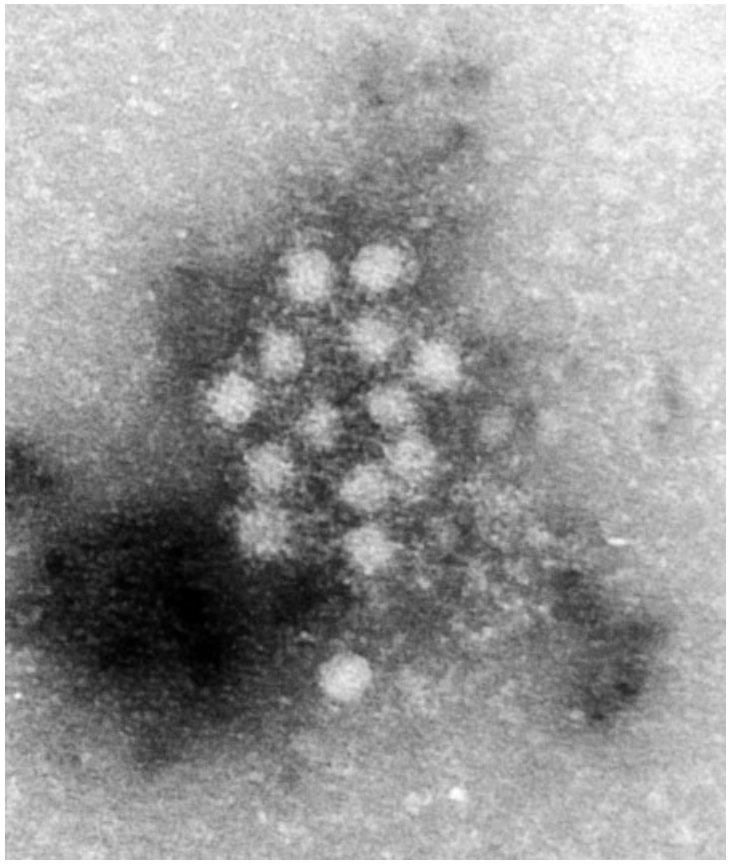

Figure 1. Immune EM of Parv4 virus particles from a patient with high titre Parv4 DNA in plasma. The micrograph shows the typical appearance of parvovirus particles coated with antibody. EM provided by Dr Hazel Appleton, Centre for Infections, Health Protection Agency

PARVOVIRUSES AND AMDOVIRUSES

The genus *Parvovirus* contains a wide range of viruses of mammals and birds, some of which cause major diseases in their animal hosts. The viruses are characterised by having dissimilar hairpins at the 5′ and 3′ ends of the genome, two mRNA promoters and a single polyadenylation site at the 3′ end [1]. Most members of the genus

Table 1. Taxonomy and characteristics of members of the *Parvovirinae*

	Parvovirus	Amdovirus	Dependovirus	Erythrovirus	Bocavirus	Parv4
5′ and 3′ Hairpin	Dissimilar		Identical	Identical	Not known	Not known
Promoters	2	1	3	1	1	1
Open reading frames (large)	2	2	2	2	3	2
Polyadenylation sites	One	Multiple	One	Multiple	Multiple	Multiple
Packaging strands	Mainly negative	Negative	Both strands	Both strands	Not known	Both strands
Human members	None	None	Several different AAV serotypes	B19V 3 genotypes	HBoV 3+ genotypes	Parv4 3 genotypes

DOI: 10.1002/rmv

package only negative stranded DNA molecules. No member of this family is known to infect humans, and they will not be considered further in this review.

Similarly there are no human members of the *Amdovirus* genus, the genus that currently only the Aleutian mink disease virus belongs to. Re-evaluation of the transcription profile has shown that this virus has a single promoter, and produces 6 different transcripts with polyadenylation signals in the mid and 3′ end of the genome [2]. The virus is unusual in that replication is enhanced by caspase activity, which is needed to cleave the non-spliced non-structural protein [3].

HUMAN DEPENDOVIRUSES

Discovery

The *Dependoviruses*, or adeno-associated viruses (AAVs) as they were originally known, were the first parvoviruses to be found in humans. Although originally identified as contaminants in adenovirus preparations they were first isolated directly from human samples in 1967 [4]. Subsequently, dependoviruses have been identified in a wide range of mammalian and avian species. Till date at least nine different dependovirus serotypes have been described in primates [5], and AAVs -1, -2, -3, 8 and 9 are common human infections [5,6].

Biology

The virions package equal numbers of positive and negative stranded DNA, and the genome has 'identical' inverted terminal repeat sequences at the 5′ and 3′ end. Efficient virus replication is dependent on co-infection with helper viruses, usually adenovirus or herpes virus, although some replication can also be detected in the absence of helper virus. The viruses have three mRNA promoters, and a single polyadenylation signal at the 3′ end.

Cell culture and tropism

Based on cell culture studies, it was originally thought that after infection of a cell the virus could enter one of two distinct but interchangeable pathways: either the lytic pathway or viral integration. In the presence of helper virus, generally adenovirus or herpes virus, then all transcripts are produced, the DNA is efficiently replicated, new capsids are produced, the DNA is packaged and the new virions are released into the environment by cell lysis. However, in the absence of helper infection, only the Rep transcripts are expressed and there is limited DNA replication. In cell culture studies with high multiplicities of infection the viral genome can become integrated into the host cell genome. This integration is not random, but dependent on shared sequences in the terminal hairpin structure and the host chromosome that the Rep protein recognises (the Rep-binding site) to give a targeted integration. For AAV2 integration this tends to be a specific region on chromosome 19 [7,8], but other sites can also be targeted. It was this propensity for targeted integration that was the reason for the initial interest in developing dependoviruses for gene therapy [9]. However, in the absence of Rep proteins, AAV integration is both inefficient and random, and increasingly it is recognised that cells can be efficiently transduced with AAV vectors without the requirement for integration, with the formation of episomal monomeric and concatameric circular DNA molecules [10]. Dependoviruses probably persist in a similar structure in adenoidal tissue following natural infection.

Different AAVs have different cell tropisms and this is mainly dependant on the different capsid structures of the virus, with heparin, sialic acid and/or the laminin receptor being used as the receptor for the human AAVs [11–13]. This is widely exploited in the gene therapy field to allow targeting of vectors to different tissues.

Epidemiology

Although original studies in the 1970's suggested that AAV infections were very common with ~80% of 14 year olds having neutralising antibody [14], these studies were done in the presence of helper virus (adenovirus 2) which may have confounded the results. More recent studies with recombinant AAV (no need for helper virus) have suggested that the seroprevalence is much lower in Europe, Australia and America, with ~30% of adults with antibody to AAV2 and 10–20% with antibody to the other human serotypes [6,15].

Disease association

Although AAV sequences have been found in genital [16] and placental tissues [17], and a role

DOI: 10.1002/rmv

in fetal loss has been suggested, these findings have not been reproducible and there is no evidence that any of the dependoviruses are pathogenic.

HUMAN ERYTHROVIRUSES

Discovery

Human B19V is the type member of the *Erythrovirus* genus. The virus was initially discovered in the serum of an asymptomatic blood donor (coded 19 in panel B, hence its name) as a cause of false-positive results in counter-immunoelectrophoresis tests for the detection of hepatitis B virus surface antigen [18]. Subsequently, related parvoviruses have been identified in cynomolgus monkeys [19], pig-tailed macaques and rhesus macaques [20]. These erythroviruses share up to 60% homology with B19V, have a similar genome organisation and share similar biological behaviour in natural hosts [21]. More recently, even closer related erythroviruses were identified in serum [22,23] and tissue samples [24] from humans. Although it was initially suggested that these might be new virus families, it is now recognised that they represent different genotypes of B19V [25]. There is no evidence for different serotypes of B19V, and the different B19V genotypes have a similar biology and pathogenicity.

Biology

Erythroviruses are characterised by packaging both positive and negative stranded DNA. They have a single promoter at the 5′ end, and polyadenylation signals in the mid genome and 3′ end. There are two small open reading frames that encode proteins of Mr approximately 7500 and 11 000. The function of these proteins is not clearly known, although the B19V Mr 11 000 protein is essential for efficient B19V replication and production of infectious virus in cell culture [26].

Cell culture and tropism

B19V is highly erythrotropic. *In vivo*, B19V replication and production of infectious virus occurs preferentially in late erythroid progenitor cells found predominantly in the bone marrow. *In vitro* the virus can be cultured in some erythromegakaryoblastoid cell lines [27,28], but replication is very inefficient, and the infection is not fully permissive. Efficient *in vitro* replication can only be observed in CD36+ erythroid progenitor cells [29].

Erythroid specificity is in part due to the tissue distribution of the receptor for B19V, the blood group P antigen [30]. This antigen, also known as globoside, is found in the erythrocyte precursors in bone marrow as well as in a variety of other tissues, including megakaryocytes, endothelial cells, placenta, fetal myocardium and fetal liver. Rare individuals who lack the blood group P antigen are naturally resistant to B19V infection [31].

Although globoside is necessary for cell infection it is not sufficient, and additional receptors are required for cell entry. Alpha5 beta1 [32] and Ku80 [33] both have been proposed as additional putative receptors. In addition to cell entry, efficient DNA processing and replication only occurs in erythroid cells, although as with most parvoviruses this can be overcome by co-infection with helper virus such as adenovirus [34].

Epidemiology

B19V infection is ubiquitous, being found in all parts of the world. Virtually all the infection is with B19V genotype 1 (the original genotype identified). Only rarely has acute genotype 2 infection been identified, although the B19V genotype 2 has been found in tissue samples [35–37]. It has been proposed that genotype 2 was more prevalent in the past, but that it was replaced by genotype 1 in the 1960's [38,39]. In contrast, B19V genotype 3 is found predominantly in parts of Africa where it seems to be the main circulating genotype [40,41]. Following acute infection B19V DNA persists in tissue, probably for the rest of the person's life, and it has been proposed that examining tissues can give a 'Bioportfolio' of the viruses an individual has been exposed to [38].

In all parts of the world B19V infection is a common illness of childhood; by 20 years of age, approximately 50% of children have detectable IgG [42–44]. In temperate climates, most infections occur in the spring, with mini-epidemics occurring at regular intervals several years apart. Secondary infection rates approach 50% in households [45] but are lower for adults in schools or other institutions [46]. Transmission is predominantly via the respiratory route, probably by droplet

DOI: 10.1002/rmv

spread, and is highest at the time of viraemia, before the onset of rash or arthralgia.

B19V infections also can occur as a result of transfusion of blood or blood products [47]. Estimates of the prevalence of B19V DNA in blood donors vary widely with the sensitivity of the assay used for detection, but in studies with sensitive PCR assays, ~1% may have low levels of virus [48], whereas 1:40 000 donors have high titres of virus [49]. Most reported cases of transfusion-related infection are due to pooled components, and virtually all pooled products contain parvovirus B19V [50]. B19V is relatively resistant to heat and is not inactivated by solvent–detergent [51]. All pooled plasma products are now screened for B19V DNA, and in Europe the European Pharmacopoeia [52] requires that pooled plasma products have a B19V viral titre of $<10^4$ IU/ml to reduce the risk of transmission.

Disease associations

Most infections caused by parvovirus B19 are asymptomatic or mild. When infection is associated with symptoms, a variety of disease manifestations, depending on the immune status of the host, can be observed (Table 1).

Erythema infectiosum

The most common manifestation of B19V infection is erythema infectiosum, a mild febrile illness with rash [53]. Erythema infectiosum is also known as 'fifth disease' as the fifth of the historical six infectious diseases of childhood associated with rash, the others being measles (first), scarlet fever (second), rubella (third), Duke's disease, now no longer recognised as a distinctive entity (fourth) and roseola subitum (sixth). The illness is also known as 'slapped cheek disease' due to the characteristic facial rash that is seen in children with infection. Studies in adult volunteers demonstrated a minor febrile illness that began approximately 8 days after nasal inoculation of virus and was associated with mild haematological abnormalities during the second week and facial rash at 17–18 days [54].

The rash usually begins on the face with the typical slapped cheek and then can spread to the trunk were it often has a lacy reticular appearance. Occasionally the rash can be more florid and rarely it can present as papular-purpuric gloves and socks syndrome (PPGSS), characterised by fever, swelling and petechiae [55].

Polyarthropathy syndrome

Although uncommon in children, arthropathy occurs in ~50% of adults and is more common in women than men [56]. The joint distribution is often symmetrical, with arthralgia and even frank arthritis affecting the small joints of the hands and occasionally the ankles, knees and wrists. Resolution usually occurs within a few weeks, but persistent or recurring symptoms can continue for years.

Transient aplastic crisis (TAC)

Transient reticulocytopenia followed by rebound reticulocytosis occurs in most subjects infected with B19V [57]. However, this transient marrow suppression is not associated with symptoms in most individuals. In individuals with increased erythropoiesis (e.g. haemolytic disorders and haemoglobulinopathies) and who depend on continual rapid production of red blood cells to prevent anaemia, B19V infection causes TAC [45,58]. TAC often requires symptomatic treatment with blood transfusions, and if not treated promptly may lead to severe complications. In one retrospective study the crude risk of cerebrovascular complications within 5 weeks after B19-associated aplastic crises in homozygous sickle cell disease was 58 times greater than expected [59]. Patients with TAC are viraemic and, therefore, infectious at the time of clinical presentation.

Chronic bone marrow failure and pure red cell aplasia (PRCA)

In patients who cannot mount an efficient immune response, B19V infection can become persistent, with high titre B19 viraemia and a resulting pure red cell aplasia (PRCA) or chronic anaemia. PRCA has been reported in a wide range of immunocompromised patients, including those with congenital immunodeficiency (i.e. Nezelof syndrome), acquired immunodeficiency syndrome and lymphoproliferative disorders (especially acute lymphoblastic leukaemia) as well as transplant recipients [60]. Along with the high levels of B19V DNA detectable in the serum, patients have a persistent anaemia associated with reticulocytopenia and absent or low levels of B19-specific antibody, bone marrow examination often reveals the presence of

DOI: 10.1002/rmv

scattered giant pronormoblasts. Temporary cessation of chemotherapy may result in an immune response to B19V and resolution of infection. Alternatively, administration of immune globulin may lead to a prompt drop in viral DNA titres in the blood and resolution of infection.

Although B19 primarily targets the red cell precursor, other haematologic lineages can be affected. Transient neutropenia, lymphopenia and thrombocytopenia have been observed during acute B19 infection [54]. Parvovirus occasionally causes a haemophagocytic syndrome [61]. Rare cases of idiopathic thrombocytopenia (ITP) and Henoch–Schönlein purpura have been reported to follow B19 infection [62], although transient erythroblastopenia of childhood (TEC) and aplastic anaemia do not appear to be caused by B19V infection.

B19V infection in pregnancy

Maternal infection with B19V during pregnancy can lead to miscarriage or the development of hydrops fetalis. Prospective studies indicate that the risk of transplacental fetal infection is about 30%, and the risk of fetal loss, predominantly in the early second trimester, about 9% [63]. The estimated risk of congenital infection after maternal B19 infection is <1% [64]. Although B19V does not appear to be teratogenic, anecdotal cases of possible eye damage and central nervous system abnormalities in infants whose mothers have experienced B19 infection during pregnancy have been reported [65]. In addition, several cases of congenital anaemia after maternal infection have been described [66].

B19V probably causes 10–20% of all cases of non-immune hydrops [67]. The pathogenesis is a combination of cardiac decomposition due to severe anaemia and B19-induced fetal myocarditis. Intrauterine blood transfusion can prevent fetal loss in some cases [68,69].

Less common presentations

A large number of clinical presentations, associated with detection of parvovirus DNA in serum and tissue, have been reported (Table 3) [62,70]. While, B19V DNA can be detected by PCR for years in normal tissue [36,71,72], with unknown clinical significance, in many cases it confounds the interpretation of B19 disease associations.

Diagnosis

Because viraemia is transient (2–4 days) in immunocompetent individuals, the diagnosis of acute B19V infection is usually based on detection of IgM antibodies [73]. Due to the importance of the conformational epitopes, commercial assays using recombinant viral-like particles expressed in insect or yeast cells are the preferred option. B19V specific IgM antibodies can be detected at the time of rash in fifth disease, and by the third day of aplastic crisis in patients with haematologic disorders. IgM antibodies remain detectable for 2–3 months after infection.

B19V specific IgG antibodies become detectable at the same time as IgM, and remain detectable thereafter for life. Because >50% of the population have B19 IgG antibodies, detection is not helpful for the diagnosis of acute infection.

In immunocompromised patients, or patients presenting with TAC, the B19V IgM and IgG may remain negative and diagnosis should be made by the detection of high titre B19V DNA in blood or serum. Diagnosis of fetal infection can be based on detection of virus in amniotic fluid and placental tissue.

In acute infection viraemia $>10^{12}$ B19V DNA genome copies per ml of serum can be detected at the height of viraemia, but titres fall rapidly within 2 days. Patients with aplastic crisis or PRCA due to B19 generally have $>10^5$ B19V DNA genome copies per ml of serum. Although most PCR-based assays

Table 2. Diseases associated with human parvovirus B19 infection

Disease	Host(s)
Fifth disease (erythema infectiosum)	Healthy children
Polyarthropathy syndrome	Healthy adults (especially women)
Transient aplastic crisis (TAC)	Patients with increased erythropoiesis
Persistent anaemia/pure red cell aplasia	Immunodeficient or immunocompetent patients
Hydrops fetalis/congenital anaemia	Fetus (<20 weeks)

DOI: 10.1002/rmv

Table 3. Unusual manifestations of parvovirus B19 infection

Body System	Disease
Hepatobiliary	Hepatitis
Cardiovascular	Myocarditis Vasculitis
Rheumatological	Arthritidies (including adult and juvenile arthritis)
Renal	Glomerulonephritis
Neurologic disease	Encephalitis Brachial neuropathy

will detect B19V genotype 1, primers specifically designed to detect genotypes 2 and 3 are required to detect all three genotypes. Low titre B19 DNA may be detectable by PCR for many months after acute infection [74], and tissues probably remain positive for the rest of the person's life [38]. Therefore, quantitative PCR should be used for diagnosis.

Prevention and treatment

No antiviral drug is available for treatment of B19V infection. However, because the humoral immune response plays a prominent role in controlling B19V infection, commercial immune globulin preparations from healthy blood donors can be used to treat, and in some cases cure, persistent B19V infection in immunocompromised patients. Generally, the dose used is 400 mg/kg/day for 5–10 days [75,76]. Treatment of patients with rash illness or polyarthropathy is symptomatic only; administration of immune globulin is not beneficial.

Currently, no vaccine is approved for B19V, although a recombinant viral preparation based on viral-like particles expressed in insect cells has been evaluated in phase 1 trials and the results were promising [77]. A second phase I/II trial was terminated in September 2008 and results are awaited.

HUMAN BOCAVIRUSES

Discovery

Bocaviruses are viruses that infect the respiratory and gastrointestinal tract of young animals, and the genus is named for the two original members of the genus bovine parvovirus (BPV1) and canine minute virus (aka canine parvovirus type 1).

A HuBoV was first identified in a study to identify novel pathogens in pooled respiratory samples of Swedish children with lower respiratory tract infections using random PCR [78]. Two libraries of DNA clones were obtained and of the >800 clones analysed, 62 contained bocavirus sequences. A specific PCR was designed, and testing individual samples confirmed a novel bocavirus in 17 of 540 (3.3%) of clinical samples. Subsequent studies have identified HBoV in respiratory samples from most parts of the world [78,79]. Similar sequences have also been identified in the faecal samples of children with gastrointestinal illness [80].

Using similar methods of virus purification and random amplification three additional HuBoV have been identified in faecal samples and named HBoV2 [81], HBoV3 [82]and HBoV4 (GenBank FJ973561), respectively. The sequences are sufficiently diverse that it has been suggested that they are different viruses, but there is no evidence to indicate whether they are antigenically different or not.

Biology

Although the nucleotide and genomic organisation of BPV1 was first characterised in 1986 [83], the transcription pattern of BPV1 was only identified more recently [84]. Both BPV and CPV1 have different palindromic hairpins at the 5′ and 3′ end [85,86], but till date the complete hairpin structure for none of the HuBoV has been characterised. In contrast to the other parvovirus genera, bocaviruses have a third 'large' open reading frame in the middle of the genome that encodes a nucleophosphoprotein (NP1). All bocaviruses appear to use a single promoter with multiple polyadenylation signals for transcription of the genome [84,87].

Cell culture and tropism

The replication cycle of BPV1 has been most often studied in primary fetal lung cells, [83] although the virus will also grow in embryonic bovine tracheal cells [88]. The virus binds to sialic acid, and glycophorin A has been proposed as a possible cellular receptor [89]. HuBoV cannot be grown in conventional culture and till date have only be cultured in a pseudostratified human airway epithelium cell culture system [87]. Although the

DOI: 10.1002/rmv

authors were able to demonstrate the production of spliced transcript and increase in viral DNA, replication was not very efficient, perhaps in keeping with the known requirement of parvoviruses for replicating cells. None of the other ocaviruses have been grown in culture, and the cellular receptor for none of the HuBoV is known.

Epidemiology

HBoV capsid proteins have been expressed in insect cells using baculovirus, and used in a number of seroepidemiology studies [90–94]. All the studies suggest that HuBoV is a common infection, but the results vary widely from studies suggesting that >90% of 5 year olds have antibody [90,91], to those suggesting that only 30% of Chinese children under 9 have detectable antibody [92]. This is surprising given that all are using very similar bocavirus viral-like particles, in a similar indirect format, and clearly reflects marked differences in sensitivity and/or specificity. So far there have been no studies using the other HuBoV as antigen, although the marked similarity in the protein sequence would suggest that there would be substantial cross-reactivity. It is presumed that it was antibody to HuBoV that was related to the earlier observation that antibody to BPV1 could be detected in human sera [95].

In contrast to the relatively small number of seroprevalence studies, there have been a large number of studies published on the detection of bocavirus DNA in respiratory samples, faecal samples, blood and/or other tissues. Based on these studies HuBoV appears to be a common infection and is found in all parts of the world. Bocaviral DNA is generally found in respiratory and faecal samples of children, but can occasionally be found in respiratory samples from adults [96–98]. Bocaviral DNA is rarely detected in blood, and it has not been detected in plasma pools.

Disease associations

There have been a large number of studies on the frequency of HuBoV DNA in respiratory secretions, especially in children with acute respiratory infection. A wide range of different assays has been used, amplifying different parts of the genome, and almost certainly with different levels of sensitivity and specificity. What is clear is that HBoV has worldwide endemicity, and can be detected in 1.5–19% of respiratory samples from symptomatic hospitalised children [99]. Positive children are generally less than 2 years old, and likely to be symptomatic with lower respiratory tract infection. However, there have been relatively few studies with asymptomatic children of this age group. When studies with control groups have been performed HBoV is detected rarely or in a significantly lower number of patients [99–104]. However, in symptomatic children, HBoV is frequently not the only pathogenic virus detected, and thus the role of HBoV as a pathogenic virus is not clear cut, especially given the propensity for parvoviruses to be detected in tissues for long periods of time, and of increased parvoviral replication by 'helper' viruses.

However, there is increase in evidence that the virus is pathogenic, and especially associated with wheezing and respiratory disease in young children [105]. In these cases, HBoV is often the only virus detected, and quantitative PCR suggests that in these cases the viral load is higher in the respiratory secretions ($>10^4$ genome copies/ml), more likely to be accompanied by detection of HBoV in blood [105], and the detection of an IgM response [106,107].

HBoV has also been detected in faecal samples, and its role as a cause of childhood gastroenteritis is also unclear. In different studies around the world, HBoV has been identified in between 0.5 and 5.5% of cases [108–113]. In most of the these studies, other pathogens have been found in the HBoV positive samples, and in one control study there was no difference in the detection rate of HboV in symptomatic and control patients [110].

Similar results have been found for the other HuBoV found in faecal samples. HBoV2 was found in 5/98 children (none with gastroenteritis) in Pakistan, but in only 3/699 of stool samples submitted to Edinburgh for enteric bacteriology [81]. In a case control study in Australia, HBoV2 was found in 17% (32/186) of cases of acute gastroenteritis, and only 8% of controls [82]. In contrast, in the one study reported so far on HBoV3, there was no difference in the detection of virus in patients with acute gastroenteritis or controls [82]. Further studies are required to determine whether any of the HuBoV are associated with some cases of childhood gastroenteritis, and also their relationship to the

DOI: 10.1002/rmv

commonly observed faecal parvoviruses remains to be established [114].

HUMAN PARV4

Discovery

Parv4 was originally identified in a patient with acute viral syndrome [115] as part of a study to identify novel viral agents using similar methods to those used to identify HuBoV. The patient was an intravenous drug user, co-infected with hepatitis C and unfortunately was lost to follow up. Parv4 and 'Parv5' (now recognised as a second Parv4 genotype) DNA have been detected in pooled plasma collections [116], and a third Parv4 genotype was more recently identified in bone marrow and lymphoid tissue of African patients with HIV infection [117].

Biology

The virions appear to package equal amounts of negative and positive stranded DNA [118]. The virus has not been grown in culture, but preliminary studies of the transcription map based on transfection of the DNA into cells suggest that Parv4 has a single promoter with multiple polyadenylation sites [119], and does not group with any of the other parvovirus genera. Although on original identification it was suggested that it should be classified as a member of the *Erythrovirus* genus, it will probably be classified as the type member of a new parvovirus genera, along with a group of similar animal viruses identified in pigs and cattle [120].

Cell culture and tropism

Parv4 does not grow in standard cell culture or bone marrow culture, and till date has not been successfully cultured *in vitro*. Like B19V, Parv4 DNA has been identified in plasma pools, and in bone marrow, lymphoid tissue, skin and liver samples [121–124] but it is not known if any of these tissues are the primary site of replication in the body.

Epidemiology

Parv4 is a common contaminant of plasma pools, and can be detected in ~4% of plasma pools [125]; the titres of Parv4 are also much lower than those seen with B19V prior to plasma pool screening. Furthermore, the virus has been found in ~3% of USA and Thailand blood donors, although unpublished data suggest that it is much lower in European volunteer blood donors. The finding of viral sequences in bone marrow and lymphoid tissue of intravenous drug users (17/24) as opposed to HIV-positive men who had sex with men (0/13), indicates that the virus can be transmitted by parenteral means [126], but whether this is the only route of transmission, is still unknown. Similarly, Parv4 sequences were detected in 13% (13/87) liver samples [124]; more than 50% of the Parv4 positive livers were co-infected with HCV. When the same 87 liver samples were tested for B19V DNA, 68% were positive.

Parv4 virus-like particles have also been expressed in insect cells and used to develop assays for seroprevalence. Although the numbers tested are small at the moment, Parv4 antibody was detected in 15/33 intravenous drug users and 15/35 haemophiliacs but could not be detected in any (0/50) orthopedic outpatient controls.

Disease associations

So far no disease has been associated with Parv4 infection. The original patient did have 'acute viral infection syndrome' [115], but it is not known if this was associated with a primary Parv4 infection, or was a co-incidental finding.

REFERENCES

1. Tattersall P, Bergoin M, Bloom ME, *et al*. Parvoviridae. In *Virus Taxonomy: Classification and Nomenclature of Viruses: Eighth Report of the International Committee on Taxonomy of Viruses*, Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds). Elsevier Academic Press: New York, 2005; 353–369.
2. Qiu J, Cheng F, Burger LR, *et al*. The transcription profile of Aleutian mink disease virus in CRFK cells is generated by alternative processing of pre-mRNAs produced from a single promoter. *J Virol* 2006; **80**: 654–662.
3. Best SM, Shelton JF, Pompey JM, *et al*. Caspase cleavage of the nonstructural protein NS1 mediates replication of Aleutian mink disease parvovirus. *J Virol* 2003; **77**: 5305–5312.
4. Blacklow NR, Hoggan MD, Rowe WP. Isolation of adenovirus-associated viruses from man. *Proc Natl Acad Sci USA* 1967; **58**: 1410–1415.

DOI: 10.1002/rmv

5. Gao G, Vandenberghe LH, Alvira MR, *et al*. Clades of adeno-associated viruses are widely disseminated in human tissues. *J Virol* 2004; **78**: 6381–6388.
6. Calcedo R, Vandenberghe LH, Gao G, *et al*. Worldwide epidemiology of neutralizing antibodies to adeno-associated viruses. *J Infect Dis* 2009; **199**: 381–390.
7. Kotin RM, Linden RM, Berns KI. Characterization of a preferred site on human chromosome 19q for integration of adeno-associated virus DNA by non-homologous recombination. *EMBO J* 1992; **11**: 5071–5078.
8. Weitzman MD, Kyostio SR, Kotin RM, *et al*. Adeno-associated virus (AAV) Rep proteins mediate complex formation between AAV DNA and its integration site in human DNA. *Proc Natl Acad Sci USA* 1994; **91**: 5808–5812.
9. Daya S, Berns KI. Gene therapy using adeno-associated virus vectors. *Clin Microbiol Rev* 2008; **21**: 583–593.
10. Penaud-Budloo M, Le GC, Nowrouzi A, *et al*. Adeno-associated virus vector genomes persist as episomal chromatin in primate muscle. *J Virol* 2008; **82**: 7875–7885.
11. Summerford C, Samulski RJ. Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. *J Virol* 1998; **72**: 1438–1445.
12. Wu Z, Miller E, Gbandje-McKenna M, *et al*. Alpha2,3 and alpha2,6 N-linked sialic acids facilitate efficient binding and transduction by adeno-associated virus types 1 and 6. *J Virol* 2006; **80**: 9093–9103.
13. Akache B, Grimm D, Pandey K, *et al*. The 37/67-kilodalton laminin receptor is a receptor for adeno-associated virus serotypes 8, 2 3, and 9. *J Virol* 2006; **80**: 9831–9836.
14. Parks WP, Boucher DW, Melnick JL, *et al*. Seroepidemiological and ecological studies of the adeno-virus-associated satellite viruses. *Infect Immunol* 1970; **2**: 716–722.
15. Halbert CL, Miller AD, McNamara S, *et al*. Prevalence of neutralizing antibodies against adeno-associated virus (AAV) types 2, 5 and 6 in cystic fibrosis and normal populations: implications for gene therapy using AAV vectors. *Hum Gene Ther* 2006; **17**: 440–447.
16. Bantel-Schaal U. zur Hausen H. Characterization of the DNA of a defective human parvovirus isolated from a genital site. *Virology* 1984; **134**: 52–63.
17. Tobiasch E, Rabreau M, Geletneky K, *et al*. Detection of adeno-associated virus DNA in human genital tissue and in material from spontaneous abortion. *J Med Virol* 1994; **44**: 215–222.
18. Cossart YE, Field AM, Cant B, *et al*. Parvovirus-like particles in human sera. *Lancet* 1975; **i**: 72–73.
19. Brown KE, Green SW, Gallinella G, Anderson S, Young NS, Anderson DC, *et al*. Isolation of a novel simian parvovirus (SPV) from cynomologus monkeys with fatal anemia [Abstract]. *Blood* 1993; **82** (Suppl 1): 311a.
20. Green SW, Malkovska I, O'Sullivan MG, *et al*. Rhesus and pig-tailed macaque parvoviruses: identification of two new members of the Erythrovirus genus in monkeys. *Virology* 2000; **269**: 105–112.
21. O'Sullivan MG, Anderson DK, Goodrich JA, *et al*. Experimental infection of cynomolgus monkeys with simian parvovirus. *J Virol* 1997; **71**: 4517–4521.
22. Nguyen QT, Sifer C, Schneider V, *et al*. Novel human erythrovirus associated with transient aplastic anemia. *J Clin Microbiol* 1999; **37**: 2483–2487.
23. Nguyen QT, Wong S, Heegaard ED, *et al*. Identification and characterization of a second novel human erythrovirus variant. *Virology* 2002; **301**: 374–380.
24. Hokynar K, Soderlund-Venermo M, Pesonen M, *et al*. A new parvovirus genotype persistent in human skin. *Virology* 2002; **302**: 224–228.
25. Servant A, Laperche S, Lallemand F, *et al*. Genetic diversity within human erythroviruses: identification of three genotypes. *J Virol* 2002; **76**: 9124–9134.
26. Zhi N, Mills IP, Lu J, *et al*. Molecular and functional analyses of a human parvovirus B19 infectious clone demonstrates essential roles for NS1, VP1, and the 11-kilodalton protein in virus replication and infectivity. *J Virol* 2006; **80**: 5941–5950.
27. Shimomura S, Komatsu N, Frickhofen N, *et al*. First continuous propagation of B19 parvovirus in a cell line. *Blood* 1992; **79**: 18–24.
28. Miyagawa E, Yoshida T, Takahashi H, *et al*. Infection of the erythroid cell line, KU812Ep6 with human parvovirus B19 and its application to titration of B19 infectivity. *J Virol Method* 1999; **83**: 45–54.
29. Wong S, Zhi N, Filippone C, *et al*. Ex vivo-generated CD36+ erythroid progenitors are highly permissive to human parvovirus B19 replication. *J Virol* 2008; **82**: 2470–2476.
30. Brown KE, Anderson SM, Young NS. Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science* 1993; **262**: 114–117.
31. Brown KE, Hibbs JR, Gallinella G, *et al*. Resistance to parvovirus B19 infection due to lack of virus receptor (erythrocyte P antigen). *N Engl J Med* 1994; **330**: 1192–1196.
32. Weigel-Kelley KA, Yoder MC, Srivastava A. Alpha5beta1 integrin as a cellular coreceptor for human parvovirus B19: requirement of functional

DOI: 10.1002/rmv

activation of beta1 integrin for viral entry. *Blood* 2003; **102**: 3927–3933.
33. Munakata Y, Saito-Ito T, Kumura-Ishii K, *et al*. Ku80 autoantigen as a cellular coreceptor for human parvovirus B19 infection. *Blood* 2005; **106**: 3449–3456.
34. Guan W, Wong S, Zhi N, *et al*. The genome of human parvovirus b19 can replicate in nonpermissive cells with the help of adenovirus genes and produces infectious virus. *J Virol* 2009; **83**: 9541–9553.
35. Hokynar K, Soderlund-Venermo M, Pesonen M, *et al*. A new parvovirus genotype persistent in human skin. *Virology* 2002; **302**: 224–228.
36. Kuethe F, Lindner J, Matschke K, *et al*. Prevalence of parvovirus B19 and human bocavirus DNA in the heart of patients with no evidence of dilated cardiomyopathy or myocarditis. *Clin Infect Dis* 2009; **49**: 1660–1666.
37. Corcioli F, Zakrzewska K, Rinieri A, *et al*. Tissue persistence of parvovirus B19 genotypes in asymptomatic persons. *J Med Virol* 2008; **80**: 2005–2011.
38. Norja P, Hokynar K, Aaltonen LM, *et al*. Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. *Proc Natl Acad Sci USA* 2006; **103**: 7450–7453.
39. Norja P, Eis-Hubinger AM, Soderlund-Venermo M, *et al*. Rapid sequence change and geographical spread of human parvovirus B19: comparison of B19 virus evolution in acute and persistent infections. *J Virol* 2008; **82**: 6427–6433.
40. Candotti D, Etiz N, Parsyan A, *et al*. Identification and characterization of persistent human erythrovirus infection in blood donor samples. *J Virol* 2004; **78**: 12169–12178.
41. Candotti D, Danso K, Parsyan A, *et al*. Maternal-fetal transmission of human parvovirus B19 genotype 3. *J Infect Dis* 2006; **194**: 608–611.
42. Centers for Disease Control. Risks associated with human parvovirus B19 infection. *MMWR Morb Mortal Wkly Rep* 1989; 38: 81–97.
43. Cohen BJ, Buckley MM. The prevalence of antibody to human parvovirus B19 in England and Wales. *J Med Microbiol* 1988; **25**: 151–153.
44. Kelly HA, Siebert D, Hammond R, *et al*. The age-specific prevalence of human parvovirus immunity in Victoria, Australia compared with other parts of the world. *Epidemiol Infect* 2000; **124**: 449–457.
45. Chorba T, Coccia P, Holman RC, *et al*. The role of parvovirus B19 in aplastic crisis and erythema infectiosum (fifth disease). *J Infect Dis* 1986; **154**: 383–393.
46. Gillespie SM, Cartter ML, Asch S, *et al*. Occupational risk of human parvovirus B19 infection for school and day-care personnel during an outbreak of erythema infectiosum. *J Am Med Assoc* 1990; **263**: 2061–2065.
47. Azzi A, Morfini M, Mannucci PM. The transfusion-associated transmission of parvovirus B19. *Transfus Med Rev* 1999; **13**: 194–204.
48. Kleinman SH, Glynn SA, Lee TH, *et al*. Prevalence and quantitation of parvovirus B19 DNA levels in blood donors with a sensitive polymerase chain reaction screening assay. *Transfusion* 2007; **47**: 1756–1764.
49. Mortimer PP, Luban NL, Kelleher JF, *et al*. Transmission of serum parvovirus-like virus by clotting-factor concentrates. *Lancet* 1983; **ii**: 482–484.
50. Saldanha J, Minor P. Detection of human parvovirus B19 DNA in plasma pools and blood products derived from these pools: implications for efficiency and consistency of removal of B19 DNA during manufacture. *Br J Haematol* 1996; **93**: 714–719.
51. Sayers MH. Transfusion-transmitted viral infections other than hepatitis and human immunodeficiency virus infection. Cytomegalovirus, Epstein-Barr virus, human herpesvirus 6, and human parvovirus B19. *Arch Pathol Lab Med* 1994; **118**: 346–349.
52. Council of Europe. Human plasma (pooled and treated for virus inactivation). # 1646. *European Pharmacopoeia* 2005; 5: 1747–1748
53. Anderson MJ, Lewis E, Kidd IM, *et al*. An outbreak of erythema infectiosum associated with human parvovirus infection. *J Hyg (Lond)* 1984; **93**: 85–93.
54. Anderson MJ, Higgins PG, Davis LR, *et al*. Experimental parvoviral infection in humans. *J Infect Dis* 1985; **152**: 257–265.
55. Smith PT, Landry ML, Carey H, *et al*. Papular-purpuric gloves and socks syndrome associated with acute parvovirus B19 infection: case report and review. *Clin Infect Dis* 1998; **27**: 164–168.
56. Woolf AD, Campion GV, Chishick A, *et al*. Clinical manifestations of human parvovirus B19 in adults. *Arch Intern Med* 1989; **149**: 1153–1156.
57. Potter CG, Potter AC, Hatton CS, *et al*. Variation of erythroid and myeloid precursors in the marrow and peripheral blood of volunteer subjects infected with human parvovirus (B19). *J Clin Invest* 1987; **79**: 1486–1492.
58. Koch WC, Massey GV. Aplastic crisis. *Pediatr Rev* 1990; **12**: 142–148.
59. Wierenga KJ, Serjeant BE, Serjeant GR. Cerebrovascular complications and parvovirus infection in homozygous sickle cell disease. *J Pediatr* 2001; **139**: 438–442.
60. Brown KE, Young NS. Persistent parvovirus B19 infection. *Rev Med Microbiol* 1995; **6**: 246–256.
61. Muir K, Todd WT, Watson WH, *et al*. Viral-associated haemophagocytosis with parvovirus-B19-related pancytopenia. *Lancet* 1992; **339**: 1139–1140.
62. Török TJ. Unusual clinical manifestations reported in patients with parvovirus B19 infection. In

DOI: 10.1002/rmv

Human Parvovirus B19, Anderson LJ, Young NS (eds). Monographs in Virology 20: 61–92. 1997; Basel, Karger. Monographs in Virology. Parks, W. P. 1997.
63. Public Health Laboratory Service. *Laboratory Investigation of Rubella*, (1st edn). Her Majesty's Stationary Office: London, 1982.
64. Miller E, Fairley CK, Cohen BJ, *et al*. Immediate and long term outcome of human parvovirus B19 infection in pregnancy. *Br J Obstet Gynaecol* 1998; **105**: 174–178.
65. Weiland HT, Vermey-Keers C, Salimans MM, *et al*. Parvovirus B19 associated with fetal abnormality [letter]. *Lancet* 1987; **i**: 682–683.
66. Brown KE, Green SW, Antunez de Mayolo J, *et al*. Congenital anaemia after transplacental B19 parvovirus infection. *Lancet* 1994; **343**: 895–896.
67. Jordan JA. Identification of human parvovirus B19 infection in idiopathic nonimmune hydrops fetalis. *Am J Obstet Gynecol* 1996; **174**: 37–42.
68. Fairley CK, Smoleniec JS, Caul OE, *et al*. Observational study of effect of intrauterine transfusions on outcome of fetal hydrops after parvovirus B19 infection. *Lancet* 1995; **346**: 1335–1337.
69. Enders M, Weidner A, Zoellner I, *et al*. Fetal morbidity and mortality after acute human parvovirus B19 infection in pregnancy: prospective evaluation of 1018 cases. *Prenat Diagn* 2004; **24**: 513–518.
70. Heegaard ED, Brown KE. Human parvovirus B19. *Clin Microbiol Rev* 2002; **15**: 485–505.
71. Soderlund-Venermo M, Hokynar K, Nieminen J, *et al*. Persistence of human parvovirus B19 in human tissues. *Pathol Biol (Paris)* 2002; **50**: 307–316.
72. Brown KE. Detection and quantitation of parvovirus B19. *J Clin Virol* 2004; **31**: 1–4.
73. Anderson LJ, Tsou C, Parker RA, *et al*. Detection of antibodies and antigens of human parvovirus B19 by enzyme-linked immunosorbent assay. *J Clin Microbiol* 1986; **24**: 522–526.
74. Musiani M, Zerbini M, Gentilomi G, *et al*. Parvovirus B19 clearance from peripheral blood after acute infection. *J Infect Dis* 1995; **172**: 1360–1363.
75. Kurtzman GJ, Cohen B, Meyers P, *et al*. Persistent B19 parvovirus infection as a cause of severe chronic anaemia in children with acute lymphocytic leukaemia. *Lancet* 1988; **ii**: 1159–1162.
76. Frickhofen N, Abkowitz JL, Safford M, *et al*. Persistent B19 parvovirus infection in patients infected with human immunodeficiency virus type 1 (HIV-1): a treatable cause of anemia in AIDS. *Ann Intern Med* 1990; **113**: 926–933.
77. Ballou WR, Reed JL, Noble W, *et al*. Safety and immunogenicity of a recombinant parvovirus B19 vaccine formulated with MF59C. 1. *J Infect Dis* 2003; **187**: 675–678.
78. Allander T, Tammi MT, Eriksson M, *et al*. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA* 2005; **102**: 12891–12896.
79. Sloots TP, McErlean P, Speicher DJ, *et al*. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. *J Clin Virol* 2006; **35**: 99–102.
80. Vicente D, Cilla G, Montes M, *et al*. Human bocavirus, a respiratory and enteric virus. *Emerg Infect Dis* 2007; **13**: 636–637.
81. Kapoor A, Slikas E, Simmonds P, *et al*. A newly identified bocavirus species in human stool. *J Infect Dis* 2009; **199**: 196–200.
82. Arthur JL, Higgins GD, Davidson GP, *et al*. A novel bocavirus associated with acute gastroenteritis in Australian children. *PLoS Pathog* 2009; **5**: e1000391.
83. Chen KC, Shull BC, Moses EA, *et al*. Complete nucleotide sequence and genome organization of bovine parvovirus. *J Virol* 1986; **60**: 1085–1097.
84. Qiu J, Cheng F, Johnson FB, *et al*. The transcription profile of the bocavirus bovine parvovirus is unlike those of previously characterized parvoviruses. *J Virol* 2007; **81**: 12080–12085.
85. Chen KC, Shull BC, Lederman M, *et al*. Analysis of the termini of the DNA of bovine parvovirus: demonstration of sequence inversion at the left terminus and its implication for the replication model. *J Virol* 1988; **62**: 3807–3813.
86. Sun Y, Chen AY, Cheng F, *et al*. Molecular characterization of infectious clones of the minute virus of canines reveals unique features of bocaviruses. *J Virol* 2009; **83**: 3956–3967.
87. Dijkman R, Koekkoek SM, Molenkamp R, *et al*. Human bocavirus can be cultured in differentiated human airway epithelial cells. *J Virol* 2009; **83**: 7739–7748.
88. Abdel-Latif L, Murray BK, Renberg RL, *et al*. Cell death in bovine parvovirus-infected embryonic bovine tracheal cells is mediated by necrosis rather than apoptosis. *J Gen Virol* 2006; **87**: 2539–2548.
89. Thacker TC, Johnson FB. Binding of bovine parvovirus to erythrocyte membrane sialylglycoproteins. *J Gen Virol* 1998; **79**: 2163–2169.
90. Endo R, Ishiguro N, Kikuta H, *et al*. Seroepidemiology of human bocavirus in Hokkaido prefecture, Japan. *J Clin Microbiol* 2007; **45**: 3218–3223.
91. Kahn JS, Kesebir D, Cotmore SF, *et al*. Seroepidemiology of human bocavirus defined using recombinant virus-like particles. *J Infect Dis* 2008; **198**: 41–50.
92. Lin F, Guan W, Cheng F, *et al*. ELISAs using human bocavirus VP2 virus-like particles for detection of antibodies against HBoV. *J Virol Method* 2008; **149**: 110–117.

DOI: 10.1002/rmv

93. Cecchini S, Negrete A, Virag T, *et al*. Evidence of prior exposure to human bocavirus as determined by a retrospective serological study of 404 serum samples from adults in the United States. *Clin Vaccine Immunol* 2009; **16**: 597–604.
94. Zhao LQ, Qian Y, Zhu RN, *et al*. Human bocavirus infections are common in Beijing population indicated by sero-antibody prevalence analysis. *Chin Med J (Engl)* 2009; **122**: 1289–1292.
95. Mengeling WL, Paul PS. Antibodies for autonomous parvoviruses of lower animals detected in human serum. Brief report. *Arch Virol* 1986; **88**: 127–133.
96. Costa C, Bergallo M, Cavallo R. Detection of Human Bocavirus in bronchoalveolar lavage from Italian adult patients. *J Clin Virol* 2009; **45**: 81–82.
97. Garbino J, Soccal PM, Aubert JD, *et al*. Respiratory viruses in bronchoalveolar lavage: a hospital-based cohort study in adults. *Thorax* 2009; **64**: 399–404.
98. Longtin J, Bastien M, Gilca R, *et al*. Human bocavirus infections in hospitalized children and adults. *Emerg Infect Dis* 2008; **14**: 217–221.
99. Schildgen O, Muller A, Allander T, *et al*. Human bocavirus: passenger or pathogen in acute respiratory tract infections? *Clin Microbiol Rev* 2008; **21**: 291–304 table.
100. Allander T, Jartti T, Gupta S, *et al*. Human bocavirus and acute wheezing in children. *Clin Infect Dis* 2007; **44**: 904–910.
101. Fry AM, Lu X, Chittaganpitch M, *et al*. Human bocavirus: a novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. *J Infect Dis* 2007; **195**: 1038–1045.
102. Maggi F, Andreoli E, Pifferi M, *et al*. Human bocavirus in Italian patients with respiratory diseases. *J Clin Virol* 2007; **38**: 321–325.
103. Kesebir D, Vazquez M, Weibel C, *et al*. Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. *J Infect Dis* 2006; **194**: 1276–1282.
104. Garcia ML, Calvo C, Pozo F, *et al*. Human Bocavirus infections in sporish 0–14 year old: clinical and epidemiological characterisations of an emerging respiratory virus. *An Pediatr (Barc)* 2007; **67**: 212–219.
105. Allander T, Jartti T, Gupta S, *et al*. Human bocavirus and acute wheezing in children. *Clin Infect Dis* 2007; **44**: 904–910.
106. Soderlund-Venermo M, Lahtinen A, Jartti T, *et al*. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. *Emerg Infect Dis* 2009; **15**: 1423–1430.
107. Lindner J, Karalar L, Zehentmeier S, *et al*. Humoral immune response against human bocavirus VP2 virus-like particles. *Viral Immunol* 2008; **21**: 443–449.
108. Nakanishi K, Tsugawa T, Honma S, *et al*. Detection of enteric viruses in rectal swabs from children with acute gastroenteritis attending the pediatric outpatient clinics in Sapporo, Japan. *J Clin Virol* 2009; **46**: 94–97.
109. Campe H, Hartberger C, Sing A. Role of human bocavirus infections in outbreaks of gastroenteritis. *J Clin Virol* 2008; **43**: 340–342.
110. Cheng WX, Jin Y, Duan ZJ, *et al*. Human bocavirus in children hospitalized for acute gastroenteritis: a case-control study. *Clin Infect Dis* 2008; **47**: 161–167.
111. Chieochansin T, Thongmee C, Vimolket L, *et al*. Human bocavirus infection in children with acute gastroenteritis and healthy controls. *Jpn J Infect Dis* 2008; **61**: 479–481.
112. Yu JM, Li DD, Xu ZQ, *et al*. Human bocavirus infection in children hospitalized with acute gastroenteritis in China. *J Clin Virol* 2008; **42**: 280–285.
113. Lau SK, Yip CC, Que TL, *et al*. Clinical and molecular epidemiology of human bocavirus in respiratory and fecal samples from children in Hong Kong. *J Infect Dis* 2007; **196**: 986–993.
114. Oliver AR, Phillips AD. An electron microscopical investigation of faecal small round viruses. *J Med Virol* 1988; **24**: 211–218.
115. Jones MS, Kapoor A, Lukashov VV, *et al*. New DNA viruses identified in patients with acute viral infection syndrome. *J Virol* 2005; **79**: 8230–8236.
116. Fryer JF, Kapoor A, Minor PD, Delwart E, Baylis SA. Novel parvovirus and a related variant present in pooled human plasma for fractionaltion. *Emerg Infect Dis* 2006; **12**: 151–154.
117. Simmonds P, Douglas J, Bestetti G, *et al*. A third genotype of the human parvovirus PARV4 in sub-Saharan Africa. *J Gen Virol* 2008; **89**: 2299–2302.
118. Fryer JF, Delwart E, Bernardin F, *et al*. Analysis of two human parvovirus PARV4 genotypes identified in human plasma for fractionation. *J Gen Virol* 2007; **88**: 2162–2167.
119. Zhi N, Wong S, Brown KE. Determination of transcriptional profile of a novel human parvovirus (Parv4) [Abstract]. *25th Annual Meeting, American Society for Virology* 2006.
120. Lau SK, Woo PC, Tse H, *et al*. Identification of novel porcine and bovine parvoviruses closely related to human parvovirus 4. *J Gen Virol* 2008; **89**: 1840–1848.

DOI: 10.1002/rmv

121. Botto S, Bergallo M, Sidoti F, *et al*. Detection of PARV4, genotypes 1 and 2, in healthy and pathological clinical specimens. *New Microbiol* 2009; **32**: 189–192.
122. Longhi E, Bestetti G, Acquaviva V, *et al*. Human parvovirus 4 in the bone marrow of Italian patients with AIDS. *AIDS* 2007; **21**: 1481–1483.
123. Manning A, Russell V, Eastick K, *et al*. Epidemiological profile and clinical associations of human bocavirus and other human parvoviruses. *J Infect Dis* 2006; **194**: 1283–1290.
124. Schneider B, Fryer JF, Reber U, *et al*. Persistence of novel human parvovirus PARV4 in liver tissue of adults. *J Med Virol* 2008; **80**: 345–351.
125. Fryer JF, Delwart E, Hecht FM, *et al*. Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from blood donors and symptomatic individuals. *Transfusion* 2007; **47**: 1054–1061.
126. Simmonds P, Manning A, Kenneil R, *et al*. Parenteral transmission of the novel human parvovirus PARV4. *Emerg Infect Dis* 2007; **13**: 1386–1388.

DOI: 10.1002/rmv